International Symposium for High-Performance Thin-Layer Chromatography

HPTLC 2011

Book of abstracts

BASEL, 06th - 08th July 2011

Some statistics

223 Abstract submissions

52 Lectures

165 Posters

333 Participants from 41 countries (16th June 2011)

- 20 % from India
- 15 % from Germany
- 13 % from Switzerland
- 11 % from France
- 4 % from Poland and Russian Foederation each
- 3 % from Brasil, Rumania and USA each
- 2 % from China, Italy and Spain each
- 18 % from residual 29 countries

41 % Academics

36 % Industry

22 % Students

60 % Women

Edited and revised by Prof. Dr. Gertrud (Gerda) Morlock

University of Hohenheim, Stuttgart and Justus-Liebig-University Gießen

Germany, June 2011

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The series of international HPTLC Symposia

6-8 June 2011, **Basel**
(21st) International Symposium for High-Performance Thin-Layer Chromatography, HPTLC 2011

11-13 June 2008, **Helsinki**
(20th) International Symposium for Thin-Layer Chromatography, HPTLC 2008

9-11 October 2006, **Berlin**
(19th) International Symposium for Thin-Layer Chromatography, HPTLC 2006

29-31 May 2005, **Siofok**
(18th) Planar Chromatography 2005

23-25 May 2004, **Visegrad**
(17th) Planar Chromatography 2004

15-18 October 2003, **Lyon**
(16th) International Symposium for TLC

21-23 June 2003, **Budapest**
(15th) Planar Chromatography 2003 (in honor of Professor Thihak)

4-6 October 2002, **Novo mesto**
(14th) Planar Chromatography Today 2002

11-13 May 2002, **Keszthely**
(13th) Planar Chromatography 2002 (in honor of Doctor Geiss)

23-25 June 2001, **Lillafüred**
(12th) Planar Chromatography 2001

11-13 May 2000, **Lillafüred**
(11th) Planar Chromatography 2000 (in honor of Professor Kaiser)

16-19 May 1998, **Visegrad**
10th International Symposium on Instrumental Planar Chromatography (60 years TLC + 10 years JPC)

9-11 April 1997, **Interlaken**
9th International Symposium on Instrumental Planar Chromatography

5-7 April 1995, **Interlaken**
8th International Symposium on Instrumental Planar Chromatography

23-26 March 1993, **Brighton**
7th International Symposium on Instrumental Planar Chromatography

23-26 April 1991, **Interlaken**
Sixth International Symposium on Instrumental Planar Chromatography

21-24 February 1989, **Brighton**
(Fifth) International Symposium on Instrumental High Performance Thin-Layer Chromatography

22–25 September 1987, **Selvino**
Fourth International Symposium on Instrumental High Performance Thin-Layer Chromatography

17–19 April 1985, **Würzburg**
Third International Symposium on Instrumental High-Performance Thin-Layer Chromatography

2-6 May 1982, **Interlaken**
Second International Symposium on Instrumental High-Performance Thin-Layer Chromatography

18-21 May 1980, **Bad Dürkheim**
First International Symposium on Instrumentalized High-Performance Thin-Layer Chromatography

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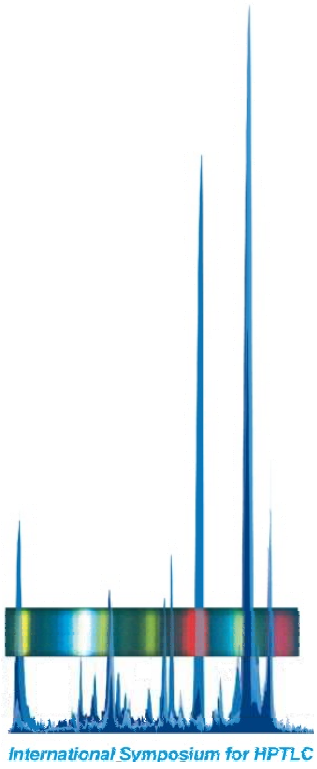

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Thursday 7th July 2011, 14:45 - 15:15

Poster Award Committee Meeting for final decision on the awardees

Thursday 7th July 2011, 19:20 - 19:40

Poster awards

Schedule of Oral Presentations

Wednesday, 6th

8.00	Registration starts
9.00	Tutorial: Hyphenations in HPTLC with UV/Vis/FLD/MS/FTIR/NMR/bioassays *Gertrud Morlock, Germany*
9.00	Tutorial: HPTLC for the analysis of botanical materials and medicinal plants *Eike Reich, Switzerland*
10.00	Coffee break (30 min)
10.30	Opening ceremony starts with the traditional and famous "Basler Morgenstraich"
10.50 – 12.30	**Session 1 – Fundamentals** ***Chair: Dr. Friedrich Geiss***
	Solvents and solvent selection for thin-layer chromatography *Colin Poole, USA*
	The latest achievements and prospects for pressurized planar electrochromatography *Tadeusz Dzido, Poland*
	The new variant of multidimensional planar chromatography *Svetlana Khrebtova, Russia*
	TLC for pharmaceutical analysis in resource limited countries *Eliangiringa Kaale, Tanzania*
	Influence of magnetic and electrostatic fields on amino acids' and biogenic amines' retention in TLC *Irena Malinowska, Poland*
	Fluorescence detection by intensity changes for AMD-TLC separations of lipids *Vincente Cebolla, Spain*
12.30	Lunch (1h)
13.30 – 15.00	**Session 2 - Miniaturization and trends** ***Chair: Prof. Dr. Mario Aranda, Chile***
	Ultra Thin Layer Chromatography Using Electrospun Nanofibers *Susan Olesik, USA*
	Ultrathin-layer chromatography on nanoengineered films *Steven Jim, Canada*
	Fabrication and Chromatographic Separations on Binder-Free, Carbon Nanotube-Fabricated Thin Layer Chromatography Plates *Matthew Lindfort, USA*
	Micro PLC to detect falsified fruit preservation *Rudolf Kaiser, Germany*

15.40 – 17.30	**Session 3 - Planar chromatography as preferred tool** ***Chair: Prof. Dr. Bernd Spangenberg and Dr. Kathrin Kabrodt, Germany***
	Planar chromatography as analytical tool in synthetic biology *Irena Vovk, Slovenia*
	HPTLC as comprehensive tool for analytical determinations *Mario Vega, Chile*
	Pharmaceutical applications of HPTLC as a problem solving technique *Clare McKinlay, UK*
	High-throughput planar solid phase extraction – a new clean-up concept in multi-residue analysis of pesticides *Wolfgang Schwack, Germany*
	Normal-phase HPTLC separation of salicylic acid, lactic acid, retinoic acid, chlorogenic acid, caffeic acid and caffeine *A. Jamshidi, Iran*
	Assessing the quality of topical formulations of Calendula officinalis with HPTLC *Snezana Agatonovic-Kustrin, Australia*
19.30	Official symposium dinner
22.00	End

Thursday, 7th

9.00 – 10.20	**Session 4 - Traditional Medicine** ***Chair: Prof. Dr. Irmgard Merfort, Germany***
	The TLC atlas of Chinese crude drugs *Zheng-Tao Wang, China*
	Marker Profiling – Evidence Based Approach for Quality Evaluation of Herbs in Indian System of Medicine *Pulok K. Mukherjee, India*
	Simple Densitometric-TLC Analysis of Non-Chromophore Containing *Wanchai De-Eknamkul, Thailand*
	Application of HPTLC for GI stability evaluation of Psoralen, Bakuchiol and Bakuchicin in simulated physiological fluids *L Sathiyanarayanan, India*
10.20	Poster session and coffee break (40 min)
11.00 – 12.20	**Session 5 - Food supplements and phytochemistry** ***Chair: Prof. Dr. Olumuyiwa Ogegbo, England***
	Utilization of HPTLC for authentication and identification of botanical specimens *Troy Smillie, USA*
	TLC with biodetection to screen plant extracts for the presence of potential drugs to be used in neurodegenerative diseases *Monika Waksmundzka-Hajnos, Poland*

	HPTLC: simple and effective tool to detect adulterated mixtures of Eurycoma longifolia root *Joseph Goh Keng Sean, Malaysia*
	Estimation of ursolic acid and luteolin in *Phyla nodiflora* (L.) Greene using HPTLC *Sangita Shukla, India*
12.20	Lunch (1h)
13.20 – 14.45	**Session 6 - Phytopharmaceutical industry** ***Chair: Dr. Mieczyslaw Sajewicz, Poland***
	The use of (HP)TLC in phytoindustrial R&D *Clemens Erdelmeier, Germany*
	Application of (HP)TLC as essential part of *Phytoneering* at Bionorica – is this analytical tool still able to compete with state of the art and novel high standard analytical tools? *Gudrun Abel, Germany*
	HPTLC and HPTLC/MS studies on some bioactive natural products *J. Madhusudana Rao, India*
	Use of HPTLC in quality control & standardisation of herbal medicinal plants & products *Mohammad Kamil, United Arab Emirates*
14.45	Poster session and coffee break (40 min)
15.25 – 17.00	**Session 7 - Validation and regulatory aspects** ***Chair: Prof. Dr. Colin Poole, USA***
	European regulatory framework and quality control of (traditional) herbal medicinal products *Werner Knöss, Germany*
	Validation of TLC procedures *Bernd Renger, Germany*
	Development of stability indicating chromatographic evaluation method for moxonidine in pharmaceutical formulations *Rajendra B. Kakde, India*
	Chemical profiling of exstesy recoverd from around Jakarta by HPTLC-spectrophotodensitometry *I M.A.Gelgel Wirasuta, Indonesia*
	RP-HPTLC estimation of benzhexol HCl and trifluperazine HCl in pharmaceutical dosage form *M.S.Charde, India*
17.00	**Panel discussion** with invited speakers (coffee available) ***Chair: Prof. Dr. Matthias Hamburger, Switzerland***
17.45 – 19.20	**Session 8 - Manufacturers session** ***Chair: Pierre Bernard-Savary, CCCM France***
	Recent instrumentation developments at CAMAG *Rolf Rolli, Switzerland*

	Future perspectives of TLC; The dominant marked leader steps ahead *Mehmet Dogan, Germany*
	HPTLC-MALDI-TOF-MS coupling for the MS and MS/MS analysis maintaining the chromatographic resolution *Martin Schürenberg, Germany*
	Phytochemical certified samples by BotaniCert, a new french startup *Do Thi Kieu Tiên, France*
	Natural compounds purification by Counter-Current Chromatography CPC/CCC *Gregoire Audo, France*
19.20	Poster award and manufacturers' cocktail
20.00	End

Friday, 8th

9.00 – 11.00	**Session 9 - Coupling with MS** ***Chair: Prof. Dr. G. Morlock, Germany and Prof. Dr. Jentaie Shiea, Taiwan***
	Coupling HPTLC and mass spectrometry: how and why *Vilmos Kertesz, USA*
	The coupling of HPTLC with DART mass spectrometry *Elena Chernetsova, Russia*
	On versatility of the TLC-MS interface and its analytical importance *Teresa Kowalska, Poland*
	Application of TLC-MS in water analysis *Wolfram Seitz, Germany*
	Improved HPTLC separation of lipids by using Automated Multiple Development (AMD) and identification with TLC-MS-Interface *Ingo Schellenberg, Germany*
	Supporting the chemist decision in small molecule leads discovery through an open and easy access automated HPTLC-MS-ELSD platform *Francis Maquin, France*
	Controlling the insulin purification process using HPTLC–MS *Michael Schulz, Germany*
11.00	Coffee break (30 min)
11.30 – 12.55	**Session 10 - Effect-directed detection** ***Chair: Dr. J. Madhusudana Rao, India***
	Bioassays as a detection tool in TLC *Irena Choma, Poland*
	Potential applicability of modern bioautography (BioArena) in the study of plant ingredients *Ágnes Móricz, Hungary*
	Free radical-scavenging activity (FRSA) of sea urchins pigments *Alexander Shikov, Russia*

	Effect-directed analysis of landfill leachates using HPTLC/AMD with bioluminescence detection *Stefan Weiss, Germany*
	HPTLC-bioluminescence detection with *Vibrio fischeri* - Method enhancements and its potential in the field of consumer goods *Vera Baumgartner, Switzerland*
13.00	Closing ceremony
13.15	Lunch
14.15	Sightseeing of Novartis Campus or Basel City
17.30	End

List of Poster Presentations

Poster group 1: Fundamentals & miniaturization

1a Fabrication and chromatographic separations on binder-free, carbon nanotube-fabricated thin-layer chromatography plates

1b Office Chromatography

1c Development of planar chromatography in 1980-2010 (scientometric study)

1d About definitions of sorption chromatography

1e A new variant of the S-chamber with an ultra small gas volume

1f The variants of circular ascending TLC

1g Circular TLC with the closed sorption layer under low pressure

1h Use of frontal and displacement chromatography in TLC to concentrate impurities

1i A new approach for multidimensional TLC evaluation

1j Determination of performance relevant parameters of HPTLC plates

1k Reproducibility studies of the peak pattern in HPTLC-spectrophotodensitometry for drug profiling propose

1l Quasi-continuous videodensitometric recording of chromatograms during the development process

1m Synthesis and purification of inflammatory radiotracer-andrographolide

1n Virtualization of HPTLC Experiments: Opportunities and Challenges

1o TLC determination of the Abraham's Solvatochromic parameters for small organic molecules

1p Determination of lipophilicity of some newly synthesized potential antimalarials by the means of reversed-phase thin-layer chromatography

Poster group 2: Detection of free radical-scavenging activity (DPPH• reagent)

2a Identification of antioxidant principles in Indian medical plant *Morinda citrifolia* using HPTLC-DPPH• method

2b Screening of antioxidant principles in green tea using HPTLC-DPPH• method

2c A method of screening the antioxidant activity of *Solanum nigrum* linn. Extracts

2d Identification of antioxidant principles in Indian medical plant *Bacopa monerei* using HPTLC-DPPH• method

2e Optimalization of a HPTLC-DPPH• method with image processing to assess the antiradical activity of phenolic plant constituents

2f Screening for antioxidant activity of the ayurvedic formulation Triphala by HPTLC-DPPH• method

2g Simultaneous quantitative determination of major phenolics in rosemary extract *via* DPPH• free-radical-scavenging activity

2h Search for substances with antioxidant activity in Amazonian plants: development of a methodology based on HPTLC

2i Screening for new cosmetic preservatives from the French Riviera: HPTLC application to antimicrobial and antioxidant assays

Poster group 3: Hyphenations, also with effect-directed detection

3a Hyphenations in HPTLC

3b Screening for bioactive secondary metabolites in sponges by HPTLC coupled with bioluminescence bacteria assay followed by HRMS

3c Performance data for the new TLC-MS Interface

3d Analytical characterisation of rhubarb fractions for application in cosmetics and pharmaceutical products by using TLC-MS Interface

3e Antimicrobial activity of *Chelidonium majus* and anticancer drug Ukrain by TLC-direct bioautography

3f Low temperature TLC-bioassay on silica gel plates for screening volatile samples for free radical scavengers and antioxidants

3g Standardized bioautographic methods for effect directed screening of samples separated on HPTLC plates

3h Potential applicability of modern bioautography (BioArena) in the study of plant ingredients

3i Development of TLC-direct bioantography tests based on *Escherichia coli* and *Bacillus sutilis* strains

3j TLC coupled with electrospray laser desorption ionization (ELDI) mass spectrometry for high-throughput analysis

3k HPTLC analysis, antioxidant activity and xanthine oxidase inhibitory activity of Indian medicinal plants

Poster group 4: Environmental analysis

4a HPLC-MS/MS, HPLC-TOF MS or simply HPTLC? Quantification of sucralose in various aqueous environmental matrices using HPTLC multidetection

4b HPTLC method for determination of active ingredient in novel insecticide formulations

4c Contribution of AMD-HPTLC fluorescence and UV scanning densitometry to the characterization of heavy petroleum products

4d HPTLC profile of substituted coumarins derived from microbial transformation

4e Development of new solvent system for the analysis of triazophos poison

4f HPTLC method for detection of chlorantraniliprole and flubendiamide

Poster group 5: Cosmetics and toxicological/forensic aspects

5a Analytical detection by TLC of methiocarb residues in biological samples

5b Identification and purification of bacterial lipids from *Enterococcus faecalis* 12030

5c Proposal for review of the quantitative monograph of Cinchonae with a better quantitative method using instrumental HPTLC and densitometry analysis

5d Extraction, isolation and detection of atropine from blood by new solvent system using HPTLC plate

5e New developments of HPTLC for the assay of detergents used for membrane proteins elucidation/crystallisation/characterisation

5f Development of a new densitometric TLC method for determination of asiaticoside content in *centella asiatica*

5g A validated quantitative HPTLC method for estimation of strychnine in *Strychnos nuxvomica* extract and marketed formulation

5h HPTLC analysis of hyoscyamine, scopolamine and their biosynthetic intermediates from *in vivo* and *in vitro* cultures of several *Solanaceae* plants

5i HPTLC evaluation of pyrrolizidine alkaloids extracted from *Tussilao farfara* and *Petasites officinalis*

5j Authentication of neutral henna leaves by HPTLC

5k Separation evaluation of selected organophosphorus fungicides by NP-TLC and RP-HPTLC

5l Quantitation of isotretinoin in topical microemulsion and microemulsion based gel formulations and their *in vitro* permeation study

5m A new HPTLC method for the assay of thiopental in postmortem blood in a fatal case of suicide

5n HPTLC: a valuable method for rapid analysis of rare orchids' plant extracts

5o Development of new solvent system for the analysis of cypermethrin

5p Screening of polyphenolic compounds in glycolic plant extract

5q Validated HPTLC method for the determination of nicotine in Tobacco (*Nicotiana tabacum* L.) extracts

Poster group 6: Pharmaceutical and clinical analysis

6a Stability study and densitometric determination of efavirenz in tablet by normal phase TLC

6b HPTLC method for simultaneous estimation of lamivudine and zidovudine in tablet dosage form

6c Normal phase TLC and simultaneous densitometric determination of rosiglitazone and glimepiride in tablet dosage form

6d Chiral assay of enantiomersof (*R,S*)-fluoxetine in pharmaceutical formulations using liquid chromatography

6e Synthesis and in vitro anticancer evaluation of the different derivative of 1-(1H-benzo[d] imidazol-2-yl)ethanone

6f HPTLC determination of nifedipine in human serum after liquid-liquid extraction

6g HPTLC determination of sertraline in human serum after liquid-liquid extraction

6h Development and validation of an HPTLC method for estimation of risperidone in bulk and tablets - comparison with HPLC

6i An interlaboratory investigation on the use HPTLC to perform assays of lamivudine-zidovudine, metronidazole, nevirapine, and quinine composite samples

6j Simultaneous estimation of anticancer terpenoids andrographolide and betullinic acid by HPTLC method

6k Quantification of andrographolide from *Andrographis paniculata* and its optimization using plant growth regulators

6l A novel HPTLC method for the separation of coexisting purines and pyrimidines

6m DE-TLC assessment of the Brazilian porcine lung surfactant lipid composition

6n Simultaneous quantitative evaluation of memantine and donepezil by HPTLC

6o HPTLC method for simultaneous estimation of antihypertensive drugs

6p A novel HPTLC analytical method for quantification and separation of mycotoxin gliotoxin and related compounds in human samples

6q Validation of a HPTLC method for quantification of cardiolipin and other phospholipids classes in mitochondria

6r Development of HPTLC method for the estimation of ondansetron in bulk drug and sublingual tablets

6s Desloratadine quantification using HPTLC

6t HPTLC method for quantification of carbamazepine in formulations and invitro diffusion study

6u Urine HPTLC-metabonomics study of soy-phytoestrogens in Africans

6v Analysis of herbal gel by HPTLC method development and validation

6x Development of an HPTLC method for simultaneous estimation of ranitidine HCl and domperidone in their combined dosage form

6y HPTLC analysis of salicylic acid in drug release media during development of an anti acne patch

6z HPTLC determination of tramadol hydrochloride in a tablet formulation in presence of related impurities

Poster group 7: Analysis of medicinal plants and traditional medicines

7a Quantification of α-asrone in *Acorus calamus e*xtract by HPTLC densitometry

7b Multivariate analysis of Radix Linderane HPTLC analysis

7c Pattern recognition in HPTLC-fingerprints of medicinal plants

7d Application of HPTLC for identification of the Chinese herbal with and without processing – *Glycyrrhizae* Radix preparata and *Glycyrrhizae* Radix

7e Identification of the Chinese herbal formulas by HPTLC – Mahuang & apricot seed combination and Mahuang & Coix combination

7f HPTLC characterization of *Salvia* species used as medicinal in Valencian community (Spain)

7g A selective determination of chrysophanol in polyherbal oil containing *Cassia fistula* using HPTLC

7h Estimation of lupeol in ayurvedic oil formulation containing *Alstonia scholaris*

7i HPTLC method for analysis of colchicine in Unani formulations containing Suranjaan Talkh and Suranjaan Seerin

7j Development and validation of an HPTLC method for quantification of anti cancer compound in *Podophyllum hexandrum* callus culture

7k A validated HPTLC method for estimation of β-sitosterol from plants used as wound healing agents

7l Detection of the decomposition of aconitine in aconitum napellus mother tinctures V.2a and identification of the main cleavage products using HPTLC-MS

7m Detection and quantitation of disulfiram in traditional medicine administered to patients with alcohol dependence

7n Analysis of drug chemical compound in traditional medicine from tasikmalaya indonesia

7o Standardisation of herbal formulations by HPTLC by the evaluation of active ingredients present with marker compounds

7p Development of HPTLC fingerprint profile of Balacaturbhadrica Curna, Hinguvastaka Curna, Triphala Curna and Trikatu Curna

7q HPTLC interventions for analysis of major bioactive chemical constituents of *Eucalyptus* foliage, cultured *Cordyceps sinensis*, *Dicentra paucinervia* tubers and *Andrographis paniculata*

7r HPTLC studies and method development of some important herbal drugs used in Indian traditional systems of medicines

7s Isolation of phytoconstituents from the chloroform fraction of the aerial roots of *Ficus benghalensis*

7t Quantitative determination of L-DOPA in seeds of Mucuna pruriens germplasm by HPTLC

7u Chemical fingerprinting of Poncirus trifoliata using HPTLC and HPLC

7v HPTLC – A simple tool to distinguish adulterants and substituents

7w Establishment of a TLC fingerprint within the Apiaceae family

7x HPTLC: Monitoring artifacts of diosgenin during traditional and modified extraction of *Dioscorea deltoidea* Wall

7y Establishment of fingerprint of different plants originate from French territory used as phytomedicame

Poster group 8: Analysis of food, feed and commodities

8a Development of a rapid analysis m*ethod* for measurement of carotenoids content in paprika fruit by HPTLC

8b Indirect evaluation of garlic lines' antibacterial activities using HPTLC

8c Study on the bioactive content of *Butia capitata* Mart, an edible fruit from Cerrado, Brazil

8d Detection and quantitation of vitamin C and the preservatives in fruit juices sold in Indian market using HPTLC

8e Detection and quantitation of caffein in nonalcoholic beverages sold in Indian market using HPTLC

8f Identification and quantification of additives in PVC foils by HPTLC

8g High-throughput determination of teflubenzuron in medicated feed fish by HPTLC

8h Determination of biogenic amines in Chilean wines by HPTLC

8i HPTLC analysis of anthocyanins in *Lonicera caerulea* L. fruits

8j New HPTLC method for analysis of flavonoids and phenolic compounds in propolis

8k Ultrafast quantitation of 5-hydroxymethylfurfural in honey using HPTLC

8l Application of planar chromatography on determination of aflatoxin B_1 in silage

8m HPTLC method for quantification of Crocetin in saffron

8n HPTLC method for the analysis of oleanolic acid in *Luffa cylindrica* L. seeds

8o Quantification of glucoraphane in florets of Broccoli

8p TLC for screening of residues of fluazuron in food of animal origin

8q Optimized separation of bioactive phenolic compounds in various salad species, using cellulose HPTLC plates and CAMAG instruments

8r TLC method for determination of phytosterols in chocolate

8s Determination of polycyclic aromatic hydrocarbons in toys by HPTLC

8t Occurrence of some mycotoxins in wheat seeds from northwest Romania using HPTLC

8u High-throughput planar solid phase extraction – application to pesticide residue analysis in tea samples

8v Validated HPTLC methods for the determination of flavour compounds in plant extracts

8w Determination of biogenic amines in wine by TLC densitometry

8x A new method for quality control of soumbala from *Parkia biglobosa*, West Africa using a group of biomarkers

Poster group 9: Analysis of food supplements

9a Quantification of isoflavones in soy-based nutritional supplements by HPTLC analysis

9b HPTLC of saponins from the seeds of *Trigonella*

9c HPTLC illustration of the USP Dietary Supplements Compendium

9d HPTLC and the determination of quality of food supplements

9e Screening and determination of sibutramine in adulterated herbal slimming supplements by HPTLC

9f Comparative determination of sibutramine in commercially available natural slimming products by HPTLC and HPLC

Poster group 10: Herbal and plant analysis

10a Estimation of piperine in *Piper longum by* HPTLC and investigation of its anticholinesterase potential

10b Determination of mahanimbine in *Murraya koenigii* by HPTLC

10c HPTLC Determination of Coumarin in Aqueous and Alcoholic Extract and Oil of *Ganoderma Lucidum*

10d Determination of rosmarinic acid - crucial aspects of quantitative validation

10e A novel sesquiterpene acid and an alkaloid from leaves of the Eastern Nigeria mistletoe with potent immunostimulatory activity on C57BL6 splenocytes

10f Identification of $GABA_A$ receptor modulators with a twist: Discovery of aristolactone in a commercial sample of *Bupleurum chinense* root

10g Isolation and characterization of anthraquinone derivatives from *Ceratotheca triloba* (Bernh.) Hook f.

10h Evaluation of *Brachylaena discolour* a potential anti-diabetic source

10i Separation of biotransformation product using enzyme from Amazonian palm (*Bactris gasipaes*) by HPTLC

10j Screening, Isolation and structure elucidation of isothiocyanates and glucosinolates from *Moringa peregrina* (Forssk) fiori

10k Comparation of HPTLC and GC-FID methods for quantification of betulinic acid in *Eugenia florida* leaves extracts

10l HPTLC for quality control of multicomponent herbal drugs: Example of Cangzhu Xianglian San

10m TLC and HPLC comparison of the phenolic acid and flavonoid levels in selected sage (*Salvia*) species

10n Quantitative determination of flavonoid glycosides in extracts of *Potentilla* species

10o Initial investigations on four species of *osmanthus* by HPTLC

10p HPTLC, a versatile quality control tool: from single plant drug to complex polyherbal formulations

10q Development and validation of an HPTLC method for evaluation of betulinic acid in *Eugenia florida* leaves

10r Seasonal variation of betulinic acid in leaves from *Eugenla florida* by HPTLC densitometry

10s Development and validation of a HPTLC method for the quantitative determination of the flavanone glycoside naringin in *Drynaria quercifolia* fronds

10t Characterization of flavonolglycosides in *Calendula officinalis* (herba) by orthogonal HPTLC and HPLC-MS

10u Quantification of phytoconstituents of the leaves of *Paederia foetida* by HPTLC

10v Development of an HPTLC method for the determination of vicine in herbal extract and formulation

10w HPTLC method for separation and simultaneous quantification of withanoloids

10x HPTLC based quality assessment of some high altitude plant species: variation with altitude

10y Development and validation of HPTLC method for estimation of umbelliferone and quercetin in *Aegle marmelos* formulation

10z HPTLC-based estimation of plumbagin in the carnivorous plant *Nepenthes khasiana*

Tutorial 1

Hyphenations in HPTLC with UV/Vis/FLD, MS, FTIR, NMR and bioassays

Morlock G.

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Initially advocated by T. Hirschfeld, hyphenated methods mean to place all of the required spectrometers into a single system so that all of the spectroscopic information is obtained in a single run. Hypernation represents a logical, rapid and efficient strategy for obtaining the maximum possible information out of a single separation.

The major problems associated with column-based hypernations are capital cost and strategies for dealing with the large amounts of data that such systems produce. The complexity of the instumentation increases, which makes them difficult to operate in a routine way. A single eluent that is optimal for all detectors is difficult to obtain. Differences in sensitivity between spectroscopic techniques and spectrometers are challenging as well.

All these problems are much less challenging in HPTLC-based hypernations because of the open system that is (1) highly adaptive to different sensitivities, (2) cost-effective by modular instrumentation compared to the status quo in analysis, (3) generating less data due to targeted access to points-of-care on the plate, and (4) directly accessible for the respective optimal solvent because the eluent is evaporated after chromatography and not impacting the different detectors [1, 2]. The latter is extremely relevant for effect-directed detection with bioassays.

Existing hyphenations are discussed like:

- HPTLC/UV/Vis/FLD
- HPTLC/UV/Vis/FLD/MS
- HPTLC/UV/Vis/FLD/bioactivity/HRMS
- HPTLC/UV/FTIR
- HPTLC/UV/Vis/FLD/FTIR ATR
- TLC/Vis/SERS

[1] G. Morlock, W. Schwack, *Trends Anal Chem* 29 (2010) 1157-1171. [2] G. Morlock, W. Schwack *J. Chromatogr. A* 1217 (2010) 6600-6609.

Tutorial 2

HPTLC for the analysis of botanical materials and medicinal plants

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Traditionally TLC plays a central role in the identification of medicinal plants and derived products. It is part of corresponding quality monographs of most pharmacopoeias. In recent years there is an international trend to modernize and improve TLC methods which often date back to the 1970s.
High Performance TLC, the modern and most advanced approach to planar chromatography is replacing the classical TLC in this process. Significantly improved reliability, the use of digital images to convey information, full compliance with cGMP and new hyphenated detection modes are some of the features that increase the acceptance of HPTLC by analysts, regulators and researchers.

What exactly is HPTLC? What are the elements of a standardized HPTLC methodology? How are methods for identification of plants developed and validated? How can TLC methods be changed into HPTLC methods? The tutorial will provide answers.

A second part of the presentation will look at possibilities and new horizons brought by HPTLC to the analysis of plants: image comparison, pattern recognition, quantitative evaluation and biological tests.

O-1a

Solvents and solvent selection for TLC

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Solvents are universal tools for method development in separation science and sample preparation. Current methods of classifying solvents to facilitate selection for a particular problem are problematic and fail to characterize their properties adequately. They either fail to take solvent cohesion into account of underestimate contributions from polar interactions. A new approach based on the solvation parameter model is more successful with respect to both problems and has allowed us to accurately characterize solvents under the categories cohesive strength, dipolarity/polarizability, hydrogen-bonding, and electron lone pair interactions.

The practical use of the solvent classification for selection and optimization of solvents in liquid-phase microextration and thin-layer chromatography will be discussed. In addition the model can be used to predict the solvent strength parameter for solvents that lack experimental data for normal phase chromatography on silica gel and alumina. Optimization of the mobile phase composition for separations by reversed-phase chromatography is also demonstrated. An interesting feature of these studies is the identification of trifluoroethanol and formamide as useful solvents for method development that do not duplicate the properties of other solvents more commonly used during screening for initial solvent selection. These studies also identify a significant contribution from hydrogen-bonding acidity for solvents such as dimethylformamide, propylene carbonate, and dimethyl sulfoxide explaining anomalies in their separation properties when conventional solvent classification scales are used.

The more we know about solvents and their behaviour the better choices can be made in identifying the best solvent for a particular application. The new solvent classification scale developed here goes some way to meeting this goal and has the potential to provide a suitable guide for separations and sample preparation.

O-1b

The latest achievements and prospects for pressurized planar electrochromatography

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Pressurized planar electrochromatography (PPEC) is the mode in which the mobile phase is driven by electroosmosis. Nurok et al. published the first paper on PPEC in 2004 [1]. Our group has also been involved in the development of this mode since 2004 [2]. PPEC is a very attractive mode for application in laboratory practice with respect to its advantages such as short separation times, high kinetic performance, and different separation selectivity relative to liquid chromatography, especially TLC. However, in spite of these attractive features this mode is still at an early stage of development. The main reason for this is the existence of many constructional and methodical challenges, which researchers are facing. In this presentation, we will discuss some aspects of the last development of PPEC by our group, such as:

- construction of contemporary devices,
- methodical solutions to electrochromatogram development,
- equilibration of the separation system,
- performance of the separation system,
- application to quantitative analysis,
- application to two dimensional separation (2D) using PPEC and HPTLC (2D PPEC/HPTLC).

This discussion confirms previously mentioned advantages of both higher separation efficiency and sample throughput, which were not achievable before. These features offer excellent opportunities for the development of the method and its application in laboratory practice.

[1] Nurok, D., Koers, J. M., Novotny, A. L., Carmichael, M. A., Kosiba, J. J., Santini, R. E., Hawkins, G. L., Replogle, R. W., *Anal. Chem.* 76 (2004) 1690. [2] Dzido, T. H., Mróz, J., Jóźwiak, G., *J. Planar Chromatogr*. 17 (2004) 404.

O-1c

The new variant of multidimensional planar chromatography

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Multidimensional TLC is a development in planar chromatography affording the full use of different mobile phases for improved separations. We propose a new approach for development of *n*-dimensional TLC (for example, the working out of 5-dimensional TLC, Fig. 1). The following new approach was used: the primary separation of the mixture into groups of compounds with similar properties. After this separation the plate (on the aluminium or polymeric support) with partially or completely separated zones containing was cut into 4 narrow rectangular sub-plates that containing three groups of partially separated compounds: I) the compounds with high R_f; II) and III) the compounds with average R_f; IV) the compounds with low R_f. Since the values of R_f. are connected with the structure and properties of separated compounds, for separation of each group the most appropriate mobile phase was used. Each sub-plate was developed at right angles to the first direction. The new variant of chromatography permitted the separation of all components of the mixture.

Multidimensional separations in planar chromatography is especially expedient for the separation of multicomponent mixtures, since it facilitates the separation of substances of different sorption characteristics with high efficiency.

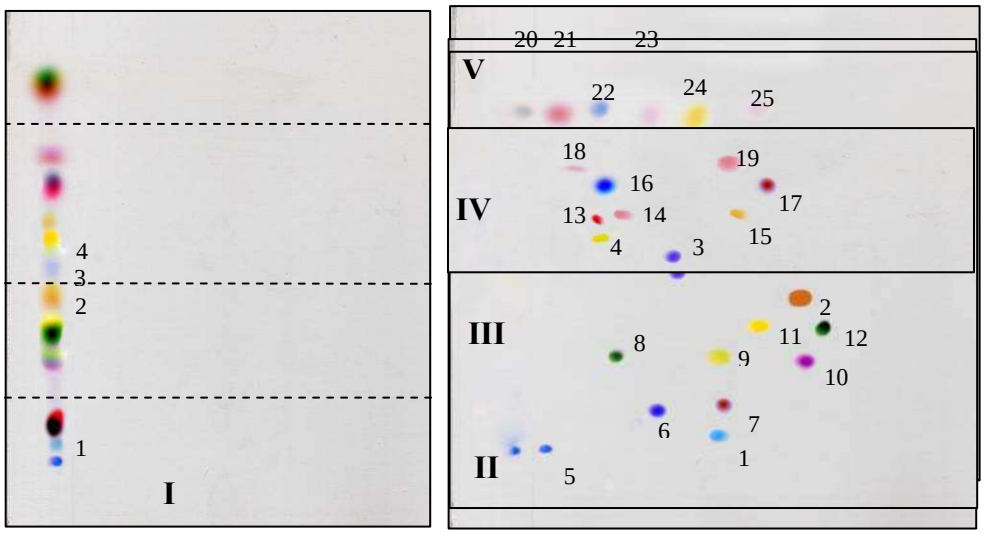

Fig 1. The chromatograms of the mixture obtained by the method of five-dimensional TLC. I- the chromatogram of the primary separation; II, III, IV, V – the chromatograms of the subsequent separations.

O-1d

TLC for pharmaceutical analysis in resource limited countries

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This paper discusses the sustainability and robustness advantages of planar chromatography that are critical to the successful performance of product quality assessments in resource limited areas including field applications. Because the robustness and ease of use, the training required for successful performance of the assessments is much lower than other technologies with comparable reproducibility such as HPLC. Some of the successful applications of this technology in resource limited countries are presented.

O-1e

Influence of magnetic and electrostatic fields on amino acids' and biogenic amines' retention in TLC

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One of the new trends in chromatography is the investigations of external factors on retention and efficiency in separation systems. From a theoretical point of view strong external electrostatic and magnetic fields can influence the separation of mixtures.

The application of magnetic and electrostatic fields to separations in planar chromatography is not complicated. The influence of the electrostatic and magnetic field on retention of biogenic amines and amino acids were investigated. In order to investigate the problem, chromatograms were developed simultaneously: without electric field, in an electric field (E = 1.4 kV/m (dry plates)) and in a magnetic field (H = 0.48 T). In figure 1 R_F values of the amino acids and biogenic amines are presented. Magnetic fields decrease retention of the investigated compounds, similar to electric fields. In magnetic and electric fields, aside the changed retention, the width of the chromatographic zone is also affected reducing efficiency.

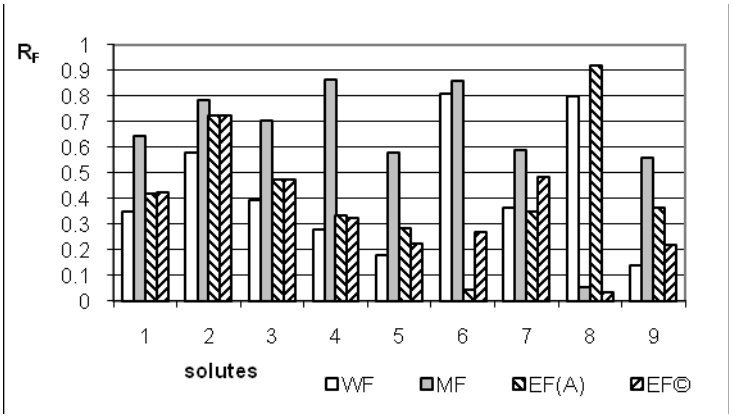

Fig. 1 R_F values of investigated amino acids (No 1-4) in mobile phase ethanol – water - ammonia 20 % (7:3:1, v/v/v) and biogenic amines (No 5-9) in mobile phase ethanol – chloroform - ammonia 20 % (7:1:1, v/v/v) obtained without electric field (*WF)*, in magnetic field *(MF)*, in electric field in anode side *(EF(A))*, in electric field in cathode side *(EF(B))*.

O-1f

Fluorescence detection by intensity changes for AMD-HPTLC separation of lipids

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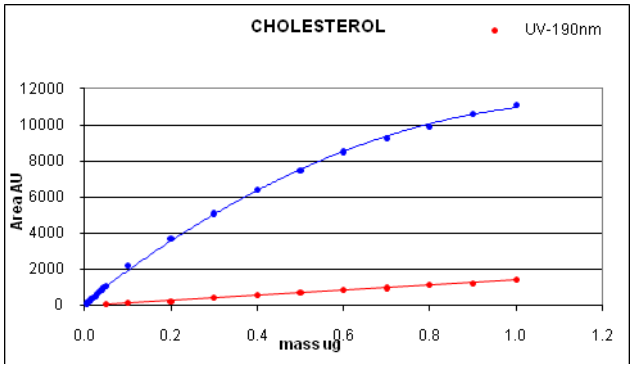
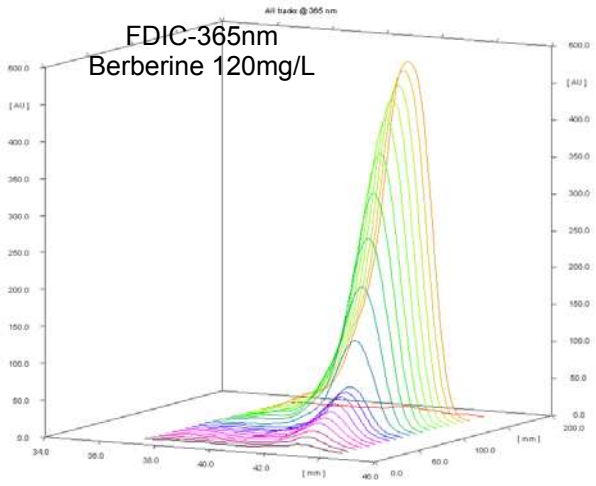
Automated Multiple Development (AMD), a gradient-based separation HPTLC technique, has been used to separate different families of lipids. Special attention is paid to the separation of sphingolipids, which are target analytes in the diagnosis and treatment monitoring of lysosomal storage diseases.

Fluorescence Detection by Intensity Changes (FDIC) allows a universal detection of lipids, including saturated lipidic structures. FDIC operates through the increases in emission experienced by the berberine cation, induced by non-specific interactions between this fluorophore and the polarizable hydrocarbon chain of the corresponding lipid, on silica gel plates when the system is excited using 365 nm.

The fluorescent molar responses of lipids and differences in response among families can be rationalized by a previously proposed model of FDIC response using computational calculations based on Molecular Mechanics. An explanation for the high FDIC response of cholesterol (LOD = 5 ng) and other cholesterol-derivatives is proposed. Unlike derivatization techniques, FDIC sensitivity can be tailored through a simple variation of fluorophore concentration.





O-2a

Ultrathin-layer chromatography using electrospun nanofibers

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Nanomaterials are currently impacting many fields of science and technology. Also, separation scientists have often postulated that increasing the homogeneity of surface functionalities and the morphology on a chromatographic support should improve chromatographic performance significantly. This talk will highlight our recent work on materials that are self-assembled at the molecular, nano and macroscopic levels. These self-assembled materials are used for the production of an array of different types of ultra thin layer chromatography plates. Marked improvements in efficiency and highly controlled selectivity will be illustrated.

Our research group recently developed a new method to provide as many as 50,000 plates/cm of separation media using nanoscale ordering [1]. To the best of our knowledge, the efficiency of this separation is higher per unit meter by at least a few orders of magnitude compared to current TLC separations and also out performs any currently available chromatographic separation media. These separations were also done in 3-5 minutes using minimal amounts of mobile phase.

More recently we have been able to separate all of the amino acids in a distance of no more than three centimeters and continue to document outstanding performance for these devices. We have just begun to scratch the surface on how this nanoscale technology can be used and the technology is far from optimized in its performance as well. This talk will highlight an expansion of applications as well as new nanoscale adsorbent materials to consider for further studies.

[1] J.C. Clark, S.V. Olesik, *J. Chromatogr. A.* 1217, 2010, 4655-4662.

O-2b

Ultrathin-layer chromatography on nanoengineered thin films

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Miniaturization has improved speed, sophistication, and overall capability in many research areas. Rapid high-sensitivity separations over short migration distances in ultrathin-layer chromatography (UTLC) [1] exemplify this trend in planar chromatography. Next-generation miniaturized chromatographic media utilizing engineered nanostructured thin films may further enhance UTLC's advantages.

The glancing angle deposition (GLAD) approach to fabricating macroporous columnar thin films has produced UTLC media ~ 5 μm thick with many intriguing characteristics ([2,3]). Unique channel-like features that are easily incorporated into anisotropic GLAD UTLC plates strongly influence both analyte migration velocity and direction. This approach can therefore tune chromatographic elution behaviours by modifying stationary phase porosity and architecture.

In this presentation, we will highlight recent efforts to improve the understanding, utility, and performance of GLAD UTLC plates. The novel elution behaviours of anisotropic stationary phases may yield new two-dimensional development modes. Concentration zones fabricated into GLAD UTLC plates have enabled large volume, low concentration sample spotting and increased separating power. We will also discuss new approaches of performing time-resolved characterization of the rapid separations on our media. Research into the miniaturized GLAD UTLC system is ongoing; recent experimental results will be presented.

[1] H. E. Hauck, O. Bund, W. Fischer, M. Schulz, *J. Planar Chromatogr.* 14, 2001, 234-236. [2] L. W. Bezuidenhout, M. J. Brett, *J. Chromatogr. A* 1183, 2008, 179-185. [3] S. R. Jim, M. T. Taschuk, G. E. Morlock, L. W. Bezuidenhout, W. Schwack, M. J. Brett, *Anal. Chem.* 82, 2010, 5349-5356.

O-2c

Fabrication and chromatographic separations on binder-free, carbon nanotube-fabricated thin-layer chromatography plates

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Patterned forests of carbon nanotubes (CNTs) were used as a template to fabricate novel silica-based thin-layer chromatography plates (TLC). The resulting CNTs are infiltrated with elemental silicon by chemical vapor deposition of silane. Silicon coated CNTs are annealed in the air, which removes the CNTs and converts the silicon to silica. The resulting material is white, which is indicative and characteristic of silica. This process produces TLC plates that are very porous and robust. SEM micrographs of the resulting microfabricated TLC plates demonstrate the precise placement of the adsorbent material.

These microfabricated plates do not require a binder to hold the adsorbent material together, and as normal phase materials give at least baseline separation of a five-component dye test mixture (CAMAG) using toluene as the mobile phase. The chromatographic efficiencies of these microfabricated TLC plates are typically 70 % higher than commercially available high-performance TLC plates, and sometimes much higher, and show a 150 % reduction in development time. That is, these microfabricated TLC plates allow for both efficiency and speed of analysis.

O-2d

Micro PLC to detect falsified fruit preservation

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Planar chromatography is highly flexible. Its methods can differ in a wide range of analytical application. New demands may call however for specialized analytical PLC-techniques. One serious reason may come up as a next threat to human health through falsified or highly unclean chemicals which are misused on agricultural products. Long time storage and long way transportation of sensitive fruits or for instance: potatoes, end up economically only if special chemicals like imazalil, thiabendazol, orthophenylphenol and other legally applicable carefully health-checked clean chemicals cover the product surface in an only minimal necessary amount. If these chemicals however are badly reproduced, unclean, oversampled or are even completely replaced by other unchecked chemicals this may result in health threats. One can avoid critical situations caused by falsified medical products simply in keeping the quality controlled drug store as the only product source instead of internet shopping. But how one can avoid intoxication by a false declared "bio lemon" impregnated with possibly critical chemicals? Prewashing normally does not work.

We used µ-PLC to enter this field. This new technique differs fundamentally from other analytical modes as it compares substances by the new “within-each-other” analysis concept instead of “after-each-other” or “parallel-to each other” runs. By this way we avoid any time- or position factor, which can cause systematic errors or large repeatability standard deviations. A next speciality in µ-PLC offers new analytical possibilities. For taking, mixing or giving samples we use micro brushes instead of precision syringes. We take surface chemicals from fruits or other agricultural products by solvent wetted micro brushes and transfer the taken sample near to the centre of a small HPTLC plate. The very soft brush tip allows for repeated sampling at the same position. This results in quantitative enrichments. Samples and test substance solutions overlap each other partially when stored on the plate surface in a special local mode. This results in the already mentioned ‘sample-within-each-other’ compare analysis by partial overlapping [1].

So we get after focussing a simultaneous two dimensional separation, where the time, the chemistry and the physics are locally absolutely identical for parts of the separated samples. This way we reach 100 % data security in case the overlapped signals differ in position and spectroscopy. If the PLC bows differ in the overlap Rp-position [1] and spectroscopy from legally accepted food preservation chemicals the corresponding substances are different for sure by 100 %. There is nevertheless comparability statistics available, but at N as large as 16 or even higher despite of the only single chromatographic run. Otherwise standard statistics is based on N = 2 for just one or two runs. Food quality tests made by µ-PLC are quick, easy, show top data security without MS *etc.* and are highly economical.

[1] For details check the internet book www.planar-chromatography-by-kaiser.com.

O-3a

Planar chromatography as analytical tool in synthetic biology

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Synthetic biology is a young discipline, introducing engineering principles into biological systems. One of the most perspective areas of synthetic biology is engineering of the biosynthetic pathways into the production organism, which may lead to improved yields as well as new compounds, produced with excellent purity due to the use of enzymes. One of the interesting new approaches was introduction of scaffolding to channel biosynthetic reaction flux.

The aim of our work was to apply planar chromatography with image analysis, densitometry and coupling to mass spectrometry as an indispensible analytical tool in synthetic biology. Different development (ascendent, horizontal and ADC chamber) and detection modes (absorption, fluorescence, image analysis, densitometry, quantitation), different derivatization reagents (anisaldehyde, phosphomolybdenic acid, H_2SO_4/ethanol) including the identification by mass spectrometry were applied to follow up the production and the yield of the synthesis of violacein, deoxichromoviridans, resveratrol, pterostilbene, lycopene, carotene, lutein, zeaxanthin, β-cryptoxanthin. Compared to the HPLC analysis, where in the cases of resveratrol and pterostilben gradient separation has to be performed, TLC provides much faster analysis. The automatic application by ATS4, the application of the samples from both sides of the HPTLC plates and simultaneous development from both sides in the horizontal developing chamber can additionally reduce analysis time and costs.

O-3b

HPTLC as comprehensive tool for analytical determinations

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HPTLC present the same principles than other chromatographic techniques but with outstanding advantages such as fastness and high throughput analysis, less labor-intensive clean up procedures, and simultaneous evaluation of standards and samples under identical conditions. HPTLC equipment have been improved during the last fifteen years achieving today fully automated system, which enhance the system reproducibility allowing reliable quantitative analysis.

These advantages have made of HPTLC a suitable tool for solving different kind of analytical task in many fields, like pharmaceutical, forensic, chemical and food industries. Important applications in food analysis have resulted extremely useful in aquaculture, e.g. dose control of quinolones, florfenicol, oxytetracycline, flubenzuron and triflubenzuron in fish feed [1]. In the same field, evaluations by HPTLC of astaxanthin and available lysine content have been implemented in fish feed quality control laboratories [2] In chemical engineering, HPTLC has been used without any other sample pre-treatment than a simple dilution for the analysis of sugars, organic acids and bio-fuels. In pharmaceutical analysis, HPTLC is a common tool for diverse evaluations such as drugs stability, content uniformity in bulk drug and pharmaceutical dosage forms, drugs plasmatic levels, etc. In forensics HPTLC has an important role in the analysis of drugs in complex matrixes like blood and urine [3].

