Hyphenation of HPTLC with NMR
for structural elucidation of bioactive compounds in medicinal herbal extracts

E.Azadniya, **G. Morlock**

Justus Liebig University Giessen, Germany

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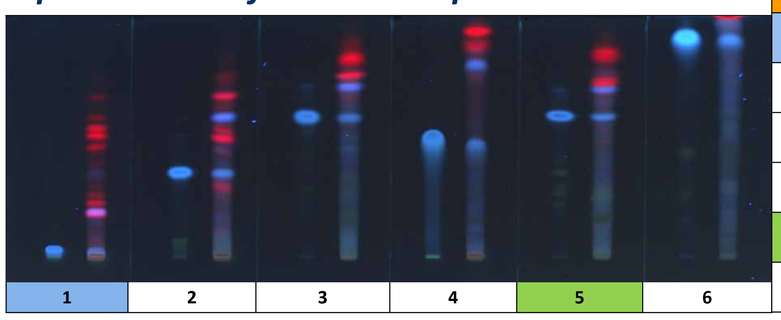
Introduction

NMR spectroscopy is one of the most powerful analytical techniques for structural elucidation of chemicals. The aim of the current project was to develop an HPTLC-NMR workflow at the analytical scale in the field of medicinal herbal extracts. The common sage (*Salvia officinalis*) and red sage (*Salvia miltiorrhiza*) were used as botanical sources, belonging to the *Lamiaceae*, which family has been studied as a source of natural antioxidants. Rosmarinic acid (RA), the most abundant natural antioxidant in *Lamiaceae* species [1-2], was used to optimize the analytical workflow. ^{1}H-NMR requires the lowest substance amount and gives quantitative information based on a linear correlation between signal intensity and sample amount. This unique property makes NMR a versatile quantitative detector. Using online zone elution via the TLC-MS Interface, hyphenation of HPTLC to NMR is less contamination-prone and more simple, without any investment in hardware, if compared to conventional scrape-off of the zone. This makes HPTLC-NMR coupling a reliable tool to investigate bioactive compounds in herbal extracts [3].

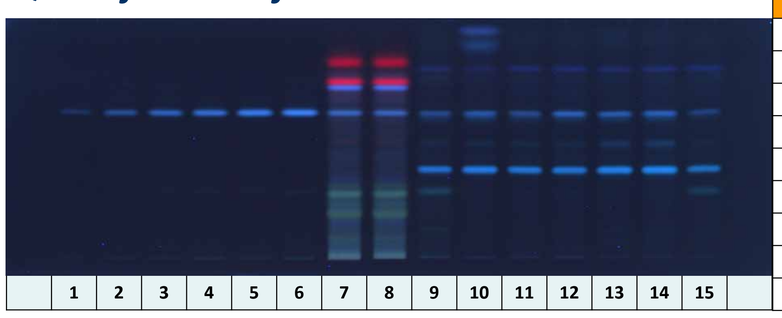
A) HPTLC method

Optimization of the mobile phase

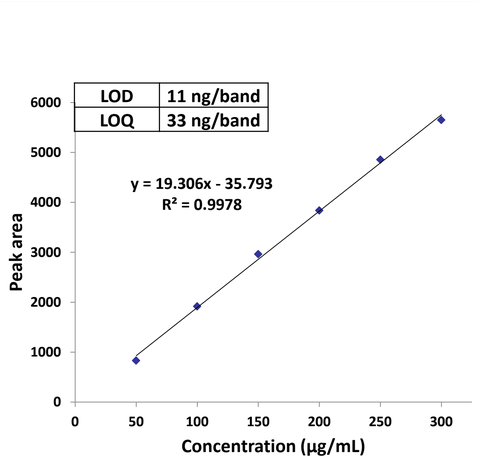