Apart of using HPTLC in food composition, this technique has resulted very useful in the analysis of xenobiotics, like mycotoxins, PAHs, and biogenic amines all this with the aim of quality insurance in food safety [4]. Thus, it is possible to conclude that HPTLC is a comprehensive tool for analytical determinations in several fields.

[1] M. Vega, E. Jara and M. Aranda. J. Planar Chromatogr. 2006, 19: 204-207. [2] M. Vega and M. Aranda. J. AOAC Int. 2009, 92: 699-702. [3] S. Mennickent, J. Contreras, C. Reyes, M. Vega and M.de Diego. J. Planar Chromatogr. 2010, 23: 81-84. [4] V. Campos, K. Muñoz, E. Jara, P. González, M. Aranda, M. Vega. The Column 2009, 5: 8-12.

O-3c

Pharmaceutical applications of HPTLC as a problem solving technique

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In the pharmaceutical industry, historically, TLC methods were developed to monitor completion of reactions, and control related impurities. The use of this technique was almost totally superseded by HPLC, GC and other separation techniques. However, more recently, HPTLC has experienced resurgence in use as a problem solving technique.

The examples presented here highlight the areas where HPTLC has been used to provide critical data and solve analytical problems to support project progression.

1. Investigations into mass imbalance issues experienced on the development of a New Chemical Entity (NCE)
2. Investigations into the cause of variations in colour observed between batches of Active Pharmaceutical Ingredient (API)
3. Analysis of compounds which have little UV absorbance and would require complex sample preparation for analysis by HPLC or GC.

In all these examples other chromatographic and spectroscopic techniques failed to give a full understanding of the problem, and HPTLC provided critical results in resolving the issues. These achievements have placed HPTLC firmly back in the limelight and it has been shown to be a useful tool available to chromatographers to help solve project problems.

O-3d

High-throughput planar solid phase extraction – a new clean-up concept in multi-residue analysis of pesticides

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Residue analysis of pesticides in fruits and vegetables requires efficient clean-up of extracts to prevent matrix effects during liquid chromatography or gas chromatography coupled to mass spectrometry (LC/MS or GC/MS). Besides gel permeation chromatography (GPC), cartridge solid phase extraction (SPE) or dispersive solid phase extraction (dSPE) using different materials have been presented in literature. Primary secondary amine (PSA) is generally used in most methods to retain fatty acids and plant phenols, while depending on the sample type graphitized carbon black (GCB) and C18-materials are applied to remove chlorophylls and lipids, respectively, from extracts.

A completely new approach of clean-up by planar chromatography was developed. QuEChERS extracts (50 µL) were applied as area onto TLC amino plates, developed with acetonitrile, dried, and then developed with acetone top down to focus all pesticides into one sharp zone (Fig. 1). Afterwards, the zones were extracted by means of the TLC-MS interface either into a vial (for GC/MS) or directly onto an HPLC column to perform TLC-HPLC-MS analyses.

With selected pesticides spiked to different matrices, the concept of planar solid phase extraction (pSPE) was proven to be very successful. As compared to dSPE procedures, pSPE resulted in total ion chromatograms nearly free of matrix compounds. About 20 extracts can be cleaned-up simultaneously, thus presenting a high-throughput concept. Regarding the multiple chances of detection in planar chromatography, the success of clean-up can easily made be visible.

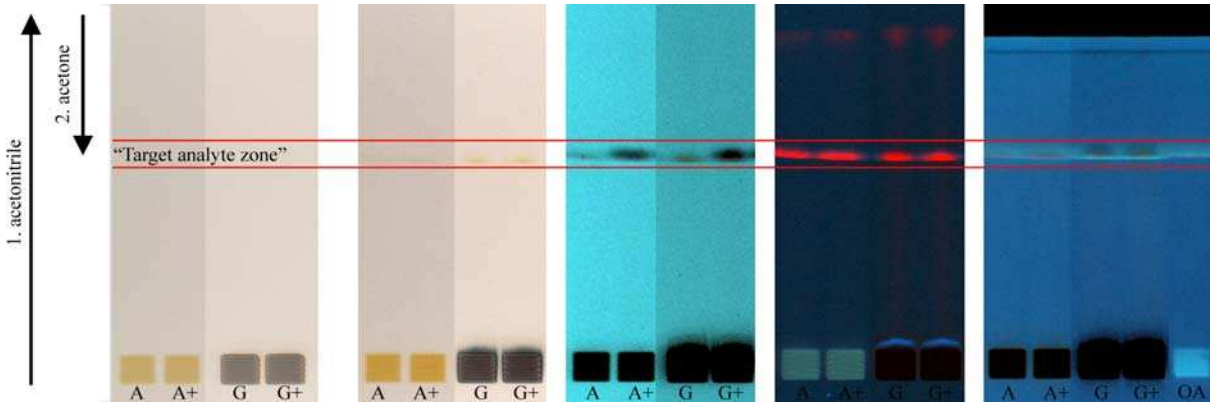

Fig. 1: Separation of matrix compounds (apple extract (A), grape extract (G), and oleic acid (FS)) from a pesticide mix (+) on TLC silica 60 NH_2 F_{254s}: a) before development, b) – e) after development (under white light (b), UV 254 (c), UV 366 (d), UV 366 after dipping into primuline solution (e)).

O-3e

Normal-phase HPTLC separation of salicylic acid, lactic acid, retinoic acid, chlorogenic acid, caffeic acid and caffeine

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There is no doubt about increasing demands for cosmetics including skin care, and makeup products. Active ingredients of lactic acid (LA), retinoic acid (RA), chlorogenic acid (CGA), caffeic acid (CA) and caffeine (C) as antioxidant and salicylic acid (SA) as a pilling agent were commonly used individually or in combination in cosmetics preparations. Numerous studies describing separation of these compounds have been done; the most common are usually based on HPLC and GC-MS. A literature search located no papers on separation reporting qualitative or quantitative TLC analysis of the above compounds simultaneously on a same plate.

The simple separation procedure using automated bandwise sample application on HPTLC plates silica gel 60 F_{254} and automated scanning of sample and standard zones is described. After comparing different binary and ternary mobile phase systems using the HPTLC Vario-System, toluene – methanol - acetic acid 7.9:2:0.1 (v/v) saturated with the mobile phase was found to be satisfactory for reproducible chromatographic separation as it was transferred to the Automated Multiple Development system (AMD2). The selective baseline separation between SA, LA, RA, CGA, CA and C were shown in figure 1. According to the chromatographic results, although CA, caffeine, SA and RA have appropriate migration distances, there is no migration for CGA and LA. Therefore good baseline separation between tow five member groups of chlorogenic acid, caffeic acid, caffeine, salicylic acid, retinoic acid and lactic acid, caffeic acid, caffeine, salicylic acid, retinoic acid has been achieved.

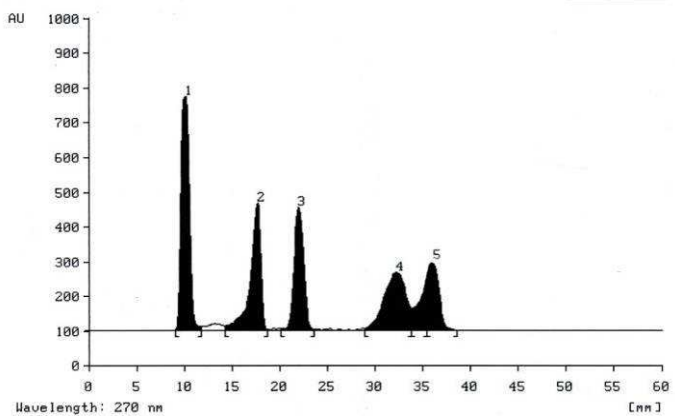

Fig. 1 Representative chromatogram obtained for chlorogenic acid and lactic acid (1), caffeic acid (2), caffeine (3), salicylic acid (4), retinoic acid (5)

O-3f

Assessing the quality of topical formulations of *Calendula officinalis* with HPTLC

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Calendula officinalis, commonly known as marygold, has long been used in both traditional and clinical medicine in wound healing and to help relieve skin inflammations and irritations. The anti-inflammatory and anti-oedematous properties have been linked to the pentacyclic mono-, di- and trihydroxy triterpenoid fatty acid esters, especially the faradiol esters. The unesterified faradiol produced by hydrolysis, has been found to have the same effect as an equimolar dose of indomethacin which is a non-steroidal anti-inflammatory drug (NSAID). However, the claimed benefits of these herbal formulations cannot be guaranteed in commercially available preparations unless standardised methods of regulation and testing are introduced.

The primary goal of this study was to develop a simple and reliable HPTLC method to quantitatively analyse active ingredients in commercially available topical creams and ointments, and to determine how different extraction methods of *Calendula officinalis* flowers affects the overall quality of the product.

The method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). This developed HPTLC method was proven to be precise, specific, sensitive, and accurate for routine quality assessment of raw material of marigold flowers extract and topical formulations.

It was found that commercially available formulations containing extracts of *Calendula officinalis* have significant variations in both composition and amounts of active pharmaceutical ingredients, due to different extraction procedures employed and the standardization requirements for extracts used in formulation. The results of this study strengthen the argument for standardised testing and regulation of commercially available herbal products to be mandatory.

O-4a

The TLC atlas of Chinese crude drugs

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The *TLC atlas of Chinese crude drugs in Pharmacopoeia of the People's Republic of China,* published in January, 2009, provided the first fingerprints of national standards for TLC authentication of herbal medicines in the world. The edition of this atlas was organized by Chinese Pharmacopoeia commission and contributed by 12 institutes. The atlas is one of the reference book series supplemented to the *ChP*. The book has Chinese version and English version including 229 monographs. At the beginning of the edition work, the related experimental operation and compilation regulation were formulated and the experimental instruments, samples collection and test method were also unified. The experiments of plotting the atlas were according to the method of *ChP*, 2005 Edition, but some methods were optimized and revised. The contents revised have been collected in the supplement edition of the *ChP*, 2005 Edition, and the new *ChP*, 2010 Edition. Shanghai R&D Center for Standardization of Chinese Medicines (SHCSCM) acted as one of the institutes involved in the research and edit of the atlas. We finished 33 monographs in which 33 monographs, 3 new reference standards were used, and 15 TLC methods were revised.

In this report, a brief introduction to this atlas and the main advances in ChP 2010 will be presented.

O-4b

Marker profiling – evidence based approach for quality evaluation of herbs in the Indian system of medicine

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The usage of natural health products is based on different traditions as well as on availability of competing medicinal products. Global trade with natural health products through usage of botanicals and popularity of traditional therapeutic systems are constantly growing. Studies on plant medicines, phytotherapeutic agents, nutraceuticals, and therapeutically important molecules derived from the plant emphasizing on the ethno medical approaches to drug discovery and features standardization and development of phytomedicines for major therapeutic indications.

The development of regulatory harmonization for natural health products in different regions created a broad spectrum of approaches for suitable regulation of such products. Given the increased use of traditional medicines, possibilities that would ensure it's successful integration into a public health framework. The links between Traditional medicine and biodiversity are exemplified by a long tradition of healing powers associated with the earth's natural systems. A key-point in choosing the appropriate method to guarantee the quality and safety of herbal products is to develop suitable methodology for their standardization, which has sufficient degree of specificity in ensuring the desired end point. The unique differences in the constituents of herbal drug shows distinctive challenges based on its identity, quality, and consistency of efficacy. The differentiation of conventional medicines and herbal drugs can be minimally considered from regulatory, economic and technical perspectives. Chemical fingerprinting of natural products and its ability to interact with physiological substrates of the human body are the mainstay of their therapeutic efficacy. This requires a multidisciplinary approach, involving analytical techniques and methodologies common to ethnomedicine, botany, pharmacology, pharmacotherapy, toxicology and pharmaco-epidemiology.

The discussion will focus on marker profiling of natural products with special reference to herbs used in Indian System of Medicine [ISM], which can depict the evidence based approach for quality evaluation of natural products. This can help in rediscovery of the connection between plant and health, which is responsible for launching a new generation of botanical therapeutics that include plant derived pharmaceuticals, multi component botanicals with health benefit, dietary supplements, functional foods and plant produced recombinant proteins. Development and evaluation of botanicals are being controlled and implemented through various agencies in different countries. The interest in natural products world wide has opened new avenues of exploration and areas of debate in developing efficacious products with quality for the healthcare. We have been working in this field for standardization and safety evaluation of herbs from Indian origin, development of value added formulation and related integrated approaches for drug development from natural resources so as to explore their benefits in therapeutics. This address will highlight various innovations in these perspectives for promotion and development of ISM through International coordination with major highlights on: (1) Different approaches on development of botanicals in ISM, (2) leveraging innovations on traditional medicines for health care, (3) HPTLC and its impact on marker analysis, and (4) quality evaluation of natural products derived from ISM through HPTLC.

O-4c

Simple densitometric TLC analysis of non-chromophore containing bioactive constituents in medicinal plant extracts

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TLC and HPTLC techniques have been used successfully in Thailand for the analysis of active constituents in medicinal plants and heath products. These include the determinations of plaunotol (an acyclic diterpenoid) in *Croton stellatopilosus* leaves [1], curcuminoids in various *Curcuma* species [2], alliin (a sulfur-containing amino acid) in garlic cloves and products, Lutein (a xanthophyll) in marigold (*Tagetes erecta*), Artemisinin (a sesquiterpene lactone) in *Artemisia annua* [3] and asiaticoside (a triterpene glycoside) in *Centella asiatica* [4] *etc*.

Among these active constituents, artemisinin and asiaticoside are the compounds that contain no chromophores in their structures. As a result, both have very weak UV absorption and are not detected easily by the commonly used UV detector. Therefore, chemical modification or derivatization of the compounds was carried out in this study in order to increase the sensitivity of their detection. By using the techniques of post-chromatographic derivatization/modification, it was found that artemisinin and asiaticoside could be converted to their chromophore-containing structures using ammonia vapor [4] and 2-napthol [5], respectively. Each of the modified products appeared to be detected sensitively (nanogram level) using its appropriate wavelength. Thus, the technique of densitometric TLC was introduced, validated and found to be used successfully in the quantitative analysis of their content in the plant materials.

[1] T. Rinthong, A. Jindaprasert, W. De-Eknamkul, *J. Planar Chromatogr . 22,* 2009, 55-58. [2] S. Tewtrakul, W. De-Eknamkul, N. Ruangrungsi, *Thai J. Pharm. Sci*., 16, 1992, 251-259. [3] T. Koobkokkruad, A. Chochai, C. Kerdmanee, W. De-Eknamkul, W. *Phytochem. Anal.,* 2007, 229-234. [4] A. Chaisawadi, W. De-Eknamkul, *International Symposium for Thin Layer Chromatogr*. 2011, Poster presentation

O-4d

Application of HPTLC for GI stability evaluation of psoralen, bakuchiol and bakuchicin in simulated physiological fluids

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HPTLC changes the scenario of herbal analysis which is nowadays replacing HPLC due to its reliability and user friendliness. In the present work, a HPTLC method has been developed and validated for the simultaneous determination of medicinally potent marker components Psoralen, Bakuchicin and Bakuchiol from *Psoralea corylifolia* using simple mobile phase consisting *n*-hexane and ethyl acetate. Quantitative evaluation was performed at 252 nm. The proposed method was applied for the estimation of the above markers in the crude extracts and marketed polyherbal formulations. The method was validated as per ICH guidelines for precision, accuracy and repeatability. The calibration curves were linear in the range of 20 - 120 ng, 30 - 130 ng, and 120 - 320 ng for Psoralen, Bakuchicin and Bakuchiol, respectively. The average recovery was 99.3 %, 99.5 % and 99.6 %, respectively for Psoralen, Bakuchicin and Bakuchiol, showing the excellent reproducibility of the method. In addition, the method was applied to evaluate the stability pattern of the markers in the simulated physiological fluids (gastric and intestinal fluids).

The *in vitro* study revealed that Psoralen is more stable in both the fluids whereas Bakuchiol and Bakuchicin is not stable in the acidic gastric fluid at pH 1.2. Psoralen is the most stable component in plant & important for treatment of psoriasis. Bakuchicin is very unstable in physiological fluids and it gets degraded within 3 min. Bakuchiol gets degraded in SIF after 45 min. Results from the study revealed that the potent DNA polymerase inhibitors (Bakuchiol and Bakuchicin) may have poor oral absorption due to their instability in the gastric environment and not suitable for oral administration. Studies are needed to improve the absorption. The proposed HPTLC method is simple, precise, specific, sensitive and accurate and can be used for routine analysis as well as for quality control of above markers in raw materials and herbal formulations.

O-5a

Utilization of HPTLC for the authentication and identification of botanical specimens

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HPTLC can be an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively and also provides a rapid visual reporting capability. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time for a series of samples. HPTLC also allows the same analysis to be viewed using different wavelengths of light providing a more complete profile of the samples in question than is typically observed with more specific types of analyses. In recent years, the HPTLC technique has been improved to incorporate the following features: HPTLC grade stationary phase, automated sample application devices, controlled development environment, automated developing chambers, computer-controlled densitometry/quantitation/reporting and fully validated procedures. These features result in methods that are not only convenient, fast, robust, and cost efficient, but also reproducible, accurate and reliable. We have utilized this classical technique extensively in our lab for qualitative and quantitative analysis of analytes in various formulation matrices. The following are a few recent examples where this technique was successfully applied [1, 2].

[1] C.S. Rumalla, B. Avula, Y.J. Shukla, Y-H. Wang, R.S. Pawar, T.J. Smillie, I.A. Khan, *J. Separation Science*, 31(22), 2008, 3959-3964. [2] C.S. Rumalla, B. Avula, J. Zhao, T.J. Smillie, I.A. Khan, *J Liquid Chromatography & Related Technologies*, 34(1), 2011, 38-47.

O-5b

TLC with biodetection to screen plant extracts for the presence of potential drugs to be used in neurodegenerative diseases

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Neurodegenerative diseases, such as Alzheimer's disease (AD) or Parkinson's disease (PD), have become major health problems globally. Thus, there is an urgent need to find new drugs that could be potentially used in the aforementioned ailments. Numerous plant extracts as well as compounds isolated from them have been investigated for their potential use in neurodegenerative diseases. As far as Alzheimer's disease is concerned drugs belonging to a class of acetylcholinesterase inhibitors are currently used to alleviate the symptoms of this ailment. However, a considerable body of evidence indicates that oxidative stress plays an important role in the development and progress of age-related neurodegenerative diseases. The continuous generation of ROS in living cells leads to cumulative damage to cellular organelle and finally to age-related pathology. It has also been proved that amyloid β peptides (Aβ) in AD brains can indirectly and directly induce oxidative stress in neurons. Thus the use of multiple antioxidants may protect against the development of severe cognitive impairment and deserves further investigations, as indicated by many research groups. TLC coupled with biodetection gives the possibility to screen plant extracts for the presence of AChE inhibitors as well as antioxidants and free radical scavengers. In this presentation the possibility of using planar chromatography in the search for new substances with the potential to be used as drugs in neurodegenerative diseases is discussed. Practical problems encountered while performing such analyses are addressed and some solutions are proposed.

O-5c

HPTLC: simple and effective tool to detect adulterated mixtures of *Eurycoma longifolia* root

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Eurycoma longifolia Jack, known locally as Tongkat Ali, is a traditional Malaysian herb associated with medicinal benefits and popularly used for energy booster, to enhance male libido and improve male fertility. Recently, the positive effects of our proprietary freeze-dried water extract of *Eurycoma longifolia* on sexual performance and well being in men with mild erectile dysfunction were clinically proven and the product was well tolerated with excellent safety profiles [1].

Its high medicinal benefits are primarily found in the root and while most of the raw materials are collected from the wild, the collection of only the authenticated root for usage in herbal preparation becomes a challenge. This can lead to unscrupulous mixing of other plant parts along with the root in the supply of raw material to the herbal drug industries.

A very practical, simply yet reliable HPTLC was developed for identification of *Eurycoma longif*olia root and detection of adulterants by fingerprint profiles. With a suitable mobile phase, mixture of *Eurycoma longifolia* root with a minimum of 5 % of the adulterant can be detected easily. The method was validated with respect to specificity, stability, precision and robustness.

[1] J. Gruenwald, M. Miller, A. George, M. Mufiza, A. Abas, J. Udani, Oral presentation in International Conference On Natural Products, 2010.

O-5d

Estimation of ursolic acid and luteolin in *Phyla nodiflora* (L.) Greene using HPTLC

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Phyla nodiflora (L.) Greene (Verbenaceae) is commonly known jalapippali in hindi. The aerial parts have been proved to be useful in diseases of heart and as diuretic. The present work deals with the estimation of two marker compounds, luteolin and ursolic acid from the aerial parts of *Phyla nodiflora* using HPTLC. Precoated HPTLC plates silica gel 60 F_{254} as stationary phase and single mobile phase toluene - ethyl acetate - formic acid (7:3:0.3, v/v/v) was used. Densitometric analysis was carried out at 254 nm for luteolin and 530 nm for ursolic acid after derivatization with natural product-PEG reagent and anisaldehyde sulfuric acid reagent, respectively (Fig. 1).

The Rf values for luteolin and ursolic acid were found to be 0.34 and 0.85, respectively. The percentage recovery of markers from the extract was carried out by standard addition method and was found to be in the range of 98.9 - 100.5 % for luteolin and 98.7 - 100.2 % for ursolic acid. The amount of luteolin was found to be 1.32 mg/g of dried powder whereas ursolic acid was found to be 1.75 mg/g. The developed method was validated in terms of accuracy, precision, repeatability and variability. The method will prove to be helpful for the market for standardization and quality control of the crude drug.

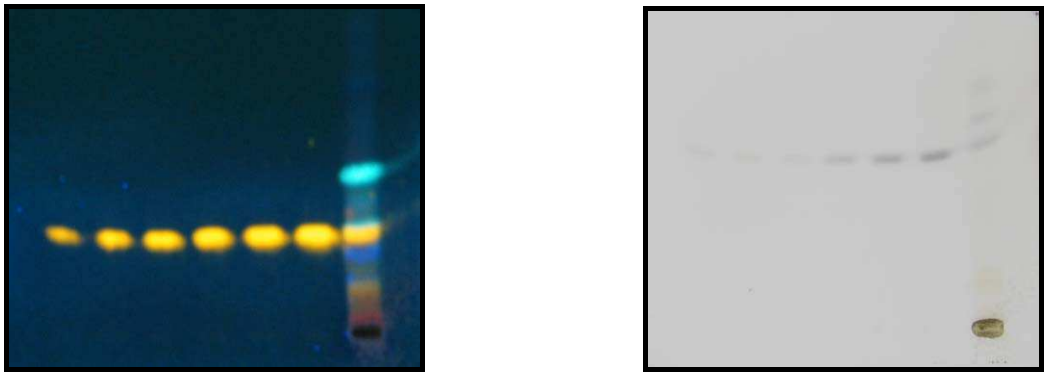

Fig. 1: HPTLC chromatogram of standard luteolin and test extract sprayed with NP-PEG reagent observed at 254 nm (left); HPTLC chromatogram of standard ursolic acid and test extracts observed in visible light

O-6a

The use of (HP)TLC in phytoindustrial R&D

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(HP)TLC over many years has become an indispensable tool in phytopharmaceutical R&D, as well as, in quality control laboratory [1]. E.g. the European Pharmacopoeia contains more than 200 monographs for the analysis of herbal drugs, extracts and essential oils, with almost all of them including HP(TLC) tests for the identification and purity checks. This reflects the high significance of this technique for phytoindustry.

Beginning with chemical screening of medicinal plants or plant extracts up to quality control procedures for herbal medicinal products, (HP)TLC can be regarded as standard technique. (HP)TLC has made much progress through the intoduction of instrumentalisation, e.g. for the sample application, development mode, detection mode and documentation possibilities. As a consequence, (HP)TLC can be run in a highly standardised way, a very important aspect for methods used in phytoindustry. One large advantage of (HP)TLC is the visualisation of the separation result. The view of a colored chromatogram offers a highly differenciated assessment of the chromatographic result. As will be demonstrated, (HP)TLC is an ideal tool for the chemical screening or fingerprinting of medicinal plant extracts for various natural product groups such as flavonoids, terpenoids, as glycosides and aglycones as well as alkaloids etc.

[1] B. Meier, D. Spriano, *J.AOAC Int.* 93, 2010, 1399-1409.

O-6b

Application of (HP)TLC as essential part of *Phytoneering* at Bionorica – is this analytical tool still able to compete with state of the art and novel high standard analytical tools?

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The *Phytoneering* philosophy of Bionorica combines *phyto* (plant-based resources) with knowledge-based *engineering.* Phytoneering covers all areas needed for development of new herbal entities (NHE) and/or improvement of Herbal Medicinal Products within the frame of Total Quality Management:

- Sourcing of Plant Raw Material
- Analytical Research (Phytomics)
- Production and Pharmaceutical Development
- Preclinical Research
- Clinical Research

At first view state of the art novel analytical tools based on separation and spectroscopy science like HPLC/UPLC, e.g. coupled with DAD, MS or NMR, or MELDI-MS seem to be superior for the research areas addressed above. Evaluation of the contribution of TLC in diverse projects, however, underlines that TLC still plays an essential role in phytoanalytics.

The presentation will focus on applications of (HP)TLC in basic research as well in daily practise in order to ensure comprehensive control from the seeds via the herbal drugs and herbal preparations (e.g. extracts) through to the herbal medicinal product. In this context representative examples will be given for evaluation of TLC as valuable analytical tool with respect to the appropriateness for different objectives, highlighting finally its competitiveness and up to dateness.

O-6c

HPTLC and HPTLC/MS studies on some bioactive natural products

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Discovery and development of new therapeutic agents is a continuous process. Of late an increasing number of people are opting for traditional/Indian medicine. Herbal drugs are preferred over synthetic drugs on account of their efficacy, easy availability and also are generally free from side effects. Tremendous efforts world over to develop new drugs based on so called rational drug design or the structure-based drug design approach has led to insignificant successes and high cost of drug development. This resulted in recent resurgence of interest in traditional medicine and plant based cost effective therapeutics all over the world. In India, we have a golden opportunity to take stock of the vast store of traditional knowledge on the healing properties of the earth's plant resources. India is known for its rich biodiversity in medicinal plants besides other plants occurring in diverse climatic regions. About 8000 species of plants are known to be used as source of medicine. The post GATT scenario provides a challenge to develop our own cost effective and innovative herbal drugs based on traditional knowledge.

Herbal drugs may be designed based on proven efficacies in ISM and modern scientific literature and generally are multiple combination of medicinal plants or their extracts. For regulatory purposes, these products are needed to be standardized and finger-printing by HPTLC or HPLC is necessary. During the last several years, IICT is engaged in the standardization of herbal drugs (both Ayurvedic medicines and modern herbal drugs) developed by various industries. The utility of HPTLC in the standardization of these herbal drugs will be discussed. Presently we are also working on HPTLC/MS for use in standardization. HPTLC combined with HPTLC/MS is also being looked into as better, viable and simple alternative to HPLC. Our experience in this direction will be presented.

O-6d

Use of HPTLC in quality control & standardisation of herbal medicinal plants & products

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There is no rational reason behind the tendency to equate *natural* with *harmlessness*. The fact that some medicine are natural does not necessarily make them safe or effective. In the recent years with evergrowing commercialization in the field of herbal medicines, there has been an instant demand for quality control of the medicines used in this system. For this standardisation is usually recommended as the solution to the problem.

In addition, a lack of knowledge of chemical standardisation with special reference to phytochemistry leads to misinterpretation and misunderstanding. It is very likely that some herbs will have side effects, interact with other medications, and be toxic. Furthermore, quite a number of these medicines are frequently adulterated cheap, less potent and spurious plant materials either posing danger to the public health or not as efficacious as expected. Many of the licenced herbal products have been found to contain non-prescription or even prescription drugs such as phosphodieterase Type 5 inhibitors, sulphonyl ureas and many other prescription drugs.

When properly employed, HPTLC provides a visual display of compounds present in the test materials. HPTLC method used here is a very powerful tool for identification of the presence of adulterants in herbal products based on the characteristic image produced and for determining the presence and the quantification of both inadvertent substitution as well as intentional adulteration of prescription drugs, *e.g.* glibenclamide, tolbutamide, glucophage, oral insulin in antidiabetic herbal preparations whereas sildenafil citrate, tadalafil, vardenafil and derivatives in sex stimulant and erectile dysfunction herbal preparations, corticoids in herbal skin ointments and sibutramine hydrochloride, rimonabant, mazindol and amphetamine and their analogues in weight loss herbal medicines, are significant as they gave almost identical and reproducible results.

In the present paper most of the above orthodox drugs have been identified in almost thirty five licenced herbal medicines using HPTLC. For detection of adulteration typically one or more zones (markers) of known or unknown identity is/are selected, these markers must be specific for the adulterants which means be present in the and absent in the unadulterated substance. To increase the analytical certainity, a chemical reference substance is used for comparision. Quantitative determinations have also been made in many cases.

O-7a

European regulatory framework and quality control of (traditional) herbal medicinal products

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The European Union has established a common regulatory framework for (traditional) herbal medicinal products. Definitions and basic principles are laid down in Directive 2001/83 EC and its amendments. Specific guidance has been developed by the Committee on Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA) in London. Specific guidance documents address for example the quality of (traditional) herbal medicinal products and the selection of appropriate specifications. A recently released guidance document is referring to the quality control of combinations of herbal substances or herbal preparations. The overall approach for the documentation of quality of (traditional) herbal medicinal products is to define in detail the whole manufacturing process and to develop a set of specifications which are suitable to control herbal substance(s), herbal preparation(s) and the finished product. (Traditional) Herbal medicinal products contain a complex mixture of constituents and consequently, the particular characteristics have to be taken into account when assessing such type of products.

Assays of marker compounds and identification of active substance(s) at different steps of the manufacturing process and in stability testing are important parts of the approach to guarantee quality control of (traditional) herbal medicinal products. A couple of analytical techniques are routinely used for assays and/or identification, e.g. HPLC, GC, TLC or HPTLC. The suitability is to be demonstrated by an appropriate validation. The choice and justification of a reasonable method in accordance with legislation and guidance on (traditional) herbal medicinal products is within the responsibility of the applicant for a marketing authorisation.

O-7b

Validation of TLC procedures

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Validation is the documented evidence of a procedure's suitability for its intended use and thus one of its key features. A prerequisite for a successful and reasonable validation is the careful consideration of the following points:

- The peculiarities of the specific analytical technique used
- The type of analytical task to be performed
- The characteristics of the product to be tested
- The information expected and the conclusions to be drawn from the analytical result
- Predefined specifications or limits to be controlled with the analytical procedure to be validated

As a consequence, validation of a HPTLC procedure must not be considered a standardized and easy to perform exercise, but is highly depending on the analytical problem.

However, within the process of harmonisation of the requirements for registration of pharmaceuticals for human use, a Guideline has been developed and adopted under the auspices of the International Conference on Harmonization (ICH) describing the characteristics for consideration during the validation of an analytical procedure for pharmaceutical analysis [1].

As this guideline has also been adopted by various Pharmacopoeias it has become some kind of a canonical model. This, however, resulted in its sometimes "blind" application and execution without considering other, sometimes more appropriate guidance like the IUPAC guideline [2].

Models will be presented on how to decide which validation approach to be considerd for different types of analytical tasks.

[1] ICH Guideline Q2(R1) *Validation of Analytical Procedures: Text and Methodology*, 2005. [2] International Union of Pure and Applied Chemistry, Pure Appl. Chem., 74, 835-855 (2002).

O-7c

Development of stability indicating chromatographic evaluation method for moxonidine in pharmaceutical formulations

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Stability indicating analytical method has been developed for estimation of newer antihypertensive moxonidine in tablet formulation by normal phase HPTLC. The developed method is simple, accurate, precise and unequivocal for quantification of moxonidine with all its possible degradation products which has been distinctly resolved on HPTLC plates silica gel 60 F_{254} with the mobile phase methanol, toluene and triethylamine in the ratio of 4:6:0.1 (v/v/v).

Densitometric quantification of moxonidine was performed at its λ_{max} 266nm. Band of moxonidine at RF value 0.58 is markedly separated from bands of reported degradation products. Linearity is justified by calibration plot of moxonidine from 400 - 1600 ng/band by height and area, too with correlation coefficient of 0.997 and 0.997, respectively. The validation evidences the method for being inevitably specific, accurate, precise and robust with prospective capability of its application in determination of these ingredients in bulk drug and/or pharmaceutical preparations.

O-7d

Chemical profiling of exstesy recovered from around Jakarta by HPTLC-spectrophotodensitometry

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Fifty four Exstesy tablets, seized from around Jakarta by narcotic police, were physically characterized and chemical profiled by HPTLC-densitometry. Shape, size, color, and weight of each table were documented. The one hundred milligrams of a homogenized drug sample were dissolved in 5 mL of pH 10.5 phosphate buffer solution and extracted with 1 mL toluene. Two micro liter of the extract were applied on the HPTLC plate silica gel GF_{254} (20 x 10 cm), then eluted in a twin-trough chamber with cyclohexane – toluene - diethylamine 75:15:5. The chromatogram was scanned by TLC-Scanner 3 at 210 nm. The *in situ* UV spectrum of each peak was scanned in the range of 190 - 400 nm. Link between tablets was determined with cosine function (C-value). The chromatograms were clustered with the single linkage method. The cluster determinate was applied for the pearson similarity value at 5 % error.

O-7e

RP-HPTLC estimation of benzhexol HCl and trifluperazine HCl in pharmaceutical dosage form

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A simple, rapid, precise and accurate HPTLC method has been developed and validated for simultaneous estimation of benzhexol hydrochloride (BENZ) and trifluperazine hydrochloride (TRIFLU) in pharmaceutical preparations. Separation was achieved on an HPTLC aluminium sheet silica gel 60 F_{254} (0.2 mm thickness) as stationary phase with methanol – acetone – toluene - ammonia (1:2:7:0.1, v/v). Densitometric quantification was performed at λ = 210 nm by reflectance scanning. The R_F values of BENZ and TRIFLU were 0.82 and 0.37, respectively. The linearity of proposed method was investigated in the range of 0.4 to 0.8 μg/spot and 1.0 to 2.0 μg/spot for BENZ and TRIFLU, respectively.

The result obtained for laboratory mixture of BENZ and TRIFLU was found to be 99.99 % ± 0.40 % and 99.83 % ± 0.18 % by peak height and 99.90 % ± 0.54 % and 99.77 % ± 0.20 % by peak area, respectively. The result obtained for the marketed formulation of BENZ and TRIFLU was found to be 99.65 % ± 0.63 % and 99.98 % ± 0.16 % by peak height and 100.10 % ± 0.54 and 99.95 % ± 0.30 % by area, respectively.

The percentage recoveries for BENZ and TRIFLU were 100.05 % ± 0.65 % and 100.06 % ± 0.23 % by peak height and 99.68 % ± 0.75 % and 99.87 % ± 0.31 % by peak area, respectively. The developed method was suitably validated for precision, accuracy, specificity and ruggedness.

O-8a

Recent instrumentation developments at CAMAG

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The TLC Scanner 4 was introduced as the successor to the very popular TLC Scanner 3. The TLC Scanner 4 represents the latest in state of the art design and functionality.

TLC-MS coupling is a powerful solution to the hyphenation of TLC and mass spectrometry and thereby opens up new possibilities for both techniques. The TLC-MS interface is a valuable complementary tool to LC-MS.

O-8b

Future perspectives of TLC; The dominant marked leader steps ahead

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Merck is largest silica gel producer for chromatography in the world, which has production capacity more than 1000 tons/year. Merck uses whole silica gel for different type of chromatography (TLC, HPTLC, HPLC, Flash and preparative chromatography). For example, customer can transfer with TLC/HPTLC developed method easily to Flash or preparative chromatography.

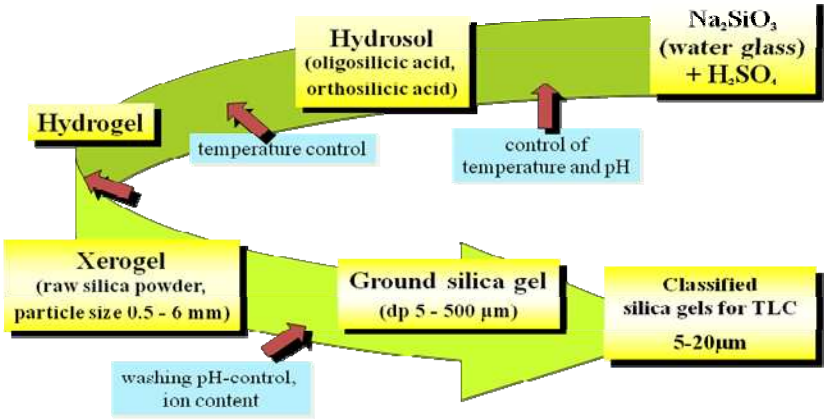

Fig.1 Production of silica gel 60

Ever since Merck KGaA pioneered the world's first pre-coated TLC plates in 1966, they have been the global leader in TLC/HPTLC plate technology. Today there are a wide range of backings, bonded phases, and specialty features in Merck's extensive TLC/HPTLC product portfolio that truly bring this mature technology into the 21st Century.

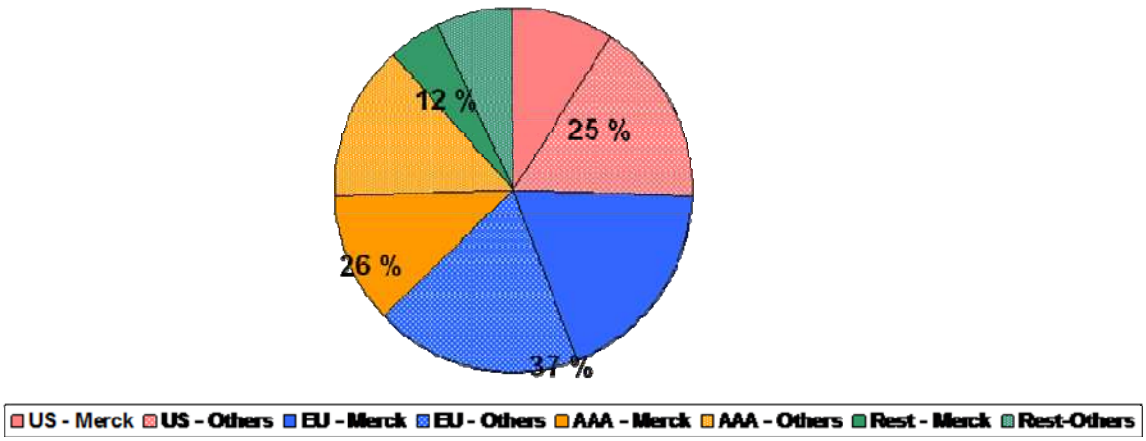

Fig.2 SDI study of the world market in the year 2007

This talk will show you what modern TLC/HPTLC is capable of and give you ideas how to apply it to your current work to solve problems and save you time and resources.

O-8c

HPTLC-MALDI-TOF-MS coupling for the MS and MS/MS analysis maintaining the chromatographic resolution

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HPTLC is a well established chromatographic analysis method that permits a robust, parallel analysis with analyte specific detection at high chromatographic resolution. However, molecular identification of TLC-separated bands, *e.g*., in a “scratch-elution” process is typically associated with loss of chromatographic resolution.

We describe a new solution that couples TLC offline with MALDI-TOF mass spectrometry to permit a high resolution molecular analysis. We use lipids to demonstrate the characteristics of the HPTLC-MALDI technology. In combination, automated sample application and HPTLC-MALDI readout offers a new powerful chromatographic analyzer that adds molecular information to high chromatographic performance.

O-8d

Phytochemical certified samples by BotaniCert, a new french startup

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BotaniCert is an independent organisation which specialises in providing confirmation of the botanical and chemical identification of species, together with conducting the relevant safety checks, all the while maintaining the highest levels of integrity.

From extractors and growers, to buyers and traders, from botanists and pharmaceutical laboratories to cosmetics & nutraceutical industries, and from manufacturers to quality controllers… At every stage of the process it is essential to have a product whose identification and botanical and chemical characteristics have been verified, validated and certified.

Following each check carried out by BotaniCert, it issues an authenticated test certificate and grants the use of its label worldwide:

1. Identification of raw materials and extracts in original state or complex matrices.
2. Establishment of “procurement” specifications for purchasing plant extracts and powders including identification, essay, checks to ensure absence of solvents and/or contaminants, adulteration detection.
3. Potential to challenge current suppliers using the HPTLC strength comparison method.
4. Verification of current portfolios for HPTLC, HPLC, GC-MS and UV strength.
5. Sale of botanical reference standards authenticated and certified by BotaniCert.
6. Repurchasing maximization by means of verifying the presence of the phytochemical complex responsible for the extract’s conventional activity.
7. Creation of a database for identification, adulteration, marker characterization, target molecule essay and metabolite degradation monitoring.
8. Studies of pharmacological activity on our ethno-botanical database: from product design through to patents and publications.

O-8e

Natural compounds purification by Counter-Current Chromatography CPC/CCC

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Screening of natural compounds in complex extract is often a fastidious process and needs several steps to obtain pure active compounds. Moreover a few mg of pure compound are needed to be able to determine their structures using spectroscopy technique.

Centrifugal Partition Chromatography (CPC) also known as Counter Current Chromatography (CCC) is a preparative, pilot and industrial liquid purification technique that does not require traditional solid supports like silica: two non-miscible phases are used, one as the mobile phase or the eluent and the other as the stationary phase maintained by the centrifugal field. The affinity of the solute for each phase can be measured by their partition coefficient that in turn dictates the order of elution for each compound.

The main aims of this technology are to isolate the maximum amount of a specific molecule at the highest purity, in a minimum of time and without using any silica column or support media.

Here is presented few application of purification of natural compounds by CPC/CCC.

O-9a

Coupling HPTLC and mass spectrometry: how and why

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A rapid advance in atmospheric pressure (AP) surface sampling and ionization techniques has provided unparalleled opportunities for direct mass spectrometric analysis of analytes on planar separation media [1,2]. This synergistic relationship is expected to push further developments from both the mass spectrometry and separations community. In this presentation, the state of the art for AP surface sampling/ionization techniques will be overviewed with emphasis on liquid extraction based methods used in our research. The flexibility in analysis the liquid extraction based methods provide in the direct analysis of analytes separated HPTLC plates will be covered in detail.

Data presented will focus on cases where the complexity of the sample dictates that mass spectrometry is an essential component for read out of the HPTLC separation. Among the examples shown will be the read out of one dimensional separations of single protein tryptic digests, a 7 protein digest, and an Ecoli digestion. Single development lanes were analyzed at millimeter resolution where extracted peptides were subjected to a nanoESI ion mapping scan function. Hundreds of proteins were identified through HPTLC-MS using database searching algorithms with the results rivalling column based LC-MS. Data showing one dimensional development of intact protein mixtures on HPTLC plates with mass spectrometric read out will also be presented. Advantages, disadvantages and future prospects to the read out of complex biomolecule mixtures on planar chromatographic media will be discussed.

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O-9b
The coupling of HPTLC with DART mass spectrometry

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The benefit of Direct Analysis in Real Time mass spectrometry (DART-MS) as compared to conventional MS techniques is the absence of the need for sample preparation, as the ionization occurs directly from the surface of solid or liquid samples [1, 2]. Due to this, the direct combination of DART mass spectrometry with high-performance thin-layer chromatography does not require any extraction dithering the spot and distorting its composition. Moreover, it is possible to detect sample components only at distinct regions of interest on the plate, which is beneficial as compared to such a more traditional method as HPLC-MS as it avoids the recording of background, matrix or negative findings.

The possibilities of the TLC/HPTLC-DART-MS combinations were demonstrated in some publications [3-7]. Due to the fixed, horizontally aligned supply of the gas flow from the DART ionization source to the MS inlet, the introduction of HPTLC/TLC plates as cut strips was inconvenient for quantitation. The repeatability was very low due to the manual positioning, but it was shown to be reliable if an internal standard was used for correction of the positioning [5].

In 2009, a new version of the DART ion source allowed adjusting the angle of the DART gas stream and the use of a motorized rail. The angled source should significantly extend the capabilities of DART-MS due to the introduction of wide surfaces like HPTLC plates. In order to select the most favorable conditions for DART-MS analysis, the proper positioning of samples is important. Therefore a simple and cheap technique for the visualization of the impact region of the DART gas stream onto a substrate was developed. The new approaches arising from these studies were applied for the analysis of complex real samples, including honey and propolis. Benefits and limitations of HPTLC-DART-MS and HPTLC-ESI-MS combinations were compared.

This work was partially supported by the Council at the President of the Russian Federation (grant MK-594.2010.3).

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O-9c

On versatility of the TLC-MS interface and its analytical importance

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Introduction of densitometric detection based on scanning of chromatographic plates in UV-Vis light revolutionized TLC and to a large extent filled the performance gap between TLC and HPLC. Introduction of the TLC-MS interface acts as a meaningful enhancement of the analytical potential of TLC, this time focused either on identification of the single separated analytes, or on a specific fingerprinting of fractionated mixtures.

There is an additional advantage inherent in the TLC-MS interface, so far not fully recognized and hardly exploited. With its aid, one can develop 2D separation systems that involve both, TLC and the TLC-MS interface. Developing of the 2D separation systems can enhance an analytical outcome, due to the following possibilities:

- Growth of the theoretical plates (*N*) number by the extension of the analytes' migration track;
- Arbitrary choice of the fractions of interest for the second separation step, performed by means of HPLC;
- Available change of analytical mode (e.g., NP vs. RP) and/or separation conditions (stationary and/or mobile phase), when switching from TLC to HPLC, in a way equivalent to gradient elution;
- Possible usage of any HPLC detection system (i.e., not necessarily the MS detector only).

All the aforementioned practical aspects of using the TLC-MS interface will be discussed in our talk and illustrated with working examples from our laboratory.

O-9d

Application of TLC-MS in water analysis

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It is of high interest for Landeswasserversorgung, one of the largest water suppliers in Germany, to monitor the occurrence of organic trace contaminants in the raw waters and to control the quality of the drinking water. Therefore, various analytical technologies such as gas chromatography and liquid chromatography coupled with mass spectrometry are applied for the routine analysis of the waters. In addition, HPTLC/AMD is used for screening purposes for the analysis of the raw and drinking waters as well as when searching for transformation products in lab-scale experiments.

The coupling of modern planar chromatography with mass spectrometry (TLC-MS) has become an interesting and powerful tool [1, 2] to provide important information for the identification of organic contaminants during the above mentioned screening procedures. This work describes different applications of HPTLC-MS in water analysis. As an example, HPTLC-MS was essential for the identification of pesticide metabolites in ground water. Also, it was possible to identify the hydrological tracer rhodamine B and its degradation products in ground water using HPTLC-MS [3]. HPTLC/AMD analysis provided distinct evidence of presence of the tracer and further fluorescent compounds (Fig. 1).

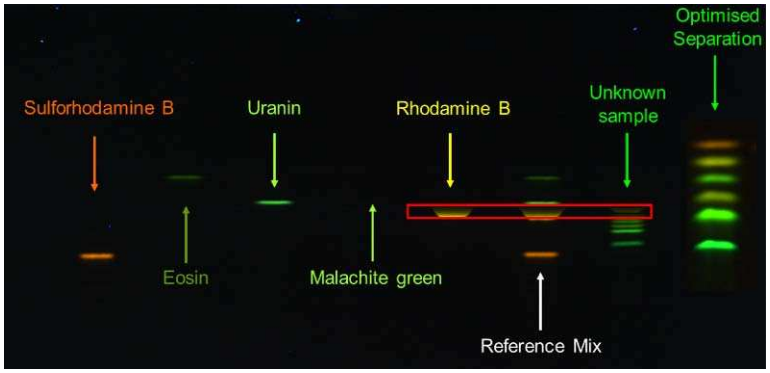

Fig. 1: HPTLC-AMD chromatogram (fluorescence, excitation at 366 nm)

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O-9e

Improved HPTLC separation of lipids by using Automated Multiple Development (AMD) and identification with the TLC-MS-Interface

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The aim of the described investigation was the development of an optimised chromatographic separation of various lipid substances, which are formed in Stratum corneum. It is well known that the main composition of Stratum corneum (SC) lipids consists of ceramides, cholesterol, cholesterol esters and free fatty acids. These components serve as a barrier function against external influences and excessive water loss. The low polarity differences between the above mentioned substances necessitate a high resolution of chromatography. By using AMD should be achieved a higher separation performance.

Various methods were successfully used for the analysis of lipid substances in the past but they are very time consuming and cost intensive concerning the amounts of solvents (1/2/3). Furthermore, for the existing AMD - methods the phenomenon of accumulation of additives from the solvents on the solvent front position is well known. This effect can be rolled back by decreasing the number of development steps and by using suitable solvents.

For all experiments as stationary phase HPTLC glass plates 20 x 10 cm, silica gel 60 F_{254}, 0,1 mm for AMD (Merck) were used, pre-washed twice with chloroform - methanol 2:1 (v/v) and activated 30 min at 120 °C in a drying oven. The application of lipids was performed bandwise using the Automatic TLC Sampler. Derivatisation was done using the Chromatogram Immersion Device with a mixture of 10 % copper sulfate in 8 % phosphoric acid followed by drying and heating (170 °C for 8 min) on a TLC plate heater. For detection a TLC Scanner and a video system were used.

First step of 8-step gradient (100 % methanol) was used to focuss on and to eluate all polar substances completely within solvent front. Steps two to six separated cholesterol-3-sulfate and the different ceramides. Step seven (100 % chloroform) was used to separate cholesteryl oleate from glyceryl trioleate. In the last step with solvent n-hexane - toluene 19:1 squalen was positioned far away from cholesteryl oleate but under the solvent front. To focus oleic acid, a chemical preconditioning of the HPTLC plate with 4 molar acetic acid was very important before step six was started. Validation of the method led to LOQs between 8.7 ng (Cer NP) and 366.0 ng (Cer AP). The identification of the lipids in real samples can be easily done by using the TLC-MS Interface in coupling with mass spectrometry which allows an identification of substances directly from the thin-layer plate. All substances could be ionized by using either ESI or APCI.

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O-9f

Supporting the chemist decision in small molecule leads discovery through an open and easy access automated HPTLC-MS-ELSD platform

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MS coupled to chromatography is a widely known and efficient support of the chemist's decision for compound identification or follow-up in a synthesis mixture. The aim of this poster is to show how an open and easy access automated HPTLC-MS-ELSD platform might be a consistent alternative and efficient complement to HPLC/UPLC-MS instrument which is, until now, the mainly used method.

TLC/HPTLC was ever widely used by the chemists as a rapid and reliable method to follow reaction processes. The reason is that compounds remain sometimes on the column, due to high polarity or weak solubility. The method is also very useful when analyte detection is poor (no chromophore). In most cases, the HPTLC/MS platform with this new TLC-MS Interface is ideal for structural analysis. The TLC-MS Interface was complemented by an MS software controlled automatic valve in order to be in real open access for chemists. The parallel connection with an Evaporative Light Scattering Detector (ELSD) enabled the analyst to distinguish between compounds that were not eluted from the plate from those that were not sufficiently ionized for MS detection. This feature might be skipped as the migration of the compound on the plate proves it's elution with the system, but this detection confirmation was also required for open access and high throughput. Besides this high-tech installation, everything was made with the classical TLC used by the chemist. The only help from HPTLC/TLC instrumentation was given by the SmartCut, who is very popular by the chemists to get tailor made plate size. All other necessary useful hints for a real practical use are also given in this poster.

This poster finally reports an experience which underlines the interest in HPTLC/TLC-MS in an advanced analytical environment, as it is the case in LCGR-AnSci (Lead Generation to Candidate Realization, Analytical Sciences) research centre from the pharmaceutical group Sanofi-Aventis. The automatization, reliability of the data, and speed were the convincing keywords for the implementation decision of the HPTLC-MS-ELSD in this analytical platform. After months of intensive use, it has confirmed a full compliance with the routine analytical needs of the authors.

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O-9g

Controlling the insulin purification process using HPTLC–MS

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Analysis of proteins and peptides is an important field in bio analysis because of the important role of proteins in cell structure and functions. Commonly used methods for protein analysis are gel electrophoresis, HPLC and mass spectrometry.

Also HPTLC can be used successfully for separation of proteins and peptides. It has been shown that peptides from tryptic digest of proteins can be separated on HPTLC silica gel 60, HPTLC cellulose plates and HPTLC RP-18 plates followed by identification with mass spectrometry [1, 2, 3].

Here we show that the combination HPTLC-MS can also be applied for the analysis of proteins on the example of Insulin. During the down stream process of insulin different by-products have to be removed. This purification process can be controlled by HPTLC and identification of insulin and by-products is possible by coupling HPTLC with MS.

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O-10a

Bioassays as a detection tool in TLC

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Bioassays (biological activity tests) are screening or semi-quantitative methods, which are based on a measurement of an effect emerging in a given biological system as a result of biological action of the substances under investigation. Usually the death or behavior/metabolic disturbance of a test organism can be observed. Test organisms can be plants or fishes, but mostly these are one-cell organisms like bacteria or fungi. It is also possible to use chemical reagents e.g. β-carotene or DPPH to visualize antioxidant or antiradical properties. Bio-detection enables searching for biologically active substances in complicated mixtures, what can be described as effect-directed analysis (EDA) [1,2].
Biological activity tests are often hyphenated with separation methods, mostly with thin layer chromatography. The method is ideal for bioassays due to an open layer, which enables evaporation of solvents used as mobile phase components. The additional advantage of TLC is possibility of analyzing many samples in the same run. Spectroscopic methods directly connected with TLC-bioassays can be used as confirmation methods giving full information about analytes [3].
Direct bioautography (TLC-DB) seems to be the most popular method linking microbiological test with planar chromatography. The method gives information on antibiotic properties of separated compounds. The developed and dried HP(TLC) plate is immersed in a suspension of bacteria in a nutrient broth, incubated and sprayed with a reagent (usually tetrazolium salt MTT). Around spots of antibacterials, inhibition zones are formed as pale areas on a purple background [4,5].
Two bioautographic tests were developed in our laboratory: one based on Gram (-) bacteria, *Escherichia coli*, and another one based on Gram (+) bacteria, Bacillus *subtilis*. These tests were used with success to determine fluoroquinolone antibiotics at their MRL (maximum residue level) in milk [6]. The test based on *E. coli* was also applied for comparison of deproteinization methods used before TLC-DB and HPLC analysis of flumequine residues in milk. Other applications are connected with analysis of essential oils in conifers and alkaloids of *Chelidonium majus* found both in the plant and in pharmaceutical preparations, like an anticancer drug, Ukrain.

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O-10b

Potential applicability of modern bioautography (BioArena) in the study of plant ingredients

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Searching for bioactive natural products requires appropriate bioassays, focused on the desired activity (e.g. antifungal, antibacterial). Different versions of layer liquid chromatography are ideal technical solutions for the chemical and biological screening of drug ingredients, because of high-throughput, low cost, easy maintenance and selectivity of detection reagents. Direct bioautography is the combined application of planar layer liquid chromatographic separation and post-chromatography bioassay. In the course of the biological detection the adsorbent layer after development is dipped into or sprayed with cell suspension and afterwards the bioautogram is visualized. This is applicable to a broad range of microorganisms that can grow directly on the chromatographic plate, for which appropriate separation conditions (without a disturbing effect on the bioassay) are also needed. Visualization of the bioautogram is usually performed by the use of yellow tetrazolium salts, reduced by living cells to bluish formazan. Another visualization method uses bioluminescent bacteria. Using the emitted light as signal, the performance of biodetection is very easy. The image of the bioautogram can be directly recorded by a cooled camera in a dark box with appropriate exposure time (depending on the intensity of the light). The light emitted by bacterial cells is closely linked to energy metabolism, so correlating with the metabolic state and the viability of the cells.

BioArena is based on direct bioautography; beyond biological detection, however, it is also appropriate for examination of the mechanism of cell proliferation inhibition and/or promotion effects. The influence of different endogenous and/or exogenous substances on the bioactivity of separated compounds can, moreover, be examined by dissolving substances in the cell suspension just before inoculation.

Overpressured-layer chromatography (OPLC), as an efficient forced flow planar layer liquid chromatographic technique, results in compact chromatographic spots and good separation efficiency especially by the use of fine particle size chromatoplate (e.g. HPTLC). OPLC is also well suited for fractionation using analytical chromatoplates providing on-line detection and subsequent peak collection. This system can be applied for efficient isolation of various substance types in general, but in this case the potential antimicrobial components from plant extracts.

The separation and isolation of antibacterial components, found previously active in a bioautographic study, of different plant extracts from species of *Asteraceae*, their re-chromatography, and identification by means of GC/LC-MS as well as modern bioautographic studies of concentrated collected peaks are demonstrated. In bioassay investigations Gram positive soil bacterium *Bacillus subtilis* (antibiotics generate white spots) and chromosomally tagged luminescence Gram negative *Arabidopsis* pathogen *Pseudomonas savastanoi* pv. *maculicola* (antibiotics generate black spots) were used.

O-10c

Free radical-scavenging activity (FRSA) of sea urchins pigments by the HPTLC-DPPH• method

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The shells of sea urchins are known to contain various polyhydroxylated naphtoquinone pigments, so-called spinochromes. The phenolic hydroxyl groups of these molecules suggested that they could participate in antioxidant activity, as was observed in other well-known antioxidant polyphenols such as catechins or gallic acid. The aim of this work was a comparison of FRSA of sea urchins *Strongylocentrotus droebachiensis* pigments by the HPTLC-DPPH• method.

The sea urchins pigments were isolated by acid demineralization of sea urchins shells and needles, followed by its extraction with organic solvent. The qualitative and quantitative analysis on acid-treated HPTLC plates with methanol - chloroform - acetic acid - water (11:50:5:2) as mobile phase. Free radical scavenging activity was examined using the DPPH• reagent [1].

For the HPTLC-DPPH• method, parameters for densitometry and post-chromatographic derivatization were developed and validated. The ID_{50} values, i.e. the dose of the compounds required to scavenge 50 % of DPPH•, were calculated for the reference substance echinochrom A and the two main zones of the pigment mixture from sea urchin shells. They were assumed to be polyhydroxylated naphtoquinones on the basis of their characteristic UV/Vis absorption spectra (Fig. 1). It was found that both polyhydroxylated naphtoquinone pigments (ID_{50} for zone with R_{st} 0.85-0.90 was 0.057 µg and ID_{50} for zone with R_{st} 0.70-0.75 was 0.043 µg) have a higher antiradical activity than echinochrom A (ID_{50} was 0.134 µg). Thus, sea urchin shells could be a new source of natural antioxidants, besides echinochrom A.

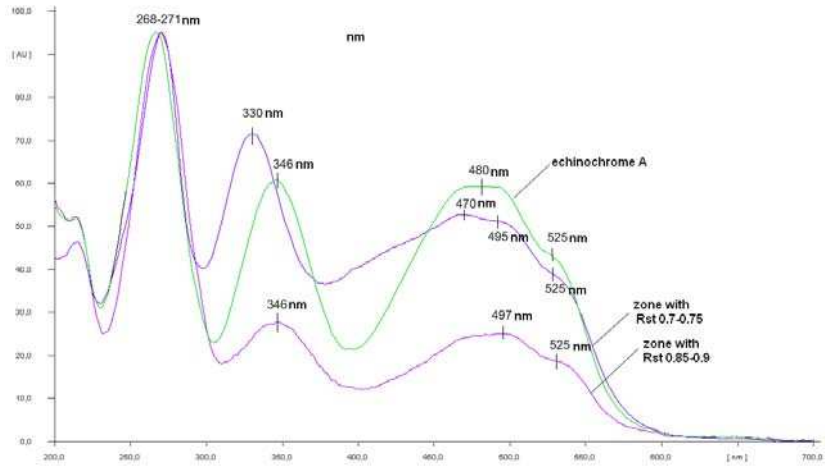

Fig. 1. UV/Vis-spectra of reference substance echinochrome A and sea urchins *Strongylocentrotus droebachiensis* pigments zone with R_{st} 0.70-0.75 and R_{st} 0.85-0.90

[1] Pozharitskaya O.N. *et al.* J Sep Sci. 30 (15) (2007) 2447-2451.

O-10d

Effect-directed analysis of landfill leachates using HPTLC/AMD with bioluminescence detection

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Anthropogenic substances can leach out from landfill sites into the environment. For this reason landfill leachates are a potential risk for the raw water resources and hence for the drinking water [1]. Therefore, a steady and comprehensive monitoring of the water resources is necessary to guarantee a high quality of drinking water.

The principle of classic screening methods is that only substances already known to the analyst are regarded. The combination of HPTLC with effect-directed detection, in particular the bioluminescence inhibition detection using *Vibrio fischeri* represents a promising complementary screening method in water analysis [2,3]. Especially the evaluation of the bioluminescence inhibition according to the approach developed by the Laboratory of Landeswasserversorgung enables a semi-quantitative comparison of samples with the so called reciprocal iso-inhibition volume (RIV). The higher the RIV-value is the higher is the concentration of the toxic substance.

The efficiency of the method was demonstrated using the example of evaluating the influence of landfill leachates towards the aquatic environment. Several sampling points were analyzed. In order to compare the results easily on a map a polar diagram illustration of the logarithmic RIV was chosen (Fig. 1). The figure shows that the pattern and intensity of the effect towards *Vibrio fischeri* for the ground water samples is altering by the influence of the landfill.

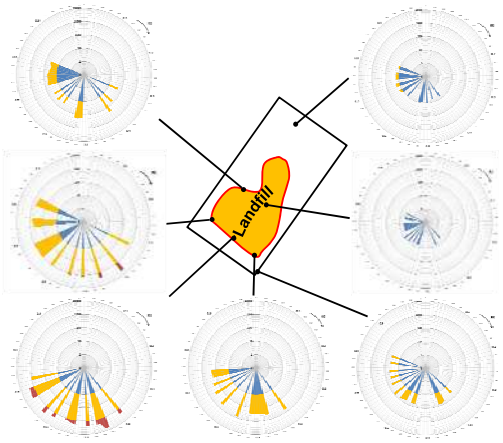

Fig. 1 Allocation of RIV polar diagrams to the sampling points of the landfill site.

[1] M. Farré, S. Pérez, L. Kantiani, D. Barceló, *Trends Anal. Chem.* 27, 2008, 991-1007. [2] G. Eberz, H. Rast, K. Burger, W. Kreiss, C. Weisemann, *Chromatographia* 43, 1996, 5-9. [3] W. Schulz, W. Seitz, S.C. Weiss, W.H. Weber, M. Böhm, D. Flottmann, *J. Planar Chromatogr*. 21, 2008, 427-430.

O-10e

HPTLC-bioluminescence detection with *Vibrio fischeri* - method enhancements and its potential in the field of consumer goods

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The luminescent bacterium *Vibrio fischeri* has been used since 1979 as an indicator organism for ecotoxicological tests. Its natural emission of light is directly linked to the bacteria's metabolism. Thus, an inhibition of the luminescence is based on a disturbance of the metabolism: The less the light emission, the more toxic the compound for the bacteria.

The HPTLC-bioluminescence coupling combines the separation ability of the HPTLC with the response of a biological system. For us, the aim of the *Vibrio fischeri* detection is to find unknown (cytotoxic) substances which have been previously overlooked using conventional detection methods and thus help to close the gap between chemical-physical detection methods and bioactivity.

The usual workflow is separating substances on an HPTLC plate using appropriate solvents, drying the plate, dipping it into the bacteria solution, taking a photo and evaluate it qualitatively or (semi-)quantitatively.

When starting with the method, the problem occurred that water-soluble substances were dissolved from the plate when being dipped in the bacteria solution. Hence, rolling as an alternative application method for *Vibrio fischeri* bacteria was developed.

Furthermore, an existing quantitative evaluation method was enhanced in such a way, that also HPLC software could be used for evaluation.

This now ready-for-use biodetection is applied for research in our laboratory, especially in the field of consumer goods.

P-1a

Fabrication and chromatographic separations on binder-free, carbon nanotube-fabricated thin-layer chromatography plates

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Patterned forests of carbon nanotubes (CNTs) were used as a template to fabricate novel silica-based thin-layer chromatography plates (TLC). The resulting CNTs are infiltrated with elemental silicon by chemical vapor deposition of silane. Silicon coated CNTs are annealed in the air, which removes the CNTs and converts the silicon to silica. The resulting material is white, which is indicative and characteristic of silica. This process produces TLC plates that are very porous and robust. SEM micrographs of the resulting microfabricated TLC plates demonstrate the precise placement of the adsorbent material.

These microfabricated plates do not require a binder to hold the adsorbent material together, and as normal phase materials give at least baseline separation of a five-component dye test mixture (CAMAG) using toluene as the mobile phase. The chromatographic efficiencies of these microfabricated TLC plates are typically 70 % higher than commercially available high-performance TLC plates, and sometimes much higher, and show a 150 % reduction in development time. That is, these microfabricated TLC plates allow for both efficiency and speed of analysis.

P-1b

Office Chromatography

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Office chromatography combines office technologies of everyday life, e.g. printers and scanners, with planar chromatography. Apart from printing on paper, printing on planar stationary phases is possible and was demonstrated for derivatization [1]. However, a new challenge is the precise application of samples on nanostructured layers of about 7 µm thickness.

Although it is possible to produce planar phases with variable nanostructure and film thickness using the glancing angle deposition (GLAD) technique, and to optimize them with regard to their separation quality [2-4], this ultra-thin phase only tolerates a minimum of solvent. Therefore a commercially available thermal ejecting Bubble Jet printer was modified for exact application whose performance was compared to other application devices.

The high performance of the automatic application was evaluated by the quantification of dyestuff mixtures. The separation process on nanostructured phases was run in parallel for many samples and took less than one minute. Repeatabilities (*%RSD*, $n = 9$, ≤ 2.6 %), coefficients of determination ($R^2 \geq 0.9969$) and relative standard deviations of the calibration curves (*%RSD* ≤ 1.3 %) showed highly reliable results. The quantification with an office scanner in combination with digital image evaluation software provides very effective performance.

Office chromatography involves several advantages such as very low reagent consumption by a clean, versatile and cheap working station. It is operated by some mouse clicks in an environment familiar to the users. Future work will focus on a fully automated chromatographic system in printer size and its hyphenations [5}.

[1] Morlock, G., Stiefel, C., Schwack, W., *J. Liq. Chromatogr. Relat. Technol.* 30, 2007, 2171-2184. [2] Bezuidenhout, L.W., Brett, M.J. *J. Chromatogr. A* 1183, 2008 179-185. [3] Morlock, G., Oellig C.,. Bezuidenhout, L.W., Brett, M.J., Schwack, W., 2010, 2940-2946. [4] S. R. Jim, M. T. Taschuk, G. E. Morlock, L. W. Bezuidenhout, W. Schwack, M. J. Brett, *Anal Chem* 82, 2010, 5349–5356. [5] G. Morlock, W. Schwack, *J. Chromatogr. A* 1217, 2010, 6600-6609.

P-1c

The development of planar chromatography in 1980-2010 (scientometric study)

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An important meaning of planar chromatography in modern analytical chemistry and also a possibility of the estimate of the prospects of its following successful and accelerated development were the basis for this scientometric investigation.

The main purpose was the identification of the main themes in the development of planar chromatography based on an analysis of the TLC literature contained in articles and abstracts published in 1980-1990 and in 2000-2010 in the following journals: Journal of Planar Chromatography, Chromatographia, Analytical Chemistry, Journal of Chromatography A, Journal of Analytical Chemistry, Russian Journal of Physical Chemistry A, Sorption and Chromatographic Processes (Russia), as well as the abstracts of articles published in CAMAG Bibliography Service database (CCBS). The following characteristics of planar chromatography were taken as the most important for the above mentioned periods: methods of chromatography, type of development chamber, version of the plate used, method of plate development, chamber and plate preparation before development, method employed for sample application, mobile phase composition, etc. The results are of interest to specialists working in different fields of planar chromatography. The change of the use of different TLC methods in the 80s and 2000s is shown below.

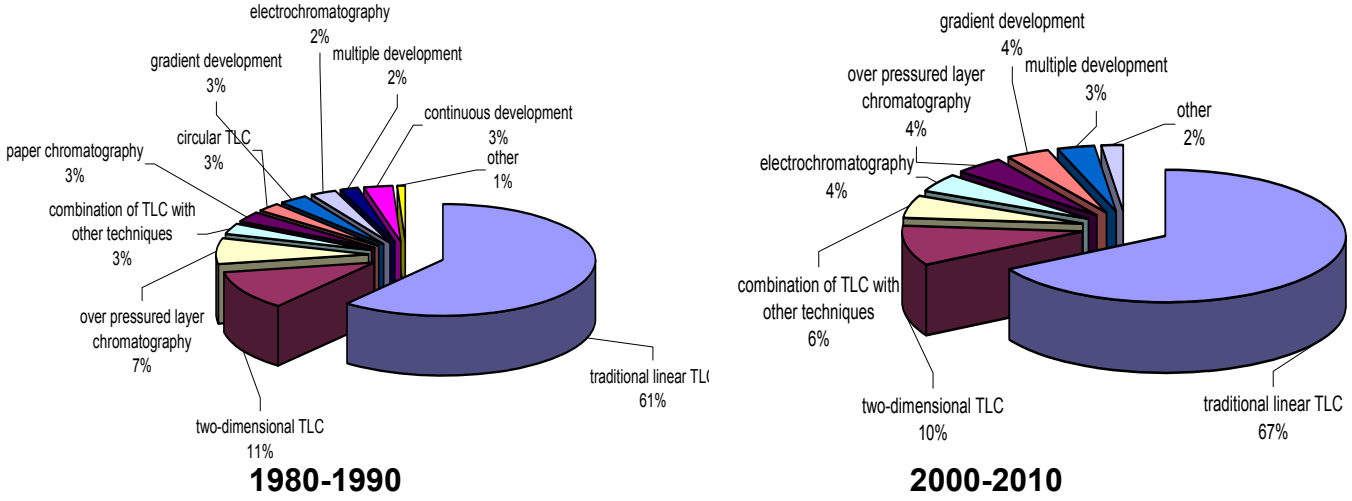

Fig. 1 The dynamics of the change of the role of the main planar chromatography methods (time span of 20 years)

[1] V.G. Berezkin, S.S. Khrebtova. *J. Planar Chromatogr*., 24, 2011 (in press).

P-1d

About definitions of sorption chromatography

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About The Present Sorption Chromatography Definition

According IUPAC Recommendations [1], “Chromatography is a physical (1-?) method of separation (2-?) on which the components to be separated are distributed between two phases (3-?), one of which is stationary (stationary phase) while the other (the mobile phase) (4-?) moves in a definite direction” [(1-?) to (4-?) *are the author's questions*].

Some propositions of this definition are questionable: 1) along with physical, also chemical factors play a significant role in many chromatographic methods: therefore chromatography is physicochemical method; 2) the chromatography is also the method of determination of physicochemical characteristic of chromatographic system and chemical compounds; 3) very often chromatography is not two phases methods but polyphases method (for example, gas-liquid chromatography actually is gas-[liquid-solid] method; 4) in chromatography there are variants in which two phases are moving. IUPAC definition is a simple operational definition, but a more general definition is an inductiive one.

Proposed Chromatography Inductive Definition

The proposed two-stage definition of chromatography was founded on using of the concept of the chromatographic phenomenon [2]:

(1) Chromatographic sorption phenomenon is formation and change of concentration zones of compounds (particles) in a flow of mobile phase relative to sorbents which selectively interact with components (particles) of the analyzed mixture.

(2) Sorption chromatography is a scientific discipline (field of science) studying chromatographic sorption phenomenon and developing methods for its practical application.

All definitions are valid for all variants of chromatography: gas, liquid column and planar.

[1] Nomenclature for Chromatography (IUPAC Recommendations 1993). *Pure and Appl. Chem*., 5, No.4, 1993, 819. [2] V.G. Berezkin, What is chromatography? (A new approach to defining chromatography), Belgium, Kortrijk, The Foundation IOPMS, 2004 (Russian edition - 2003).

P-1e

A new variant of the S-chamber with an ultra small gas volume

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For implementing TLC it is necessary to use a closed chamber, while the increasing of the chamber volume decreases repeatability of chromatographic data. A simple S-chamber (Sandwich-chamber) (Fig.1) proposed by Stahl has a gas volume not less than 30 cm^3.

The authors expected that the decrease in the value *d* (the distance between the adsorption plate layer and the chamber) would allow a decrease the role of transitional processes and accelerate equilibrium of the mobile phase – vapor in the S-chamber. The experimental data obtained corroborated this supposition (Fig. 2). The figure is given in the coordinates *t=f(d)*, as well as in the coordinates *N=f(d)*, where N is the number of theoretical plates.

As it follows from figure 2, the decrease in the distance d in the S-chamber to 0.1 mm permitted an increase the mobile phase velocity, corresponding to shorter separation times by 25 % for Merck TLC plates as well as an increase in the separation efficiency on average by 20 % compared with an S-chambers with d=2 mm.

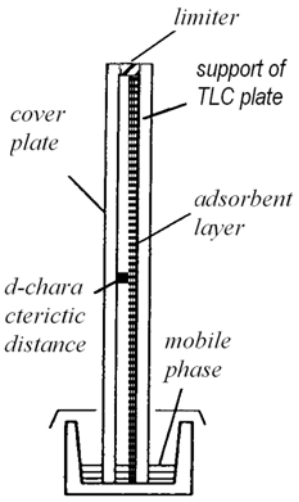

Fig. 1. The scheme of S-chamber

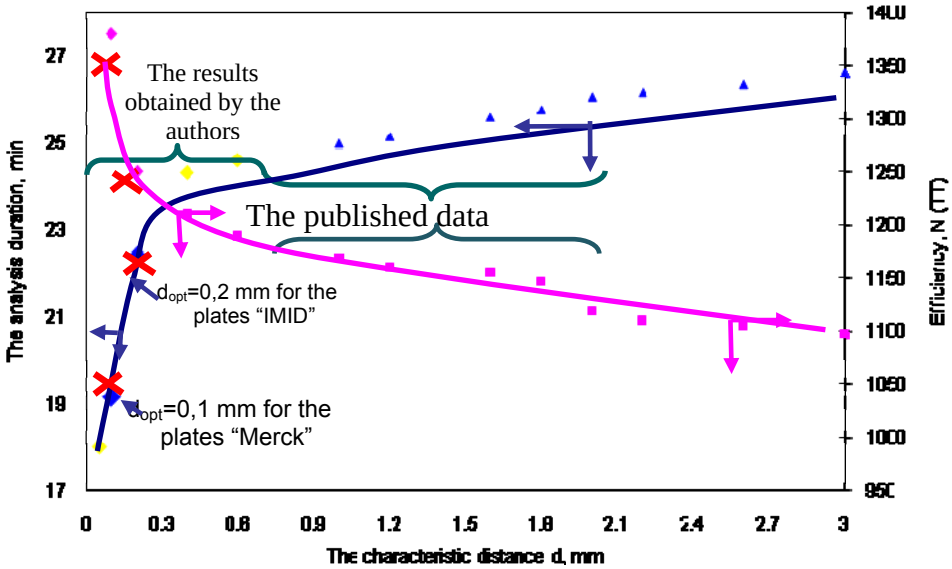

Fig. 2 [1]. Dependence of the duration of mobile phase migration (t, min) and efficiency (N) on the characteristic value of *d* for the plate of 10x10 cm (toluene is the mobile phase).

[1] V.G. Berezkin, S.S. Khrebtova. *Zavod. Lab.*, 77 (1) 2011, 4-7 (Russia).

P-1f

The variants of circular ascending TLC

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Circular TLC is characterized by higher efficiency compared with linear TLC. However, the use of circular TLC in analytical practice is restricted by the insufficient development of the variants of this method. The known circular TLC variants are characterized by, first, that the mobile phase supply is conducted in the central area of a TLC plate, and, second, that separation is carried out in a horizontal chamber.

The main drawback of the circular TLC method is, in our opinion, the relative complexity of the apparatus (in comparison with traditional linear TLC) when the TLC plate positioned horizontally. That is why it seemed more expedient to implement the new variants of ascending circular TLC: corner and lateral TLC using only standard N-chambers for ascending linear TLC (Fig 1).

In the given work the new simple variants of circular TLC (circular, corner and lateral TLC) for the ascending variant of mobile phase supply to the plate are studied, as well, their comparison with traditional ascending linear TLC has been carried out. The given variants are characterized by higher separation efficiency compared with linear TLC (not less than by 2 times), by the simplicity of its implementation, but with longer separation times.

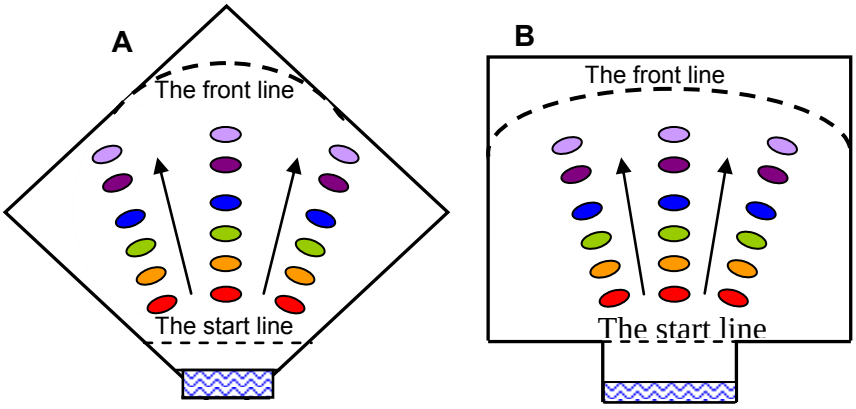

Fig. 1 The schematic of the experiment when implementing circular ascending TLC, (A) the corner position of supply area, (B) the lateral position of supply area.

P-1g

Circular thin-layer chromatography with the closed sorption layer under low pressure

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The velocity of separation is one of the most significant characteristics of the chromatographic method. The low and steadily declining mobile phase velocity is the most significant drawback of all the traditional variants of TLC. To overcome this drawback the Hungarian chromatographers proposed to use forced flow of the mobile phase through a TLC plate temporarily turned into a flat column (TLC under pressure). The given variant has been successfully used in TLC for many decades, however, its practical use is reduced by the high cost of the equipment.

That is why IPS RAS has not decreased its efforts to work out simpler systems in which only a very small overpressure is needed [1,2]. The complications of TLC under pressure are substantially simplified if circular development is used. Using the idea of R.Kaiser we have devised a simple arrangement with a low pressure mobile phase (Fig.1). In this variant of TLC a high pressure applied to a polymeric film in contact with the sorption layer is not required to evenly distributed the mobile phase over the whole TLC plate area.

The use of the proposed method permits a decrease the duration of separation (by 40-50%), as well providing higher efficiency.

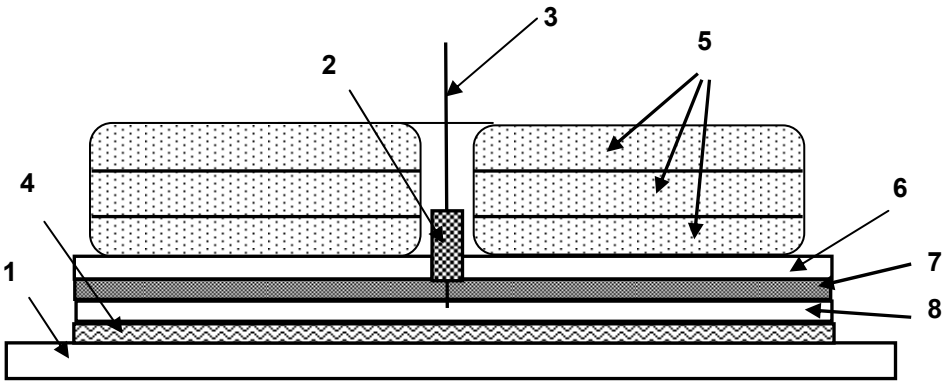

Fig. 1 The schematic of the device for circular TLC under small pressure: 1 base, 2 clamp of the capillary, 3 capillary for the mobile phase supply, 4 plate support, 5 load, 6 protective glass, 7 seal, 8 plate sorption layer

[1] V.G.Berezkin, V.V.Buzaev, *Doklady Physical Chemistry*, 347, 1996, 481-485. [2] V.G.Berezkin, E.V. Kormishkina. *J. Planar Chromatogr.*, 19, 2006, 81-85.

P-1h

Use of frontal and displacement chromatography in TLC to concentrate impurities

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Modern chromatography is, in general, eluent chromatography, although it is often expedient to use frontal and displacement methods, especially when identifying impurities. It is interesting to note, that frontal-eluent variant of circular TLC, that is the variant in which the sample and the eluent move to one point on a TLC plate, was described by N.A. Izmailov and M.S. Shraiber in the first work on TLC . Use of the two-stage frontal-eluent variant in TLC (Fig. 1) is expedient, since when separating the compounds by frontal chromatography not only primary (rather rough) separation takes place, but also concentration of sample components, which permits more reliable identification of impurities. When using circular TLC the chromatogram is in the form of narrow concentric circles that provides an opportunity to increase the efficiency of the separation. Using displacement development in TLC also leads to concentration of diluted samples.

Thereby, the combined consecutive use of frontal, displacement and eluent methods when identifying the impurities is expedient to be applied in TLC. In the given work the estimate of the possibility of using frontal-eluent and frontal-displacement methods in circular TLC as an example of the identification of impurities has been carried out. Frontal-displacement TLC allows the separation and analysis of diluted solutions.

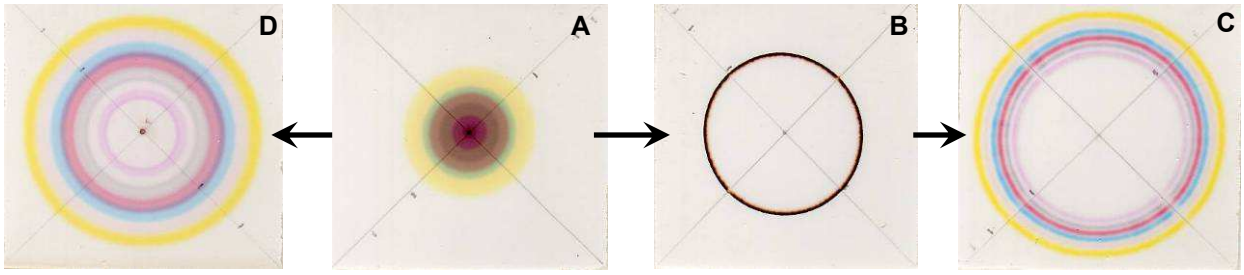

Fig. 1 Schematic of frontal-eluent and frontal-displacement TLC: A - the chromatogram at the frontal application of the sample (frontal TLC); B - the chromatogram after carrying out of the concentration using displacement TLC; C - the chromatogram after the separation of concentrated sample B by the method of eluent TLC; D - the chromatogram after the separation of frontal chromatogram A (eluent TLC).

P-1i

A new approach for multidimensional TLC evaluation

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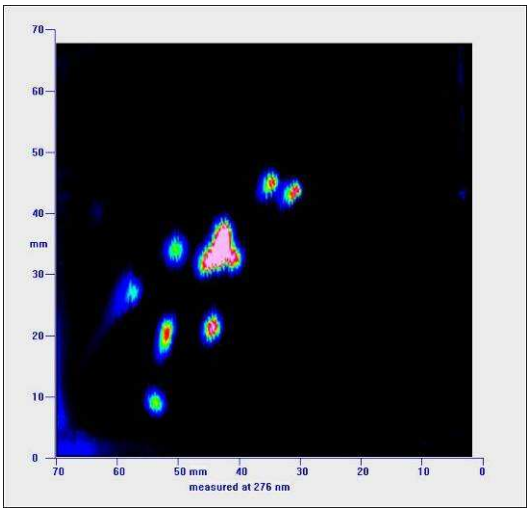
Sulfonamides were the first synthetic drugs with antimicrobial properties and some compounds from this group are still in use. All antimicrobial sulfonamides contain a sulphonamide- and an NH_2-group. Worldwide large amounts of highly diluted sulfonamides are released into the environment. The purpose of this paper is to specify ways for a specific and sensitive quantification of sulfonamides in the effluent of wastewater treatment plants.

TLC is a flexible quantification method. Commonly a separated track is scanned by a TLC-scanner or a CCD-device. This results in a two dimensional evaluation (2D-evaluation), because sets of date pairs (2-tuple data) are generated from the measurement data. Commonly, the x-data represent the separation distance in mm (or calculated as Rf-values) and the y-values represent the more or less correctly transformed light intensities.

A three dimensional evaluation is possible if light intensities from a single TLC-track are measured at different wavelengths. The resulting CCD contourplot is specified as a set of data triplets, called a set of 3-data tuples. Such a set of 3-data tuples can also be generated by scanning a two dimensional TLC separation at a given wavelength. In this case a set of two data files representing distances refer to a single set of light intensity data.

This concept can be extended to a data set of 4-tuples (quaternions) representing light intensities from a 2D-TLC separation measured at different wavelengths. The characterisation of a 2D-TLC-plate is also possible as quinternions when spectral resolved light is measured in absorption and in fluorescence. A set of sexternions or even septernions is possible when the two-dimensional diode-array technique is combined with a specific staining step and bioeffective-linked analysis.



Example of a quaternion set of data. A 2D-separation of 11 sulfonamides separated on a cyano-propyl plate is measured in absorption at different wavelengths. The plot is measured at 276 nm.

P-1j

Determination of performance relevant parameters of HPTLC plates

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Usually the theoretical plate number per meter of a test system is used to describe the efficiency of a HPLC column. For HPTLC an indication of efficiency per meter is not useful because the migration distance is limited to an optimum range of some centimeters. The term separation number is an established parameter to characterize the separation efficiency of TLC plates. The separation number indicates the maximum number of theoretical chromatogram zones that can be separated in a particular separation system. The higher the separation number the higher is the separation performance of the plate.

For Merck, as producer of TLC plates, it is very important to guarantee a consistently high quality of HPTLC plates. Here we show how the separation number is used in quality control for industrial production of HPTLC plates.

P-1k

Reproducibility studies of the peak pattern in HPTLC-spectrophotodensitometry for drug profiling propose

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Influences of developing technique, plate drying, elution and extraction on the reproducibility of peak pattern chromatograms in spectrophotodensitometry were investigated. The reproducibility of peak pattern chromatogram was presented as distribution of C-cosine coefficient function. The clustering of chromatograms of three different brands paracetamol tablet has been carried out for drug profiling. The aim of this study was to investigate the analytical method for drug profiling based on HPTLC-spectrophotodensitometry.

Variation of hR_F values due to elution time differences resulted in wide range of C values. This range of C values can be reduced by using the corrected- hR_F values (hR_{Fc}) in the calculation. Drying plate on 60 ^oC for 10 min or 70 ^oC for 5 min were the optimum one for evaporating mobile phase from plates, and gave the minimum amount of non-analyt peak. Therefore, for the purpose of drug profiling, HPTLC spectrophotodensitometry data base is suggested to use hR_{Fc} values in the calculation. The horizontal development technique presented better clustering than vertical development in the twin-trough chamber.

P-1l

Quasi-continuous videodensitometric recording of chromatograms during the development process

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Traditional TLC is known to have some drawbacks: 1) processes of separation and detection are divided in time and space, and 2) recording of chromatograms is carried out after the separation on plate, that is after the complete wetting of the plate by the mobile phase and its subsequent drying.

The new TLC method with quasi-continuous registration of separation results has been proposed by us [1]. The TLC variant proposed is based on simultaneous conducting of separation on a plate and on registration of chromatograms obtained in a real time during their development using a videodensitometer. In the variant implemented recording of the separation results simultaneously with the progress of the separation up to the stage of the complete wetting of the plate by a mobile phase and its drying before detection. In figure 1 the example of chromatograms obtained at quasi-continuous recording at the different distances of a mobile phase front during the process of separation is given.

Using the proposed method of videodensitometric TLC detection permitted, first, to continuously obtain the information on the process of separation, second, to sharply reduce the duration of the experiment as the compounds of interest are separated earlier than the time required for normal development with a fixed distance. What is especially significant when analyzing colorless compounds is that a signal at UV-light is obtained.

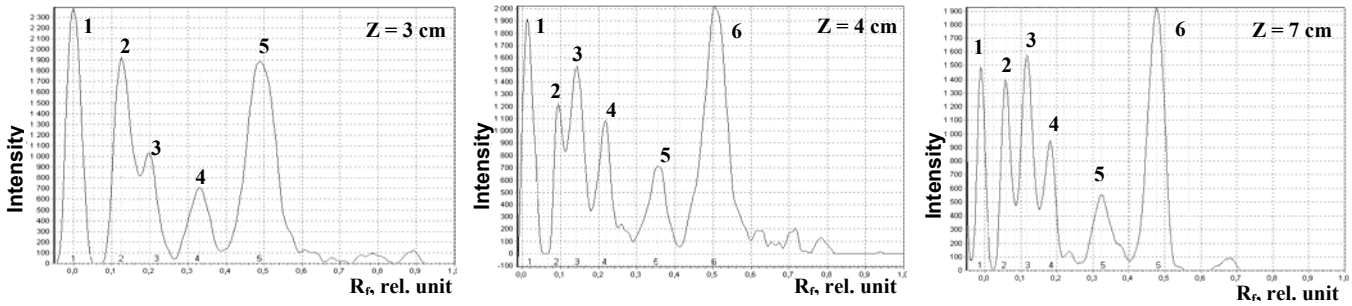

Fig. 1 The change of chromatograms at the continuous results registration in linear TLC (eluent: toluene, 1 Ciba F II, 2 indophenol, 3 Ariabel Red, 4 Sudan Blue, 5 Sudan II, and 6 dimethyl-aminoazabenzene).

[1] V.G. Berezkin, A.V. Chausov, *Doklady Physical Chemistry* 433 (2), 2010, 137-141.

P-1m

Synthesis and purification of inflammatory radiotracer-andrographolide

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Andrographolide, a bioactive component of *Andrographis paniculata* (Burm.F) Nees, is the major lactone diterpenoidal bicyclic constituent in this plant which has proven to exert many bioactivities including anti-inflammatory [1, 2, 3, 4].

The labeling with radionuclides are often used for therapy, detection and quantification of metabolites in the body. Even though the metabolites are very low in concentration they can be detected by the energy they emitted. Radionuclide can be used as radiotracer to detect whether drug really interacts with its target.

The objective of this study was to synthesize ^{131}I-labelled andrographolide for inflammatory radiotracer and to study the chromatographic profiles of the radiolabeled compound by using iTLC-SG plate and RP-HPLC with a fraction collector attached to the instrument. The fractions were collected and measured their gamma emission by using a gamma counter. The highest fractions were further purified by column chromatography and the product was injected to LPS-induced mice to study its biodistribution.

Indirect radioiodination of andrographolide with ^{131}I radionuclide yielded 72.6 % purity of the labeled compound. The compound was distributed in all organs with the highest accumulation occurred in the stomach (16.9 %/g organ).

[1] Chiou, W-F., Chen, C-F., and Lin, J-J., (2000), *British Journal of Pharmacology*, 129: 1553-1560. [2] Shen, Y.C., Chen, C.F., Chiou, W.F., (2002), *British Journal of Pharmacology,* 135: 399-406. [3] Hidalgo, M.A., Romero, A., Figueroa, J., Cortes, P., Concha, I.I., Hancke, J.L., and Burgos, R.A., 2005, *British Journal of Pharmacology,* 144: 680-685. [4] Levita, J., Nawawi, A., Mutholib, A., Ibrahim, S., (2010), *J. Applied Sci*. 10(14): 1481-1484.

P-1n

Virtualization of HPTLC Experiments: Opportunities and Challenges

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Physical distances and the lack of resources make us unable to perform experiments, on sophisticated instruments. Also, good experiment hand experts are always a scarce resource. Therefore, virtual demonstration of sophisticated machines would provide a feel of real hand on the instrument and help in sharing the unaffordable instruments among the students enhancing their skills, knowledge and their contribution to the field.

Fig. 1 A screen shot of the VCL Website

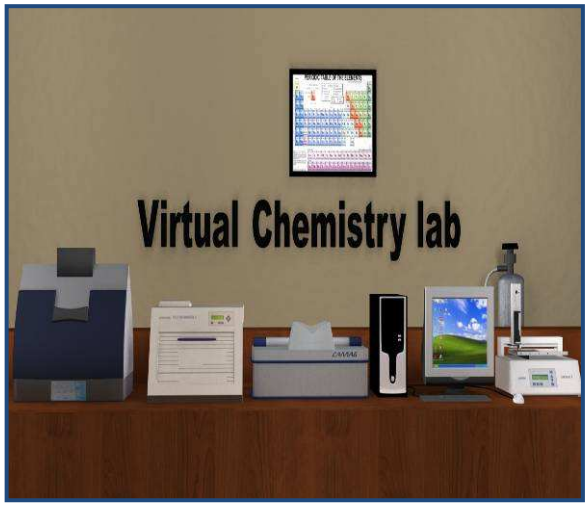

Fig. 2 Animated model of the HPTLC

In the present paper, we share our experiences in setting a Virtual Chemistry Lab demonstration of step by step procedure related to the HPTLC separation of some organic compounds in 3D animations including overview of the instrument, basic principle, theory, experimental manual, precautions, self evaluation quiz and suggested readings. The experiments include standardization of HPTLC method for amino acids, sugar molecules, cholesterol and caffeine as reference and extended to their estimation in market available coconut water, milk samples, edible oils, fruit juices, tea and coffee. The presentation provides a better understanding of concepts of theoretical background of the technique and a platform of a real lab view. In order to obtain optimum performance the development of virtualization software is needed which should be multi-core enabled and allow the use of multiprocessor configurations by running multiple tasks and operating systems on a single physical machine. The concept of virtualization has a major role in helping the organizations to reduce the operational cost, improved efficiency, better utilization and flexibility of existing hardware.

P-1o

TLC determination of the Abraham's Solvatochromic parameters for small organic molecules

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Abraham's solvatochromic parameters have been widely used as a tool for the assessment of several partition constants such as octanol-water, soil-water, blood-brain barrier or skin to blood permeation constant, for many pharmaceuticals as well as ecologically important compounds. These parameters have been determined using various partitioning systems, mostly employing modern chromatographic techniques. Since up to date there is no data about using TLC to determine the Abrahams solvatochromic parameters. The goal of the present study was to try to employ several reversed-phase and normal-phase TLC systems in order to map interactions that occur in chromatographic environment.

Nine reversed-phase systems comprising methanol, acetonitrile, dioxane, or acetone as organic modifiers, on both C-18 and CN-modified silica and six normal-phase systems including silica gel combined with nonpolar solvent systems were employed. The total number of 51 compounds with known values of Abraham's parameters was used for calibration of each system. The dominant terms in LSER equations in the case of reversed-phase chromatography are hydrogen bond donating properties and vacancy formation while the last one is omitted in the case of studied normal-phase systems. Each system was further used to obtain Abrahams solvatochromic parameters for 36 unknown organic compounds of low molecular mass and among them for 12 benzodiazepines. Based on these parameters, several partition constants were assessed such as: octanol-water (log K_{OW}), soil-water (log K_{OC}) and blood brain barrier (logBB).

P-1p

Determination of lipophilicity of some newly synthesized potential antimalarials by the means of reversed-phase thin-layer chromatography

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Lipophilicity is important parameter regarding biological activity of a compounds; it determines the passage of a drug trough cell membranes, its metabolisms and excretion. The common measure of lipophilicity is octanol-water partition coefficient (log*P*). It is based on partition of a neutral substance between aqueous and non-aqueous phases under equilibrium conditions. Traditionally it can be determined by a "shake-flask" method. However, this method is demanding, tedious and time consuming. In addition, it cannot be applied for determination of lipophilicity for both extremely polar and extremely lipophilic compounds. Due to its efficiency and simplicity, reversed-phase TLC (RPTLC) becomes method of choice for the lipophilicity estimation. Determination of lipophilicity by TLC is based on relationship between retention of a substance, expressed as R_M value, and its log*P* value.

In the scope of this study the lipophilicity of twenty nine newly synthesized potential antimalarials (derivatives of 4-amino-7-chloroquinolines and adamantane) was determined by means of RPTLC. Thirteen standard compounds with known log*P* values were used to calibrate the chromatographic systems. They are chromatographed together with studied substances on RP-18 HPTLC plates with a mobile phase containing tetrahydrofurane as organic modifier and ammonia buffer (pH = 9). Lipophilicity parameters of studied compounds were assessed based on their R_M values and calibration relationship previously established for standards. Obtained values were further compared with in-silico predicted lipophilicity parameters.

P-2a

Identification of antioxidant principles in Indian medical plant *Morinda citrifolia* using HPTLC-DPPH• method

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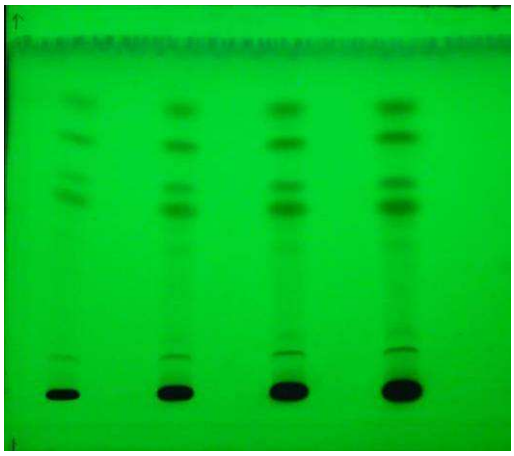
Morinda citrifolia L. (Noni) has been used in folk remedies by Polynesians for over 2000 years, and is reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelmin, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects.

A number of major components have been identified in the Noni plant such as scopoletin, octoanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin-1-methyl ether, anthraquinone glycoside), b-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid,Alizarin, amino acids, acubin, *L*-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and a putative proxeronine.

In the present study HPTLC is used to screen the various phyto constituents present, and specify the phyto principle which are responsible for antioxidant activity using DPPH• method. In order to search for antioxidant principles and the extract was prepared with water. This extract was screened for antioxidant activity using silica gel GF_{254} as a stationary phase and toluene - ethyl acetate - formic acid 6:6:1 as mobile phase at different concentrations. The developed plate was sprayed with 0.5 % DPPH• (2, 2-diphenyl-1-picryl hydrazyl) reagent.



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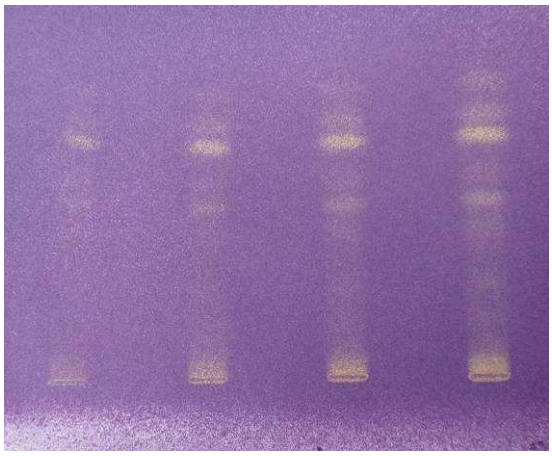

Visible (DPPH•)

P-2b

Screening of antioxidant principles in green tea using HPTLC-DPPH• method

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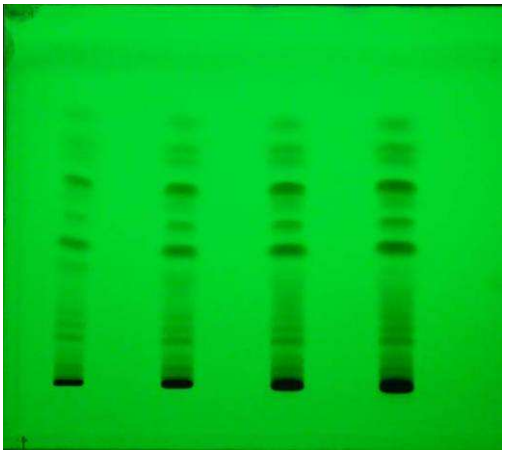
Green tea is made solely with the leaves of *Camellia sinensis* which is rich in antioxidant principles. It helps in the prevention and treatment of cancer and certain neurodegenerative diseases such as Alzheimer's and Parkinson's. In present work we developed a new method for screening the antioxidant principles present in green tea. The developed method is also used to identify and estimate the phyto principles responsible for free radical scavenging activity present in the aqueous extract using HPTLC-DPPH• method.

The aqueous extracted was treated with ethyl acetate and the sample was applied at various concentrations. The developed chromatogram was scanned at 254 nm and 366 nm. The chromatogram was sprayed with 0.5% methanolic DPPH• (2, 2-diphenyl-1-picryl hydrazyl) reagent. The free radical scavenging zones were identified immediately as yellow areas against a light violet/ purple background.



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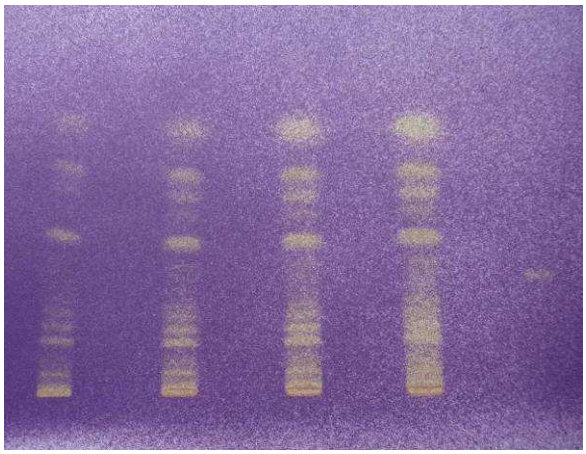

Visible (DPPH•)

P-2c

A method of screening the antioxidant activity of *Solanum nigrum* linn. extracts

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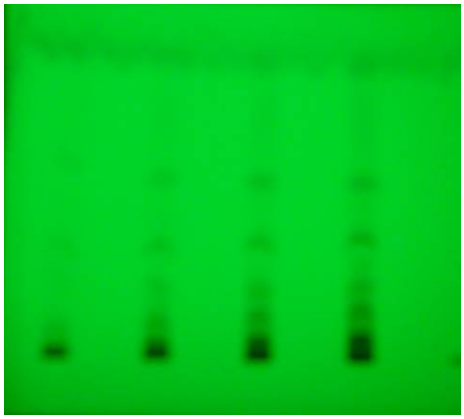
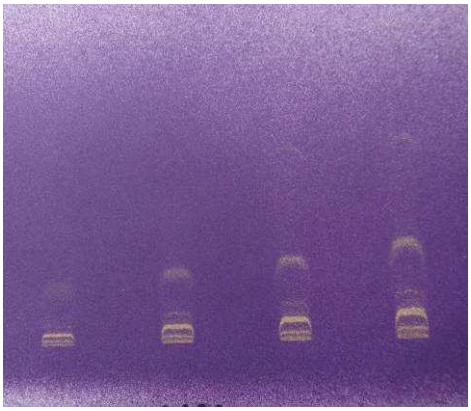
Solanum nigrum Linn. (Solanaceae) commonly known as “Black nightshade” has been extensively used in traditional medicine in India. Extracts of the plant are used as analgesic, antispasmodic, anti-inflammatory, antidiabetic and vasodilator. We screened this plant for antidiabetic and *in-vitro* antioxidant activity [1].

In the present study the leaf extract was taken for *in-vitro* antioxidant study by using HPTLC followed by DPPH• method [2]. In this method, the various phytoconstituents which are responsible for the antioxidant activities can be identified easily and quantified. Further it is possible to isolate the above said antioxidant exhibiting phytoconstituents for the isolation by preparative HPTLC method [3].

The prepared extract was chromatographed using TLC plates silica gel GF_{254} as a stationary phase and chloroform - methanol (9:1) as the mobile phase. Sample extracts were applied as 5, 10, 15, 20 µL at a concentration of 1 mg/mL. The developed chromatogram was scanned at 254 nm and 366 nm using the TLC Scanner 3. The plates were sprayed with DPPH• reagent (0.5 % solution in methanol) in order to identify the phytoconstituents responsible for antioxidant activity.



254 nm



Visible (DPPH•)

[1] A. Dafni, Z. Yaniv, *J. Ethnopharmacol.* 44, 1994, 11-18. [2] E. Reich, A. Blatter, *Thin Layer Chromatography:* Instrumentation. 2nd ed., 2005, 91-99. [3] M. Athar, S. Sultana, S. Perwaiz, *J. Ethnopharmacol*. 45: 1995, 189-192.

P-2d

Identification of antioxidant principles in Indian medical plant *Bacopa monerei* using HPTLC-DPPH• method

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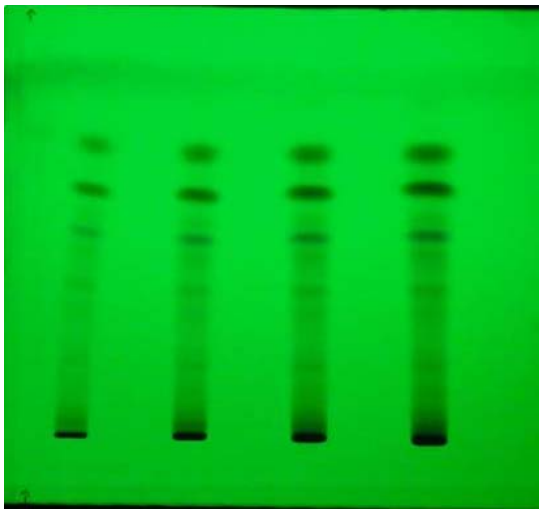
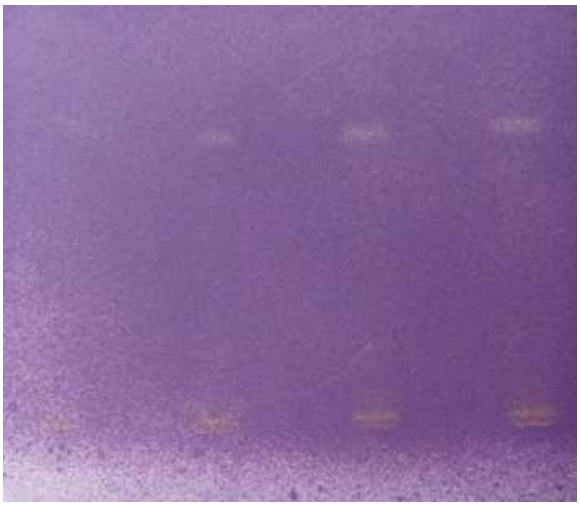
The role of antioxidants in the management of chronic disease is well defined and well established [1]. The available spectroscopic and *in-vivo* assay methods of antioxidants are expensive and time consuming. In the present study HPTLC is used as a tool to confirm the presence of phyto constituents and specify the phyto principles which are responsible for free radical scavenging activity using DPPH• method, in *Bacopa monerei*, a medicinal plant used in the Indian system of medicine as a memory enhancer.

The aqueous extract was screened for antioxidant activity using TLC plates silica Gel GF254 as a stationary phase and toluene - ethyl acetate - formic acid (6:6:1) as mobile phase [2]. The developed plate was sprayed with 0.5 % DPPH• (2, 2-diphenyl-1-picryl hydrazyl) reagent in methanol [3]. These spots were further confirmed for flavonoids by using Folin-Ciocalteu as spray reagent.



254 nm



Visible (DPPH•)

[1] K. N. Udupa, R. H. Singh, *Clinical and Experimental studies on Rasayana Drugs and Panchkarma Therapy*, 1995. [2] R. L. Prior, G. Cao, *Free Radic. Biol. Med*. 27, 1999, 1173-1181. [3] O. N. Pozharitskaya, S. A. Ivanova, A. N. Shikov, D. V. Demchenko, *J. Sep. Sci*.29, 2007, 2245-2250.

P-2e

Optimalization of a HPTLC-DPPH• method with image processing to assess the antiradical activity of phenolic plant constituents

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Chromatographic methods are one of the most popular techniques applied in the screening of plant extracts for the presence of biologically active compounds. The purpose of this study was to develop a novel method for preliminary quantitative evaluation of antiradical activity, based on HPTLC. Moreover, we present new simple technique for handling the data, obtained during analysis.

Chromatographic conditions for complete resolution and anti-radical activity screening were optimized for mixture of phenolic standards, commonly encountered in plant extracts. The best results were achieved on HPTLC plates RP-18W and the following mobile phase: methanol and water (45:54, v/v) with 1 % *o*-phosphoric acid. After drying, plates were immersed in methanolic DPPH• radical solution and immediately documented. The active antiradical constituents appeared as yellowish spots, produced by bleaching the purple color of the DPPH• reagent. A stronger radical scavenging capacity resulted in more intensive area around the bleached spot on the TLC plate. The images were documented (jpg-files) and ImageJ, a free and open source image processing program was used for processing the results obtained and for quantitative measurements. For evaluation of assay efficiency, the antiradical activity of *Rosa rugosa* Thunb. flower extracts was determined. The activity of extracts was expressed as Standard Activity Coefficients (SACs), which are relative measures of the activity to the pure standard.

The proposed method is simple, fast, cheap and easily applicable – only a digital camera or TLC Visualizer and freely available open source software is required. Moreover this technique enables separation and detection of active constituents simultaneously.

P-2f

Screening for antioxidant activity of the ayurvedic formulation Triphala by HPTLC-DPPH• method

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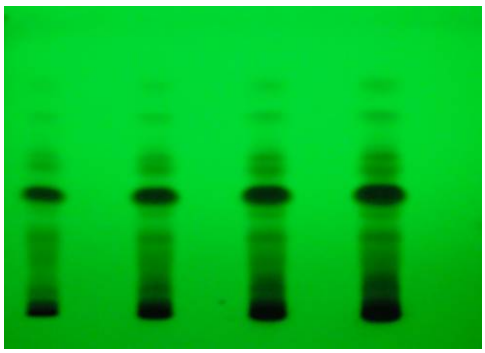
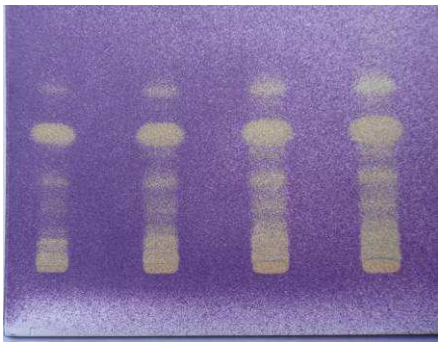
Triphala is a classical ayurvedic preparation that has three main ingredients, Emblica officinale, Terminalia chebula and Terminalia belarica. It is considered the most potent herbal preparation due to its multiple applications. It acts as an antioxidant agent preventing free radical formation in the body. This property of triphala helps it to prevent early aging signs like wrinkles and premature graying of hairs. It is very effective in stimulating the liver for its functioning to the optimized level. More over it also helps in secretion of the bile juices that are very much required for fat metabolism.

In the present study we carried out the identification of the antioxidant phyto principles which are present in the formulation by HPTLC followed by spraying DPPH• reagent since this method is rapid, accurate and economical when compared to other spectroscopic and in vivo methods. Triphala extract was dissolved in water and hydrolyzed to liberate the bounded flavonoids and extracted with ethyl acetate and concentrated. This solution (1 mg/mL) was subjected to HPTLC analysis using silica gel GF_{254} as stationary phase and toluene - ethyl acetate - formic acid (6:6:1) at 5, 10, 15, and 20 µL. The experimental conditions were 25 °C and a relative humidity of 40 %.

The developed chromatogram was scanned using TLC Scanner 3 with winCATS software. Peak areas were measured at 254 nm and 366 nm. Further the developed chromatogram was sprayed with 0.5 % (w/v) methanolic DPPH• reagent. After optimal time of reaction (30 min), the antioxidant principles appeared as yellow -pale color on a violet background.



254 nm



Visible (DPPH•)

[1] The Ayurvedic Formulary of India, part-I Ist edition (Ministry of Health and family Planning ,Dept of Health,Govt.of India,Delhi),1978,85-95,183-193. [2] Lalla J K, Harampurkar P D, Application of HPTLC to Alternative Medicines-qualitative and quantitative evaluation of the Ayurvedic formulation Triphala Churna, J Planar Chromatography 13(5) (2000) 390-392. [3] Kowalska T, Kaczmarski K, Prus W. In: Sherma J, Fried B, Handbook of Thin-Layer Chromatography. 3rd ed. New York, Marcel Dekker, 2003 p. 52-56.

P-2g

Simultaneous quantitative determination of major phenolics in rosemary extract *via* DPPH free-radical-scavenging activity

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Rosemary extracts are convenient sources of natural antioxidants particularly phenolic constituents. This group is mainly constituted by several minor flavonoids, while the main components are rosmarinic acid and the diterpenoid compound known as carnosic acid. Consequently the quality of commercial extracts depends on the claimed antioxidant content calculated as rosmarinic acid (RA) and carnosic acid (CA). Therefore a rapid and selective method aiming to evaluate the above mentioned features should be highly desirable.

A HPTLC-densitometric method, based on external standard approach combined with DPPH radical in situ derivatization, was developed for routine analysis of RA and CA in rosemary extracts. This latter compound is reported to rapidly degrade toward the oxidized form of carnosol quinone in water media [1] and carnosol in methanol [2]. Therefore the problem of the chemical stability of the carnosic acid during the HPTLC analysis has been weighed up.

[1] T. Masuda, T. Kirikihira, Y. Takeda, J. Agric. Food Chem. 53 (2005) 6831-6834. [2] C.T. Ho, T. Ferraro, Q. Chen, R.T. Rosen, M.T. Huang ACS Symposium series 547, Washington DC: American Chemical Society (1994) 2-19.

P-2h

Search for substances with antioxidant activity in Amazonian plants: development of a methodology based on HPTLC

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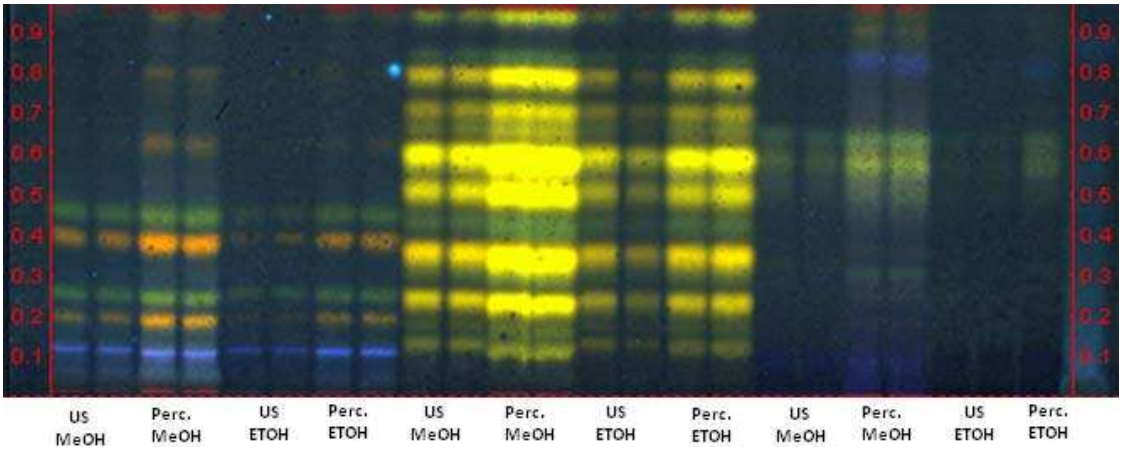
The study of Amazonian plants has been carried out systematically by researchers from the Graduate Program in Chemistry, UFPA-Brazil. Among the families studied, the Leguminosae/Fabaceae, the second largest family in agricultural and economic importance and which has a higher number of genera and species. The leaves of the species *Apuleia leiocarpa*, *Abarema jupumba* and *Parkia pendula*, were chosen as the focus of this work, because they have potential antioxidant activity based on the HPTLC/DPPH• method. The experimental methods developed had as primary target the phytochemical screening, involving the extraction and analysis of polar substances, as flavonoid glycosides, since they are substances with recognized antioxidant activity. The developed methodology proposed to evaluate the extraction power of four solvents: methanol, ethanol, ethyl acetate and dichloromethane, and also select the best method of extraction: Percolation or ultrasound, using HPTLC as a tool of analysis. Starting from the same sample amount, we found that the methanol and ethanol were more efficient for the extraction of interest compounds, based on TLC analysis. When we comparing the method of extraction (percolation or ultrasound) using the selected solvents (ethanol and methanol), there was no difference in the chemical profile, only in the intensity of bands, suggesting different concentrations. The percolation using methanol was the most efficient method.



P-2i

Screening for new cosmetic preservatives from the French Riviera: HPTLC application to antimicrobial and antioxidant assays

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The current trend in cosmetic formulation is the use of natural products as preservatives instead of parabens and other preservatives. In this context, a project named NATUBAVAL has been recently launched. This project aims to promote the plant diversity of the French Mediterranean region by developing new natural preservatives for cosmetics.

In a first step, our team of ethnobotanists selected 200 Mediterranean plants on the basis of their use in traditional medicine, and ethnopharmacological studies. Ethanol-water plant extracts were performed by Soxhlet continuous extraction or by maceration. These aqueous extracts were then screened to highlight those that could be used as preservatives.

Due to the large number of extracts, we used HPTLC to quickly compare the samples and obtain a chemical fingerprint of the corresponding plant metabolites. We tested various solvent systems to separate extract components, such as polyphenols, terpenoids and phytosterols. To reveal these components, we used different derivatization reagents. We focused our study on two biological activities: antioxidant and antimicrobial. The antioxidant activity was assayed using a methanolic diphenylpicrylhydrazyl (DPPH•) solution as free-radical scavenger reagent. Antimicrobial activity, especially antifungal activity against *Aspergillus niger*, was revealed by spraying a spore solution on the plate and after incubation for 24 - 48 hours. One original plant (A) presented interesting activities, both as antioxidant and antimicrobial. Consequently, in-depth analyses were performed to characterize the bioactive compounds.

Our presentation reports the results obtained from our bioguided screening of plant extracts. The analytical study of the A plant is also developed.

[1] J.L. Rios, M.C. Recio, *Journal of Ethnopharmacology* 100, 2005, 80–84. [2] Sagar B. Kedar, R.P. Singh, *Journal of Food Science Technology* 48 (4), 2011, 412–422. [3] J.L. Rios, M.C. Recio, A. Villaw, *Journal of Ethnopharmacology* 23, 1988, 127–149.

P-3a

Hyphenations in HPTLC

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Initially advocated by T. Hirschfeld, in 2007 the term hypernation (super-hyphenation) was coined by I.D. Wilson and T.A. Brinkman to place all of the required spectrometers into a single system so that all of the spectroscopic information is obtained in a single run. Hypernation represents a logical, rapid and efficient strategy for obtaining the maximum possible information out of a single separation. The major problems associated with column-based hypernations are capital cost and strategies for dealing with the large amounts of data that such systems produce. The complexity of the instumentation increases, which makes them difficult to operate in a routine way. A single eluent that is optimal for all detectors is difficult to obtain. Differences in sensitivity between spectroscopic techniques and spectrometers are challenging as well.

All these problems are much less challenging in HPTLC-based hypernations because of the open system that is (1) highly adaptive to different sensitivities, (2) cost-effective by modular instrumentation compared to the status quo in analysis, (3) generating less data due to targeted access to points-of-care on the plate, and (4) directly accessible for the respective optimal solvent because the eluent is evaporated after chromatography and not impacting the different detectors. The latter is extremely relevant for effect-directed detection with bioassays.

Existing hypernations are for example [1, 2]:

- HPTLC/UV/Vis/FLD
- HPTLC/UV/Vis/FLD/MS
- HPTLC/UV/Vis/FLD/bioactivity/HRMS
- HPTLC/UV/FTIR
- HPTLC/UV/Vis/FLD/FTIR ATR
- TLC/Vis/SERS

Several examples are given in the field of natural products search, pharmaceutical analysis and food analysis [3-5].

Literature: [1] G. Morlock, W. Schwack, *TrAC* 29/10, 2010, 1157-1171. [2] G. Morlock, W. Schwack, *J. Chromatogr. A* 1217, 2010, 6600-6609. [3] A. Klöppel, W. Gasse, F. Brümmer, G. Morlock, *J. Planar Chromatogr.* 21, 2008, 431-436. [4] A. Klöppel, F. Brümmer, A. Kolm, G. Morlock, *CAMAG Bibliogr. Service CBS* 102, 2009, 4-7. [5] G. Morlock, C. Oellig, *J. AOAC Int*., 2009, 745-756.

P-3b

Screening for bioactive secondary metabolites in sponges by HPTLC coupled with bioluminescence bacteria assay followed by HRMS

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The direct coupling of HPTLC and bioluminescence bacteria assay with *Vibrio fischeri* was used for compound separation and monitoring potential pharmaceutical effects in several marine sponge extracts. Sponges were collected in the Mediterranean Sea (Adriatic Sea) and screened for bioactive metabolites. Separated bioactive compounds inhibited the bacterial luminescence and could be identified as darkened zones directly on the HPTLC plate.

For structure elucidation of unknown substances the application of online high resolution mass spectrometry (HRMS) was used. The compounds could easily be investigated by rapid extraction directly from the analytical plate using an extractor based interface (ESI-HRMS) or “open air” from the plate’s edge via DART.

Thus, a *Vibrio fischeri* bioactive zone was identified as Avaron, an antiviral metabolite synthesized by the sponge *Dysidea avara*. Further, we detected an increase of productivity in sponges from *in vitro* culture (Primmorphs) as well as the additional synthesis of toxic metabolites. Following investigations with *Crambe crambe*, that disclosed to have one of the most toxic metabolites compared to other sponges in the bioluminescence assay, showed antitumoral properties.

The combination of planar chromatography with luminescent bacteria bioassay and following structure elucidation via HRMS proofed to be an effective, robust and economic system for bioactivity screening in complex mixtures. Parallel analysis of 30 extracts was possible and much faster compared with rival column techniques (*e.g.*, HPLC). This method is particularly suitable for drug discovery giving first hints of potential pharmaceutical applications and was used in the field of bioactive natural products for the first time.

P-3c

Performance data for the new TLC-MS Interface

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Since the middle of 2009 an upgraded elution-head based TLC/HPTLC-MS interface has been available for online elution of zones of interest from the plate (TLC-MS interface, [1]). This small add-on module, which can be plug-and-play connected to any given HPLC-MS instrumentation in the laboratory, was shown to be universal and versatile [2], and is expected to grow in interest. In contrast to the forerunner model ChromeXtract [3], which was constructed for flexible foils, the whole plate can evenly be placed on the carrier. This is helpful for proper sealing, especially of zones on the outer tracks. It is suited for operation on glass plates as shown for the modified ChromeXtract in 2006 [4]. Zone tightening is performed pneumatically, which is much more convenient for the user, and a laser light cross aids in proper positioning and exact centering of the elution head onto the zone. In addition, the cleaning of the elution head is automated, which eases the performance and accelerates analysis time.

Performance data for this new interface has not been reported thus far, although it is assumed that they might be similar to, or even better than the modified manually operated ChromeXtract. Hence, the present study focuses on such performance data using a commercially available 6 component dye mixture. The repeatability *(%RSD*, n = 5) was around 10 %, the limit of detection was in the low ng/band range and the coefficients of determination of the calibration curves were better than 0.993. The proof of the correct assignment of some lipophilic dyes was also demonstrated, and the study clearly showed the reliability of the elution-head based HPTLC-MS and the support by planar hyphenations in general [5].

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P-3d

Analytical characterisation of rhubarb fractions for application in cosmetics and pharmaceutical products by using the TLC-MS Interface

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Both the pharmaceutical and cosmetics industry underline their need for new active ingredients or ingredient mixes from natural sources. Interest is focussed, in particular, on polyphenolic compounds from plant sources with their well-documented free-radical scavenging activity, immunomodulatory, antiviral as well as general antiphlogistic properties.

This work, therefore, investigates whether the *Rheum* genus, a member of the polygonacea family, could be a potential alternative raw material. Defined extracts from different *Rheum* species, that were available as ethyl acetate phase as well as aqueous phase, were fractionated using column chromatography. This means that it was possible to produce a defined limitation of the range of contents of these fractions. Thus, it was easier to identify the polyphenols contained, on the one hand. On the other hand, during the functional tests of the fractions obtained by this method to determine their antioxidant, antiviral and immunostimulatory properties, a clear increase in their medical action compared with those of the total extracts was noted. The fractions were analytically characterised using high performance liquid and high performance thin layer chromatography combined with spectroscopic and mass spectroscopic detection (metabolit profiling). TLC-MS-Interface allows an identification of substances directly from the thin layer plate. All substances could be ionized by using ESI.

A combination of functional and analytical characterisation techniques were used to determine links between the presence or absence of specific compounds and an increase or decrease in the above mentioned free-radical scavenging activity, antiviral or immunomodulatory properties.

P-3e

Antimicrobial activity of *Chelidonium majus* and anticancer drug Ukrain by TLC – direct bioautography

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The greater celandine (*Chelidonium majus L*.) is an herbaceous perennial plant common in Europe, western Asia and North America. The main alkaloids present in the herb are: berberine, chelidonine, coptisine, sanguinarine and chelerythrine [1]. The fresh extract from the plant inhibits the growth of fungi, bacteria, viruses and protozoa. Celandine related drugs have calming, analgesic, anticonvulsant and hypnotic effects. One of the therapeutic agents based on *Chelidonium majus* extract is Ukrain, a semi-synthetic thiophosphoric acid derivative of chelidonine alkaloids. Ukrain is a selective cytostatic, which is toxic only against cancer cells. It has antymitotic action against tumors, inhibits angiogenesis and stimulates immune system. Ukrain is used for the treatment of pancreatic, colorectal, breast, rectum, bladder, prostate, ovarian, endometrial cancer as well as leukemia.

In the present work antimicrobial activity, both of Ukrain and of its components (isoquinoline alkaloids from *C. majus L.),* against two strains of bacteria: *Escherichia coli* and *Bacillus subtilis* were investigated. The method of choice was TLC combined with microbiological detection, called direct bioautography (TLC–DB) [2]. In this method bacteria grew directly on the chromatogram containing separated substances. After staining with MTT dye solution, antibacterial activity was observed as white inhibition zones on a purple background.

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P-3f

Low-temperature TLC-bioassay on silica gel layers for screening volatile samples for free radical scavengers and antioxidants

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Recently it has been shown that oxidative stress can play an important role in the development and progress of some neurodegenerative diseases (*e.g.,* Alzheimer's and Parkinson's disease, myastenia gravis, *etc.*). Thus there is a growing interest in finding chemical compounds that can act as natural free radical scavengers and can easily cross the blood-brain barrier. Volatile samples contain a variety of chemical compounds that are characterized by a wide range of biological activity. Most of the chemical constituents of essential oils are terpenoids, that have been shown to posses antioxidant, anti-inflammatory or anticholinesterase activity. Due to their low molecular weight and high lipophilicity they can easily cross the biological membranes and induce the aforementioned activities. Unfortunately the lipophilic character of these samples causes difficulties in assessing their free radical scavenging ability by means of routine spectrophotometric tests.

In this study low-temperature TLC is proposed as a suitable technique for the detection of free radical scavengers and antioxidants in selected essential oils of the *Lamiaceae* family. Silica gel effectively immobilizes essential oil constituents and the direct access to the partially separated compounds gives the ability to screen the samples with antiradical properties. One of the greatest advantages of the proposed technique is the ability to screen several samples simultaneously. It has been shown that it is possible to screen the samples' constituents for the possible synergistic and antagonistic interactions. This methodology also enables the identification of compounds with prooxidant properties. It was possible to identify the most potent free radical scavengers and antioxidants from among the analyzed samples by means of the developed technique.

P-3g

Standardized bioautographic methods for effect directed screening of samples separated on HPTLC plates

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Effect directed bioautographic screening on HPTLC is an important tool in the search for new bioactive compounds from medicinal plants. It is also an additional detection mode for unknown constituents of samples. The advantage of in-situ bioautographic methods on HPTLC results from the hyphenation of a separation of complex samples with subsequent biological tests. A simplified sample preparation and instant results are additional benefits of the techniques.

In this study we adapted three bioautographic methods for in-situ detection and combined them with the advantages of state of the art HPTLC for reproducible results. The different types of bio-assays can be performed directly on the plate without or with just a short incubation time. Several method parameters had to be standardized, above all the immersion or spraying procedure, as well as the exposure and incubation time. We studied following bioautographic assays:

- Enzyme assay: inhibition of α- and β-glucosidase
- Cellular detection process: inhibition of bioluminescent bacteria *Vibrio fischeri*
- Physico-chemical detection: reduction of DPPH radical to DPPH

The inhibition of α- and β-glucosidase is an important target for the search of pharmaceutical ingredients active against type 2 diabetes and anti-viral infections [1]. The bioluminescent bacteria *Vibrio fischeri* used for activity-related detection reveal toxicological properties of plant species. This bio-assay is also suitable for the monitoring of drinking and waste water [2]. The radical scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) is suitable for detecting antioxidant properties of substances from medicinal plants [3]. The screening for antioxidative compounds may also be of interest to manufacturers of new cosmetic formulations.

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P-3h

Potential applicability of modern bioautography (BioArena) in the study of plant ingredients

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Searching for bioactive natural products requires appropriate bioassays, focused on the desired activity (e.g. antifungal, antibacterial). Different versions of layer liquid chromatography are ideal technical solutions for the chemical and biological screening of drug ingredients, because of high-throughput, low cost, easy maintenance and selectivity of detection reagents. Direct bioautography is the combined application of planar layer liquid chromatographic separation and post-chromatography bioassay. In the course of the biological detection the adsorbent layer after development is dipped into or sprayed with cell suspension and afterwards the bioautogram is visualized. This is applicable to a broad range of microorganisms that can grow directly on the chromatographic plate, for which appropriate separation conditions (without a disturbing effect on the bioassay) are also needed. Visualization of the bioautogram is usually performed by the use of yellow tetrazolium salts, reduced by living cells to bluish formazan. Another visualization method uses bioluminescent bacteria. Using the emitted light as signal, the performance of biodetection is very easy. The image of the bioautogram can be directly recorded by a cooled camera in a dark box with appropriate exposure time (depending on the intensity of the light). The light emitted by bacterial cells is closely linked to energy metabolism, so correlating with the metabolic state and the viability of the cells.

BioArena is based on direct bioautography; beyond biological detection, however, it is also appropriate for examination of the mechanism of cell proliferation inhibition and/or promotion effects. The influence of different endogenous and/or exogenous substances on the bioactivity of separated compounds can, moreover, be examined by dissolving substances in the cell suspension just before inoculation.

Overpressured-layer chromatography (OPLC), as an efficient forced flow planar layer liquid chromatographic technique, results in compact chromatographic spots and good separation efficiency especially by the use of fine particle size chromatoplate (e.g. HPTLC). OPLC is also well suited for fractionation using analytical chromatoplates providing on-line detection and subsequent peak collection. This system can be applied for efficient isolation of various substance types in general, but in this case the potential antimicrobial components from plant extracts.

The separation and isolation of antibacterial components, found previously active in a bioautographic study, of different plant extracts from species of *Asteraceae*, their re-chromatography, and identification by means of GC/LC-MS as well as modern bioautographic studies of concentrated collected peaks are demonstrated. In bioassay investigations Gram positive soil bacterium *Bacillus subtilis* (antibiotics generate white spots) and chromosomally tagged luminescence Gram negative *Arabidopsis* pathogen *Pseudomonas savastanoi* pv. *maculicola* (antibiotics generate black spots) were used.

P-3i

Development of TLC-direct bioautography tests based on *Escherichia coli* and *Bacillus subtilis* strains

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The popularity of effect directed analyses (EDAs) has increased rapidly in the last decade. The main reasons for this are: good sensitivity and selectivity of EDA methods, as well as their simplicity, speed, and low cost. Among these methods, thin-layer chromatography hyphenated with direct bioautography (TLC-DB) plays an important role [1].

The principle of TLC-DB is as follows: 1) dipping a developed TLC plate in a bacterial suspension growing in a proper medium, 2) incubation of the TLC plate in a humid atmosphere, and 3) visualization of antimicrobial compounds on the TLC plate, usually using tetrazolium salts, e.g. MTT. The dye is reduced by dehydrogenase of living bacteria into colored formazan. The cream-white spots on the purple background indicate the presence of antimicrobial agents on the TLC plate [2, 3].

Two newly developed TLC-DB tests for Gram-negative bacteria, *Escherichia coli* and Gram-positive bacteria, *Bacillus subtilis*, are presented. Various parameters influencing bacterial growth during the optimization procedure were studied, such as: various times of pre-incubation and incubation of the bacteria as well as various times of incubation of TLC plates before and after spraying with MTT solution [4]. First, the methods were tested for flumequine standards and then applied to the determination of antibiotic residues in milk.

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P-3j

TLC coupled with electrospray laser desorption ionization (ELDI) mass spectrometry for high-throughput analysis

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Electrospray laser desorption/ionization (ELDI), an ambient ionization method which combines laser desorption with electrospray ionization, has been developed to characterize chemical compounds on the surface of the sample. [1] Analytes are desorbed from the sample surface using a pulsed laser beam. Post-ionization of the desorbed analytes in an electrospray plume produces high-quality mass spectra characteristic of electrospray ionization, including organic, inorganic and biological compounds. Since sample pretreatment is usually unnecessarily, ELDI/MS can be used to rapidly characterize the chemical compounds on the surface of various solids and liquids including those on a thin-layer chromatography (TLC) plate. [2]

In this study, we have developed a TLC-ELDI/MS system for the purpose of performing high throughput sample analysis. A prototype TLC-ELDI/MS system was built by using the parts purchased mostly from LEGO include building blocks, gears, belts, conveyer, light sensor, electronic controller and motor etc. One of the advantages of using building blocks to build the system is that it is extremely easy to take apart, switch, or change the units. The TLC-ELDI/MS system includes (1) a TLC plate storage box with a plate dealing system, (2) plate conveyer system, (3) light sensor and electronic control system, (4) TLC plate collecting box and (5) ELDI system. As the first TLC plate moved out of the storage box, it entered a conveyer and passed over the light sensor, this would trigger the dealing machine in the storage box to deliver the second TLC plate out of the box. A pulse UV laser was used to irradiate the surface of TLC plate on the conveyer. The chemical compounds desorbed by the pulse laser were subsequently entered an electrospray plume and ionized by the charged methanol species in it. The TLC plates were delivered through the laser spot sequentially. At the end of the conveyer, the TLC plate entered a collecting box. As the LEGO based TLC-ELDI-MS system was proven to work, we replaced the LEGO units with Teflon parts and tested the system again. Finally, we replaced some Teflon parts with stainless steel units for the concern of long term use.

A drop of ink and cold formula were used as the samples for testing. The sample was applied on the commercial alumina-based silica gel TLC plate. The chemical compounds were then developed. After drying in ambient, the TLC plate was set in the storage box and analyzed by TLC-ELDI/MS. At least six chemical compounds in the ink were successfully detected including the ions of m/z 372, m/z 344, m/z 227, m/z 213, m/z 358, and m/z 329. Nine chemical compounds were detected in the cold formula including the ions of m/z 203, m/z 192, m/z 275, m/z 354, m/z 180, m/z 195, m/z 383, m/z 221, and m/z 152. The time required to complete the analysis of a TLC plate with a distance of 4 cm was approximately 3.5 min. This suggests that the TLC-ELDI/MS system allows the screening up to 400 TLC plates per day.

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P-3k

HPTLC analysis, antioxidant activity and xanthine oxidase inhibitory activity of Indian medicinal plants

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A chemical fingerprint was developed for roots of six Indian medicinal plants by a simple, sensitive, selective, precise, and robust HPTLC method for determination of flavonoids. The antioxidant capacities were estimated by using *in vitro* and *in vivo* assay. In order to find *in vitro* antioxidant properties, plant extract were studied for ABTS, DPPH and FRAP methods expressed as TEAC (Total equivalent antioxidant capacities). The *in vivo* evaluation of oxidative stress (LPO) and antioxidant defenses (concentration of GSH, as well as CAT and SOD activities) were measured in CCl_4 induced toxic rats. All plant extracts was found to inhibit the toxicity as seen from the decreased LPO and increased GSH, SOD and CAT levels.

The plants qualitatively revealed predominant amount of flavonoids like Quercetin, Rutin, Luteolin and Vitexin, also with high levels of phenolics. The TEAC values of the these medicinal plants ranged from 46 - 140 µM trolox/100 g dry weight (dw), from 85 - 430 µM trolox/100 g dw, and 185 - 560 µM trolox/100 g dw for ABTS, DPPH and FRAP respectively. The total phenolic and flavonoid content ranged from 10.2 - 28.2 and 5.8 - 10.1 mg of gallic acid equivalents (GAE)/100 gdw respectively. Xanthine oxidase inhibitory activity was assayed for the aqueous, methanol – water mixture and methanolic extract. All plant roots extracts demonstrated significant xanthine oxidase inhibitory activity at 100 g/mL, plant extracts revealed an inhibition greater than 50 % and IC_{50} values below 100 g/mL and showed more than 50 % inhibition. This effect is almost similar to the activity of allopurinol against xanthine oxidase (90.2 ± 0.4 %).

This study indicates that the high content of flavonids and phenolics with good antioxidant activity may be helpful for treatment of inflammation and gout.

P-4a

HPLC-MS/MS, HPLC-TOF MS or simply HPTLC? Quantification of sucralose in various aqueous environmental matrices using HPTLC multidetection

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Sucralose, a persistent chlorinated substance used as sweetener in Europe since 2005, can already be found in waste water, and various countries focused on the release of sucralose into the aquatic environment. HPLC-MS(/MS), HPLC-TOF and GC-MS were preferably used as analytical methods in the ultra-trace range after solid phase extraction.

A quantitative HPTLC method, which was orthogonal to the given analytical methods with regard to separation principle and detection, was highly suited for screening, as up to 17 samples were separated in parallel on a HPTLC plate within 15 min. The availability of post-chromatographic derivatization of sucralose was another benefit, which allowed its selective detection in sewage effluent and surface water (Fig. 1). The sucralose content determined in four water samples of an interlaboratory trial was in good agreement to the mean laboratory values of that trial, analyzed by HPLC-MS/MS or HPLC-TOF-MS with the use of mostly isotopically labeled standards. The good accuracy, cost-efficiency and high sample throughput capacity proved HPTLC as preferable method.

Fig. 1 Selective detection of sucralose at the ultratrace level in sewage effluent and surface water after derivatization

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P-4b

High-performance thin-layer chromatographic method for determination of active ingredient in novel insecticide formulations

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A new, simple, sensitive, selective, precise, robust and rapid HPTLC method was developed for the detection and quantification of commercial formulations of fipronil, indoxacarb, thiacloprid, thiamethoxam, imidacloprid, spiromesifen and spirotetramat. The active substances were extracted from commercial formulations with acetone - acetonitrile and chromatographed on HPTLC plates silica gel 60 F_{254} in horizontal chambers with acetone - acetonitrile - ethyl acetate - methanol - chloroform as mobile phase. A known amount of reference grade of these novel insecticides and its formulation was subjected to TLC, and the amount of pesticide present in the active ingredient spot was estimated by densitometry in a single beam, single wavelength reflectance mode.

The method was validated in terms of linearity, precision, accuracy, and sensitivity. Calibration curves of these novel insecticides were linear in the range of 50 - 200 ng, and the correlation coefficient for the calibration equation ranged between 0.91 and 0.98. Recoveries from laboratory-prepared test samples of the formulation were in the range of 93 - 97%. The method has been validated by further analyses using gas liquid chromatography (GLC) using electron capture detector (ECD) for fipronil, indoxacarb, thiamethoxam and high performance liquid chromatography (HPLC) for thiacloprid, spirotetramat and gas chromatography-mass spectrometry (GC-MS) for spiromesifen, and results were comparable. The method was found to be reproducible and convenient for quantitative analysis of these compounds. The proposed HPTLC method has application for quality control and determination of the shelf life of commercial formulations.

P-4c

Contribution of AMD-HPTLC fluorescence and UV scanning densitometry to the characterization of heavy petroleum products

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Heavy petroleum cuts cover a wide range of products which consist of hundreds of compounds varying in molecular structure and size, polarity and functionality. Long-chained, linear and cyclic saturated hydrocarbons are also present in these products together with high-MW polyaromatic and heterocyclic structures. This presentation demonstrates the potential of HPTLC using Automated Multiple Development (AMD) for the characterization of these kinds of products.

For a given heavy petroleum product, different schemes of AMD separation can be envisaged to obtain information with increasingly levels of complexity. The use of elution modes (sequential elution with solvents, refocalization using isocratic elution, or solvent gradient elution) and different stationary phases allows several levels of information:

- a simple or more complex hydrocarbon type-analysis
- a detailed separation
- a distribution of aromatics according to their ring number.

Densitometry with UV and fluorescence is used to determine UV-absorbing compounds. FDIC using the berberine cation is used for detecting and quantifying saturated compounds. Examples are shown for standard substances that model heavy hydrocarbon products and real samples.

P-4d

HPTLC profile of substituted coumarins derived from microbial transformation

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In the present study, microbial biotransformation, one of the tools of Green Chemistry, is used for preparing certain substituted derivatives of coumarins as biologically useful compounds where, the usage of the hazardous chemicals and solvents were fairly minimised. Nine biotransformation experiments were performed, where, random combination of four micro-organisms and five substrates (JSN-1 to JSN-9) showing optimum recovery of the final compounds were selected. The experiments were performed by Shake Culture technique, with two-stage fermentation procedure and the conditions suitable for the growth of the organism required for both the substrates and the product formation were standardised.

The characterisation of the transformed products was done mostly on the basis of HPTLC. Further, to support their final structure, mass spectra and NMR were obtained. The samples were loaded on HPTLC plates silica gel G 60 F_{254}, 20 cm x 10 cm, using the Linomat 5 and detected at 254 nm and 366 nm with TLC Scanner 3. Out of the nine biotransformation experiments performed, two transformations were found to be successful. These transformations are as follows; O-deethylation of 7-ethoxy-4-methyl coumarin which gave 7-hydroxy-4-methyl coumarin using *S. griseus* with a yield of 4.2 % (JSN-8) and O-methylation of 7-hydroxy-4-methyl coumarin which gave 7-methoxy-4-methyl coumarin using *C. tropicalis* with a yield of 5.5 % (JSN-3). The crude biotransformed extracts were then screened for their anti-oxidant activity by DPPH scavenging method and nitric oxide scavenging method. Further, the test extracts were subjected for their routine anti-bacterial activity.

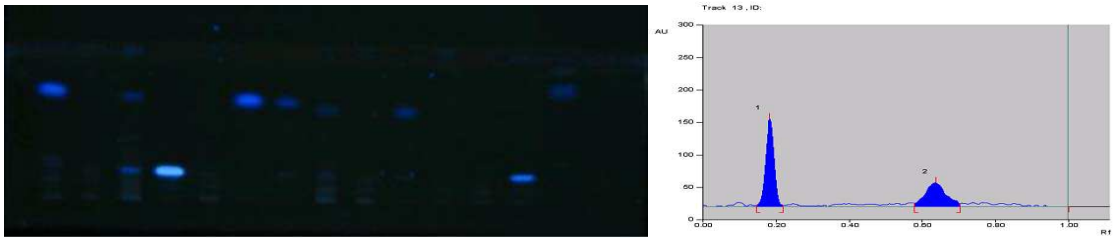

Fig. 1 HPTLC plate developed in benzene - MeOH 9:1 loaded with crude test extracts, substrate and standards, illuminated and scanned at 366 nm.

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P-4e

Development of new solvent system for the analysis of triazophos poison

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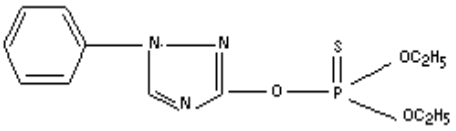
Triazophos is an organophosphorus pesticide, which was most recently evaluated toxicologically by the 1993 JMPR (Annex 1, reference 68), when an ADI of 0–0.001 mg/kg bw was established. It was re-evaluated by the *Pesticide Residues in Food-2002-Joint FAO/WHO Meeting on Pesticide Residues.*

In India, generally triazophos 40 % EC is used. Triazophos is a phosphoric acid ester compound with contact and stomach action. It controls pests like Bollworms, Caterpillar, Leafhoppers, Stem borers, Cutworms Armyworm & mites of Cotton, Cereals, Rice, Potatoes, Citrus & Vegetable Crops. Although several instrumental methods like HPLC, GC, UV-Visible, HPTLC are used for separation and identification of triazophos. Since these methods are very costly and time consuming, therefore in the present study an attempt was made to analyze it using TLC. The advantages of TLC are the low cost, less time consuming, analysis of large number of samples simultaneously and minimum sample preparation. Therefore in the present study an attempt was made to develop new solvent system for triazophos (61.1 %). The detection of spots on the developed TLC plates was performed by using Bromophenol Blue followed by 4 % acetic acid as spraying reagent. There were 20 different solvent systems with different volumetric ratios used in separation and identification of triazophos.



Structural formula of triazophos

P-4f

HPTLC method for detection of chlorantraniliprole and flubendiamide

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A method was developed for the detection and quantification of active ingredients in soluble concentrate (SC) formulations of chlorantraniliprole (Coragen 18.5 SC) and flubendiamide (Fame 480 SC). Known concentrations of the reference grade standards and the soluble concentrate of chlorantraniliprole and flubendiamide dissolved in acetonitrile were applied on HPTLC plates silica gel 60 F_{254} using a Linomat syringe (100 µL). These plates were developed in horizontal chambers with ethyl acetate and methanol as mobile phase for chlorantraniliprole and flubendiamide, respectively. The amount of active ingredient present in formulations of chlorantraniliprole and flubendiamide were estimated by densitometry in a single beam, single wavelength reflectance mode at 270 and 252 nm respectively. The method was validated in terms of linearity, precision, accuracy, and sensitivity. Calibration curves of these insecticides were linear in the range of 50 - 200 ng/zone, and the correlation coefficients for the calibration equation were 0.999 and 0.982 for chlorantraniliprole and flubendiamide, respectively. The limits of detection for both insecticides were 50 ng/zone. Recoveries from laboratory-prepared test samples of the formulation were in the range of 95 - 98 %. The method has been validated by further analysis using HPLC with photo diode array (PDA) detector, and results were comparable. The method was found to be reproducible and convenient for quantitative analysis of these compounds. The proposed HPTLC method has its application for quality control and determination of the shelf life of commercial formulations of pesticides.

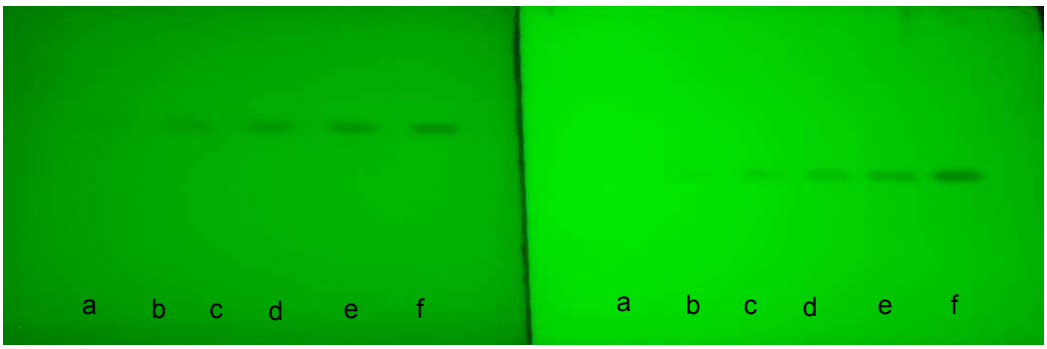

Fig. 1 HPTLC plate left: **1a** solvent acetonitrile, **1b** standard flubendiamide (100 ng), **1c** standard flubendiamide (200 ng), **1d** standard flubendiamide (1000 ng), **1e** Fame 480 SC (850 ng), **1f** Fame 480 SC (1700 ng); right: **2a** solvent acetonitrile, **2b** standard chlorantraniliprole (100 ng), **2c** standard chlorantraniliprole (200 ng), **2d** standard chlorantraniliprole (1000 ng), **2e** Coragen 18.5 SC (850 ng), **2f** Coragen 18.5 SC (1700 ng)

P-5a

Analytical detection by TLC of methiocarb residues in biological samples

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Suspected poisoning of animals by pesticides is investigated routinely in the Pharmacology and Toxicology Laboratory. Past years results shows an important increase on methiocarb intoxication in Portugal. Methiocarb (or mercaptodimethur), also know as mesurol, is a carbamate pesticide used as a molluscicide, insecticide and acaricide. This compound can be the cause of poisoning in domestic animals and wildlife, including birds, which are natural mollusc predators.

In our laboratory a thin layer chromatography methodology has been used for multiresidue detection. However, it has been observed, that methiocarb can be visualized with the same spray reagent used for the organophosphates chemical group, so the aim of this study was to find a chemical reagent that could be sprayed on TLC plates to allow a correct identification of methiocarb in suspicious samples.

Analytical standard solution of methiocarb was applied onto TLC plates silica gel G 60 F_{254}, eluted with a combination of n-hexane - acetone (40:10 v/v) and visualized at 254 nm. Reagent sprayed for methiocarb identification was *p*-anisaldehyde in methanol and glacial acid acetic (85:10 v/v). The plates sprayed were also heated at 110 ºC, and in these conditions a pink spot was obtained. This spray reagent was also tested in suspicions samples (liver, stomach contents) from domestic wild species and bates.

The results indicated that this method allows a double confirmation of the presence of methiocarb in samples and a greater power on the identification. This methodology is suitable for routine control of suspicions methiocarb intoxication of wild and domestic animals.

P-5b

Identification and purification of bacterial lipids from *Enterococcus faecalis* 12030

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Bacterial compounds from the outer cell membrane of bacteria can induce cytokine production and may play an important role in inflammatory immune responses. For gram-negative bacteria Lipid A is known as cytokine inducing factor. However, the equivalent components of gram-positive bacteria have not been well investigated. We therefore studied membrane lipids extracted from *Enterococcus faecalis* and from two isogenic targeted deletion mutants, and investigated these extracts regarding cytokine induction in cell cultures, and through different stainings (α-naphtol, Mo-staining and ninhydrin). In mutant EF 2890 the gene *bgsB* is deleted, and therefore the synthesis of the two glycolipids monoglycosyl-diacylglycerol (MGlcDAG) and diglycosyl-diacylglycerol (DGlcDAG) is blocked (Fig. 1). In the deletion mutant EF 2891 the gene *bgsA* is deleted, and therefore this mutant can not produce the glycolipid DGlcDAG.

The lipids were extracted by two different extraction procedures (Bligh-Dyer and butanol extraction). Preliminary data show higher extraction yields by use of Bligh-Dyer and also differences in the composition of the extracted lipid compounds. In addition, different staining methods were tested to identify the functional lipid groups. Differences could not only be seen in glycolipids but the mutants also showed differences in the compositions of the produced lipoproteins and phospholipids (Fig. 2).

To identify cytokine inducing lipids the extracted lipid fraction was separated by PLC. The purified compounds were investigated in a cell culture system with RAW264.7 cells and measurement of the cytokine TNF-α by ELISA. These procedures will eventually help to identify lipid compounds in gram-positive bacteria that cause inflammation in the host during infection.

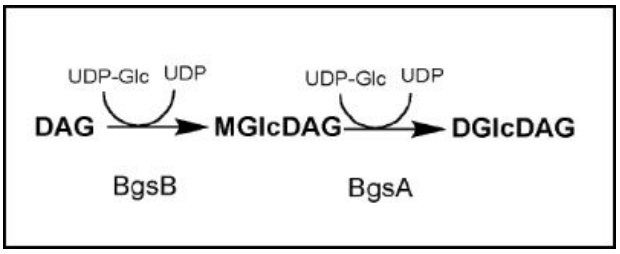

Fig. 1 Pathway for glycolipid synthesis in *E. faecalis*

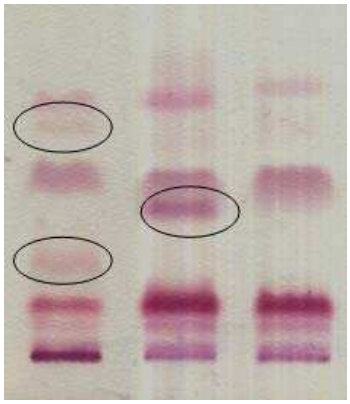

Fig. 2 lane 1 Wildtype, lane 2 EF2890, lane 3 EF 2891 stained with ninhydrine

P-5c

Proposal for review of the quantitative monograph of Cinchonae with a better quantitative method using instrumental HPTLC and densitometry analysis

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Since the first ultra-chromatograms from Ismaïlov & Schreiber in 1936, TLC/HPTLC was ever widely used by the phytochemists for plant identification, by fingerprinting. Quantitative HPTLC in this field has a very limited development, despite it shows a very good adaptation to this task with new HPTLC plates, instrumentation improvements and a certain knowledge of the development of such methods.

This poster reports the first step of HPTLC method setup for the evaluation of alkaloids quantification in plants. The method is completely described in the poster, shows already interesting results, and appeals for final optimisation. The selected example of Cinchonae has the advantage of being representative of this kind of evaluation problem and its current Ph. Eur. and Ph. Fr. [1, 2] monograph contains a complex protocol with several extraction steps using toxic solvents.

With the given first results, a quantitative method seems to be possible within the requirement of the authorities. Further developments will be made in this direction to have a proposal with a good chance of success, and to adapt other quantitative methods for other similar requests.

[1] European Pharmacopoeia VIIth edition for herbal substance section, monograph 0174: Cinchona Bark**,** 01/2011. [2] French Pharmacopoeia Xth edition for mother tincture section, monograph: China rubra for homeopathic preparations, 2002-2007.

P-5d

Extraction, isolation and detection of atropine from blood by new solvent system using high-performance thin-layer chromatography plates

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Atropine is a basic constituent of Dhatura and is non-volatile in nature. Atropine is considered to be a cerebral deliriant. Although several instrumental methods like UV, GLC and HPLC are available for separation and identification of atropine, the cost of analysis is very high. Therefore a simple, rapid and reliable HPTLC method for separation of atropine has been presented.

The proposed system is well suited for day-to-day analysis. The main advantage of TLC is the low cost, simultaneous analysis of large number of samples, and a minimum sample preparation is required. Atropine was first extracted from blood and then identified on HPTLC plates using various new solvent systems. A total of 40 solvent systems were selected, in which 20 solvent systems were giving good results. The R_F values in these 20 solvent systems ranged between 0.06 and 0.89. The migration time was between 27 and 146 min. In case any of 20 solvent systems is not available, another mentioned system can be used for the analysis of Atropine. For the detection of atropine on the developed plates, the Dragendorff spraying reagent was used.

P-5e

New developments of HPTLC for the assay of detergents used for membrane proteins elucidation/crystallisation/characterisation

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Detergents are amphipathic molecules needed for solubilising membrane proteins during purification steps and subsequent biochemical or biophysical analysis. Knowing the exact composition of the protein-detergent complex is an important prerequisite for crystallization. Indeed, techniques used to increase protein concentration affect also detergent concentration which in turn could prevent the formation of high-quality crystals.

Inspired by a quantification of detergent with TLC [1], we developed a sensitive method, using HTPLC, for the detection of small amounts of detergent bound to membrane proteins during purification and crystallization steps. Thanks to a strategy based on Snyder triangle, a dichloromethane – methanol - acetic acid (42:7.6:0.4) solvent was determined to separate two detergents. Then, we optimized the migration and revelation conditions with one of these: the dodecyl-β-maltoside (DDM), the most detergent used in membrane proteins crystallization. With this detergent, we found a linear relationship between detergent amount and staining band ranged from 0.1 g to 0.8 g which is the most powerful analysis described so far, for routine measurements of detergent concentration. Assays performed with our method on purified membrane complexes show that the analysis requires no prior treatment and allow us to assess the amount of detergent bound to the protein. The HPTLC method is also a promising technique to analyse the exchange of detergent during purification techniques.

The sensitivity of the method is 10 times greater than classical techniques and other advantages such as the need for minimal amounts of unconcentrated samples and the absence of samples treatments make this method useful for analysis and quantification of detergents.

[1] Laura R. Eriks, June A. Mayor, Ronald S. Kaplan. Anal Biochem 323 (2003) 234-241.

P-5f

Development of a new densitometric TLC method for determination of asiaticoside content in *Centella asiatica*

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Asiaticoside is a major active compound in *Centella asiatica* L. which has many pharmacological activities, such as wound healing, anti-inflamatory, memory enhancing, immunomodulatory activity and etc. It is an ursane-type triterpene glycoside which has weak UV absorption (≤ 200 nm) due to lacking of chromophore in its structure. Various analytical methods have been developed for determination of asiaticoside content in raw materials or finished products. Presently, HPLC is the only standard analytical method used for the analysis, but it is not so efficient owing to the weak UV absorption of asiaticoside.

In this study, derivatization of asiaticoside with 2-naphthol on a silica gel plate was developed for direct determination of the triterpenoid glycosides in *C. asiatica* crude extracts using the UV–visible range. The developed densitometric TLC appeared to be simple, accurate, precise and fast. Eighteen chromatographic runs could be performed simultaneously per plate within 15 minutes.

In practice, crude extracts of *C. asiatica* are prepared from the plant materials under reflux with 80 % methanol and partially purified by dichloromethane and butanol. Analysis was performed on a TLC plate silica gel 60 F_{254} (20 x 10cm.) with chloroform – methanol - water as the mobile phase. Densitometric analysis was performed at 530 nm after post-chromatographic derivatization with 2-naphthol sulfuric acid reagent (brownish band for glycoside) [1]. This new method showed good sensitivity and selectivity. The linear range for the analysis of asiaticoside was 100 - 1000 ng/band ($r^2 \geq 0.99$) with good precision *(%RSD*, 1.15 - 1.9%).

[1] M. Vega, G. Morlock, *J. Planar Chromatogr.* 20, 2007, 411-417.

P-5g

A validated quantitative HPTLC method for estimation of strychnine in *Strychnos nuxvomica* extract and marketed formulation

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A new, simple, sensitive, selective, precise and stability-indicating HPTLC method for analysis of strychnine in *Strychnos nuxvomica* extract, and in marketed formulation was developed and validated. The method was employed on TLC aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of toluene - ethyl acetate - diethyl amine (7:2:1, v/v/v). Densitometric analysis of strychnine was carried out in the absorbance mode at 254 nm. This system was found to give compact spots for strychnine (R_F value of 0.42 ± 0.01, for six replicates).

Strychnine was subjected to acid, alkali and neutral hydrolysis, oxidation, dry and wet heat treatment and photo and UV degradation. The drug undergoes degradation under all stress conditions. Also, the degraded products were well resolved from the pure drug with significantly different R_F values. The method was validated for linearity, precision, robustness, LOD, LOQ, specificity and accuracy. Linearity was found to be in the range of 100 - 1000 ng/spot with significantly high value of correlation coefficient r^2 = 0.9928. The limits of detection and quantification were 12.0 and 36.4 ng/spot, respectively. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of strychnine in *Strychnos nux vomica* extract, and in market formulation.

The developed method effectively resolved the strychnine in *Strychnos nuxvomica* extract, and in marketed formulation hence; it can be employed for routine analysis as a stability indicating.

P-5h

HPTLC analysis of hyoscyamine, scopolamine and their biosynthetic intermediates from *in vivo* and *in vitro* cultures of several *Solanaceae* plants

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Tropane alkaloids, such as hyoscyamine and scopolamine, are important pharmaceuticals used in medicine because of their anti-cholinergic activity. They are still isolated from intact plants, as their chemical synthesis is still unprofitable. Therefore, from many years an effort has been made to develop the biotechnological method for their production [1].

At each stage of the biotechnological research it is necessary to monitor the results of the experiment by identifying not only the content of final products but also of their intermediates [2]. In case of alkaloids the method of choice is TLC which enables rapid analyses of many samples simultaneously. More than 100 chromatographic systems were described for tropane alkaloids analyses in various plant matrices. Most papers deal only with separation of two main compounds, namely hyoscyamine and scopolamine and there is no literature data on simultaneous TLC separation of littorine and anisodamine, the important precursors in tropane alkaloids biosynthesis [3].

In our study we have tested almost 30 different chromatographic systems for tropane alkaloids separation. The best resolution was achieved on HPTLC plates silica gel 60 F_{254} with the mobile phase chloroform – methnol – acetone – 25 % ammonia (75:15:10:2, v/v/v/v). The plates were developed twice with 5 min preconditioning with the mobile phase vapors. In the developed conditions the separation of hyoscyamine, scopolamine, littorine, anisodamine and cuscohygrine was possible in 12 different plant matrices. The optimized method was than applied to carry out quantitative analyses (densitometric detection) of the above compounds. The method was validated for linearity, limits of detection and quantification, repeatability, intermediate precision, and recovery.

[1] B. Drager, J. Chromatogr. A. 978, 2002, 1-35. [2] S. Berkov, A. Pavlov, Phytochem. Anal. 15, 2004, 141-145. [3] T. Mroczek, TLC of tropan alkaloids, in: M. Waksmundzka-Hajnos (Ed.) Thin layer chromatography in phytochemistry, CRC Press, Boca Raton, 2008.

P-5i

HPTLC evaluation of pyrrolizidine alkaloids extracted from *Tussilago farfara* and *Petasites officinalis*

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Characterisation of the phytochemical composition of plant extracts is a difficult task due to the complexity of the chemical structures generally found. Even powerful separation procedures can fail to discriminate between chemically-related compounds and often several procedures have to be adopted to give a reasonably well defined extract.

HPTLC evaluation of pyrrolizidine alkaloids (PAs) extracted from *Tussilago farfara* and *Petasites officinalis* was performed on silica gel F_{254} plates with different mobile phases to find the best conditions to characterize the extracts. The results indicate that many different compounds are found in the extracts despite the isolation procedures performed as following: pulverised dried plant was refluxed with acidified methanol 50 % (pH = 2-3), then extracted in two steps with dichloromethane and ether; the aqueous phase was alkalinized with ammonia (pH = 9-10) and the PAs were extracted in dichloromethane.

In order to differentiate between the compounds, a series of similar alkaloids (atropine, codeine, nicotine, caffeine and scopolamine) were also spotted on the plate, the densitometric analysis was performed and graphs of the molecular weight, number and position of eluted spots as a function of the mobile phase polarity relative index were plotted. Certain pattern recognition-type methods for the characterisation of the extracts were also performed, resulting in a better characterisation of the extract meant to be further used in toxicological studies.

P-5j

Authentication of neutral henna leaves by HPTLC

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Neutral henna (*Cassia italica*) is a traditional plant used in cosmetics for hair care applications or in medicine as a laxative. The laboratory chose to develop a plant extract for cosmetic application from neutral henna leaves.

Identification of neutral henna leaves is difficult because there is no monograph. Thanks to HPTLC, a method has been developed for the identification of the vegetal material through a phytochemical profile. The analysis of polyphenols has been realised before and after hydrolysis of the extract.

Thereby, the raw material can be validated according to an internal reference profile, established with the plant used to develop the extract in the first step. The method has been tested on plants from different suppliers. According to them, leaves could have different aspects (whole or ground into fine powder). After extraction of these raw materials, the extracts were applied for validation in HPTLC. Some profiles show deviations from the reference profile and therefore a non-conforming plant.

HPTLC is thus a good method to highlight the falsification of plant material and in our case to choose the right supplier according to our required specifications.

P-5k

Separation evaluation of selected organophosphorus fungicides by NP-TLC and RP-HPTLC

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Poisoning by organophosphorus fungicides (OPFs) is although uncommon; recently, a few cases of deaths owing to the ingestion of OPFs were reported. Literatures on chromatographic separation of these fungicides are minimal. The evaluation of the efficacies of separation is of primary interest when it is contemplated from a forensic matrix. Hence, a study was undertaken to evaluate the efficacies of separation of three OPFs, namely ditalimfos D, edifenfos E, and tolclofos-methyl TM by NP-TLC on silica gel 60 F_{254} and RP-HPTLC on silica gel 60 RP-18 WF_{254}.

The relationship between the mobile phase composition (n-hexane - acetone for NP-TLC, and methanol - water for RP-HPTLC) and R_F values affirmed that with the increase in the acetone content in case of NP-TLC and decrease of the water content in RP-HPTLC resulted in the increase in R_F values for the three OPFs. The three OPFs are completely separated from each other with $\Delta R_F \geq 0.04$, except for the pair of compounds E/D in NP-TLC with a mobile phase composition of hexane - acetone 10:0, and for the pair of compounds E/TM in case of RP-HPTLC with mobile phase compositions of 10:0, 7:3, 6.5:3.5 and 6:4. Under the chromatographic conditions used, D and TM were found to have higher R_M values in normal phase and reverse phase, respectively. Ditalimfos was therefore adsorbed strongly in NP-TLC and tolclofos-methyl in RP-HPTLC. The peak resolution (R_S) values in NP-TLC were greater than 1.5 for the pair of compounds E/D, TM/E, and TM/D in solvent composition ratio of 9:1, 8.5:1.5, 8:2, and 7.5:2.5. R_S values in RP-HPTLC were also higher for all the pair compounds, except for E/TM.

The study revealed that the system of NP-TLC with mobile phase n-hexane - acetone (9:1) provided the optimum conditions for the separation of D, E, and TM as compared to RP-HPTLC system. Complete separation of E from TM was not achieved in RP-HPTLC.

P-5l

Quantitation of isotretinoin in topical microemulsion and microemulsion based gel formulations and their *in vitro* permeation study

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A new, simple, and rapid HPTLC method was developed and validated for quantitative determination of isotretinoin. Isotretinoin was chromatographed on TLC plates silica gel 60 F_{254} using toluene - methanol (9:1, v/v) as mobile phase. Isotretinoin was quantified by densitometric analysis at 340 nm. The method was found to give compact spots for the drug (R_F = 0.43 ± 0.01). The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9995 in the concentration range 100 – 500 ng/spot. The method was validated for precision, recovery, repeatability, and robustness as per the ICH guidelines. The minimum detectable amount was found to be 13.7 ng/spot, whereas the limit of quantitation was found to be 41.6 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of isotretinoin. The method was successfully employed for the estimation of equilibrium solubility, quantification of isotretinoin as a bulk drug, in commercially available preparation and in-house microemulsion formulations.

P-5m

A new HPTLC method for the assay of thiopental in postmortem blood in a fatal case of suicide

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Thiopental is an ultra-short-acting barbiturate, used as an induction agent during general anesthesia and to manage intra cranial pressure in traumatic brain injuries. Because of its rapid onset of action, the potential for accidental or intentional abuse of thiopental is high. In this paper, we present a case in which a 25-year-old female deliberately injected a fatal dose of thiopental.

A method is developed for the evaluation of thiopental levels in the postmortem blood by a simple and rapid HPTLC. The extraction procedure was optimized by using acetonitrile, dichloromethane, and ethyl acetate. The effects of pH over the range of 5 to 6.5 were examined for the optimum recovery. An average analytical recovery of 90.5 % was achieved from an ethyl acetate extract at pH 5.5 with a R.S.D. of ±2.5 %.

Chromatographic separation was achieved on silica gel 60 F_{254} plates with an optimized mobile phase consisted of hexane – dichloromethane - ethyl acetate in the ratio 7.5:2:0.5 (v/v). Densitometric detection was carried out at 290 nm in the absorbance mode. No significant chromatographic interference was observed from other drugs used to diagnose the brain death. Calibration curve for thiopental in blood were linear from 1 to 100 µg/mL with r^2 = 0.994. The detection limit was 0.44 µg/mL and its lower limit of quantification 1.4 µg/mL. The method showed excellent intra-assay precision *(%RSD* 1.1 to 6.3 %) and inter-assay precision (*%RSD* 0.4 to 1.4 %) for spiked blood samples at concentration of 1, 10, and 50 µg/mL.

The toxicological analysis revealed high concentrations of thiopental in postmortem blood (204.75 µg/mL ± 0.34), which is of immense help to conclude that the death occurred due to fatal doses of thiopental.

P-5n

HPTLC: a valuable method for rapid analysis of rare orchids' plant extracts

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Plant extracts offer great biodiversity in phytochemical compounds and deserve special attention in their characterization. The great biodiversity of plants can be found in their primary and secondary metabolites. HPTLC is a very unique method to get a rapid and complete overview of the molecular families present in various plant extracts.

LVMH Recherche has developed a multi-step phytochemical screening using HPTLC (specific elution techniques and staining reagents) in order to define a 'phytochemical identity card' allowing the scientists to have a clear phytochemical approach of the plant extracts they are working on [1].

The developed method allows the determination of specific components called 'phytochemical markers' and can be used to guide the biological activity screening, to determine the toxicological risk and to anticipate an extract analytical data sheet useful for an industrial development.

The preliminary method can be completed by others in order to quantify compounds by densitometry, to characterize the UV spectrum (Wincats spectrum database) of the different compounds and to define optimized separation (AMD).The objective is also to obtain information which could then lead to rough molecular structure of these components before the use of more powerful analytical studies (GC, MS, RMN).

Our technique was applied to rare epiphyte Orchids from Yunnan, South China. In the process of selection of Orchids for cosmetic or pharmaceutical use, the phytochemical identity card is useful to confirm the genus and species, to differentiate the organs extracted and to confirm the traceability of the materials. We found especially that some terpenoïds are more specific 'markers' for stems from genus *Vanda.*

Secondary and primary metabolites of *Vanda* orchids family identification, help to select and control specific molecular fractions having pharmacological anti-inflammatory or anti-ageing properties.

[1] Darnault S, Lamy C, Cailleaud Y, About N, Andre P: HPTLC: a useful support for research and development of active ingredients from selected plants – International Symposium for TLC, Lyon, 2003.

P-5o

Development of new solvent system for the analysis of cypermethrin

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Cypermethrin is a synthetic pyrethroid primarily used as an insecticide. Cypermethrin poisoning cases are most common in India and most commonly come to Forensic Science laboratories for analysis. So far it is analyzed by instrumental methods such as HPLC, GLC, HPTLC, *etc*. which are not only costly but also time consuming and required more sophisticated instruments.

Therefore, a more simple TLC technique was used for separation and identification of cypermethrin which was extracted from blood by a solvent extraction method and identified using various solvent systems. The spots were visualized by spraying with Bromophenol Blue reagent followed by 4 % acetic acid.

P-5p

Screening of polyphenolic compounds in glycolic plant extract

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Glycolic plant extracts are part of the cosmetic ingredients. They are viscous and that leads to difficult HPTLC analysis. A specific sample preparation by Solid Phase Extraction (SPE) was developed using a polymeric reverse phase cartridge to remove glycol. Glycol is eluted and polyphenolic compounds are trapped. Then, screening of phenolics acids or OPC (oligomer procyanidolics) can be easily performed on HPTLC.

Phenolic acids such as ferulic acid separated on HPTLC plates silica gel with toluene – ethylformate - formic acid 5:4:1 (v/v/v) with the Automatic Development Chamber (ADC 2) in a saturated twin trough chamber. Detection was performed after immersion in Natural Product–PEG reagent and evaluation under 366 nm.

Oligomer procyanidolics (OPC) with DP<3 (degree of polymerization) separated with ethylacetate – toluene - formic acid 9:9:2 (v/v/v) in the unsaturated ADC2 chamber and OPC with DP>3 using ethylacetate – methylethylketone - formic acid - water 15/9/3/3 (v/v/v) in the saturated ADC2 twin trough chamber. OPC detected after immersion in vanillin-HCl solution and evaluation under daylight.

P-5q

Validated HPTLC method for the determination of nicotine in Tobacco (*Nicotiana tabacum* L.) extracts

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Nicotine is found in a wide variety of plant, especially in tobacco leaves where it constitutes the main alkaloid. Highly toxic, nicotine contributes to various human diseases [1]; its content in formulated product and thus in tobacco extracts as to be known. The aim of this poster is to present a sensitive method for nicotine quantification in non-processed and processed tobacco extracts.

TLC/HPTLC was mainly used by chemists as a rapid and reliable technique to follow reaction processes and is largely employed for plants screening and botanical certification. TLC densitometry has emerged as an efficient tool for the phytochemical evaluation of herbal drugs [2] because of its simplicity and minimum sample clean-up requirement. Many studies have reported nicotine quantification in biological samples (blood, serum, urine…), gaseous samples (air samples or tobacco smokes) or formulated products using gas chromatography, liquid chromatography or other analytical techniques and no papers report nicotine quantification in tobacco extracts. A HPTLC method was developed and validated to quantify nicotine in various tobacco extracts (alcoholic extract, concrete, absolute and “low nicotine” extract). Sample preparation and analytical conditions were optimised in order to extract all nicotine from tobacco extracts and to obtain weak limit of detection with high sample throughput. Stabilization of nicotine under its neutral form during the plate development was studied. All validation data were presented in the poster with optimisation parameters.

This poster finally reports nicotine quantification in tobacco extracts with comparison of the results with those obtained by HPLC. Time and solvent consumption were the convincing keywords for the implementation decision of the HPTLC/UV in analytical platform or quality control service.

[1] Lin MS, Wang JS, Lai CH. Electrochim Acta 53, 2008, 7775. [2] Ravishankara MN, Srivastava N, Padh H, Rajani M. Planta Med. 67, 2001, 294.

P-6a

Stability study and densitometric determination of efavirenz in tablet by normal phase thin-layer chromatography

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A simple, precise and accurate stability indicating normal phase HPTLC method for determination of efavirenz in tablet preparation has been developed and validated. Efavirenz from the formulations in presence of its degradation product was separated on HPTLC plates silica gel 60 F_{254} with chloroform, methanol and toluene in the proportion of 7:1:2 (v/v/v) as mobile phase.

Densitometric quantification was performed at 252 nm. Well resolved bands were obtained for efavirenz with R_F value of 0.70 and for degradation products. The method was validated for specificity, accuracy, precision and robustness. The calibration curve was found to be linear in the concentration range of 500 - 1000 ng per band both by area and height with correlation coefficients of 0.999 and 0.998, respectively. The method is selective and specific with potential application in pharmaceutical analysis of these drugs in bulk and formulations.

P-6b

HPTLC method for simultaneous estimation of lamivudine and zidovudine in tablet dosage form

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A simple, precise and accurate normal phase HPTLC method for determination of lamivudine and zidovudine in tablet preparation has been developed and validated. Lamivudine and zidovudine from the formulations were separated with chloroform, methanol and toluene in the proportion of 7.5:1.5:1 (v/v) as mobile phase. Well resolved bands were obtained with R_F values of 0.19 ± 0.02 and 0.68 ± 0.02 for lamivudine and zidovudine, respectively, at 278 nm with application volume of 7 μL. Specificity study showed that lamivudine and zidovudine were found to undergo degradation (nearly 5 - 21 %) under all these conditions with exception that both drug were stable in elevated thermal conditions.

Calibration curve was found to be linear in the concentration range of 300 - 1500 and 600 - 3000 ng per band by area evaluation with correlation coefficients of 0.999 and 0.999 for lamivudine and zidovudine, respectively. The linear regression equations were found to be y = 7.285x + 466.103 for lamivudine and y = 2.782x + 4306.437 for zidovudine. Mean recovery near to 100 % were indicative of the accuracy of the method and showed that the method was free from interference of excipients present in the formulation. By height, LOD were 84.98 and 491.83, while LOQ were 257.51 and 1490.39 µg/mL for lamivudine and zidovudine, respectively. By area, LOD were 49.83 and 385.51, while LOQ were 150.99 and 1168.22 µg/mL for lamivudine and zidovudine, respectively. The method is selective and specific with potential application in pharmaceutical analysis of these drugs in bulk and formulations.

P-6c

Normal phase TLC and simultaneous densitometric determination of rosiglitazone and glimepiride in tablet dosage form

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A simple, precise and accurate normal phase HPTLC method for simultaneous determination of rosiglitazone and glimepiride in multicomponent pharmaceutical preparations has been developed and validated. Rosiglitazone and glimepiride from the formulations were separated on HPTLC plates silica gel 60 F_{254} with toluene, methanol, ethyl acetate and formic acid solution in the proportion of 7:3:1:0.01 (v/v) as mobile phase.

Densitometric quantification was performed at 245 nm. Well resolved bands were obtained with R_f values of 0.28, 0.75 for rosiglitazone and glimepiride, respectively. The method was validated for specificity accuracy, precision and robustness. The calibration curve was found to be linear in the concentration range of 500 - 2000 and 250 - 1000 ng per band by area with correlation coefficients of 0.999 and 0.997 for rosiglitazone and glimepiride, respectively. The method is selective and specific with potential application in pharmaceutical analysis of these drugs in bulk and formulations.

P-6d

Chiral assay of enantiomersof (*R,S*)-fluoxetine in pharmaceutical formulations using liquid chromatography

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Fluoxetine {N-methyl-γ-[4-(trifluoromethyl)-phenoxy]benzenepropanamine}(the most prescribed drug in USA) [1], a drug of the selective inhibitor of serotonin reuptake class, is used in treating a variety of psychiatric and metabolic derangements. Among the (R)-(-) and (S)-(+)-forms of fluoxetine, there is a small but distinguishable stereospecificity in the serotonin reuptake inhibition with the (S)-form being slightly more potent [2]. The (S)-isomer is effective for the treatment of migraine headaches while the (R)-isomer is used for treatment of depression. Thus, the enantio-separation of the racemic fluoxetine becomes significant.

Chiral assay of enantiomers of fluoxetine was achieved in pharmaceutical formulations using direct and indirect methods. A chiral selector is used as chiral mobile phase additive in thin-layer chromatography; enantiomers were separated and isolated and were used to determine the elution order in HPLC. (R,S)-flouxetine was derivatized with (S)-N-(4-nitrophenoxycarbonyl)phenylalanine methoxyethyl ester [(S)-NIFE] [3-4], Marfey's reagent [5-7] and its variants. The diastereomers were separated using RP-HPLC. The effect of flow rate and TFA concentration on resolution was studied. The diastereomers obtained by derivatization with Marfey's variants were also separated by RP-TLC.

The direct TLC method used is very successful and useful to obtain the native enantiomers of the racemic fluoxetine, which could be subsequently used. There is a clear evidence of TLC being a complimentary technique to HPLC. The CDRs provided a high sensitivity with limits of detection at nanogram level that could be applied for the trace analysis in pharmaceutical and biological samples. Under the reaction conditions of derivatization no racemization was observed and the diastereomers were stable for several weeks.

[1] http://en.wikipedia.org/wiki/Fluoxetine#cite_note-1 (Top 200 Brand Drugs by Units, 2007). [2] Wong DT, Fuller RW, Robertson DW. *Acta Pharmaceutica Nordica* 1990; 2: 171–180. [3] Péter A. *et al. J Chromatogr A* 2002; 948: 283–294. [4] Hess S *et al. J Chromatogr A* 2004; 1035: 211–219. [5] Marfey P. *Carlsberg Research Communications* 1984; 49: 591–596. [6] Bhushan R, Kumar V. *Biomedical Chromatography* 2007; 21:1064–1068. [7] Bhushan R, Kumar V. *Acta Chromatographica* 2008a; 20: 329–347.

P-6e

Synthesis and in vitro anticancer evaluation of the different derivative of 1-(1H-benzo[d] imidazol-2-yl)ethanone

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1-(1H-benzo[d]imidazol-2-yl)ethanone synthesized and to react with 1,2-dibromoethane to get desired intermediate, then these intermediate condensation with secondary and primary amine in the presence of sodium acetate and con. H_2SO_4 using ethanol as a solvent yielded two separate series of 1-{(1-substitutedethyl)-1H-benzo[d]-imidazol-2-yl}ethanone and 1-(2-bromoethyl)-2-(1-substitutedhydrazonoethyl)-1H-benzo[d]imidazole, respectively. The purity of the compounds was checked by TLC and melting point. Spectral data ^{1}HNMR, FTIR and mass spectra of synthesized compounds were recorded and found in full agreement with the proposed structures. The elemental analysis results within ± 0.4 % of the theoretical values. Among all the synthesize compounds (15), eleven compounds were selected for *in vitro* anticancer evaluation at the National Cancer Institute (NCI), USA, according to their applied protocol at a single dose (1×10^{-5}M) in full NCI 60 cell panel. Among the selected compounds, some compound show significant anticancer activity.

[1] Shadia AG, Khaled HH, Ahmed SK, Mireya LR, Sean MK, Abdel MA, Khamry El, Hoda ED, Euro. J. Med. Chem. 44, 2009, 1500–1508. [2] Francisca PA, Helgi JC, Jaime PV, Juan CP, Sergio RM, Guadalupe PH, Nayeli LB, Alicia HC, RC, Francisco HL, Euro. J. Med. Chem. 44 (2009) 1794-1800.

P-6f

HPTLC determination of nifedipine in human serum after liquid-liquid extraction

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A simple, rapid, specific, precise and accurate HPTLC method for quantitation of nifedipine in human serum was developed and validated. The method includes a liquid-liquid extraction of the analyte from the matrix using dichloromethane - *n*-hexane (30:70, v/v) as extraction solvent, and carbamazepine as internal standard.

In the developed method, chromatography was performed on pre-coated HPTLC plates silica gel 60 F_{254} (10 cm x 10 cm and 10 cm x 20 cm, layer thickness 0.2 mm) prewashed with methanol, with chloroform - ethyl acetate - cyclohexane (19:2:2, v/v/v) as mobile phase. The developing solvent was run up to 80 mm in a vertical trough chamber previously saturated with the solvent mixture for 20 min. Densitometric detection was done at 238 nm.

The regression data for the calibration plots showed a good linear relationship (r = 0.996) in the range of 2 and 25 ng/band, corresponding to 0.02 and 0.25 ng/µL of nifedipine in human serum after the extraction process and applying 10 µL to the chromatographic plates. The *%RSD* of intra-assay and inter-assay precision were in the range of 0.6 % to 3.6 % (n = 3) and 1.2 % to 3.6 % (n = 9), respectively. The limit of detection and limit of quantitation were found to be 0.72 ng/band and 0.86 ng/band. The recovery values were between 93 % and 102 % (*%RSD* ≤ 4.3), and the Rf for nifedipine and carbamazepine were 0.31 and 0.10, respectively. Patient serum samples were analyzed by this HPTLC method successfully. Therefore, this HPTLC method is suitable for quantitative determination of nifedipine in human serum.

P-6g

HPTLC determination of sertraline in human serum after liquid-liquid extraction

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A simple, rapid, specific, precise and accurate HPTLC method for quantification of sertraline in human serum was developed and validated. The method includes a liquid-liquid extraction of the analyte from the matrix using a mix of organic solvent as extraction solvent, and the respective internal standard.

In the developed method, chromatography was performed on pre-coated HPTLC plates silica gel 60 F_{254} (10 cm x 10 cm and 10 cm x 20 cm, layer thickness 0.2 mm) prewashed with methanol. The developing solvent was run up to 80 mm in the Horizontal Developing Chamber. Chromatographic separation was performed with a mixture of toluene - ethyl acetate – methanol - ammonia solution 25 % (45:15:10:1.5, v/v) as mobile phase. The method was validated for linearity, precision and accuracy. Densitometric detection was done at 220 nm. The regression data for the calibration plots showed a good linear relationship in the range of therapeutic levels. Patient serum samples were analyzed by this HPTLC method successfully. In conclusion, the method is rapid, precise, accurate, selective and sensitive for quantitative determination of sertraline in human serum.

P-6h

Development and validation of an HPTLC method for estimation of risperidone in bulk and tablets - comparison with HPLC

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A simple and sensitive HPTLC method has been developed for the quantitative estimation of risperidone in bulk and tablets. Chromatography was performed on HPTLC plates silica gel 60 F_{254} and a mobile phase constituted with chloroform – methanol - triethylamine (55:45:0.1, v/v/v) as mobile phase. Quantification was made at 280 nm. The developed method was fully validated according to ICH guidelines. Specificity, linearity, precision, accuracy and quantification limit were established.

On the other hand, the quantitative HPTLC results were compared to those obtained by an RP-HPLC method. In the liquid chromatographic method, the determination of risperidone was performed on an Ascentis C8 column (250 × 4.6 mm) and a photodiode array detector operating at 280 nm. The mobile phase was a mixture of phosphate buffer 0.05 M - acetonitrile (40:60, v/v) adjusted to pH 5. Good agreement in the quantitative results was found between the two techniques. The proposed HPTLC method can be used for the determination of risperidone in bulk and tablets.

P-6i

An interlaboratory investigation on the use HPTLC to perform assays of lamivudine-zidovudine, metronidazole, nevirapine, and quinine composite samples

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Two laboratories extensively investigated the use of HPTLC to perform assays on lamivudine-zidovudine, metronidazole, nevirapine, and quinine composite samples. To minimize the effects of differences in analysts' technique, the laboratories conducted the study with automatic sample application devices in conjunction with variable-wavelength scanning densitometers to evaluate the plates. The HPTLC procedures used relatively innocuous, inexpensive, and readily available chromatography solvents used in the Kenyon or the Global Pharma Health Fund Minilabs TLC methods. The use of automatic sample applications in conjunction with variable wavelength scanning densitometry demonstrated an average repeatability or within-laboratory RSD of 1.90%, with 73% less than 2% and 97% at 2.60% or less, and an average reproducibility or among-laboratory RSD of 2.74%.

P-6j

Simultaneous estimation of anticancer terpenoids andrographolide and betullinic acid by HPTLC method

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Bettulinic acid (pentacyclic triterpenoid) is obtained from *Betula alba* and from other plants also, whereas Andrographolide (labdane diterpenoid) is obtained from *Andrographis paniculata*. Both the drugs are reported as anticancer agent, and exert direct anticancer activity on cancer cells by cell cycle arrest and by DNA damage.

The aim of present study was to develop and validate a simultaneous method for quantification of andrographolide and betulinic acid in pharmaceutical formulation. HPTLC method was developed on aluminium TLC plate silica gel 60 F_{254} using toluene - ethyl acetate - formic acid (5:4:1, v/v/v) as mobile phase. The method was validated for accuracy, precision, recovery, specificity LOD and LOQ. The solvent system was found to give compact spot at R_F 0.25 ± 0.02 and 0.67 ± 0.02 for andrographolide and betulinic acid, respectively (Fig. 1). The densitometric analysis was carried out the absorbance mode at 540 nm and method was found to give good linear relationship, $r^2 = 0.99$, in the concentration range of 10 - 1500 ng/spot for both the drugs. The developed method can be used for quality control and stability testing of several anticancer formulations containing bettulinic acid and or andrographolide.

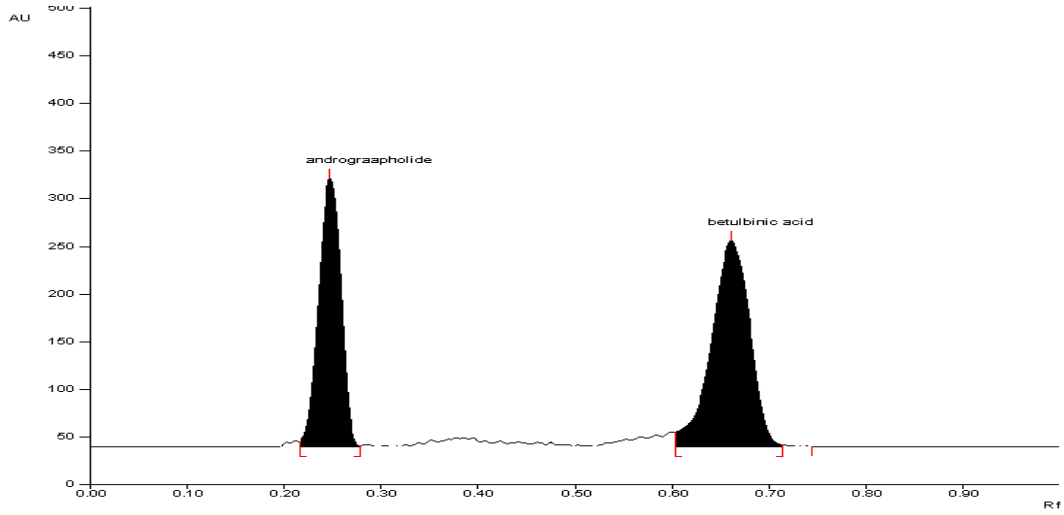

Fig. 1 Chromatogram showing seperation of bettulinic acid and or andrographolide

P-6k

Quantification of andrographolide from *Andrographis paniculata* and its optimization using plant growth regulators

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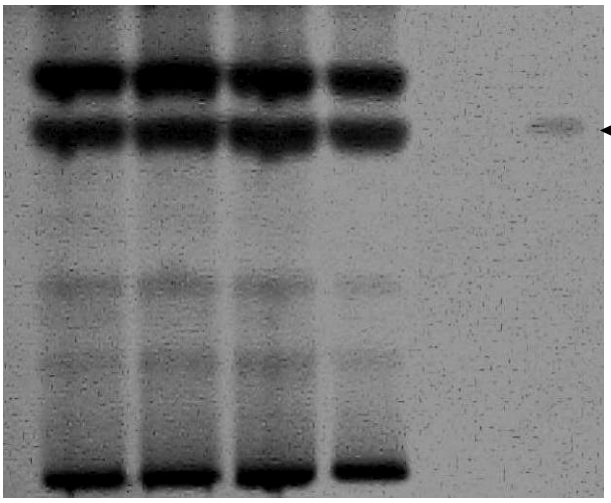
Andrographolide, a diterpenoid lactone, is the main active principle of *Andrographis paniculata* (Kalmegh) and possess antityphoid, antihepatotoxic, antibiotic, antithrombogenic, antisnake venom, antipyretic and anti-HIV properties beside its general use as an immunostimulant. Keeping in view, the importance of andrographolide, the present study was aimed at improving the content of andrographolide by foliar application of plant growth regulators (PGRs). Field grown plants of *Andrographis paniculata* were foliarly sprayed with indole-3yl acetic acid (IAA, 5, 10 and 25µgml^{-1}) and salicylic acid (SA, 50, 100 and 150 µg/mL) at 30 and 45 days after transplanting (DAT). Plants sprayed with water only served as control. Leaf samples harvested from control and treated plants after 90, 105 and 120 days of transplanting were harvested at three different stages of growth i.e. 90, 105 and 120 DAT were subjected to HPTLC analysis for quantification of andrographolide. The analyses revealed that maximum accumulation of andrographolide in leaves occurred during 90 to105 DAT and about 1.2 mg andrographolide per gram of dry weight of leaves was estimated in control plants at 105 DAT. The application of IAA and SA enhanced the andrographolide content over control. 25 µg/mL IAA and 100 µg/mL SA were found to be more effective in improving the content of andrographolide.

Based on these studies, tested PGRs appear to be effective in improving the content of andrographolide and their use could be exploited in future for upregulating quantity and quality of this chemical.



Chromatograms of andrographolide at 230 nm: IAA 5 µg/mL (a), 25 µg/mL (b), 100 µg/mL (c); control (d); solvent (methanol, e); standard 5000 ng/zone (f)

P-6l

A novel HPTLC method for the separation of coexisting purines and pyrimidines

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A novel HPTLC method has been developed for the resolution of coexisting two purines (adenine, and guanine) and three pyrimidines (cytosine, uracil, and thymine). The nucleobases were separated on aluminum-backed HPTLC plates cellulose 60 F_{254} with the aid of 5.0 % aqueous sodium deoxycholate-acetonitrile 1:3 (v/v) as mobile phase. All the nucleobases (two purines and three pyrimidines) were viewed on HPTLC plates under 254 nm UV light. The order of R_F value was guanine < adenine < cytosine < uracil < thymine. The effect of pH (acidity or basicity) of the mobile phase on the retention of individual nucleobases was examined.

Furthermore, the effect of interference of mono- (Li^+, Na^+), and bivalent (Mg^{2+} Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}) cations; mono- (Br^-, CH_3COO^-, NO_3^-), and bivalent (CO_3^{2-}, IO_4^-, SO_4^{2-}, MoO_4^{2-}) anions, and complexing ligands (urea, and EDTA) on the retention behavior of nucleobases were also assessed. The chromatography of nucleobases was also performed on HPTLC plates silica gel 60 F_{254} and RP-18 F_{254}. These plates failed to separate the coexisting purines and pyrimidines. The detection limit of all nucleobases on cellulose 60 F_{254} layers was also determined. The proposed method is rapid, easy, and reliable. It can be applied for routine analysis of DNA, and RNA nucleobases.

P-6m

DE-TLC assessment of the Brazilian porcine lung surfactant lipid composition

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Among analytical methods for drug substances separation TLC is so far the most used for biological lipids separation due to its practical, easy and low cost features. The natural lung surfactant is a mixture of 90% lipids and 10% of proteins. 80% of total lipids are phospholipids (PL) and among them, phosphatidylcholine (PC) is the major component. The surfactant deficiency at birth caused by premature delivery is responsible for the development of neonatal respiratory distress syndrome (NRDS). The surfactant replacement therapy is the adopted clinical care. Surfactant preparations are generally obtained from natural sources by organic solvent extraction. A low cost surfactant from porcine lungs was developed and for its PL profile characterization, conventional TLC, on silica gel 60 using chloroform – methanol - water as the solvent system was performed. PLs were visualized on the plate with the ammonium molybdate spray and used for subsequent determination of the phospholipids content. The combination of digital photography with regular TLC (DE-TLC) associated to a public dominion computer program (TLC Analyzer) that analyzes digital images of TLC plates and produces multispectral scans, densitograms, and calibration curves improved quantitative determination [1]. The experience on this TLC (DE-TLC) modality will permit future investments in more professional and sophisticated equipments (application device, scanner and documentation system). Our results showed good correlation values comparing with those obtained by phosphorus dosage. The mass error value calculated from surfactant PC spot after the optical density analysis was around 15 % (64 μg) from the expected mass value (57-66 μg).

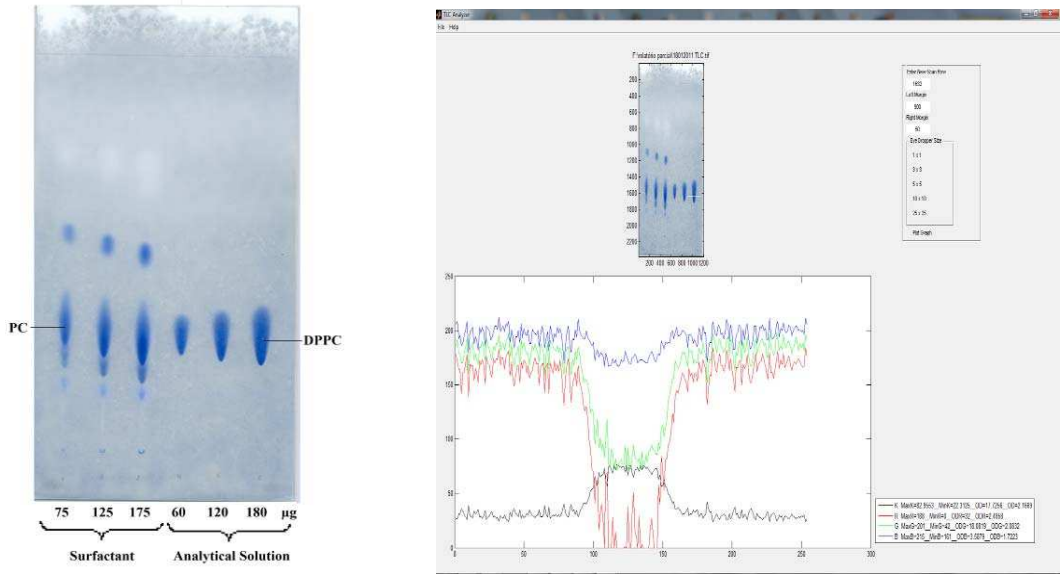

Fig. 1 Lung surfactant spots on TLC-Analyzer software

Supported: FAPESP, Fundação Butantan, Sadia,CNPq

[1] A.V.I. Hess *J. Chem. Education* 84, 2007, 842-847.

P-6n

Simultaneous quantitative evaluation of memantine and donepezil by HPTLC

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Alzheimer disease is among the most investigated maladies, but the existing medication can only decrease the rate of symptoms on set. Specific drugs (including memantine and donepezil) are often co-administered and the chronic toxicity data on their use are still missing.

The paper intends to present a method for the simultaneous evaluation of donepezil and memantine from a synthetic mixture (1:1, w/w) in view of their further quantification from biological samples. Memantine hydrochloride and donepezil hydrochloride have been assayed on HPTLC plates silica gel 60 using as mobile phase a mixture of chloroform - cyclohexane - methanol (2:2:1) and densitometric detection in the reflectance/absorption mode.

The two drugs are well separated (determined R_f values were 0.58 for memantine and 0.65 for donepezil), thus permitting their evaluation as co-administered drugs. The spectral evaluation performed on the separated spots resulted in well-defined maximum absorption peaks, characteristic to the tested substances (four local maxima for memantine at 201, 231, 274 and 315 nm and three for donepezil at 235, 285, 396 nm). Best wavelength to determine both substances was set at 274 nm.

The quantitative assay led to linear regression curves for 5 - 30 µg memantine and 1 - 20 µg for donepezil. The method can be used to therapeutic monitoring of both substances co-administered in Alzheimer disease therapy.

P-6o

HPTLC method for simultaneous estimation of antihypertensive drugs

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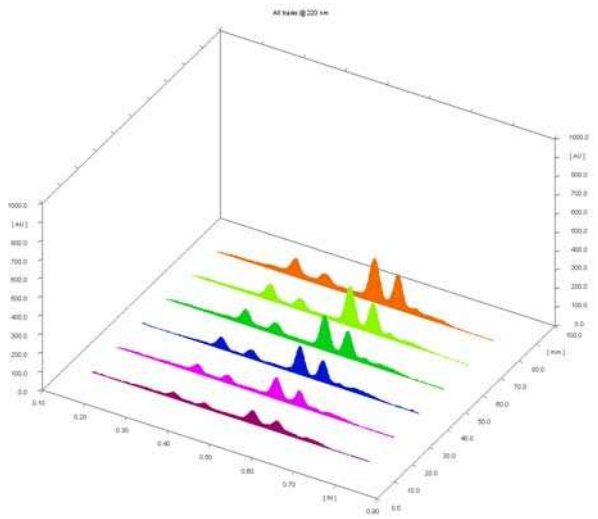
A simple and versatile HPTLC method was developed for simultaneous analysis of irbesartan, telmisartan, ramipril and hydrochlorothiazide in bulk and tablets. Chromatography was performed on TLC aluminium foils silica gel 60 F_{254}, 20 cm × 20 cm with 0.2 mm layer thickness. Samples were applied as 4 mm bands, 5 mm apart, by means of a Linomat 5. Linear ascending development to a distance of 80 mm was performed with an optimized mobile phase in a twin trough glass chamber previously saturated with mobile phase vapor for 20 min at room temperature (25 °C). After development the plates were scanned at the selected wavelength by means of the TLC Scanner in the absorbance mode using the deuterium lamp. A variety of mobile phases were investigated and the suitability of the mobile phase was decided by study of the sensitivity of the assay, time required for analysis and the use of readily available solvents. The drugs were satisfactorily resolved with a mobile phase mixture consisting of acetonitrile – toluene – methanol – formic acid in the ratio 8:10:2:0.6 (v/v/v/v) with Rf values of 0.35, 0.42, 0.54 and 0.60, respectively. The accuracy and repeatability of the proposed method was ascertained by evaluating various validation parameters like linearity, precision, accuracy and specificity according to ICH guidelines. HPTLC method provides a faster and cost effective quantitative control for routine analysis of the antihypertensives in bulk and its formulations.



Three dimensional densitogram showing the linearity and resolution of drugs

P-6p

A novel HPTLC analytical method for quantification and separation of mycotoxin gliotoxin and related compounds in human samples

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Invasive aspergillosis, a nosocomial opportunistic infection caused by pathogens of the genus Aspergillus (mainly by Aspergillus fumigatus), is associated with significant mortality. This is due in part to the absence of optimal diagnostic modalities, which hamper early disease detection. Gliotoxin (GT) and bis-methylthio-gliotoxin (mGT) are secondary metabollites produced by pathogens of the genus Aspergillus. We have previously shown that GT is a virulence factor during experimental aspergillosis induced by Aspergillus fumigatus that helps mold to establish infection and survive within the host. Thus, we hypothesise that GT and mGT should be produced during the first stages of infection, representing a potential early diagnosis marker to reveal mold presence. A method to help with early diagnosis of aspergillosis based on the detection of GT and mGT during invasive aspergillosis is presented.

Our detection system is based on quantitative HPTLC. In order to establish the detection limit of this system**,** samples of serum from healthy donors are spiked with known amounts of GT and mGT, extracted with dichloromethane and separated on HPTLC plates. GT and mGT are detected and quantified by using fluorescent dyes or under UV light exposure. Subsequently this method is used to analyse GT and mGT presence in serum samples of patients with probable and proven aspergillosis. Because of its simplicity, speed and accuracy, it could be easily implemented in clinical diagnosis laboratories and would complement or even improve current diagnosis methods of aspergillosis.

P-6q

Validation of a HPTLC method for quantification of cardiolipin and other phospholipids classes in mitochondria

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Few studies suggest that cardiolipin, a specific phospholipid of mitochondria, could be involved in the hypermetabolism associated to cancer cachexia. Although several methods to quantify phospholipids and especially cardiolipin exist, none are sufficiently sensitive, precise, rapid and cost effective.

The aim of this study was to validate the HPTLC-densitometry technique, according to the ICH guidelines, to quantify phospholipids (phosphatidylethanolamine, sphingomyelin, phosphatidylcholine, phosphatidylinositol) and especially cardiolipins in mitochondria. Samples were applied on HPTLC plates using a Linomat 5. The analysis of phospholipids classes was performed with densitometric scanning with a Reprostar and Videoscan. The amount of each phospholipid classes was determined with the Wincats software ver.1.4.1. and Videoscan. The method was tested by evaluating validation parameters like linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), inter-day and intra-day assay precision, repeatability of measurement, and repeatability for sample application. Validation of the method was performed by statistical analysis using one-way ANOVA (Statplus software).

For cardiolipins, LOD and LOQ were 7.5 ng/spot and 18.0 ng/spot, respectively. The linearity range was 400 - 800 ng/spot with regression coefficient of 0.998. CVr and CVR were from 6.53 to 8.26 % and 6.67 to 8.54 %, respectively. All statistical tests were significant to validate the method for all phospholipid classes. Therefore, the HPTLC-densitometry was successful to demonstrate that cardiolipin content was increased in liver mitochondria of cancer rats. In conclusion, the HPTLC-densitometry method is a new, selective, precise and cost effective for the determination of cardiolipins and other phospholipids classes.

[1] FM. Helmy. *Cell Biochem Funct* 25, 2007, 233-243. [2] "*Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement",* M. Thompson, S. Ellison, A. Fajgelj, P. Willetts, R. Wood, J. Pure & Applied Chemistry 71(2), 1999, 337-348. [3] JI .Ruiz B.Ochoa. *Journal of lipid research* 38, 1997, 1482-1489.

P-6r

Development of HPTLC method for the estimation of ondansetron in bulk drug and sublingual tablets

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Ondansetron is a serotonin subtype 3 (5-HT_3) receptor antagonist used in the management of nausea and vomiting after cancer chemotherapy, radiotherapy and surgery [1]. Various analytical methods used for estimation of ondansetron include HPLC method in human plasma [2] and spectrophotometric method in solid dosage form [3]. Hence, it was thought worthwhile to develop a simple, economic and validated HPTLC method for the quantification of ondansetron in bulk drug and pharmaceutical dosage forms. The mobile phase composition was chloroform - ethyl acetate - methanol - ammonia (9:5:4:0.1, *v/v*).

Spectrodensitometric analysis of ondansetron was carried out at 254 nm and obtained symmetrical, well-resolved, well-defined peak at R_F 0.52 ± 0.02. The calibration plot was linear in the range 200 - 1200 ng/spot and showed good linear relationship with coefficient of regression, R^2= 0.9952 with respect to peak area. The method was validated according to the ICH Q2(R1) guidelines. The limit of detection and quantitation were 14.8 and 44.9 ng/spot, respectively. The recovery study was carried out by standard addition method and the percentage recovery was found to be 99.34 ± 1.08. Therefore it was concluded that the proposed developed HPTLC method can be applied for identification and quantitative determination of ondansetron in bulk drug and dosage forms.

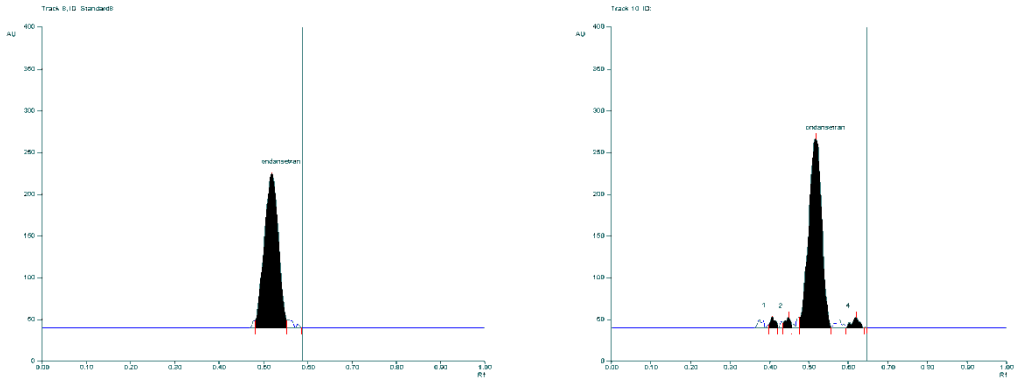

Fig. 1 A typical HPTLC chromatogram of standard ondansetron (Rf = 0.52 ± 0.02, left and ondansetron and the excipients in sublingual tablets formulation (right)

[1] M.E. Butcher, *Oncology*. 50, 1993, 191–197. [2] M. Deport, S. Leroux, G. Caille, *J Chromatogr B*. 693, 1997, 399-404. [3] SP. Sastry, T. Rao, *Indian J Pharm Sci*. 64, 2002, 482-485.

P-6s

Desloratadine quantification using HPTLC

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A new, simple, and rapid HPTLC method was developed and validated for quantitative determination of desloratadine. Desloratadine was chromatographed on HPTLC plates silica gel 60 F_{254} using methanol - chloroform - toluene - ammonia (5:5:1:0.3) as mobile phase. Desloratadine was quantified by densitometric analysis at 254 nm. The method was found to give compact spots for the drug (R_f = 0.6 ± 0.01). The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9997 in the concentration range 150 – 750 ng/spot.

The method was validated for accuracy, precision, recovery, repeatability, linearity, specificity and robustness as per the ICH guideline. The minimum detectable amount was found to be 21 ng/spot, whereas the limit of quantitation was found to be 65 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of desloratadine. The method was successfully employed for the quantification of desloratadine as a bulk drug and in commercially available preparation.

P-6t

HPTLC method for quantification of carbamazepine in formulations and *invitro* diffusion study

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A new HPTLC method was developed and validated for quantitative determination of carbamazepine. Carbamazepine was chromatographed on TLC plates silica gel 60 F_{254} using ethyl acetate – toluene - methanol (5.0+4.0+1.0, v/v/v) as mobile phase. Carbamazepine was quantified by densitometric analysis at 285 nm. The method was found to give compact spots for the drug (R_f = 0.47 ± 0.01). The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9995 in the concentration range 100 – 600 ng/spot.

The method was validated for accuracy, precision, recovery, repeatability, and robustness as per the ICH guideline. The minimum detectable amount was found to be 16.7 ng/spot, whereas the limit of quantitation was found to be 50.4 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of carbamazepine. The method was successfully employed for the estimation of equilibrium solubility, quantification of carbamazepine as a bulk drug, in commercially available preparation, in-house developed mucoadhesive microemulsion formulations, solution and in vitro diffusion study using sheep nasal mucosa.

P-6u

Urine HPTLC-metabonomics study of soy-phytoestrogens in Africans

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HPTLC has been explored in various analyses, including pharmaceutical and biomedical analysis, environmental and forensic analysis, plant and herbal analysis and food analysis. In this study, HPTLC was used in urinary metabolic profiling 'metabonomics' studies following a phytoestrogen-rich diet intervention in human volunteers. The objectives of this study were to evaluate the differences in metabolic changes of phytoestrogens during a short-term soya-milk intervention and investigate possible factors influencing these differences in phytoestrogen metabolism. To date, no study has been carried out on assessing phytoestrogens and their metabolites in urine using HPTLC and multivariate analysis.

The methodologies involved were enzymatic hydrolysis, sample-clean-up using solid-phase extraction followed by HPTLC analysis. On silica HPTLC plates, urine samples were run alongside mixture of the chemical reference standards of genistein, daidzein, equol, o-desmethylangolensin, seco-isolariciresinol, matairesinol, enterolactone and enterodiol (Fig. 1). Optimal separation was achieved using a developing solvent of n-heptane and ethyl acetate over 80 mm migration distance and post-derivatisation of the developed plates was carried out. The derivatised-HPTLC fingerprint was "*binned*" using VideoScan and statistically analysed using SIMCA-P+ multivariate analysis: Principal Component Analysis (PCA) and (orthogonal-) Partial-Least Square – Discriminant Analysis ((O-) PLS-DA).

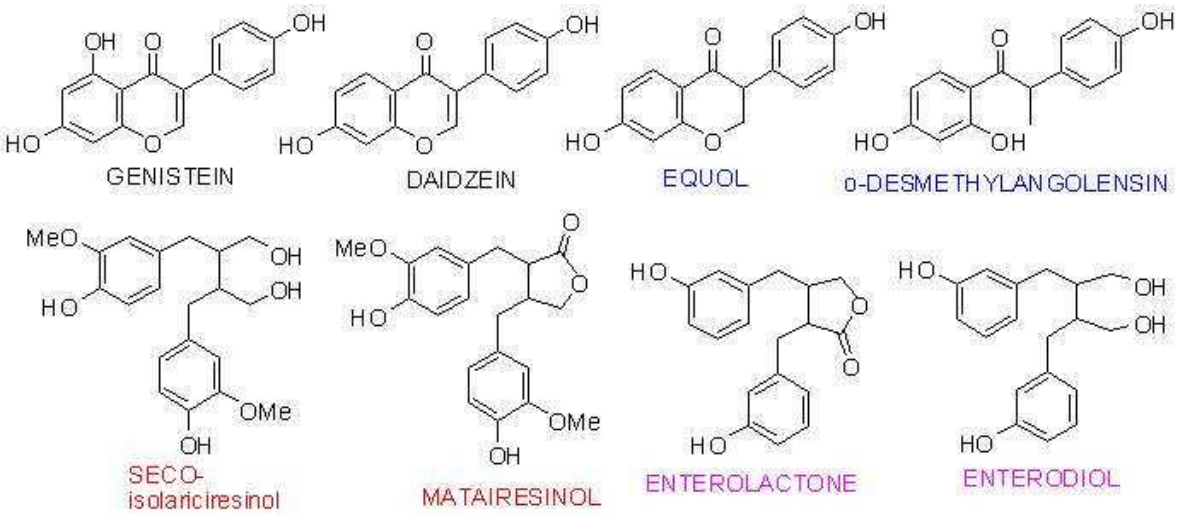

Fig.1: Phytoestrogens and metabolites analysed in this study

P-6v

Analysis of herbal gel by HPTLC method development and validation

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The alcoholic seed extract of *Albizzia lebbeck* exhibited a significant spermicidal activity in the in vitro bioassays. Contraceptive gels were formulated using this bioactive extract. The alcoholic seed extract was incorporated into vaginal gels using various gelling agents Preliminary phytochemical screening of this extract indicated the presence of carbohydrates, gums, alkaloids, amino acids and saponins.

A HPTLC method was developed and validated for the estimation of the alcoholic seed extract. HPTLC studies of the seed extract were performed with n-butanol - glacial acetic acid - water 5:1.5:2. The HPTLC fingerprint was recorded at 254, 366 and 540 nm and the developed plates were sprayed with the anisaldehyde sulfuric acid reagent which indicated the presence of saponins.

The various validation parameters included studies on linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). Accurately weighed amounts of different concentrations (0.5 %, 2 %, 5 %, 10 % and 20 %) of seed extract gels were used in the analysis. The developed plates were scanned at 254 nm. The calibration curve of the component at R_F 0.18 was linear in the concentration range of 40 - 120 µg/mL with the correlation coefficient of 0.9907. The LOD and the LOQ was found to be 10 µg and 40 µg, respectively. The percent assay was evaluated using the peak area. The content of the extract in the formulated gels (percent assay) was determined to be in the range of 97 – 101 %.

P-6x

Development of an HPTLC method for simultaneous estimation of ranitidine HCl and domperidone in their combined dosage form

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An accurate and precise HPTLC method for simultaneous estimation of Ranitidine HCl and domperidone in their combined dosage form has been developed on aluminium foil silica gel 60 GF_{254} with methanol - 1,4-dioxane 4:6. The detection was carried out at 232 nm. The detector response was linear for ranitidine HCl and domperidone between 3.0 to 50.0 µg/mL. The percent estimations (% ± SD) of both drugs in the laboratory mixture were found to be 100.2 ± 0.7, 99.8 ± 0.1 and 100.1 ± 0.2, 100.2 ± 1.6 by peak height and peak area.

The percent estimations (% ± SD) of both drugs in marketed formulation were found to be 101.1 ± 0.9, 99.4 ± 0.5 and 99.6 ± 0.7, 99.5 ± 0.7 by peak height and peak area. The recovery study was carried out by standard addition method. The recovery was found to be 100.3 ± 0.9, 100.8 ± 0.6 for ranitidine HCl and 100.3 ± 0.4, 99.5 ± 0.4 for domperidone. The method was validated according to the ICH guidelines. The accuracy of the method was evaluated by the recovery. The results of the method fulfilled the prescribed limit of 98 – 102 % and showed that the method is free from interference of excipients.

P-6y

HPTLC analysis of salicylic acid in drug release media during development of an anti acne patch

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Salicylic acid (SA) is a key ingredient in many skin-care products for the treatment of diseases such as acne, corns and warts. SA is one of the main antiseptic components in acne formulations. Anti-acne patches formulated with SA in the adhesive for application to sensitive skin areas was under development. Optimization of the release profile requires many determinations of SA levels in the release media over a wide range of concentrations. Numerous studies describing quantitation of SA have been done. No HPTLC procedure has been evaluated for determination of SA in release media.

In this study a simple high throughput HPTLC procedure was developed for determination of SA in release media of the under development anti acne patch. Chromatographic separation between the released SA and the sample matrix components was achieved on HPTLC plates silica gel 60 F_{254} with toluene - methanol - acetic acid 75:24:1 (v/v) as mobile phase. Quantitative investigations were performed by densitometry at 270 nm (Fig. 1, left). First order polynomial calibration function (r^2 = 0.996) over the range 50-400 ng/zone was found. Residuals are distributed at random around the zero-line without any trend (Fig. 1, right). Therefore the adequacy of the regression model calibration was confirmed, in the linear working range.

The procedure was validated according to ICH guidelines. Specificity, linearity, detection limit (DL), quantitation limit (QL), precision, accuracy and robustness of the procedure were characterized. This chromatography system resulted in well baseline separation between SA and sample matrix component zones. The well-resolved signals demonstrate the specificity of the procedure.

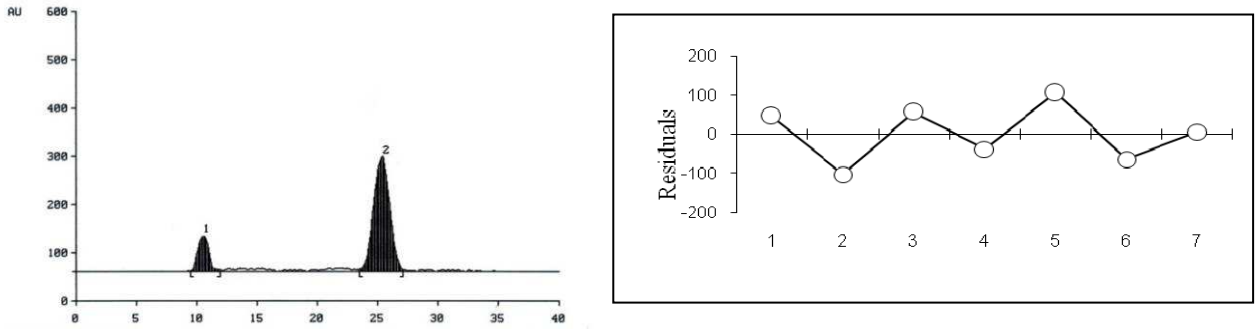

Fig. 1 left: Baseline separation between SA (2) and matrix components (1); right: Plot of residuals for SA in the linear working range

P-6z

HPTLC determination of tramadol hydrochloride in a tablet formulation in presence of related impurities

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Tramadol is an opioid analgesic drug that is used for treatment of moderate to sever pain. It also has noradrenergic and serotonergic properties that may contribute to its analgesic activity. In this study a simple high throughput HPTLC procedure was developed for determination of tramadol hydrochloride (TH) in bulk drug and pharmaceutical dosage. Chromatographic separation between the TH, related impurities and the sample matrix components was achieved on HPTLC plates silica gel 60 F_{254} with toluene – methanol - trifluoroacetic acid 70:29.9:0.1 (v/v). Quantitation was performed by densitometry at 290 nm. First order polynomial calibration function (r^2 = 0.997) over the range 52-312 ng.zone^{-1} was found. Residuals are distributed at random around the zero-line without any trend (Fig. 1, right); the calibration function can therefore be regarded as linear between the upper and lower amounts of the range.

The procedure was validated according to ICH guidelines. Specificity, linearity, detection limit (DL), quantitation limit (QL), precision and accuracy of the procedure were characterized. This chromatography system resulted in well-separated compact zones (Fig. 1, left). Excellent baseline separation was obtained between TH, available impurities B, C, D, E and the placebo components. The well-resolved signals demonstrate the specificity of the procedure.

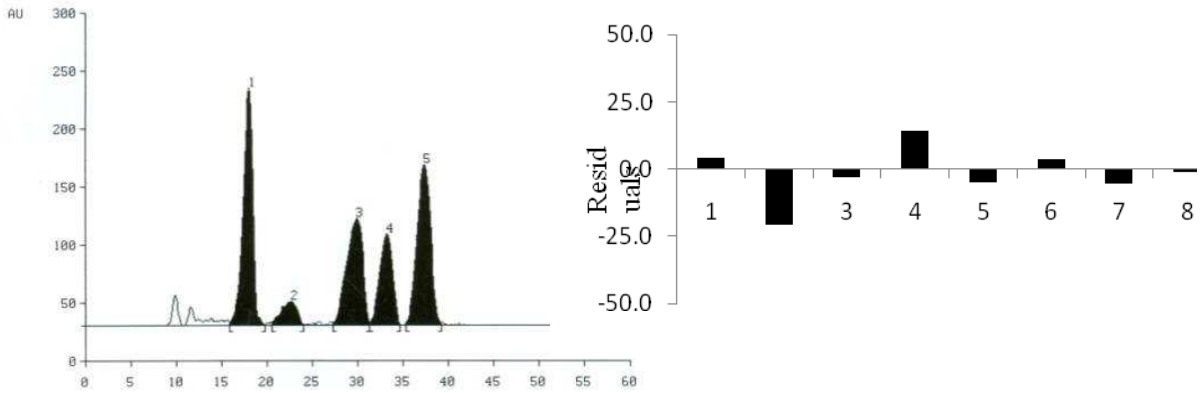

Fig. 1 left: Baseline separation between TH (3), impurities B (4), C (5), D (1), E (2) and the placebo components remained on the start; right: Plot of residuals for TH in the linear working range

P-7a

Quantification of α-asrone in *Acorus calamus* extract by HPTLC densitometry

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Acorus calamus L. (Araceae) commonly known ‘‘sweet flag’’ or ‘‘calamus’’ is used in Indian System of Medicine and Traditional Chinese Medicine for its potential effect on memory disorder, lipid peroxide content, anti-aging and anti-cholinergic activity. α-Asarone is one of its major bioactive components.

A simple HPTLC method has been developed to control the quality of raw herbs through analysis of the markers' profile. The solvent system was optimized to be toluene - ethyl acetate (8:3, v/v). Five different amounts of α-asarone (2, 4, 6, 8, and 10 µg) and 50 µg of extract were applied on a TLC plate using Linomat 5. The ascending development was carried out in a pre-saturated twin-trough chamber (10 cm × 10 cm). The developed plate was scanned at 254 nm using the TLC Scanner 3 with winCATS software. Presence of α-asarone (Fig. 1) in the extract (Fig. 2) was confirmed by the corresponding Rf value of the standard (0.71). The linearity range of the calibration curve was found to be 200 - 1000 ng/spot. The linearity relationship was described by the equation y = - 23.304 + 23.842 x with r = 0.996 (peak area). The amount of α-asarone in the extract was found to be 2.13 % (w/w). The present method was simple, accurate, specific, precise and reproducible. It may be useful for rapid and cost-effective development of quantity measurement and marker analysis of *A. calamus*.

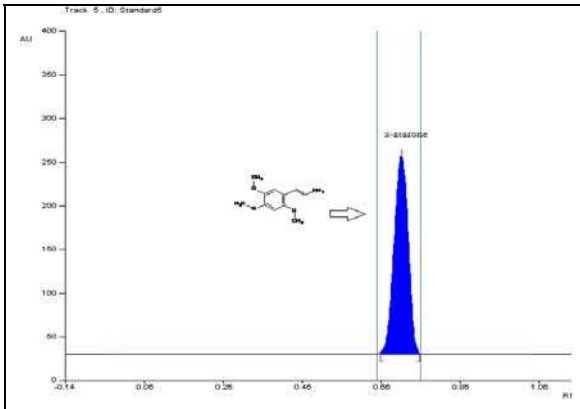

Fig. 1 α-Asarone chromatogram

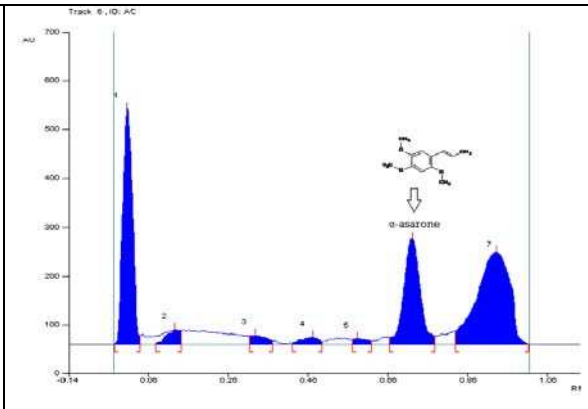

Fig. 2 Extract chromatogram

P-7b

Multivariate analysis of Radix Linderane HPTLC analysis

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Multivariate analyses have been used in hyphenated chromatographic and detection techniques for reducing the dimensionality of data. However, no study has yet integrated this statistical analysis into HPTLC. This study incorporates the use of multivariate analysis (supervised and unsupervised) in HPTLC for the authentication and discrimination of different sources of a traditional Chinese medicine (TCM) herb, Wuyao: Radix Linderane commonly known as combined spicebush root. This is the dry roots of plants in Lauraceae family *lindera stryvhnifolia (Sieb. Et Zucc.) Villar.* In China, *Radix linderae* is mainly grown and cultivated from Zhejiang, Henan, Anhui, Guangdong, Guangxi provinces.

HPTLC fingerprints of the plant extract at 366 nm illumination (Fig. 1) was analysed using VideoScan to reduce the multi-dimensionality of the tracks. This was achieved by binning the chromatograms (area) into ~0.05 - 0.1 R_F variables. The results (Fig. 2) showed that good reproducibility and predictibility (>>0.5) in both supervised and unsupervised MVA. Thus, this MVA analysis can be applied to HPTLC.

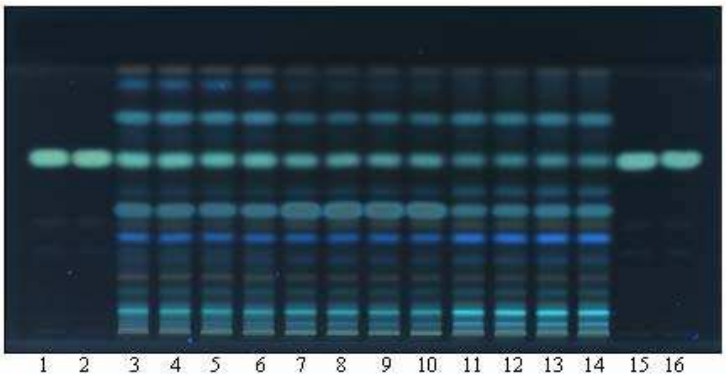

Fig. 1. HPTLC chromatogram of *Radix linderae*.

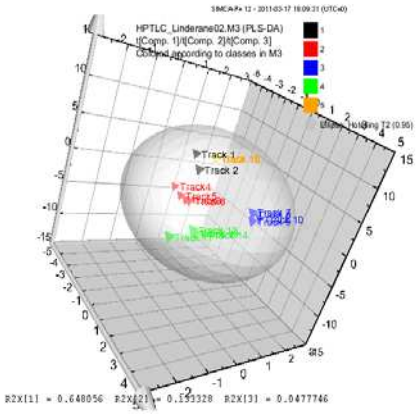

Fig 2. Partial least square-discriminant analysis of HPTLC data. **1**: Tracks 1-2; from different provinces: 3-6: Province 1; **2**: Tracks 3-6; **3**: Tracks 7-10; **4**: Tracks 7-10: Province 2; 11-14: Province 3. 11-14; **5**: Tracks 15, 16.

P-7c

Pattern recognition in HPTLC-fingerprints of medicinal plants

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HPTLC is an established tool for the analysis of medicinal plants. HPTLC fingerprints are used for identification and characterization of herbal drugs and products, thus they are utilized for quality control [1]. Identification of a plant material is usually performed by comparing its fingerprint with that of a botanical reference material. Our aim was to make this comparison more objective by employing chemometric methods. A principal problem that had to be addressed is the variance of fingerprints of multiple samples of the same plant species. It is based on natural variability due to climate conditions, soil composition, time of harvesting and storage conditions.

For the analysis of herbals standardized HPTLC methods and qualified equipment has been used in combination with a novel software for taking and evaluating high dynamic range images (HDRI). First a visual evaluation of fluorescent images was performed. Then images of the individual sample tracks of a plate were converted to profiles describing three individual color channels. Finally principal component analysis (PCA) was performed with these profiles to build clusters of similar profiles that represent the variability of the same species.

More than 50 samples of thyme leaf (*Thymus vulgaris*, L.) were analyzed to characterize their variability of the fingerprints. We compared them with other thyme species, various samples of oregano (*Origanum vulgare* L.) and sage (*Salvia officinalis* L.). The analyzed species showed similar chemical profiles (flavonoids) nonetheless with considerable variances within each kind. Nevertheless it was possible to assign species – related clusters to the species with PCA. Pretreatment of chromatographic data including normalization of peak intensity as well as correcting shifts in Rf values from plate to plate leads to well defined clusters representing defined plant species [2].

[1] WHO, General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines, Geneva 2000, p.23. [2] C-J. Xu, Y-Z. Liang, F-T. Chau, Y. V. Heyden, *J. Chrom. A.* 1134, 2006, 253-259.

P-7d

Application of HPTLC for identification of the Chinese herbal with and without processing – *Glycyrrhizae radix preparata* and *Glycyrrhizae radix*

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Pao Zhi (processing) is a very important procedure in Chinese herbal medicine. As well-known, processing procedures such as stir-baking, steaming, and calcining may modify many properties of the herbs, such as efficacy and safety. According to the pharmacopoeia and related literatures, till now, no difference can be found via Thin-Layer Chromatography (TLC) in herbals treated with and without processing. Only few fluorescent spots of the same position and colors between sample and reference in the TLC were selectively compared.

Chromatography was performed on an HPTLC silica gel 60 F_{254} plate as stationary phase with toluene - ethyl formate - formic acid - dichloromethane (10:12:6:12, v/v/v/v) as mobile phase. The chromatogram was examined at UV 254 nm, UV 365 nm and after derivatization with the sulfuric acid reagent (10 % in ethanol) under white light illumination and at UV 365 nm.

In this report, a simple, rapid and cost-efficient HPTLC method with high precision for identification of *Glycrrhizae radix preparata* (Zhi Gan Cao, processing) and *Glycrrhizae radix* (Gan Cao, unprocessing) will be presented. In comparison with the whole spectrum of the extract, the chromatogram provides a characteristic pattern and possible differentiation between herbals with and without processing.

P-7e

Identification of the Chinese herbal formulas by HPTLC – mahuang & apricot seed combination and mahuang & coix combination

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The traditional Chinese herbal medicine combines different kinds of herbs. This combined recipe with interrelated medical substances makes the formulas effective, such as accentuate the strengths and reduce the side effects. Several formulas are comprised of many same single herbs with only one or two different single herbs, for example, Mahung & Coix C. (Ma Xing Yi Gan Tang) and Mahung & Apricot Seed C. (Ma Xing Shi Gan Tang). It is not always easy to distinguish these similar formulas by TLC. In this report, a simple HPTLC procedure for identification of the formulas with a specific mobile is described.

Chromatography was performed on an HPTLC plate silica gel 60 F_{254} as stationary phase using four different mobile phases (v/v):

1. dichloromethane - ethyl acetate – methanol - dest. water 15:40:22:10
2. petroleum ether - ethyl acetate - acetic acid 10:3:0.1
3. toluene - ethyl formate - formic acid - dichloromethane 10:12:6:12
4. *n*-butanol - acetic acid - dest. water 7:1:2

The chromatogram was then examined at UV 254 nm and UV 365 nm and after derivatization with the sulfuric acid reagent (10 % in ethanol) or molybdophosphoric acid reagent under white light illumination and at UV 365 nm. For identification of the two formulas – Mahuang & Coix C. and Mahang & Apricot Seed C, petroleum ether - ethyl acetate - acetic acid 10:3:0.1 was shown to be the best mobile phase as well as derivatization with the sulfuric acid reagent.

P-7f

HPTLC characterization of *Salvia* species used as medicinal in Valencian community (Spain)

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The Mediterranean and Irano-turanic region has *ca*. 40 species of *Salvia*. In the Valencian community five wild and cultivated species are used traditionally as medicinal. Quality control of the herbal drugs appears as an essential step for an eventual commercialization. The aim of the work was to develop a method of identification by HPTLC to distinguish the different species of *Salvia* used in the region.

Essential oils obtained by hydrodistillation and hydroethanolic (EtOH 50%) and dichloromethane extracts of air-dried aerial parts of *S. microphylla, S*. *officinalis*, *S.* x *auriculata*, *S. blancoana* subsp. *mariolensis* and *S. lavandulifolia* subsp. *lavandulifolia* were analyzed by HPTLC to compare chromatographic profiles for the oil and the two extracts, respectively. Analyses were carried out under controlled conditions for sample application and plate development using HPTLC instrumentation.

As it can be seen in Fig. 1 (left), the polyphenol chromatographic patterns of the hydroethanolic extracts allow differentiating between all species with the exception of *S. lavandulifolia* subsp. *lavandulifolia* and *S. blancoana* subsp. *mariolensis*, two closely related taxa which show similar profiles. However, the dichloromethanolic extracts show some differences between these two taxa (Fig. 1, right). From the point of view of essential oils, the presence of thujone in the oils of *S. officinalis* and *S. x auriculata* distinguishes these taxa from the other three, among which the essential oil of *S. michrophylla* appears as the most different.

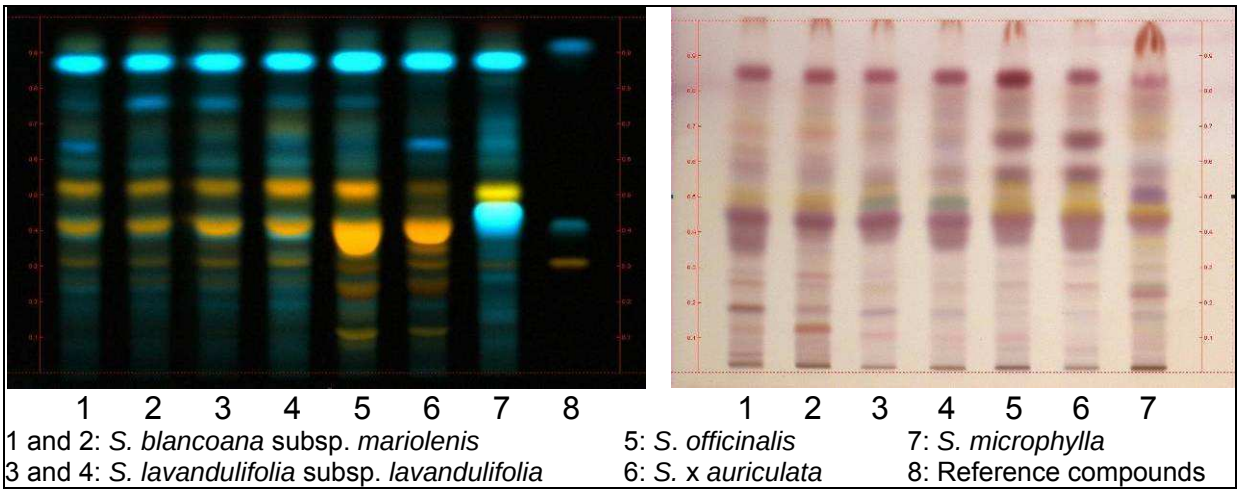

Fig. 1 HPTLC chromatograms of the hydroalcoholic (left, UV light at 365 nm, derivatization: NPR + PEG 400) and dichloromethane (right, day light, derivatization: anisaldehyde reagent) extracts of the aerial parts of several *Salvia* species.

P-7g

A selective determination of chrysophanol in polyherbal oil containing *Cassia fistula* using HPTLC

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Many polyherbal oil formulations in traditional system of medicine contain *Cassia fistula* Linn. (Family:Leguminosae). Chrysophanol (1,8-Dihydroxy-3-methylanthraquinone) is an natural anthraquinone and a major therapeutically important constituent of the seeds of *Cassia fistula*. In the present studies attempt is made to develop a validated simple, rapid and sensitive HPTLC method for identification and quantification of chrysophanol from polyherbal oil formulation (*Kustharaksasa Taila)*.

Chromatography of methanolic extract of these formulation was performed on silica gel 60 F_{254} aluminum-backed TLC plates of 0.2 mm layer thickness. The plates were developed up to 85 mm with the binary-mobile phase containing hexane - ethyl acetate (9.5:0.5, v/v) at 22 ± 2 ºC with 20 min chamber saturation. The system produced compact band of chrysophanol at a R_F value of 0.28. The marker chrysophanol was quantified at its maximum absorbance of 290 nm. The limit of detection and limit of quantitation values were found to be 5 ng/band and 15 ng/band, respectively. The linearity with respect to peak area was found to be in the range of 50 – 300 ng/band with a correlation coefficient of 0.9997.

Poly-herbal oil formulation was analyzed with reasonable accuracy and no matrix interference was observed. The developed HPTLC method is accurate, precise, and cost-effective .The method developed can be used for marker based quality assurance of poly-herbal oil formulation (*Kustharaksasa Taila)* containing *Cassia fistula* as one of the active ingredient.

P-7h

Estimation of lupeol in ayurvedic oil formulation containing *Alstonia scholaris*

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Lupeol (20(29)-Lupen-3ß-ol, 3ß-Hydroxy-20(29)-lupene) is a major therapeutically important constituent of the bark of *Alstonia scholaris*. A sensitive, simple, rapid, and efficient HPTLC method was developed and validated for the analysis of Lupeol in marketed Ayurvedic oil formulation (*Kustharaksasa Taila)* containing *Alstonia scholaris* (Llnn.) *R. Br.* (Apocynaceae). Chromatography of methanolic extract of this Ayurvedic oil formulation was performed on aluminum-backed TLC plates silica gel 60 F_{254} of 0.2 mm layer thickness. The plate was developed up to 85 mm with the ternary mobile phase toluene – chloroform - ethyl acetate - 0.1 % glacial acetic acid (10:2:1:0.03, v/v/v/v) and at 22 ± 2 °C with 20 min chamber saturation.

After derivatization with vanillin sulphuric acid reagent a well resolved symmetric band for lupeol from its oil formulation was observed at R_F 0.28 with UV absorbance at 254 nm. The limits of detection and quantitation were 10 and 30 ng/spot, respectively. The linear regression analysis data for the calibration plot showed a good linear relationship with a correlation coefficient of 0.9993 in the concentration range of 50 – 300 ng/spot for lupeol with respect to peak area. The repeatability (*%RSD*) of the method was 0.97 %. Recovery values from 98 to 102 % indicate excellent accuracy of the method. The developed HPTLC method is accurate, precise, and cost-effective, and it can be successfully applied for the determination of lupeol in marketed Ayurvedic oil formulation (*Kustharaksasa Taila)* containing *Alstonia scholaris*.

P-7i

HPTLC method for analysis of colchicine in Unani formulations containing suranjaan talkh and suranjaan seerin

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Colchicine (Fig. 1) is the major bioactive alkaloid present in suranjan talkh (Bitter variety of *Colchicum luteum* Bak) and suranjan seerin (Sweet variety of *Colchicum luteum* Bak), which have been reported to possess anti-rheumatic, anti-gout [1], and anticancer potential [2]. It has also been prescribed for its cathartic and emetic effects [3]. Since, the suranjaan is major ingredient of several important Unani and herbal formulations like majoon-e-suranjan, roghan-e-suranzan, suffoof-e-suranjan, habb-e-suranjan, aujai capsule, rhemartho gold, and byna tablet etc. Quantification of colchicines will play a great role in quality control of these formulations.

Hence, HPTLC method has been developed and validated for analysis of colchicine in unani formulations of various dosage forms like safoof (powder), majoon (semisolid sugar based dosage form), hubb (tablet), roghan (oil) and capsules. The analyte was applied on aluminum TLC plates silica gel 60 F_{254} and developed using the mobile phase toluene – dichloromethane - methanol in equal proportion. Quantification was performed by densitometric scanning at 350 nm, which showed a linear response in the range of 50 - 500 ng/spot. The developed method was validated as per ICH guidelines for linearity, precision, accuracy, Specificity, Robustness, LOD and LOQ. The method developed was applied for quality-control of different Unani and herbal formulations containing Suranjaan Talkh and Suranjaan Seerin.

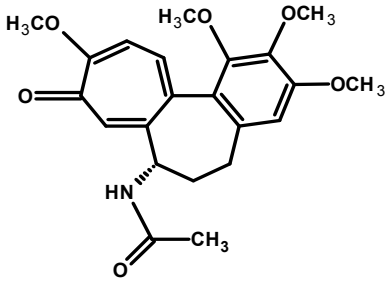

Fig. 1 Chemical structure of colchicine

[1] S. Sweetman (ed.), Martindale, The Complete Drug Reference, 33rd edn, 2002, p. 400. [2] F. Alali, K. Tawaha, R.M. Qasaymeh, *Phytochem. Anal.* 15 (2004), 27–29. [3] V. Mirakor, V. Vaidya, S. Menon, P. Champanerker, A. Laud, *J Planar Chromatogr.* 21, 2008, 187–189.

P-7j

Development and validation of an HPTLC method for quantification of anti cancer compound in *Podophyllum hexandrum* callus culture

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Podophyllum hexandrum Royle (Berberidaceae), a perennial herb, native of India is known to contain the highest content of anti cancer compound podophyllotoxin [1]. Callus culture was successfully developed and maintained on MS basal medium supplemented with plant growth hormones.

A new, simple, cost effective and rapid HPTLC method was developed and validated for quantitative determination of anti cancer compound podophyllotoxin. Standard podophyllotoxin was chromatographed on silica gel 60 F_{254} TLC plate using acetonitrile - water (4:6) as mobile phase. Podophyllotoxin was quantified by analysis in the absorbance mode at 210 nm. The method was found to give compact spots for the drug (R_f = 0.51 ± 0.02). The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.99909 in the concentration range 100 - 1000 ng/spot. The method was validated for precision, recovery, repeatability, and robustness as per ICH guidelines. The minimum detectable amount was found to be 5.0 ng/spot, whereas the limit of quantization was found to be 15.3 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of podophyllotoxin. The method was successfully employed for the estimation of podophyllotoxin in bulk drug and callus culture of *P. hexandrum*. The developed HPTLC method would be an important tool in the quality control method of bulk drug and callus culture of *P. hexandrum*.

[1] R. Chartterjee, *Econ. Bot*., 6, 1952, 343–354.

P-7k

A validated HPTLC method for estimation of ß-sitosterol from plants used as wound healing agents

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Medicinal plants constitute a source of raw materials for both traditional systems (e.g. Ayurvedic, Chinese, Unani, Homeopathy, and Siddha) and modern medicine. Nowadays, plant materials are employed throughout the industrialized and developing world as home remedies, over-the-counter drugs and ingredients for the pharmaceutical industry. Chemical fingerprinting and selection of chemical markers using HPTLC has been demonstrated to be a powerful technique for the quality control of traditional herbal medicines.

In present work, phytochemical fingerprinting and quantitative estimation of a biologically active steroidal compound ß-sitosterol was achieved from wound healing plants like *Blumea lacera* (Roxb.) DC (Asteraceae) leaves, *Limonia acidissima* Linn (Rutaceae) fruit rind, *Tridax procumbens* L. (Asteraceae) leaves using HPTLC. Aluminum foils precoated with silica gel 60 F_{254} were used with a mobile phase comprising of toluene - ethyl acetate – methanol - glacial acetic acid (8:1:0.5:0.6, v/v/v/v) and densitometric determination was carried out at 366 nm in reflectance/absorbance mode. The linear regression data for the calibration plots showed a good linear relationship with r^2 of 0.9991. A recovery study was carried out to check the accuracy of the method. At two different levels the average recovery of ß-sitosterol was found to be 101.3 %. The developed method was also applied for quantitation of ß-sitosterol from a complex matrix based polyherbal Unani formulation *Sufoof–e–Ziabetes Dulabi* and its ingredients. The developed HPTLC method is simple reliable and fast to establish the quality of raw materials with respect to ß-sitosterol.

P-7l

Detection of the decomposition of aconitine in *Aconitum napellus* mother tincture V.2a and identification of the main cleavage products using HPTLC-MS

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In contrast to the conventional medicine where their use is regressive because of their high toxicity, preparations of *Aconitum napellus* play a major role in homeopathic and traditional Chinese medicine. Common indications include neuralgia, coughs and sneezes, fever, rheumatic pain, and it is also used as an anti-inflammatory drug.

Aconitum napellus contains numerous diterpene alkaloids in concentrations of typically 0.5 - 1.5 % dry weight. The toxicity decreases from its main component aconitine to the cleavage products benzoylaconine and aconine, induced by the hydrolytic cleavage of acetate and/or benzoate groups. Aconitine is one of the most toxic phytochemicals, highly lipophilic and absorbable through the skin. It opens the Na^+-channels to activate the peripheral nerve endings before disabling them. Toxic concentrations of Aconitum napellus cause numbness, cardiac arrhythmia, and finally death by apnoea.

The analytical intention is to show the decomposition of aconitine in aged mother tinctures by using a HPTLC method that makes this visible in its chromatographic fingerprint. Using an innovative TLC-MS interface coupled to an ion trap mass spectrometer employing an LTQ Orbitrap XL (Thermo Fisher Scientific), the identification of the relevant decomposition products is realized by multistep mass spectrometric fragmentation.

P-7m

Detection and quantitation of disulfiram in traditional medicine administered to patients with alcohol dependence

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Traditional treatments like acupuncture, herbal powder and different ayurvedic medicines are popular in rural areas of India as cure for alcohol dependence. Even in a clinical setting, patients have reported the use of traditional medicine in alcohol abstinence and validated its use. Few samples of traditional medicine obtained from different but unauthentic sources were received from caregivers. These powders either green or white in color stored in sealed pouches or wrapped in paper weighed approx 1.5 to 2.5 g. Patients consumed it once in the morning or twice in a day for 10 days and can continue it for months if craving persists. Beneficial effects of the traditional medicine were similar to those seen with modern drugs. This prompted us to carry out chemical analysis of these medicines.

HPTLC with excellent resolution, sensitivity and reproducibility offers the best option for screening and quantitation of the suspected material. After screening by color tests, samples were analyzed by HPTLC using ethyl acetate – methanol - ammonia 8.5:1.5:0.5 as mobile phase. Scanning was done with TLC Scanner 3 at 254 nm. Rf for disulfiram was 0.80. The data for calibration plots showed good linear relationship r^2 = 0.99454 and the minimum detectable limit was 15 ng/µL. The recovery of disufiram in the powder was 99.8 - 101%.

The concentration of disulfiram in the unknown samples was approximately four fold higher than the daily dosage prescribed. Psychiatric complications due to disulfiram over-use are common in India. Higher concentrations can cause hepatotoxicity, psychosis, peripheral neuropathy, and optic neuritis. It also explains the necessity to examine medications from unauthorized and inauthentic sources that could affect the patients' health. However, the inference should not be misconstrued as an attempt to undermine the contribution of herbal medicine and traditional drugs in treating alcohol dependence.

P-7n

Analysis of drug chemical compound in traditional medicine from Tasikmalaya Indonesia

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Drug chemical compound were not allowed to be added in traditional medicine because of the differences in indication, contra indication, and doses. One of the traditional medicine that usually been added with drugs was headache jamu or pain killer. This study was conducted to analyze drugs chemical compound that may be added into Jamu as traditional medicine.

The analysis consist of sample collection, extraction, and mefenamic acid, acetaminophen and acetosal identification using three different instruments. Sample was taken from Tasikmalaya West Java Indonesia. TLC and UV-Vis spectrophotometry showed that three samples of headache jamu positively contain mefenamic acid. HPTLC analysis at 290 nm also showed that the jamu has the same retention time with mefenamic acid standard at 11.3 min.

P-7o

Standardisation of herbal formulations by HPTLC by the evaluation of active ingredients present with marker compounds

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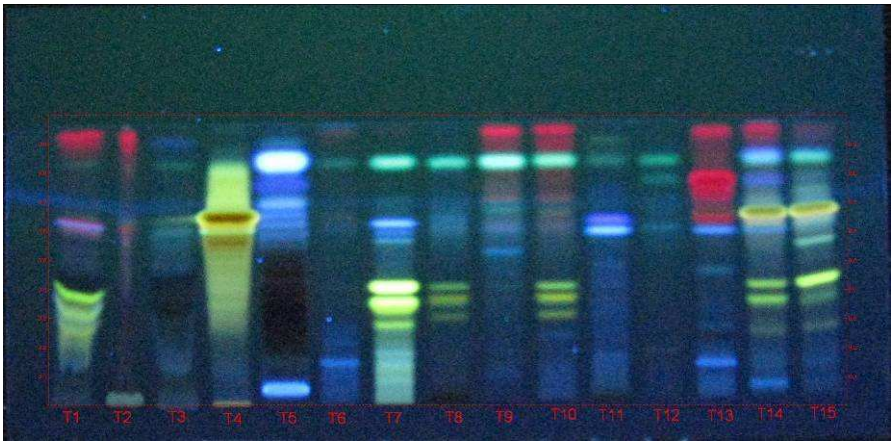
A traditional Ayurvedic poly herbal tablet “Mukkadi Gutika” is used for external application to reduce the Pain, Swelling and Redness of Eye as per ancient Ayurvedic texts. This was taken for standardisation. It is prepared out of herbs like *Hemidesmus indicus, Symplococus racemosa, Pterocarpus santalinus, Curcuma longa, Terminalia belerica, Terminalia chebula, Emblica officinalis etc.*

Published data is already available regarding the active ingredients present in the various herbs. The herbs were extracted in methanol individually and spotted on silica gel plates. Different solvent systems were selected for each of the herbs. Two major ingredients, *i. e.* gallic acid and curcumin, were used as marker compounds.

The paper describes a simple HPTLC method for the standardization of the herbal formula by accurately estimating the quantity of these marker compounds present in two different batches.



HPTLC photograph of “Mukkadi Gutika” and its ingredients under 366 nm

[1] Sahasrayogam, (Vaidyapriya Vyakhyanam, p. 524), TLC Atlas of Ayurvedic Pharmacopoeia Drugs Vol 1, pp. 5, 33, 59, 61, 105, 111; Vol 2, p. 124. [2] Quality Standards of Indian Medicinal Plants, Vol 2, pp. 122-123; 3, p. 78. [3] Ayurvedic Pharmacopoeia of India, Part I, Vol III, pp. 155, 207, p. 219. [4] HPTLC Fingerprint Atlas of Ayurvedic Single plant drugs, p. 30.

P-7p

Development of HPTLC fingerprint profile of Balacaturbhadrica curna, Hinguvastaka curna, Triphala curna and Trikatu curna

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The Traditional System of Medicine has a profound history. It is indeed a wealth to be explored. The raw materials employed are mostly of plant origin and some are from animal and mineral origin. Since, the raw materials are of natural origin it is susceptible to variations due to many of environmental factors. Thus, standardization forms an inevitable role in the development of drugs. It further envisages pathway to the Traditional System to make it acceptable in the global scenario. The raw materials and the finished product need to be standardized pharmacognistically and chemically. As far as identification of raw drug is concerned, there are several techniques, methods and parameters available depending on the nature of raw drugs for determining the quality. However, HPTLC is an important modern quality assessment tool for evaluating the plant materials. It helps in qualitative and quantitative analysis of the sample. Once the HPTLC fingerprint profile is developed for authentic raw material, it would be feasible to compare the finished product to ascertain whether the formulation is prepared with correct raw material and to study the batch-to-batch variation. Adulteration and substitution can be easily differentiated. The test can be performed at high speed with low cost revealing results in a short period of time.

There are several herbal formulations listed in the ayurvedic formulary of India for treating various conditions. Among the curna category of drugs, Balacaturbhadrica curna, Triphala curna, Trikatukuc curna and Higuvastaka curna are reported to be useful for a wide range of ailments. Earlier reports on the preliminary physico-chemical standards for these drugs are available. In the present paper, the concept of HPTLC has been employed to standardize the formulations along with physico -chemical parameters. The study had revealed that the concept explained above were very suitable for analyzing the raw materials employed in a preparation. The HPTLC fingerprint profile developed for the formulations, viz., Balacaturbhadrica Curna, Triphala curna, Trikatu curana and Hingvastaka curna would help in standardizing the formulation and analyzing the raw materials employed.

P-7q

HPTLC interventions for analysis of major bioactive chemical constituents of *Eucalyptus* foliage, cultured *Cordyceps sinensis*, *Dicentra paucinervia* tubers and *Andrographis paniculata*

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HPTLC technique was applied to evaluate the content of major bioactive chemical principles of *Eucalyptus*, *Cordyceps sinensis*, *Dicentra paucinervia* and *Andrographis paniculata*. *Eucalyptus* leaves are important byproducts from biomass produced for the pulp and paper industry and contain ursolic acid, a therapeutically active triterpene, as the major constituent. In relation to the commercial utilization of the leaves from *E. hybrid, E. citriodora* (EC)*, E. torelliana* (ET) and the hybrid bred from EC and ET, ursolic acid content in the leaves of these species was determined and found to be 0.88, 1.07, 0.99 and 1.08 %, respectively.

Cordyceps sinensis (CS), the entomopathogenic fungi, is one of the most famous traditional Chinese medicines and health foods. Production of cordycepin, the active principle of the fungi, was investigated in the CS cultured on barnyard millet (*Echinochloa crusgalli*) and content of it was much higher in the mycelia of the cultured CS (0.029 %) than the natural CS (0.004 %).

The tubers of *Dicentra paucinervia,* a highly potent ethno medicinal herb, are used by tribals living in the eastern Nagaland and adjoining Manipur states of India in the treatment against various diseases. Quantity of proptopine and allocryptopine, the main alkaloids of the plant, was determined in the tubers grown in two different agro climatically distinct locations and found to be comparable (protopine 0.50, 0.55 %; allocryptopine 0.69, 0.70 %).

Influence of intercropping of *Andrographis paniculata,* a revered medicinal plant, with *Morus alba* on concentration of andrographolide, a diterpene lactone, was studied. No favorable effect of intercropping on andrographolide content was observed.

P-7r

HPTLC studies and method development of some important herbal drugs used in Indian traditional systems of medicines

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There has been an increasing realization that the green medicine is safer and this has led to the spurt in the use of plant based medicines across the world and in India too. The global herbal market is about US$ 90 billion which is growing at the rate of 10-15 % annually and is expected to cross 5 trillion US$ by 2030. The traditional medicine in India functions through two streams i.e. the folk stream and the classical organized stream that includes the Ayurveda, Unani, Siddha etc. The folklore medicine is again routed either through the rural village based or the tribal based. Thus the use of medicinal plants amounts to around 8000 wild plants in these medicines.

Indian herbal drug industries generally face the problem of adulteration & substitution. It is observed that in herbal markets of the country, sometimes not only the various species of particular genus but entirely different taxa are being sold under the same vernacular name. For example in the name of 'Talispatra' an important Ayurvedic drug, different leaves of *Taxus wallichiana*, *Abies spectabilis* and *Rhododendran anthopogan* are being sold in Dehradun, Kolkatta & Amritsar market respectively. Similarly on the name of Pittapapra different plants viz. *Fumaria parviflora*, *Peristrophe bicalyculata* and *Oldenlandia corymbesa* and *Rungia* are being sold in various crude drug markets.

Therefore, there is a need to develop quality parameters of raw drugs, proper collection and processing along with HPTLC finger printing to get desirable quality of raw material so that same may be included in Ayurvedic Pharmacopoeia of India (AYUSH).

P-7s

Isolation of phytoconstituents from the chloroform fraction of the aerial roots of *Ficus benghalensis*

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Ficus benghalensis is a large tree, 20 - 30 meters high with widely spreading branches bearing many aerial roots. Studies carried out in our laboratory have confirmed the immunostimulant activity of the aerial roots. The aqueous extract was found to enhance the cell mediated and antibody mediated immune responses in animal experiments. Phytochemical screening of the aqueous extract of *Ficus benghalensis* indicated the presence of flavonoids and phenolics. The bioactive aqueous extract was fractionated using solvents of varying polarity like toluene, chloroform, ethyl acetate and n-butanol. HPTLC studies of the chloroform fraction of the aqueous extract indicated the presence of many simple coumarin compounds having greenish blue fluorescence and furanocoumarins having intense blue fluorescence.

Preparative TLC and circular chromatography were used to isolate these compounds. Preparative TLC using toluene - ethyl acetate 4:6 as the mobile phase did not give very good resolution, was time consuming and required large amounts of mobile phase. Hence normal phase circular chromatography (a cyclograph) using a 1000 µ circular plate was tried. This technique was much faster with each run being completed in 15 minutes and did not consume more than 250 mL of mobile phase. A gradient elution using various solvent combinations resulted in separation of three phytoconstituents. These were isolated and subsequently characterized by various spectrometry techniques. Circular TLC proved to be a faster and more solvent efficient isolation process. This could be used routinely for separation and isolation of phytoconstituents.

P-7t

Quantitative determination of L-DOPA in seeds of *Mucuna pruriens* germplasm by HPTLC

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Mucuna pruriens Linn. (Fabaceae), commonly known as Kewanch or Kaunch, Atmagupta and Velvet bean, is a climbing annual legume, endemic in India and in other parts of the tropics including Central and South America. About fifteen species of *Mucuna* are found in the forests and plains of India. All the parts of *Mucuna* contain valuable medicinal properties and there is heavy demand of *Mucuna* in Indian drug market. It is an important medicinal plant used for treatment of Parkinson's disease and many others in ancient Indian medical system. *M. pruriens* seed powder contains high amount of L-DOPA (L-3,4 dihydroxy phenylalanine), which is a neurotransmitter precursor and effective remedy for the relief in Parkinson's disease. L-DOPA extracted from seeds of *Mucuna* is a constituent of more than 200 indigenous drug formulations and is more effective as drug than the synthetic counterpart.

A densitometric HPTLC method was developed for quantification of L-DOPA content present in the seeds extract. The method involves separation of L-DOPA on HPTLC plates silica gel 60 GF_{254} using n-butanol – acetic acid - water 4:1:1 (v/v/v) as mobile phase. Quantification was done at 280 nm using absorbance reflectance mode. Linearity was found in the concentration range of 100 to 1000 ng/spot with the correlation coefficient value of 0.9980. The method was validated for accuracy, precision and repeatability. Mean recovery was 100.9 %. The LOD and LOQ for L-DOPA determination were found to be 3.41 ng/spot and 10.35 ng/spot, respectively.

The proposed HPTLC method was found to be precise, specific and accurate for quantitative determination of L-DOPA. It can be used for rapid screening of large germplasm collections of *Mucuna pruriens* for L-DOPA content. The method was used to study variation in fifteen accessions of *Mucuna* germplasm collected from different geographical regions.

P-7u

Chemical fingerprinting of *Poncirus trifoliata* using HPTLC and HPLC

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Poncirus trifoliata is popular herb used in traditional Korean medicine. According to traditional practice, it is used to relieve fever, promote salivation and relieve thirst, facilitate eruption and dispel pathogenic factors from the superficial muscles and to top diarrhea. The aim of this study was to evaluate the quality of *P. trifoliata* using HPTLC and HPLC method that is commercially available as a raw plant material in order to guarantee good quality products.

Samples of root of *P. trifoliata* were extracted with 70 % methanol. Two chromatographic methods were developed to determine the chemical fingerprinting of *P.trifoliata*. The first was based on HPTLC identification followed by densitometric measurement at 365 nm. The second was based on RP-HPLC separation with gradient elution and photodiode array detection at 280 nm.

The simplicity of the sample preparation, and the possibility of analysing several samples of herbal products simultaneously in a short time, make HPTLC the method of choice. The HPTLC method was feasible for the comprehensive quality evaluation of herbal products. From the comparison of their “fingerprint”, it was possible to detect substitution of plants that are different form those declared on the label.

The HPTLC may be used as a rapid method by which to control the quality of raw plant materials and formulations based on the title plant.

P-7v

HPTLC – A simple tool to distinguish adulterants and substituents

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Herbs are major source of Indian system of medicine. As the herbal drug industry faces an increase in demand all over the world, the possibility of using adulterants and substitute are also increasing.

Four plants, viz., Puskkaramulam - *Inula racemosa* Hook f. (root), Atimaturam - *Glycyrrhizha glabra* Linn. (root), Nila-vembu - *Swertia chirayita* (Roxb. ex. Flem) Karsten (whole plant) and Venkunkiliyam - *Shorea robusa* Roxb. ex. Gaertn. f. (resin) were analysed for their authenticity through HPTLC. The evolved HPTLC finger prints and photo documentations at UV 254 nm, UV 366 nm and after derivatization with vanillin-sulphuric acid are useful for their identification and quality control of these drugs.

P-7w

Establishment of a TLC fingerprint within the Apiaceae family

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The TLC fingerprint is commonly used in the control of medicinal plants. The major interest of this method is the comparison of the chemical profile between species of different provenances and the discovery of chemotypes which are non-usable as phytomedicament. This method is also usable to establish intra-family rankings on the basis of common chemical components and to establish many phylogenetic chemical families.

For homogeneous Apiaceae families it is possible to make an under-classification using the presence or absence of compounds or major flavonoids. One of this subfamily is constituted for the following plants Anise, Fennel, Cumin, Coriander, Dill, Caraway and Angelica.

The extract of each plant is prepared by dry powder in a methanolic solution. The samples were separated on HPTLC plates with toluene - ethyl acetate - methanol 6:3:1 (v/v/v). The detection was achieved by spraying with sulphuric acid anisaldehyde reagent.

P-7x

HPTLC: Monitoring artifacts of diosgenin during traditional and modified extraction of *Dioscorea deltoidea* Wall

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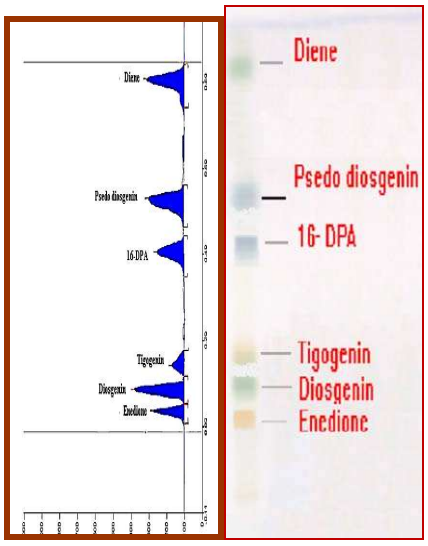
Diosgenin (steroidal saponins) is extensively used as a steroid drug precursor and accounts for about 50 % of the total steroid drugs output in the world. The present study deals with monitoring artifacts of diosgenin during its extraction procedure from *dioscorea deltoidea* tubers and development of validated stability indicating method for simultaneous estimation of artifacts of diosgenin (Enedione, Tigogenin, 16 DPA, Psedodiosgenin, Diene) [1-6] using TLC plates silica gel 60 F_{254} with chloroform - methanol - hexane 30:0.4:20 (v/v/v) as mobile phase. The chromatogram was scanned at 540 nm after derivatisation with anisaldehyde reagent.

Monitoring these artifacts are major task for improve the yields and purity of diosgenin. The developed method is found to be simple, specific, precise and stability indicating. The specificity of the method was confirmed the impurity profile of diosgenin during the extraction process of *dioscorea deltoidea tubers.*

Chromatographic fingerprint and linearity equations of diosgenin artifacts



#	Derivative compounds	R_F	Linear equations
1	**Enedione**	0.12	Y= 1.285+ 1.03x , r = 0.995, Sdv = 2.55, LOD= 60ng , LOQ= 400 ng
2	**Diosgenin**	0.18	Y = 2882.7 + 1.751, r = 0.991, Sdv + 2.73, LOD= 60ng , LOQ= 400 ng
3	**Tigogenin**	0.24	Y= 1371.31 + 1.458x ,r = 0.972,Sdv= 4.23, LOD= 80 ng, LOQ= 500 ng
4	**16 DPA**	0.43	Y= 1830.9 + 3.468 , r = 0.995, Sdv = 4.89 , LOQ= 550ng , LOD= 65ng
5	**Psedodiosgenin**	0.57	Y= 1171.31 + 2.598x ,r = 0.992,Sdv= 4.93, LOD= 40 ng, LOQ= 300 ng
6	**Diene**	0.82	Y= 620.5 + 20.14x , r = 0.998 ,Sdv = 3.49, LOQ= 0.3 ng, LOD= 20 ng

[1] Rahul Kasar, Ph.D thesis, Mumbai University, India, 2007. [2] Denis Drapeau, Yves sauvaire, Harvey W. Blanch, Planta medica, 1986, 474-477. [3] Gulliver Lopez Sanchez, Jorge C. Medina Acevedo, Analyst, 97, 1972, 973-976. [4] Pushpa Khanna, Aditya Kumar Rathore, S.K. Manot, Indian Journal of Pharmaceutical Sciences, May-June 1980, 98-99. [5] Indian Herbal pharmacopoeia, vol-II, A joint publication of RRL and IDMA, 1999, 44. [6] M L Vishin, Current Science,1962, 7, 284-285.

P-7y

Establishment of a TLC fingerprint of different plants from the French territory used as phytomedicament

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The full suspension of fresh plants was obtained through the S.I.P.F. method. This method integrates 3 phrases: cryogenics (cooling in liquid nitrogen at -120 °C), cryo-grinding and ultra molecular pressure. Fresh herbs are then packed in a hydroalcoholic solution (30 % of alcoholic strength) which stops the enzymatic activity of the chosen plant. The active principles cannot therefore undergo modification or degradation.

Echinacea, nettle, burdock, Passion flower, hawthorn, valerian, queen of meadows, dandelion, artichoke, lemon balm, cassis, horsetail, sweet clover… all these plants originate from the French territory and these extracts must meet the standards of Pharmacopoeia. Different TLC fingerprints are established and related to different classes of compounds.

The different chromatographic methods are described for research of chemotypes and for the possibility of a densitometric determination in agreement with the different pharmacopoeias.

P-8a

Development of a rapid analysis method for measurement of carotenoids' content in paprika fruit by HPTLC

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Various analysis methods are used to measure the content of carotenoids in paprika fruit, however, each analysis method requires different conditions such as equipment, cost and time. The most rapidly and conveniently used pigment measurement for breeding new varieties and quality evaluation for goods related to chili and paprika was to measure the ASTA (American Spice Trade Association) value, and this method has aided in identifying the objective level of pigment. However, while the existing ASTA method is a convenient method to present the level of total pigment, it has limitations in rating the individual content of various carotenoids that represent the paprika's color.

To make up for such shortcomings, an HPTLC method was applied to separate the carotenoid components of paprika, and an image analysis method was used to measure the content of red and yellow pigments. As a result of separating the pigments using heptanes and *tert*-butyl methyl ether (1:1) as mobile phase, the typical 5 types of red pigment and 3 types of yellow pigment could be seen. The separated red pigments of paprika were capsanthin, capsanthin 5, 6-epoxide, and capsorubin. The yellow pigments were ß-carotene, ß-cryptoxanthin, cucurbitaxanthin A, zeaxanthin, and violaxanthin. All these showed Rf values of between 0.3 ~ 0.9. The result of carotenoids analysis for paprika germplasm (39 samples) using the developed method was that the ratio of red pigment to yellow pigment (R/Y) ranged from 0.9 ~ 3.1, and the correlation (r) with the existing ASTA method was 0.93.

P-8b

Indirect evaluation of garlic lines' antibacterial activities using HPTLC

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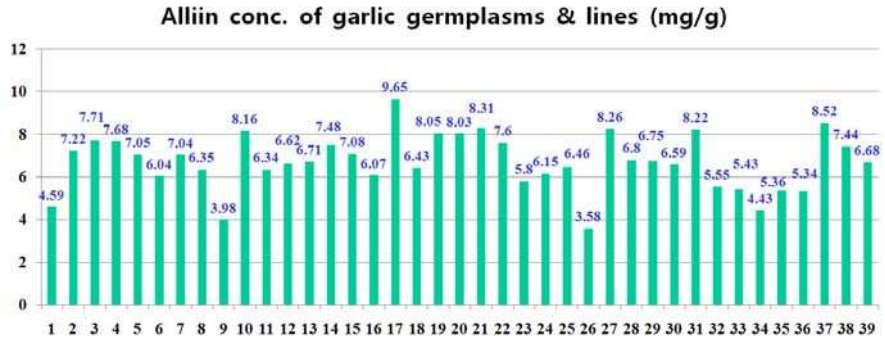
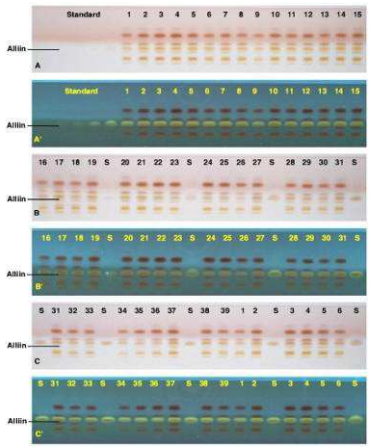
In Korea, garlic is one of the most important seasoning vegetables to make Kimchi. Owed to garlic's functionality in human health, its secondary products, such as fermented garlic and various extracts are explosively increasing in recent days.

Among these functionalities, the most interesting part is antibacterial effect to kill harmful food poisoning bacteria such as *E.coli* and *S. aureus*. The prime goal of this study is to provide basic information to screen the most promising garlic lines for a high concentration of alliin.

By the comparison of 39 garlic lines' alliin concentrations with HPTLC, we found the highest one to be 9.65 mg/g (00-77-2), and the lowest one to be 3.58 mg/g differing almost by a factor of 2.7. To evaluate the actual antibacterial effect of these lines, *E. coli* and *S. aureus* were cultured with garlic extracts. Although the actual antibacterial effects against both food poisoning microorganisms were not identical, the general trends of the effect were similar. The highest antibacterial effect was found in 00-77-2 (9.65 mg/g) followed by 8613 (7.44 mg/g) and 01-25-1 (7.6 mg/g). The garlic line 00-77-2 showed the highest alliin concentration and the other two lines were also categorized as high alliin concentration group.





P-8c

Study on the bioactive content of *Butia capitata* Mart, an edible fruit from Cerrado, Brazil

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Coquinho-azedo is the name of both the edible fruit and the tree of *Butia capitata* (*Palmae*). The use of *Butia capitata* fruit is attested in Cerrado region, Brazil, since 2500 years ago; nowadays natives widely use this fruit for the nice taste and its high nutritional value. Coquinho harvesting has a great social and economic importance to the communities located close to its distribution areas, and its trade could give great enterprise perspectives for the inhabitants [1]. Some previous studies on coquinho fruit [2-5] reported a high content of antioxidants like polyphenols and flavonoids in fresh and frozen pulp. This data support the value of the fruit, commonly used in Brazil as snacks or energy drinks [1].

In this work, developed within the Italian-Brazilian study on Cerrado biodiversity, we present the possibility to obtain a fingerprint of the coquinho pulp, focalized on phenolic and flavonoidic compounds. The use of a HPTLC video-densitometric approach, both on silica and C18 phases, provides a really versatile method to control the content of these bioactive molecules.

Moreover, an evaluation of the typical UV-Vis spectra of the flavonoidic compounds obtained *in situ*, was developed. Mass spectrometry was used to confirm the identity of the prominent components. The HPTLC approach allows the quality control by application of the method as stand-alone testing in remote areas with minimal facilities.

[1] P.A.D. Da Silva, Doctorial Thesis. Biological science Inst - University of Brasilia (2008). [2] A.E. De Souza SchmidtGoncalves, F.M. Lajolo, M.I. Genovese, J. Agric. Food Chem. 58 (2010) 4666. [3] A.C. Jacques, P.B. Pertuzatti, M.T. Barcia, R.C. Zambiazi, Braz. J. Food Technol. 12 (2009) 123. [4] M.I. Genovese, M. Da Silva Pinto, A.E. De Souza Schmidt Goncalves, F.M. Lajolo, Food Sci. Technol. Int. (London, U. K.) 14 (2008) 207. [5] J.P. Faria, F. Almeida, L.C.R. Da Silva, R.F. Vieira, A.-C. T.D., Rev. Bras. Frutic. 30 (2008) 827.

P-8d

Detection and quantitation of vitamin C and preservatives in fruit juices sold in Indian market using HPTLC

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Fruit juices are considered healthy liquid food all over the world. The readymade juices sold in cans as well as tetra packs are very handy and consumed in large qualities in order to seek the health benefits of the fruits. The readymade fruit juices claim to be as similar as the fresh juices, this claim always remains doubtful. A project was undertaken to detect and quantitate the vitamin C content in various fruit juices sold in Indian market, which is the important ingredient in the claims of such juices.

The juices were also analyzed for presence of any preservatives using HPTLC. The technique of HPTLC proved very convenient and easy especially because no sample preparation was needed and non interference of other ingredients from the juices in this technique. The results were significant and at times the claims made by the manufacturers were found to be misleading. The results will be discussed in details in the main paper.

P-8e

Detection and quantitation of caffein in nonalcoholic beverages sold in Indian market using HPTLC

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HPTLC became one of the important tools capable of giving fast results, high resolution and separation of more accurate and precise quantitative results with many advantages over other techniques. TLC is widely used for food analysis and quality assessment throughout the developing world. Applications of HPTLC are wide spread in the field of pharmaceuticals as well as food industry. Most of the non alcoholic beverages do not mention the caffeine content on the label of the bottle.

Our survey of all the non alcoholic drinks available in India also revealed the same fact. From children to adults all consume these beverages very freely and thus get caffeine in the body which may not be always good for all who consume it. A project was undertaken to detect and quantify the amounts of caffeine in various non alcoholic beverages manufactured and sold in India.

P-8f

Identification and quantification of additives in PVC foils by HPTLC

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Plastic foils of polyvinyl chloride (PVC) are often used for wrapping cheese, meat and vegetables. A suitable flexibility is obtained by adding various plasticizers and other additives. Mainly used plasticizers are citrates, adipates and phthalates. Epoxidized soybean oil (ESBO) is also known for plasticizing flexible foils.

In contact with food these compounds tend to migrate because they are not bonded to the polymer. In regulation (EU) 10/2011 in conjunction with regulation (EG) 1935/2004 [1,2] the European legislation defined substances, allowed to be added to plastic materials with food contact. To check the compliant composition, migrating studies must be performed.

Therefore, planar chromatography on silica gel was used to separate additives in ethanol extracts of different PVC foils. The primuline reagent was employed for detecting the various additives. Quantification was performed by TLC Scanner 3 with fluorescence measurement at 366 nm.

Due to this chromatography ESBO delivered numerous zones on the plate. A lipase based treatment and chromatography on modified silica layers enabled quantification of ESBO via characteristic components.

[1] Comission Regulation (EU) No. 10/2011 of January 2011 on plastic materials and articles intended to come in contact with food. [2] Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC.

P-8g

High-throughput determination of teflubenzuron in medicated feed fish by HPTLC

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Sea lice (*Caligus rogercresseyi)* parasites have become in a major concern for Chilean aquaculture due to the important damages produced to farmed salmon. For treating this infestation, antiparasitic agents such as teflubenzuron are frequently used. Since the drug is administered via fish feed, the dose-control is relevant to ensure the appropriate dose to the fish. Thus, the objective of this work was to develop a high-throughput thin-layer chromatographic method for monitoring the teflubenzuron dose in fish feed.

Teflubenzuron was extracted from fish feed using a simple extraction with acetonitrile. Chromatography was performed on HPTLC plates silica gel 60 F_{254} using a mixture of toluene – methanol - acetic acid (9:1:1, v/v/v) as mobile phase. Detection was carried out by UV absorbance at 256 nm. Calibration data in the range from 40 to 200 ng/band fit a linear regression model with a determination coefficient (R^2) of 0.996. Repeatability and intermediate precision showed *%RSDs* of 2.2 % and 2.7 %, respectively.

Recoveries of spiked samples at three levels by triplicate ranged from 101.4 to 104.3 % with intermediate precisions from 2.7 to 7.2 %. Because of its high throughput capacity, fast sample preparation and good validation results, this method was already implemented in one of the major Chilean industry of salmon feed industry. Consequently, it is possible to indicate that this simple and reliable thin-layer chromatographic method is a good alternative for teflubenzuron dose control in fish feed.

P-8h

Determination of biogenic amines in Chilean wines by HPTLC

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Biogenic amines are well known organic nitrogenous compounds generally formed by bacterial decarboxylation of free amino acids. The presence of biogenic amines in foods and feeds, especially histamine, is a typical indicator of spoilage. In the case of wines this kind of amines are mainly formed along the vinification stages, either during the alcoholic and/or during the malolactic fermentations, however, the later is the most important stage regarding the amount produced. Biogenic amines are a health risk for sensitive individuals, symptoms include: nausea, hot flushes and headaches.

Chilean wine quality is well recognized in the world, which makes imperative the establishment of analytical methods to evaluate the presence of biogenic amines in wines. The objective of this work was to establish and validate an analytical methodology to determine the principal biogenic amines in Chilean wines by thin-layer chromatography.

Wine samples were first treated with polyvinylpolypyrrolidone and then derivatized with dansyl chloride using an experimentally optimized procedure. Chromatography was performed on HPTLC plates silica gel 60 extra-thin (100 µm) using an automatic developing chamber (ADC2) to carry out three successive developments with the following mobile phases: benzene – chloroform - triethylamine (10:6:2, v/v/v), benzene – chloroform - triethylamine (10:6:7, v/v/v) and chloroform - diethyl ether - triethylamine (6:4:1, v/v/v). Detection was done in fluorescence mode at 366/>400 nm.

The method proposed was capable to achieve a reliable quantification of histamine, tyramine, putrescine, cadaverine, spermidine, spermine and phenylethylamine in Chilean wines. After analytical validation, 20 wines samples were evaluated showing biogenic amines levels from 4.2 to 65.1 mg/L.

P-8i

HPTLC analysis of anthocyanins in *Lonicera caerulea* L. fruits

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Lonicera caerulea L. (blue-berried honeysuckle or sweetberry honeysuckle) fruits are widely used in Russia, China and Japan as nutritive food. Moreover, blue-berried honeysuckle has been reported to be one of the richest sources of antioxidants [1]. *L. caerulea* fruits and their polyphenolic fraction are responsible for superoxide scavenging activity, inhibit microsome peroxidation in rat livers, reduce the biofilm formation and adhesion to the artificial surface of *Staphylococcus epidermaidis, Escherichia coli, Enterococcus faecalis and Streptococcus mutans* [2]. Additionally, the phenolic fraction of the above plant suppress UVB-caused injury of keratinocytes [1]. Cyanidin glycosides, including cyanidin 3-glucoside, are the main constituents of polyphenolic fraction. The content of other anthocyanins is significantly lower [1, 2].

HPLC is the most widely used method of qualitative and quantitative analysis of anthocyanins in plant material. However, the progress in the visualization techniques makes TLC as a method of choice in the analysis of the above group of secondary metabolites. The aim of the work was to optimize TLC conditions for qualitative and quantitative analysis of anthocyanins in *L. caerulea* fruits. The best separation was achieved on HPTLC plates RP 18 W using water - acetic acid - hydrochloric acid (76:20:4, v/v/v) up to a migration distance of 5 cm. The humidity was 75 %. The elaborated method was validated in accordance with standard procedure.

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P-8j

New HPTLC method for analysis of flavonoids and phenolic compounds in propolis

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A normal phase HPTLC method for analysis of propolis was newly developed. The intention was to establish a new method suited for screening of the still unknown chemical profile of German propolis sorts, for differentiation between different types of propolis and for assignment of the origin of the propolis samples. As flavonoids are of interest in medicinal and cosmetic preparations, the flavonoid pattern was focused in the propolis samples.

The separation was performed on HPTLC plates silica gel 60 with a mixture of n-hexane – ethyl acetate – acetic acid and an acidic conditioning of the plate. The separation of up to 20 samples lasts less than 30 min. Detection was performed in the multi-wavelength scan before and after derivatization with Neu`s reagent and enhancement of various zones by polyethylene glycol. Advantages of the multi-detection in HPTLC are discussed.

Propolis extracts were compared with reference substances and, after recording and comparison of mass spectra, marker compounds were assigned. The mass spectra obtained by DART-MS and ESI-MS were compared and discussed. Finally, quantitation of marker compounds of each type of propolis is demonstrated.

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P-8k

Ultrafast quantitation of 5-hydroxymethylfurfural in honey using HPTLC

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The 5-hydroxymethylfurfural (HMF) concentration is a very important factor reflecting the quality of honey. Nowadays quantitation of HMF in honey is traditionally performed using spectrophotometric determination after White or after Winkler and using HPLC methods [1]. To our opinion, HPTLC is suited for prompt and cost-effective analysis of honey. However, the number of publications in this field is still very limited. For example, no publications on quantitation of 5-hydroxymethylfurfural (HMF) in honey using HPTLC were found.

We propose the ultrafast determination of HMF in honey using HPTLC instead of the traditional methods. The HPTLC separation lasts only 5 minutes, and up to 23 honey samples can be analyzed simultaneously on the same plate, providing the increase of the analysis throughput in more than 20 times as compared to HPLC. Using the simplest sample preparation (just dissolving 1 g honey in 10 mL water), performing a 5-minute separation and scanning at 288 nm, it was possible to quantify HMF in honey at the level of 8 mg/kg or even lower.

Thus, this method was suited to quantify concentrations of 5-hydroxymethylfurfural in honey according to different regulations with limit values in the range between 15 and 80 mg/kg. When necessary, coupling HPTLC with ESI-MS using the TLC-MS Interface provides further confirmation on the identity of the analyte.

This work was financially supported within a joint DAAD-Rosobrazovanie program "Mikhail Lomonosov" - "The development of a scientific potential of a higher school (2009-2010)", project 01201058913.

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P-8l

Application of planar chromatography on determination of aflatoxin B_1 in silage

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Aflatoxin B_1 is a naturally occurring mycotoxin that is produced by many species of Aspergillus, a fungus, most notably *Aspergillus flavus* and *Aspergillus parasiticus*, bifurano-coumarin derivative. Aflatoxins B_1 is toxic and is one of the most potent hepato-carcinogens mycotoxins known. It appears to occur much frequently in silage.

This presentation deals with the planar chromatography method for determination of aflatoxin B_1 in silage. Dried and ground silage (20 g) was mixed with 10 mL and 100 mL chloroform and shaken for 10 min, decanted through filter paper into 50 mL glass-stopper and ca. 10 g anhydrous Na_2SO_4 were added to 50 mL filtrate. A florisil column was prepared for cleanup (glass wool in bottom of chromatographic column, 5 g anhydrous granulated Na_2SO_4, 0.7 g florisil, and 0.5 g anhydrous granulated Na_2SO_4), washed with 30 mL chloroform - hexane (1:1) and 20 mL chloroform - methanol (9:1) and eluted with aflatoxin B_1 with 30 mL acetone - water (99:1).

Chromatography was performed on HPTLC plates silica gel 60, 20 x 10 cm. Calibration standard of aflatoxin B_1 and sample extracts were applied with Linomat 5. Anhydrous diethyl ether was performed to remove contaminants in the reverse development. The plate was cut 1 cm below the point of application. The plate was developed with chloroform – acetone - water (88:11.9:0.1) in the AMD 2 system. Aflatoxin B_1 was measured by fluorescence densitometry by means of a TLC Scanner 3 with a mercury lamp and K400 secondary filter. The excitation wavelength was 366 nm. The R_F value of aflatoxin B_1 under these conditions was 0.61.

The study was performed to produce a selective, sensitive and accurate HPTLC method for determination of aflatoxin B_1 in silage. Linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and repeatability were tested. The LOD of aflatoxin B_1 was found 2 µg/kg and the LOQ was found 8 µg/kg in silage. The mean recovery was 74 % in the range 10 - 30 µg/kg of silage. The repeatability was tested according to the Eurachem Guide.

In summary, an HPTLC method for quantification of aflatoxin B_1 in silage was developed. The method is useful for inspection of silage, and it is able to use its modification for determination of aflatoxin B_1 in other matrices.

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P-8m
HPTLC method for quantification of crocetin in saffron

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A simple, selective, precise, accurate and cost effective HPTLC method for the analysis of crocetin (Fig. 1) in crude drug has been developed and validated. Aluminium TLC plate precoated with silica gel 60 F_{254} was used as the stationary phase whereas hexane - ethyl acetate - formic acid (5:4:1, v/v/v) was used as the mobile phase. A compact and well resolved peak of crocetin was observed by densitometric analysis in the absorbance mode at 433 nm (Fig. 2). Calibration curve revealed a good linear relationship between the peak area and concentration.

Validation of the developed method also carried out and found to be accurate, precise, specific and reproducible. The proposed method provides a faster and cost effective quality control tool for routine analysis of crocetin in crude as well as traditional herbal formulations which contains saffron as one of the ingredient.

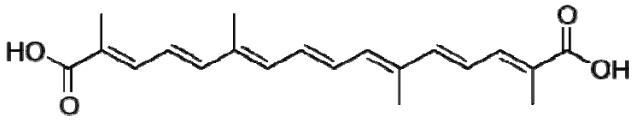

Fig. 1 Structure of crocetin

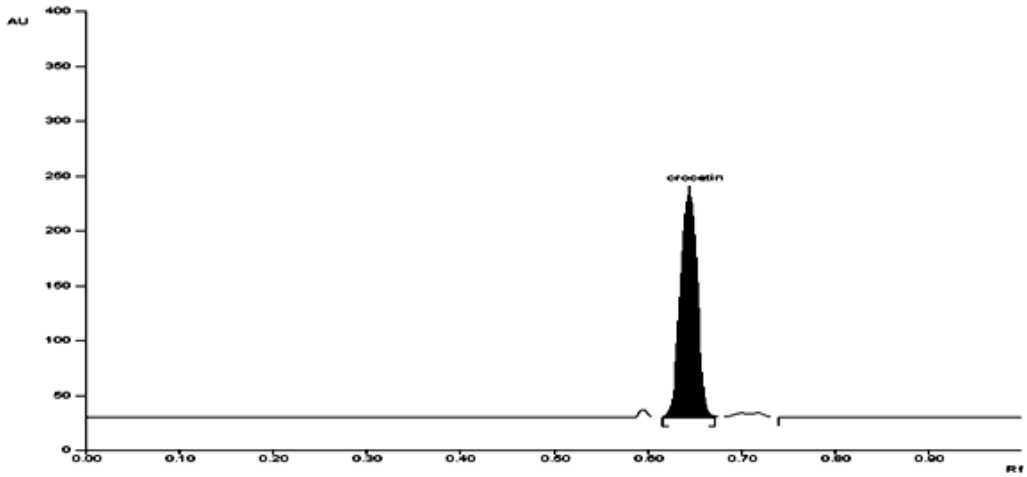

Fig. 2 Densitogram of crocetin standard at 433 nm

P-8n

HPTLC method for the analysis of oleanolic acid in *Luffa cylindrica* L. seeds

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Luffa cylindrica (LC) of the family Cucurbitaceae is also known as Sponge Gourd. It is found throughout India, in wastelands and is also cultivated. It is used as a vegitable after cooking, throughout the country. Different parts of the plant including the seeds are reported to have different medicinal uses [1]. Literature reports the presence of aglycone [2], oleanolic acid [3].

Oleanolic acid, 3 ß-hydroxyolean-12-en-28- oic acid, is a naturally occuring triterpenic acid present in various plants. Pharmacological activities include hepatoprotective action, anti-ulcer activity, anti-hyperlipidemic, anti-inflammatory activity, anti-fertility activity etc.

This paper describes the development of an analytical method for the estimation of oleanolic acid from seeds of LC. The n-hexane extract of dried seeds of LC was processed and HPTLC method developed to estimate the oleanolic acid present. There is no reported method for the estimation of oleanolic acid from LC chromatographically.

The method developed was validated for its linear range, LOD, LOQ, accuracy, precision, repeatablity, largely following the ICH guidelines. The recovery of the method was found to be between 98.7 ± 0.76 to 100.8 ± 0.88 %. The intra-day precision was found to be 0.85 - 1.01 % and the inter-day precision was 1.04 - 1.19%. The developed method was found to be simple, accurate and precise.

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P-8o

Quantification of glucoraphane in florets of broccoli

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Broccoli is one of the most widely consumed cruciferous vegetables worldwide. This plant contains high levels of glucosinolates, a group of secondary metabolites involved in cancer prevention. In their analysis HPLC has been used in many studies due to its repeatability, accuracy and reliability. Nevertheless, the limiting factor of this method is the high cost solvents and accessories involved in the technique. As an alternative, it is possible to use TLC coupled with densitometry; this technique enables quantitative and qualitative analysis of different compounds with a precision similar to that obtained with HPLC. In addition to the low cost of inputs, short analysis times and small solvent volumes, make this a highly competitive technique.

In this study a quantification of glucoraphane in broccoli florets extracts was approached using TLC coupled to densitometry as an alternate method to HPLC. The retention coefficient of commercial standards had a value of 0.60. Densitometric analysis of the TLC plates of the glucoraphane standard showed that the highest of absorption intensity was found between 226 and 230 nm. Its R_f value was the same as the value shown by a fraction of crude broccoli extract, making possible to detect the presence of such compound. Because the system had adequate resolution and was able to determine the zone of maximum UV light absorption, it was possible to use these data to construct models that allowed the reliable prediction of glucoraphane concentration of broccoli crude extracts.

P-8p

TLC for screening of residues of fluazuron in food of animal origin

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Due to the increasing resistance of ticks to the pesticides in use in New Calédonia sanitary authorities planed the generalization of the use of fluazuron against bovine tick *Boophilus microplus*.

To ensure the protection of consumers maximum residues levels have been implemented for fluazuron in bovines edible products. Therefore for the safety of exported bovine production a residues monitoring method is necessary.

Due to the lack of published methods for the analysis of fluazuron residues and the very few laboratories performing this type of analysis, New Caledonia direction for veterinary, alimentary and rural affairs decide to develop and validate a first step qualitative screening method using planar chromatography.

The interest of planar chromatography as a modern analytical method in the field of drug residue monitoring and the obtained results will be presented.

P-8q

Optimized separation of bioactive phenolic compounds in various salad species, using cellulose HPTLC plates and CAMAG instruments

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The specific content in phenolic compounds [1, 4] of various salads is shown regarding their medicinal properties. We have recently optimized the parameters with the use of HPTLC plates (Merck, # 16092) and CAMAG instruments [2, 3]. The use of high performance plates and of spray-application obtained with an automated sample applicator ATS3 gave use sharper bands and a higher sensitivity. This method was very easy to setup and the results very comprehensive. We might now use antioxidative tests directly on the plate to confirm and ensure the real activity and compare its level in our samples.

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P-8r

TLC method for determination of phytosterols in chocolate

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Phytosterols are steroidal alcohols with chemical structure similar to cholesterol. Many epidemiological studies show that phytosterols have potential protective role against cardiovascular diseases in a way that they bind on substrates for cholesterol in a human body [1]. Chocolate is a good source of phytosterols [2].

Phytosterols are usually quantitated by gas chromatography, but TLC is also appropriate technique for screening as well as densitometric quantification of the phytosterols. TLC plates silica gel 60, *n*-hexane - ethyl acetate 5:1 (v/v) as developing solvent and molydbophosphate acid as detection reagent were commonly used in TLC methods for separation of different lipid classes. However, in such chromatographic conditions the obtained resolution between the phytosterol fraction (ß-sitosterol, stigmasterol, campesterol as the major representatives) and other compounds present in the chocolate test solution is not good enough for densitometric measurements. Therefore, the proportion of ethyl acetate was increased. The best separation of sterol fraction was achieved by using *n*-hexane - ethyl acetate 5:3 (v/v). In addition the time and the temperature of the derivatisation were also optimised. The content of free phytosterols in chocolate was estimated by using ß-sitosterol as standard. The analysed chocolates contained from 44.7 to 107.7 mg/100 g of free phytosterols.

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P-8s

Determination of polycyclic aromatic hydrocarbons in toys by HPTLC

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The presence of polycyclic aromatic hydrocarbons (PAH) in toys represents a great problem due to their strong carcinogenic properties combined with easy dermal penetration. As a result of the recently developed EU toy directive including new maximum levels for all CMR compounds, the determination of PAH is a highly topical subject. For the German certification mark, each of the 16 EPA-PAH in toys has to be under the limit of determination of 0.2 mg/kg.

PAH in toys are generally quantified by time consuming GC-MS and HPLC-FLD methods. The aim of this study was to develop a fast and economic HPTLC method.

A German norm (DIN 38407-7) already describes a procedure to determine 6 PAH in drinking water by HPTLC on caffeine-impregnated silica plates [1]. However, only these 6 PAH are detectable, while all others do not give a fluorescent signal on caffeine plates.

Therefore, RP18 HPTLC plates were used, when best separation was obtained by a three-fold development wih acetonitrile - water 9:1 (v/v), using the AMD2 system to prevent the analytes from light and oxidation. Still co-eluting pairs of PAH (benzo[a]-pyrene/dibenzo[a,h]anthracene, benzo[a]anthracene/chrysene, phenanthrene/anthracene, fluorene/acenaphthene) were determined by selective excitations/by-pass filters and by fluorescence quenching of alternant PAH with nitromethane.

The extraction of toy samples was performed by carbon tetrachloride. An SPE clean-up on SiO_2 catridges was essential to separate polar fluorescent co-extractives and polymers. Limits of determination limits were between 0.25 and 1 ng/zone.

The determination of recoveries and the application to different toys is currently under study.

[1] G. Morlock, S. Kopacz, *J. Liq. Chromatogr. Rel. Technol.*, 2008, 1925-1942

P-8t

Occurrence of some mycotoxins in wheat seeds from northwest Romania using HPTLC

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The contamination level of Aflatoxins (B1, B2, G1, G2) and Ochratoxin A in wheat seeds produced in northwest Romania during the last two years (2009 and 2010) was investigated using different HPTLC revealing techniques. The first step in mycotoxins isolation and identification was to establish the most efficient method. In this purpose we have chosen the protocol using the chloroform extraction for the simultaneous detection of aflatoxines B1, B2, G1, G2 and Ochratoxin A, described by Braicu *et al.* [1] and Morar *et al.* [2] and modified by our research group. We compared the obtained results with those obtained using the imunoaffinity column purification.

120 samples were investigated and 27 % were positive. For analytical reasons, the first step was to establish the presence of mycotoxins by confirmatory screening and then densitometry was used for quantification.

This study has been financed by Romanian Ministry of Education and Research, PN II Project 52132/2008.

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P-8u

High-throughput planar solid phase extraction – application to pesticide residue analysis in tea samples

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Residue analysis of pesticides in plant samples requires efficient clean-up of extracts to prevent matrix effects during liquid chromatography or gas chromatography coupled to mass spectrometry (LC/MS or GC/MS). With selected pesticides spiked to different fruit and vegetable matrices, a new concept of planar solid phase extraction (pSPE) was proven to be very successful (see O-3d). As compared to dispersive SPE procedures, pSPE resulted in LC/MS chromatograms nearly free of matrix compounds, thus free of interferences.

As rather challenging matrices, QuEChERS extracts of tea samples were next chosen and applied to HTpSPE. Besides chlorophylls and polyphenols, high amount of caffeine is co-extracted resulting in strong matrix effects both in LC/MS and GC/MS. However, caffeine could not be separated from pesticides on amino modified TLC layers, formerly used for extracts of fruits and vegetables. Therefore, the clean-up strategy had to be changed:

1. A pre-clean up by dSPE (PSA + C18 silica) was essential to generally reduce the huge amount of co-extracted matrix compounds.
2. Normal phase silica TLC plates were used for pSPE, first developed with acetonitrile, then in back direction with acetone - water (87.5:12.5).

These modifications led to absolutely colorless extracts free of caffeine. As compared to only dSPE clean-up (PSA + C18), the additional pSPE resulted in a background signal, almost free of interferences. After HTpSPE, spiked tea extracts showed nearly the same profile as a pesticide solvent standard (Fig. 1). Identical results were obtained for both black and green tea samples.

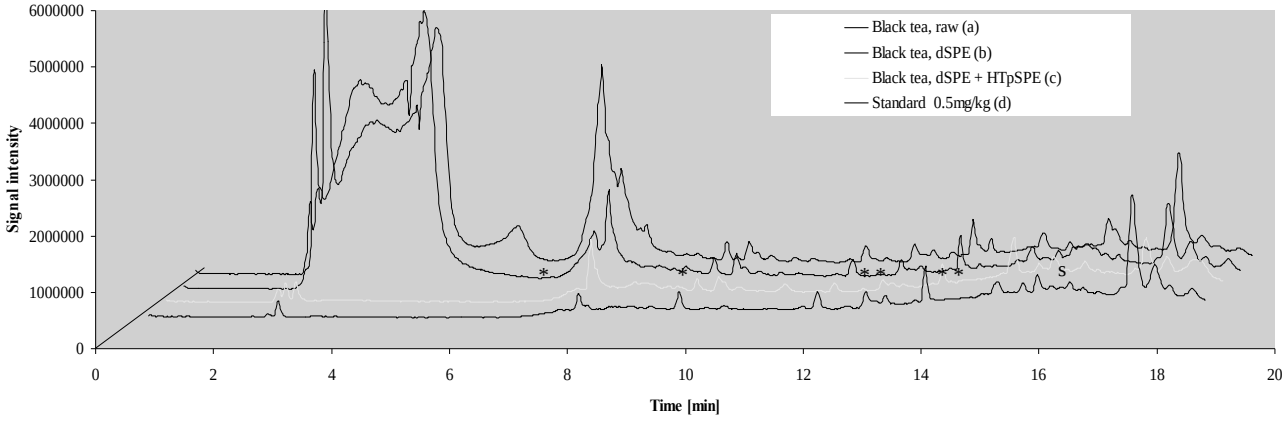

Fig. 1 LC/MS total ion chromatograms of raw tea extracts, extracts after dSPE, and extracts after dSPE + HTpSPE.

P-8v

Validated HPTLC methods for the determination of flavour compounds in plant extracts

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Flavour compounds are of major importance in flavour and fragrance industries. In some cases target compounds quantification is necessary to control raw materials quality and/or authenticity. The aim of this poster is to show how HPTLC can be used to authenticate vanilla products (extracts and beans) by phenolic compounds ratio determination [1] and how it can be used to control vetiver essential oil quality and its acetylated form through the quantification of vetiver alcohols and acetates.

TLC was initially used by chemists to follow reaction processes or to screen plant extracts. Very useful when analytes do not respond in UV (no chromophore), this technique is convenient for high sample throughput. HPTLC plates and scanner densitometer development with TLC steps automation make it a quantitative tool faster than the other techniques. Usually vetiver alcohols and acetates contents are determined using GC/FID and GC-MS; however, these techniques give only relative percentage of each compound in the oil and due to vetiver matrices complexity (more than 300 sesquiterpenoids [2]), it is sometimes difficult to assign and integrate all alcohols or acetates signal. The developed HPTLC method allows vetiver compounds separation based on their functionality (all alcohols are under two spots and all acetates migrates in a single spot); with appropriate standards (purified from essential oil), alcohols and acetates were quantified in different samples after derivatization. The second method was developed for simultaneous quantification of vanilla's phenolic compounds and the vanillin precursor, vanillin β-D-glucoside in vanilla fruits, beans and extracts. Major steps of both methods are automated (sample application, development, detection) or fully controlled (derivatization of the plate, plate drying).

The present poster reports validation data for both methods. The first method can be used as quality control method with quantification of alcohols in vetiver essential oil and acetates in acetylated oil. It was also used to study the impact of roots drying step on alcohols content and to determine the kinetic of acetylation reaction. The second method was applied to authenticate vanilla beans and extracts and to control their quality through the determination of vanillin content.

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P-8w

Determination of biogenic amines in wine by TLC densitometry

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Biogenic amines are small-molecular-weight organic bases that can be encountered in a number of different metabolic processes. In wine, biogenic amines result from the decarboxylation of amino acids, operated by lactic acid bacteria [1]. Ingestion of food containing biogenic amines (and especially histamine) can result in health nuisances, such as nausea, vomit, blushing and palpitations [2]. Histamine levels in wine are relatively low, especially in comparison to other fermented foods (e.g. cheese), nevertheless the toxic effects of this molecule are augmented by ethanol. Researchers and professionals normally rely on instrumental techniques (*i.e.* HPLC, UPLC) for biogenic amine analysis. Due to the high cost and low-throughput of the current analytical methods monitoring of biogenic amine levels of wines is not performed on a regular basis.

In the present work a new method, based upon TLC/densitometry, was developed and validated. The technique allowed determining the four most abundant biogenic amines in wine (*i.e.* putrescine, histamine, tyramine and cadaverine) at concentrations as low as 1 mg/L. To our knowledge, this is the first time quantitative TLC is successfully applied to wine analysis. The method is simple and inexpensive and offers performance standards comparable to those of HPLC. Other than densitometric analysis TLC plates were submitted to visual evaluation. The latter approach permitted to assign a score for each amine based upon comparison with reference samples. A very good correlation (R^2=0.966) was observed between histamine concentrations measured by densitometric analysis and scores issued by visual evaluation. This indicates that the method could be employed for a reliable estimation of the histamine content of wine in all facilities that are not equipped for instrumental analysis, as is often the case in wineries and oenological centers.

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P-8x

A new method for quality control of soumbala from *Parkia biglobosa,* West Africa using a group of biomarkers

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In West Africa, soumbala is made from fermented seeds of *Parkia biglobosa* (Jacq.) Benth. (Fabaceae). It is a common flavouring additive in food production and high quality soumbala is important for marketing and subsequent sale. Today no method for evaluation of quality of soumbala is available.

Quality was determined by local women producing soumbala in rural Burkina Faso. High and low quality of soumbala were extracted with different solvents and screened for different groups of biomarkers.

Color and color intensity of filter-sticks dipped in a mixture of extracts and reagent were compared to a PANTONE® formula guide uncoated scale. In addition analyses using advanced image evaluation equipment were performed. Here the color intensity of each stick was measured in a 5 mm (diameter) cardboard shape in a cabinet with white light. The intensity (height) correlates with concentration of group of biomarkers in the extract [1]. The results showed that one reagent was found to detect the group of biomarkers in the extracts of high and low quality of soumbala. This was found to correlate with the quality assessment made by the local women on high or low quality.

This method has a potential to be used as a felt-test kit for quality control of soumbala in Africa.

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P-9a

Quantification of isoflavones in soy-based nutritional supplements by HPTLC analysis

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Preparations of soy (*Glycine max* L.) are used in western countries as an alternative to hormonal therapy in the treatment of menopausal problems such as hot flushes. This can be attributed to the present isoflavones, mainly genistin, daidzin and glycitin which show a weak estrogenic activity and are therefore also called phytoestrogens.

An HPTLC method for the identification and quantification of these three main isoflavones in soy containing nutritional supplements was developed. Validation was performed according to the ICH guidelines. For the quantitative determination of the isoflavones, a mobile phase of acetic acid - ethyl acetate (2:3, v/v) was used which afforded sufficient separation on HPTLC plates silica gel 60. HPTLC allowed simultaneous and quick quantification of the isoflavones and efficient distinction between different botanical supplements. Therefore, this inexpensive method could be used in the quality control of nutritional supplements of soy.

P-9b

HPTLC of saponins from the seeds of *Trigonella*

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Fenugreek seed is a source of phytoestrogens, including diosgenin derivatives [1] and isoflavones [2]. Presence of diosgenin derivatives is variable and depends on the origin of plant material [3]. Using HPTLC method, the conditions for analysis of steroidal saponins in plant material cultivated in Poland have been established. TLC analyses were performed in the ACD 2 chamber at 33 % humidity. During the process of optimization, binary and tertiary mixtures of solvents were used, namely: *n*-hexane/acetone, n-hexane/ethyl acetate, cyclohexane/acetone, chloroform/acetone, chloroform/methanol, chloroform/methanol/formic acid, cyclohexane/ethyl acetate/water, cyclohexane/acetone/water, chloroform/acetone/formic acid.

Separation was performed on TLC plates silica gel 60 F_{254} and HPTLC plates silica gel 60 F_{254}. The best separation of steroidal saponins was obtained on HPTLC plates silica gel 60 F_{254} (preconditioning time 5 min) using chloroform – methanol - formic acid (30:20:2, *v/v/v*) as mobile phase in a saturated chamber (saturation time 10 min). In the analyzed extract, eight bands, identified as saponins after derivatization with Ehrlich reagent were observed. In the optimized conditions, the complex of saponins present in seeds of *Trigonella coerulea* was compared with the one present in *T. foenum-graecum* seeds.

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P-9c

HPTLC illustration of the USP Dietary Supplements Compendium

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The United States Pharmacopoeia (USP) Dietary Supplements Compendium (DSC) is the new comprehensive reference for dietary supplements. The compendium features full-color illustrations of typical TLC and HPTLC chromatograms – for the first time ever in the history of official compendia monographs.

CAMAG laboratory has been cooperating with the USP DSC for three years. In the beginning our work focused on the development of a standardized HPTLC methodology as well as a suitable standard format for the HPTLC chromatograms to be printed in the compendium. As a basis for future monograph revisions new definitions for TLC and HPTLC have been proposed. While continuing with the illustration of existing methods on TLC plates we also contributed HPTLC methods as supplementary information. This permits the user to choose between the “old” official TLC method and a new, equally suited HPTLC method, while still complying with the cGMP rules for dietary supplements. Based on our research these alternative methods can be rated as “scientifically valid” as required by the cGMP rules.

For the 2011 revision of the DSC the monographs for Ashwagandha, Red Clover, Pygeum, Centella, Bacopa and Senna are being evaluated. Using Ashwagandha as example this poster illustrates the process of evaluation and optimization of existing official methods and transferring them into standardized HPTLC methods. We simplified the tedious sample preparation procedure, and adjusted the sample application parameters for use on HPTLC layers. Optimum chromatogram development was achieved in the Automatic Development Chamber under controlled relative humidity. The detection step was optimized by changing the derivatization reagent to sulfuric acid reagent and by immersing the plate instead of spraying.

P-9d

HPTLC and the determination of quality of food supplements

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What actually are the main targets of HPTLC? Looking to the published papers, about 1/3 are dedicated to standardization and identification of pharmaceutical products and drugs, but more than 2/3 are focused on studies of medicinal plants and natural products mainly in the pure phytochemical tradition. This is confirmed by the papers distribution among the different classes of natural substances: terpenes are predominant (33 %), followed by phenols (26 %) and alkaloids (15 %), whereas primary metabolites account only for 10 %.

However, it is possible that this situation will change rapidly. As already for TLC, the main importance of HPTLC could be not inside the research, but in the applications, according to the requirements of the moment. Therefore, we present a series of applications of HPTLC in the food supplements sector performed in our Centre for Control of Quality of the University "Sapienza" of Rome.

We focused on determination of quality of market food supplements, in order to compare variations in composition, derived from harvesting and post harvesting treatments. HPTLC resulted an excellent approach, very useful to get visualized results and answers to the commercial needs. We report analyses of different extracts of medicinal plants (eg. *Cassia senna*, *Cynara scolymus*, *Arpagophytum procumbens*, *Echinacea* ssp.), obtained by different techniques, and also of neem (*Azadirachta indica*) oil and exhausted materials obtained from different origins.

However, in some cases HPTLC had to be supported by other techniques in order to complete or check the obtained results, as observed in the analysis of *Arctostaphylos* products.

P-9e

Screening and determination of sibutramine in adulterated herbal slimming supplements by HPTLC

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Botanicals and botanical preparations intended for human consumption as food supplements and related products are widely marketed with various healthclaims. Such phytopreparations are easily available to consumers through several distribution channels: OTC in pharmacies, in supermarkets, herbalist's shops or via the Internet. There are some general concerns with respect to botanicals and botanical preparations mainly relating to quality and safety issues.

In this context, the problem of undeclared drugs in plant medicines is of growing importance. The case of sibutramine is timely and of particular interest. This molecule was an approved drug used as an appetite suppressant. However in 2010, this drug was withdrawn from European [1] and US [2] markets because of its unacceptable risk over benefit ratio.

In this study, an HPTLC method was developed for the screening and determination of sibutramine in herbal dietary supplements. Capsules were extracted in methanol by sonication. Crude extracts were directly applied (automatic TLC sampler ATS4) on HPTLC silica gel plates and developed with a toluene-methanol mixture (automatic developing chamber ADC 2). The quantity of sibutramine was measured at λ_{max} 223 nm (TLC scanner 3 and winCATS software), over the range 300 to 3000 ng.
With this method, amongst the 39 weight-loss supplements screened, 17 were positive for sibutramine with 3 up to 24 mg of adulterant per capsule. These results were compared with conventional HPLC.

[1] European Medicines Agency, *press release*, 21 January 2010, EMA/39408/2010.
[2] Food and Drug Administration, *FDA Drug Safety Communication*,8 October 2010

P-9f

Comparative determination of sibutramine in commercially available natural slimming products by HPTLC and HPLC

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Obesity, a worldwide health problem, may have negative impacts on life quality and on life span. In addition to dietary precautions and physical exercise, drug therapy is implemented for weight loss. The consumption of natural slimming products has been preferred due to the misbelieve that they are safe. While the efficiency of these products is rather weak compared with synthetic drugs, they are frequently adulterated with chemical slimming agents such as sibutramine to increase their potency.

New validated methods for the identification and quantitation of the sibutramine were developed by HPTLC-densitometry (225 nm) and HPLC equipped with a fluorescence detector (225 nm and 316 nm). These methods were applied to the analysis of three natural slimming products. Separations were performed on HPTLC plates silica gel 60 F_{254} using a mobile phase of *n*-hexane – acetone - ammonia (10:1:0.1). For HPLC analysis, a phenyl column (5.0 µm, 150 x 4.6 mm) and an isocratic mobile phase of acetonitrile - water (45:55, pH 3.0) was used. The developed methods were validated for accuracy, precision, linearity, selectivity and recovery. As a conclusion, both methods were found to be useful for the routine analysis of illegally added sibutramine in these products.

P-10a

Estimation of piperine in *Piper longum by* HPTLC and investigation of its anticholinesterase potential

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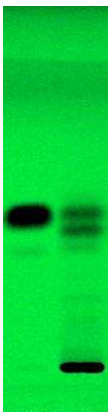
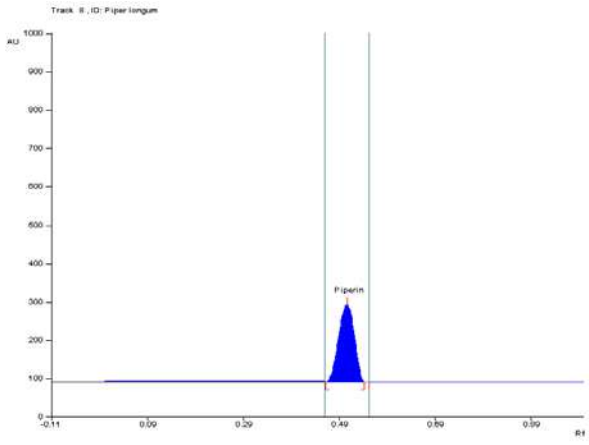
The long pepper, *Piper longum* (Piperaceae) popularly known as long paper is a well known spice used in Indian subcontinent. Beside its edible uses, it is an integral part of the Indian system of medicine, for central nervous system disorders such as Alzheimer's disease. Piperene the major phytoconstituent in it has been proved to contribute for its biological activities. The present study was an effort to estimate the content of piperine in *P. longum* through HPTLC and its acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activity by in vitro bioautography and 96-well microtiterplate method. Estimation of piperine was done by HPTLC method using Linomat 5. Toluene - methanol in the ratio of 9:1 (v/v) was used as mobile phase. Densitometric scanning was then done at 343 nm with the TLC Scanner 3 equipped with winCATS software.

The HPTLC method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision according to ICH guidelines. Linear relationship was found to be y = 5.225 + 0.264x, with correlation co-efficient (r) = 0.99585. The piperine content (R*f* value of 0.50) in *P. longum* extract was found to be 7.5 mg/g. LOD and LOQ was found to be 1.9 ng and 5.7 ng, respectively. The %RSD was found to be <2 %. *P. longum* and piperene, both showed anticholinesterase activity (IC50 values of piperine were; AChE: 43.47 ± 2.12 µg/mL and BChE: 73.08 ± 1.49 µg/mL respectively). The present methods were reproducible and simple for standardization of *P. longum* with potential anticholinesterase activity.



A B

HPTLC chromatogram of piperine (A) and *P. longum* extract (B)



HPTLC densitogram of the standard piperine

P-10b

Determination of mahanimbine in *Murraya koenigii* by HPTLC

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This paper describes a rapid, accurate, specific, repeatable HPTLC method for the determination of the carbazole alkaloid mahanimbine from *Murraya koenigii*. The mobile phase consisting of hexane and chloroform (6.5:3.5, v/v) was used and the chromatogram was scanned at 254 nm. The linear regression analysis data for the calibration plots showed good linear relationship with a correlation coefficient of 0.9953 and this method was validated for precision, repeatability, and accuracy. The proposed HPTLC method was found to be precise, specific and accurate and may be used for identification and quantification of mahanimbine in the herbal extract and its formulations.

P-10c

HPTLC determination of coumarin in aqueous and alcoholic extract and oil of *Ganoderma Lucidum*

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The present study has been carried out to determine coumarin by HPTLC in alcoholic (70 % ethanol), aqueous extracts and oil of *Ganoderma Lucidum* of Indian Himalayan Region (IHR). *Ganoderma Lucidum* known as the *king of herbs* has been reported to have a number of novel biological activities and it contains several bioactive components such as adesnosine, alkaloids, coumarin, ergosterols, ganoderic acids, lactones, mannitol, nucleotides, organic germanium, polysaccharides, protein, triterpenoids, unsaturated fatty acids, vitamins, minerals *etc.* which are well known for their neutraceutical properties. Coumarins and their derivatives are a group of important natural compounds, which are known to have multi-biological activities such as anti-HIV, anti-tumor, anti-hypertension, anti-arrhythmia, anti-osteoporosis, pain relief, preventing asthma, antisepsis, anti-inflammatory as well as antioxidant activities.

The aqueous and alcoholic extracts of *Ganoderma Lucidum* have been prepared using conventional maceration (M) and modern Accelerated Solvent Extraction (ASE) techniques and oil was extracted using cold extraction method. A comparative study has been carried out in terms of coumarin content using HPTLC as well as phytochemical analysis in terms of total phenolics, total flavonoids, total reducing power and antioxidant activity using ABTS, DPPH• and FRAP assay. The lyophilized alcoholic and aqueous extracts and oil of *Ganoderma Lucidum* have been dissolved in methanol and applied onto the HPTLC plate silica gel 60 F_{254} with the Linomat 5. The HPTLC plate was developed in dichloromethane - methanol 9:1. Then the plate was derivatized by dipping in iced cold methanol - sulfuric acid (9:1) and dried. Spots were visualized at UV 254 and 366 nm and scanned at 254 nm.

The abundance of bioactive constituents and consistent fingerprint pattern has been observed in different extracts and oil of *Ganoderma Lucidum*. HPTLC analysis confirmed segregation of bioactive compounds with individual Rf values and peak area percentage. However, alcoholic extracts and oil have shown comparatively higher concentration of the coumarin.

P-10d

Determination of rosmarinic acid - crucial aspects of quantitative validation

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Rosmarinic acid (RA), a natural phenolic compound found in many Lamiaceae herbs expecially in rosemary and sage, has a number of interesting biological activities, e.g. antiviral, antibacterial, antiinflammatory and antioxidant [1]. Moreover rosmarinic acid has been recently reported to inhibit the hemorrhagic effect of snake venoms [2] and for its effects on Alzheimer's Disease [3]. The main source of this compound is *Rosmarinus officinalis* L. However, reports have been published on the TLC determination of RA in a variety of herbal extracts [4-8] but none provided reliable quantitative results as the proposed methods are impaired by some methodologycal weakness. Our work is focused on the analytical aspects of HPTLC quantitative validation. Here we present the pre-validation procedure, the linearity claiming and the calibration matrix effect as focal points in developing a validated HPTLC method.

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P-10e

A novel sesquiterpene acid and an alkaloid from leaves of the Eastern Nigeria mistletoe with potent immunostimulatory activity on C57BL6 splenocytes

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In our continued efforts to identify the immunoactive constituents of a local mistletoe species in Eastern Nigeria, a novel sesquiterpenoidal acid, 2, 3-dimethoxy-benzo [a, b] cyclopentenyl-3', 3', 5'-trimethyl pyran-4-carboxylic acid (**1**), and a known alkaloid, lupinine (**2**) were isolated. These compounds were screened for immunostimulatory activities on isolated C57BL/6 mice splenocytes at concentrations of 10, 25 and 100 µg/ml. Their effects on splenocytes proliferation and on the expression of CD69, an early immune cells activation marker, were determined using flow cytometry techniques and compared to Lipopolysaccharide (LPS; 10 µg/ml) and Concanavalin A (ConA; 2 µg/ml) which were included as standards. The compounds (**1** and **2**) at a concentration of 25 µg/ml showed statistically significantly (p<0.05) stimulatory activity on the isolated splenocytes compared to the non-stimulated control cells with values of 56.34 ± 0.26 % and 69.84 ± 0.19 % respectively compared to 7.58 ± 0.42 % recorded for the control.

Similarly, the CD69 expression assay at the above dose showed that the compounds were stimulatory with statistically significant values ($p<0.05$) of 2.31 ± 0.07 % and 2.71 ± 0.03 % respectively compared to 1.69 ± 0.05 % recorded for the non-stimulated control. The compounds were characterized using a combination of TLC, UV/visible, IR, NMR (^{13}C-NMR and ^{1}H-NMR) and DEPT, MS and detailed 2-dimensional correlation (H-H COSY, HSQC, HMBC, NOE, and NOESY) studies. These compounds may be responsible in part, for the immunostimulatory activities already established for the Eastern Nigeria mistletoes.

P-10f

Identification of $GABA_A$ receptor modulators with a twist: Discovery of aristolactone in a commercial sample of *Bupleurum chinense* root

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Gamma-Aminobutyric acid type A ($GABA_A$) receptors are the major mediators of fast synaptic inhibition in the central nervous system. In a screening of a plant extract library for $GABA_A$ receptor modulatory activity with a two–microelectrode voltage clamp assay using *Xenopus laevis* oocytes expressing the receptor subtype $\alpha_1\beta_2\gamma_{2s}$, a petroleum ether extract prepared from a commercial sample of the traditional Chinese herbal drug *Chaihu* (*Bupleurum chinense* DC. roots) showed significant activity. By means of an HPLC–based activity profiling approach combined with on-line and off-line spectroscopy, the germacranolide aristolactone was identified as one of the main active compounds (EC_{50} 56.02 ± 5.09 µM). However, aristolactone has been reported as a taxonomic marker of the genus *Aristolochia* (Aristolochiaceae). This suggested a possible adulteration or replacement of *Bupleurum* roots by *Aristolochia* roots. *Aristolochia* species are being used in traditional Chinese medicine but contain highly nephrotoxic aristolochic acids. These drugs are not permitted for sale in Europe for safety reasons. Using a validated HPTLC protocol for aristolochic acids, the herbal sample was confirmed to be a mixture of *Aristolochia manshuriensis* root and *Bupleurum chinense* root. This finding was corroborated by macroscopic and microscopic inspection of the drug.

P-10g

Isolation and characterization of anthraquinone derivatives from *Ceratotheca triloba* (Bernh.) Hook. f.

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Following a complete chemical profile of methanolic extracts of *Ceratotheca triloba* roots, stems, flowers and leaves, the predominant compounds were isolated, and characterized by preparative TLC and identified by UV, ^{1}H NMR, ^{13}C NMR and EI-LC-MS. Three anthraquinone derivatives; 9, 10 anthracenedione; 1-hydroxy-4-methylanthraquinone; and 5, 8-dimethoxy-2, 3, 10, 10a-tetrahydro-1H-phenanthrene-4, 9-dione and also asteroid; androst-5-ene-3, 17, 19-triol were isolated from the roots of *C. triloba*. Of these, 9, 10 anthracenedione and 1 hydroxy -4-methylanthraquinone showed antibacterial and anti-oxidant activity, and showed potent inhibition of the human topoisomerase II enzyme. This enzyme, in a normal cell causes the unwinding of the DNA and is also structurally similar to commercial anti-cancer compound mitoxanthrone which is also an anthracenedione.

P-10h

Evaluation of *Brachylaena discolour* a potential anti-diabetic source

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Diabetes mellitus is a metabolic disease which occurs as a result of insulin deficiency and/or insulin resistance and is a major cause of disability and hospitalization. There are many known therapeutic strategies for the treatment of diabetes, with conventional treatments including the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo- and disaccharides [1]. One group of drugs introduced in the management of Type 2 diabetes is represented by the inhibitors of б-glucosidase and б-amylases. These enzymes are responsible for the breakdown of oligo- and/or disaccharides to monosaccharides. The inhibition of these enzymes leads to a decrease of blood glucose level, because monosaccharides are the form of carbohydrates which are absorbed in the small intestine [2]. The treatment of Type 2 diabetes is complicated by several factors inherent to the disease and elevated post prandial hyperglycemia (PPHG) is one of the risk factors [3]. Therefore, it becomes necessary to identify the amylase inhibitors from natural sources having lesser side-effects.

The traditional African herbal medicinal system practiced for thousands of years have reports of anti-diabetic plants with no known side effects. Such plants and their products have been widely prescribed for diabetic treatment all around the world with less known mechanistic basis of their functioning. Therefore, these natural products need to be evaluated scientifically in order to confirm claims for their anti-diabetic properties. This study will investigate potential phytochemicals from *Brachylaena discolor* as possible dietary adjuncts or therapeutics for diabetes therapy. Methanol and aqueous extracts were evaluated for their alpha amylase inhibition potential, and compared results obtained from methanol and ethanol extracts of *Psidium* sp. which has been documented as having anti-diabetic properties in traditional medicine. From results obtained *Brachylaena discolor* showed great potential as an anti-diabetic drug. The aqueous crude extracts of three of the five *Psidium* species as well as *Brachylaena discolor* yielded better inhibition properties as compared to their methanolic counterparts. *Brachylaena discolor* also exhibited a 4 fold increase over the *Psidium* sp. exhibiting the highest values with respect to alpha amylase inhibition.

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P-10i

Separation of biotransformation product using enzyme from Amazonian palm (*Bactris gasipaes*) by HPTLC

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A Brazilian palm (*Bactris gasipaes)* with high peroxidase activity was able to be used as biocatalyst in the biotransformation of organic compound. The mixture of the product and reagent was separated by HPTLC with simple strategy and few steps. Various analytical tools such as HPLC, GC, GC-MS and MS, and infrared spectroscopy progress in natural product chemistry have always liked with innovation in analytical technology. Among the chromatographic techniques, HPTLC, more popular in quality control and standardization of traditional herbs, was applied in the separation of biotransformation product 6,6´-bis[5-hydroxy-2-hydroxymethyl-4-pyrane-4-ona], the goal of the present study, and the results showed an efficiency in analytical separation as though preparative separation of the product. Quantification and characterization of the product were performed and confirmation of the product was done by LC/MS and NMR.

This work is part of a systematic investigation of plant source of peroxidase ready to biotransform HMP into HMPD, an important molecule dimer able to be assayed as new antileishmaniasis agent. Recently our group described that HMP presented high activity as macrophage inducer, eliminating pathogen in the cell. Our intention is to produce a derivative with higher activity than HMP. In this study analytical and preparative HPTLC was performed to separate both products from residual reagent.

P-10j

Screening, Isolation and structure elucidation of isothiocyanates and glucosinolates from *Moringa peregrina* (Forssk) fiori

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Moringa peregrina is a native Iranian shrub which grows in the south parts of Iran. It is one of the 13 xerophytic species from genus Moringa distributed from tropical Africa to the East India. *M. peregrina* were collected from Nikshahr, Sistan va Balouchestan province, Iran at the April of 2011. Different extracts and fractions with different polarity from hexane to water were prepared.

The presence of isothiocyanates (ITC) and glucosinolates (GLS) in *M. peregrina* was preliminary screened using the TPF (trichloroacetic acid potassium hexacyanoferrate iron-III-chloride) reagent. By spraying with the TPF reagent, blue spots for ITC and GLS were observed and also for some other secondary metabolites. However, some recognized compounds were separated from the others using preparative TLC. Further isolation and purification were carried out in preparative HPLC. 1D and 2D NMR and MS were used for structure elucidation in final stages. Their biological activitiy like antimicrobial and antidiabetical properties is in progress.

P-10k

Comparation of HPTLC and GC-FID methods for quantification of betulinic acid in *Eugenia florida* leaves extracts

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Betulinic acid (BA) has been identified in several plants including *Eugenia florida* (EF). This compound has been shown inhibiting melanoma-specific cytotoxic in both cell culture: *in vitro* and *in vivo* studies. The present work describes the comparison of GC-FID and HPTLC methodology for quantification of BA extracted from *Eugenia Florida leaves*.

The determination of the limit of detection (LOD) and the limit of quantification (LOQ) of the method by HPTLC and GC-FID were determinate to be 0.1 µg/µL and 1 µg/µL, respectively.

A representative calibration curve (polynomial regression for HPTLC and linear for GC-FID) was obtained by plotting peak area of BA against the concentration over the range of 1 – 50 µg/µL, for HPTLC and 10 - 100 µg/µL for GC-FID. A third-order polynomial function: $y = -0.908x^3 + 181.330x + 672.120$ ($r^2 = 0.9992$; *sdv* = 3.0 %) was obtained for HPTLC and a linear function: y = 2.008638x + 2,903333 ($r^2 = 0.9989$; *sdv* = 2.0 %) for GC-FID.

Both GC-FID and HPTLC offer advantages for BA quantification analysis. Whilst there is no statistical difference in the results obtained, HPTLC can be used for cheaper and faster determinations of large sample batches, whilst the superior sensitivity and the greater number of analytes separated make GC-FID an ideal tool for monitoring studies at the trace level.

P-10l

HPTLC for quality control of multicomponent herbal drugs: Example of Cangzhu Xianglian San

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Due to their complexicity, multicomponent herbal drugs pose enormous analytical challenges for quality control (QC). Although they may have traditionally been used for hundreds of years, the information about their chemical composition is often still limited. Selecting markers to monitor the identity and potency of the mixture is, therefore, difficult.

Using as an example the traditional Chinese veterinary combination medicine Cangzhu Xianglian San (CXS), composed of the herbal drugs Coptis rhizome, Aucklandia root, and Atractylodes rhizome, a systematic approach to improve the quality control of multi-herbal drugs by the use of HPTLC fingerprints was elaborated. HPTLC allowed determination of the presence of all three components in the drug after derivatization with anisaldehyde reagent. The same method can also be used to quantitatively determine the content of berberine by scanning densitometry. Hence, HPTLC proved to be a rapid and powerful technique for proper identification, detection of adulteration as well as quantitative analysis of complex herbal mixtures.

P-10m

TLC and HPLC comparison of the phenolic acid and flavonoid levels in selected sage (*Salvia*) species

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About twenty different sage species from the *Salvia* genus have been compared by means of TLC and HPLC for their phenolic acid and flavonoid content, using a multi-step experimental procedure.

Firstly, the dried plant material underwent two selective extractions according to the pharmacopoeial regulations and total contents of phenolic acids and flavonoids were evaluated by means of UV-Vis spectroscopy. Based on these preliminary results, several sage species were selected which had the highest abundance of the investigated phenolics for more detailed evaluation.

The selected sage species underwent a second-step selective extraction for phenolic acids and flavonoids, which included em acid/basic hydrolysis, in order to extract phenolic acids and flavonoids free of aglycones and glycosides.

Finally, these secondary extracts were analyzed and fingerprinted by means of TLC and HPLC, using a variety of detection systems, i.e., MS (TLC-MS), DAD and ELSD (HPLC).

The ultimate goal of this study was to explore the curative potential of the different sage species as efficient free radical scavengers and to select these species which might be suitable for medicinal purposes.

P-10n

Quantitative determination of flavonoid glycosides in extracts of *Potentilla* species

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The genus *Potentilla* L. (Rosaceae) comprises about 500 species of herbs or rarely shrubs, some of which have medicinal properties [1]. In our previous studies, we have achieved satisfactory separation of three polyphenols: tiliroside, methyl brevifolin-carboxylate and ellagic acid on HPTLC silica gel, using a toluene - ethyl formate - formic acid (6:4:1 v/v/v) [2]. In a recent study we optimized the separation of different polyphenolics, in selected extracts from *Potentilla* species, including flavonoid derivatives in both free (aglycone) and glycoside-bound forms, and two hydrolysable tannins: ellagic acid and its derivative [3]. Therefore, the aim of our current study was to determinate eight flavonoid glycosides: apigenin 7-glucoside, luteolin 7-glucoside, kaempferol 3-glucoside, kaempferol 3-glucuronide, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-glucuronide as well as quercetin 3-rutoside in ethyl acetate extracts obtained from aerial parts of selected *Potentilla* species: *P. anserina*, *P. argentea*, *P. erecta*, *P. fruticosa*, *P. grandiflora*, *P. nepalensis*, *P. recta*, *P. rupestris* (*Drymocallis rupestris*) and *P. thuringiaca*.

The analysis was performed according to a previously developed HPTLC method [3]. The compounds were separated on HPTLC diol phases F_{254} with ethyl acetate - methyl ethyl ketone - diisopropylether - formic acid (3:10:4:1, v/v/v/v) as mobile phase. Chromatographic separation was performed using the Automatic Development Chamber (ADC2), and the content of the analyzed compounds was determined using the TLC Scanner 4. The proposed HPTLC method was found to be simple, reproducible and can be easily applied as a routine analytical method for the quantification of these compounds in plant materials.

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P-10o

Initial investigations on four species of *osmanthus* by HPTLC

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Osmanthus is a genius of about 30 species of flowering plants in the family oleaceae.

They are mostly native of warm temperate Asia. The most commonly used species of this genius are *Osmanthus fragrance* Lour. in fragrances and *Osmanthus heterophyllus* (G. Don) P.S. Green in herbal drugs.

For these reasons we have decided to focus our analysis on the leaves of these two species. We also intended to broden our research on *Osmanthus armatus* and *Osmanthus decorus* (Boiss. & Bal.) Kasapligil in order to differentiate each species, to allow for adulteration and to perform traceability analysis.

[1] Freddy Ghozland, Xavier Fernandez: Histoire humaine des plantes à parfum: l'herbier parfumé; ed.Plumedecarotte; France; 2010; 148-149. [2] Chun-Di Hu *et al:* Essential oil composition of *Osmanthus fragrans* varieties by GC-MS and heuristice volving latent projections; *Chromatographia* 2009; 70, 1163-1169. [3] Eike Reich, Anne Schibli: High-performance Thin-layer chromatography for the analysis of medicinal plants; Thieme; China, 2006; 180 - 182. [4] Shigeaki Sakamoto,Koichi Machida,Masao Kiluchi: Lignan glycosides from the leaves of *Osmanthus heterophyllus*; *J. Nat. Med*., 2008, 62, 362 - 363.

P-10p

HPTLC, a versatile quality control tool: from single plant drug to complex polyherbal formulations

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Ayurveda is the most ancient health care system practiced widely in India. Due to their complex nature, Ayurvedic medicines are prone to contamination, deterioration and variations in composition. Therefore, the need arises to evaluate and ensure their quality so that the right materials are selected and incorporated into the formulation. HPTLC fingerprint analysis represents a rational approach for the assessment of quality of Ayurvedic medicines since multiple components in a single plant drug or a polyherbal formulation can be detected and quantified. The current paper represents several case studies, where the versatility of HPTLC has been successfully applied by developing suitable validated methods for various bioactive markers.

Single plant drug: A TLC method has been applied for quantitation of marmelosin from *Aegle marmelos* and for simultaneous quantitation of ß-sitosterol and lupeol from *Asteracantha longifolia*. A RP-HPTLC method has been developed for quantitation of Mimosine from *Mimosa pudica*. Kaempferol, quercetin, ß-sitosterol and lupeol were simultaneously quantitated from *Cuscuta reflexa*.

Polyherbal Formulation: Kaempferol and karanjin has been quantified from polyherbal oil based traditional formulations, *Sadbindu Taila* and *Jatyadi Taila,* respectively. A polymarker based TLC has been applied for simultaneous quantitation of eugenol, piperine and ß–sitosterol from *Lavangadi Vati*, a traditional pill formulation.

Biological Matrix: HPTLC has been successfully used to detect and quantitate piperine, ß–sitosterol, marmelosin and karanjin from animal blood plasma. The absorption elimination curves for these phytochemical markers have been used to evaluate bioavailability of these phytochemicals from their respective traditional formulations.

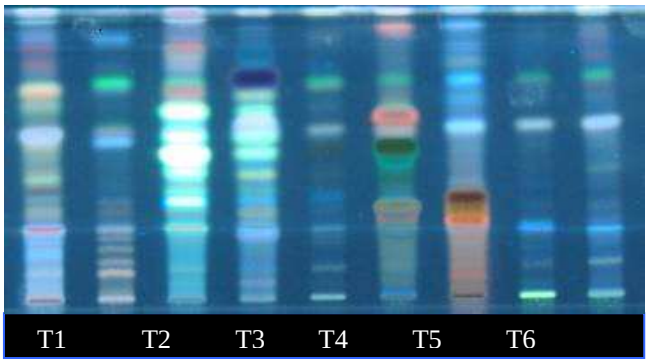

Fig. 1 TLC Fingerprint of DCM extract of *Jatyadi Taila* and its ingredient plant raw materials (fluorescence at 366 nm): T1: Leaf powder of *Azadirachta indica,* T2: Whole plant powder of *Trichosanthes dioica,* T3: Leaf powder of *Pongamia pinnata,* T4: Jatyadi Taila, T5: Heart wood powder of *Prunus cerasoides,* T6: Root powder of *Saussurea lappa,* T7: Rhizome powder of *Curcuma longa,* T8: Stem powder of *Berberris aristata,* T9: Rhizome powder of *Picrorhiza kurruoa*

P-10q

Development and validation of an HPTLC method for evaluation of betulinic acid in *Eugenia florida* leaves

Lessa B.M., Nóbrega A.B., Santos J.B., de Oliveira R., Coutada L. C. M., Lopes C.C., Lopes R.S.C., Slana G.B.C.A.

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Betulinic acid (BA) has been identified in several plants including *Eugenia florida* (EF). This compound inhibits melanoma-specific cytotoxicity in cell culture *in vitro* and *in vivo*. The present work describes the development and validation of an HPTLC method for identification and quantification of BA isolated from *Eugenia Florida leaves* extract.

The limit of detection (LOD) and limit of quantification (LOQ) were determinate to be 0.1 µg/µL and 1 µg/µL, respectively. A representative calibration curve (polynomial regression) was obtained by plotting peak area of BA against the concentration over the range of 1 – 50 µg/µL at 210 nm. A third-order polynomial function: $y = -0.908x^3 + 181.330x + 672.120$ ($r^2 = 0.9992$; sdv = 3.0 %) was obtained.

P-10r

Seasonal variation of betulinic acid in leaves from *Eugenla florida* by HPTLC densitometry

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FIOCRUZ/Farmanguinhos, Rua Sizenando Nabuco, 100, Manguinhos, CEP 21041-250; Rio de Janeiro, RJ, Brasil, abnobrega@gmail.com

Betulinic acid (BA) has been identified in several plants including *Eugenia florida* (EF). This compound inhibits melanoma-specific cytotoxic in cell culture *in vitro* and *in vivo*. The present work describes the development and validation of an HPTLC method for identification and quantification of BA isolated from *Eugenia florida leaves*.

HPTLC plates silica gel 60 (5 x 10 cm, 0.2 mm in thickness) were developed to a height of 45 mm (10 min) in a saturated vertical developing chamber using $CHCl_3$ - MeOH (9:1, v:v). The samples were applied as 10 mm bands using Linomat IV. The HPTLC plates were dried at room temperature. Betulinic acid seasonal concentration in the crude extract from *E. florida* were measured at the absorption maximum of 210 nm. Each determination was carried out in triplicate.

Seasonal determination of BA in *Eugenia florida* leaves

Month/Year	Conc. (%)	RSD (%)	Month/Year	Conc. (%)	RSD (%)
January/2008	11.59	1.0	July/2008	21.24	1.8
February/2008	22.29	1.5	August/2008	14.51	0.7
March/2008	25.95	1.2	September/2008	21.23	0.8
April/2008	22.98	1.6	October/2008	33.21	1.6
May/2008	27.23	1.9	November/2008	28.01	1.3
June/2008	31.63	1.2	December/2008	16.49	1.7

P-10s

Development and validation of a HPTLC method for the quantitative determination of the flavanone glycoside naringin in *Drynaria quercifolia* fronds

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A rapid, simple and reliable quantitative densitometric HPTLC method was developed for the determination of flavanone glycoside (naringin) in *Drynaria quercifolia* fronds. The sample preparation was carried out by refluxing the plant material with 70% (v/v) aqueous methanol for 30 min. The extracts and standard solutions was applied on TLC aluminium foils silica gel 60 F_{254} using Linomat 5. Naringin was separated from the plant extract using a saturated mixture of ethyl acetate – chloroform – methanol - formic acid (7:3:2:0.1, v/v/v/v). The plates were developed vertically up to a distance of 80 mm in the twin-trough chamber. Spectrodensitometric scanning was carried out by TLC scanner 3 at the wavelength of 286 nm in the absorption mode. The solvent system was found to give compact spots for naringin (Rf value 0.22 ± 0.03). The linear regression analysis data for the calibration curve showed a linear relationship (r^2 = 0.9938) in the concentration range 200 - 1200 ng/zone with respect to peak area. The HPTLC method was successfully validated for precision, recovery and robustness with respect to ICH guidelines. The statistical analysis of the data showed that the proposed method is precise, reproducible and accurate, and can be employed for the standardization of the plant on basis of naringin content.

P-10t

Characterization of flavonolglycosides in *Calendula officinalis* (Herba) by orthogonal HPTLC and HPLC-MS

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Due to its anti-inflammatory, antipyretic, anticancerogenic, and healing effects, *Calendula officinalis* L. is one of the most commonly used plants for herbal drugs and their respective medicinal preparations. In particular, the calendula flowers have been the focus of investigations of their phytochemical constitution. Flavonolglycosides are the main constituents of Calendula. Numerous scientists have reported on the investigation of flavonolglycosides in calendula flowers, and the whole calendula plant after isolation and purification of these compounds. However, the scientific literature seems to lack reports on the flavonoids present in Calendula herba.

According to the German Homeopathic Pharmacopoeia (HAB), the mother tincture Calendula herba V. 2a is prepared from the fresh, aerial parts of the plant. Harvested during the time of blossom it thus contains the phytochemicals of the stems and leaves as well as those of the flowers. The compounds found in the Calendula herba tincture could therefore be related to those reported in Calendula flowers.

The aim of this work is to compare the separation of flavonolglycosides in Calendula herba V.2a by normal phase and reversed phase TLC and structure characterization of the flavonoids by HPLC coupled with negative electrospray ionization (ESI) ion trap mass spectrometer employing an LTQ Orbitrap XL.

P-10u

Quantification of phytoconstituents of the leaves of *Paederia foetida* by HPTLC

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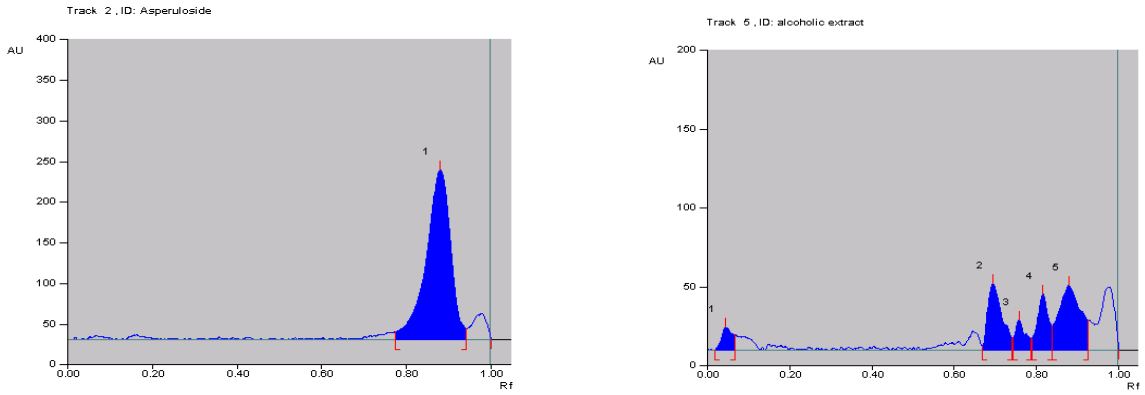
The present study aims at identification and quantification of bio-active phytoconstituents in the leaves of *Paederia foetida* by HPTLC. Various extracts of the leaves of *Paederia foetida* were prepared and subjected to preliminary phytochemical screening. Asperuloside (an irridoid glycoside), lupeol & ß-sitosterol were qualitatively confirmed to be present in ethanol and aqueous extracts as major phyto-constituents.

These constituents were quantified on HPTLC plates silica gel G F_{254}. Asperuloside was quantified using chloroform – methanol - water (6:6:1) as mobile phase while lupeol & ß-sitosterol were simultaneously quantified using ethyl acetate - benzene (0.5:9.5). Developed plates were sprayed with 10 % methanolic sulphuric acid and the absorbance was measured at 366 nm (asperuloside) and 500-600 nm range (lupeol & ß-sitosterol). The content of asperuloside, lupeol and ß-sitosterol in the leaves was found to be 0.095 %, 0.183 % and 0.151 %, respectively. Validation parameters for the estimation method were performed as per ICH guidelines and all the parameters were found to be well within the prescribed limits.



HPTLC chromatograms

P-10v

Development of an HPTLC method for the determination of vicine in herbal extract and formulation

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In the present work a HPTLC method was developed and validated for the determination of vicine in herbal preparations containing *Momordica caharantia* fruits. The study was performed on TLC aluminium foils silica gel 60 F_{254}. The chromatograms were developed in twin trough glass chamber saturated with mobile phase comprised of ethyl acetate – methanol – water - formic acid (7.5:3:1:0.1, v/v/v/v) at room temperature (25 ± 2 °C). The chromatograms were analyzed in absorbance/reflectance mode at 278 nm using the TLC Scanner 3.

The developed system had given specific peak (R_F value of 0.31 ± 0.02) for vicine. The data obtained from linear regression analysis for the calibration plots showed good linear relationship (r^2 = 0.9990) with respect to peak area in the concentration range of 400 - 1400 ng/spot. The method was validated as per the guidelines of ICH. The limits of detection (18.8 ng) and quantification (50 ng) were also established. Statistical analysis of the obtained data showed the reproducibility and selectivity of the method for vicine estimation in the herbal preparations.

P-10w

HPTLC method for separation and simultaneous quantification of withanoloids

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An easy, economic, accurate, precise, less time consuming and reproducible HPTLC method has been developed for separation and simultaneous quantification of withaferin A, withanolide A and withanone on silica gel. The solvent system chloroform - ethyl acetate in equal ratio was found to achieve separation of withaferin A, withanone and withanolide A at R_F values 0.10 ± 0.01, 0.16 ± 0.01 and 0.21 ± 0.01, respectively (Fig. 1). The proposed method was validated as per ICH guidelines for accuracy, precision, robustness, LOD and LOQ. The calibration parameters showed good linearity with r^2 values >0.9950 over a wide range and excellent sensitivity. The results demonstrated its simplicity, reliability, economicity and suitability over other reported methods for quality control of withania as well as herbal formulations containing it as an ingredient.

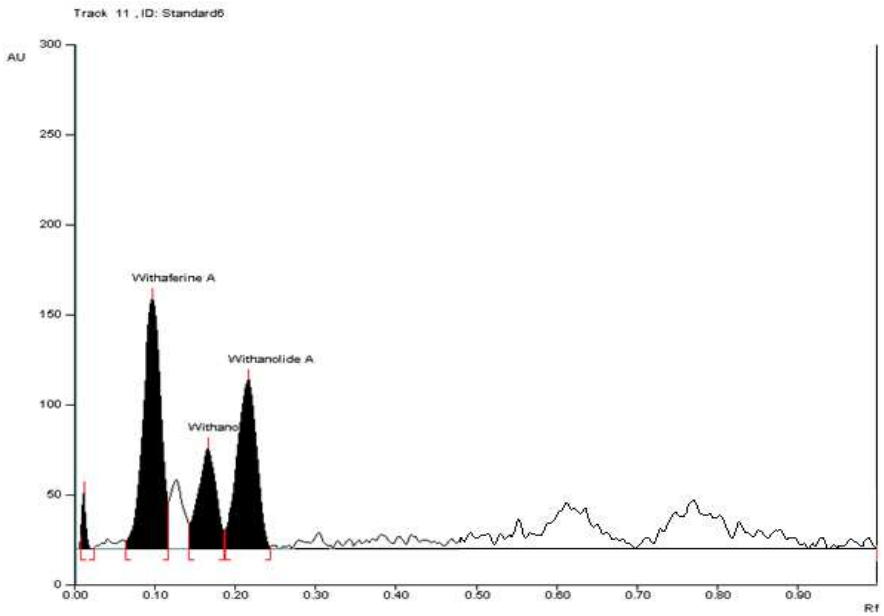

Fig. 1 HPTLC chromatogram showing separation of withaferine A, withanone and withanolide A

P-10x

HPTLC based quality assessment of some high altitude plant species: variation with altitude

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HPTLC is a quality assessment tool for the evaluation of herbal/herbal products. It allows the analysis of broad spectrum and wide range of compounds, both efficiently and cost effectively. As compared to other chromatographic techniques, in HPTLC based separation, modification of TLC/HPTLC plates and pre- and post-chromatographic derivations are most common followed by viewing through different range of wavelengths (254 nm, 366 nm and white light).The desired compound, if needed, can be extracted and identified. This technique provides a complete chemical profile of the plant which can be used further for specific studies. Chromatogram thus generated can be digitally stored in the form of pictures and reproduced for comparison when the need arises. HPTLC method of some high altitude plants species have been developed and location based variation have been validated.

P-10y

Development and validation of HPTLC method for estimation of umbelliferone and quercetin in *Aegle marmelos* formulation

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Aegle marmelose (L) (Rutaceae) generally acknowledged as bael and contains aegeline, agelinine, rutin, lupeol, tannins, phlobatannins, flavonoids, umbelliferone, quercetin and volatile oils [1]. In the present investigation the methanolic extract of four marketed *Aegle marmelos* formulations (F_1, F_2, F_3, and F_4) was investigated for the quantification of umbelliferone, quercetin, total flavanoid and phenolic contents. A simple HPTLC method was developed for the quantification of umbelliferone and quercetin. The method was validated for precision, repeatability, and accuracy in accordance with ICH Q2 guidelines. Maximum amount of the umbelliferone (3.413 ± 0.3 µg/100 mL) and quercetin (3.854 ± 0.4 µg/100 mL) was noticed in formulation F_4. Likewise, highest flavonoid (8.134 ± 0.044 mg/kg) and phenolic (6.145 ± 0.05 mg/kg) content were also noticed in formulation F_4. The HPTLC method developed was found to be repeatable, selective, showed good resolution, separation and accurate for the quantification of umbelliferone and quercetin in different formulations of *A. marmelos* which could be used for the routine quality testing of the formulation.

[1] A. Banerjee, SS. Nigam, *J. Am. Oil Chem. Soc*. 56, 1979, 647-651.

P-10z

HPTLC-based estimation of plumbagin in the carnivorous plant *Nepenthes khasiana*

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This study reports the first detection of the biologically active naphthoquinone plumbagin (PLU) in the Indian carnivorous plant *Nepenthes khasiana* Hook. GC-EIMS analysis of the dichloromethane wash of *N. khasiana* pitcher top found 92.2 % of PLU in it. Subsequently, PLU was detected in its root, stem, leaves and pitchers. PLU in the roots and aerial parts of *N. khasiana* field and potted plants were systematically estimated by HPTLC densitometry. PLU peaks in *N. khasiana* methanol extracts (R_F 0.49; toluene - glacial acetic acid 9.5:0.5; 265 nm) were well resolved from other peaks.

A linear calibration curve of PLU was generated in the range 0.05 to 0.90 μg (y = 11380 + 3.721, R^2 = 0.997). The HPTLC method was validated in terms of accuracy, precision, repeatability and linearity. PLU contents in both field and potted *N. khasiana* plant parts were also estimated on chitin-induction and prey-capture. PLU contents in *N. khasiana* pitchers of uninduced field and potted plants were as low as 0.03 ± 0.00 % and 0.11 ± 0.00 % (dr. wt.), respectively. On chitinase induction, these values were enhanced to 0.22 ± 0.00 % and 0.38 ± 0.00 % (dr. wt.), respectively, in field and potted samples. Chitin induction and prey capture produced droserone (DRO) and 5-O-methyl droserone (M-DRO) in the enzymatic pitcher fluid of *N. khasiana*. The formation of DRO and M-DRO in the pitcher fluid on chitin induction was confirmed by DART-HRMS and HPTLC profiles. The molecular roles of PLU, DRO and M-DRO in prey capture in *N. khasiana* will also be presented.

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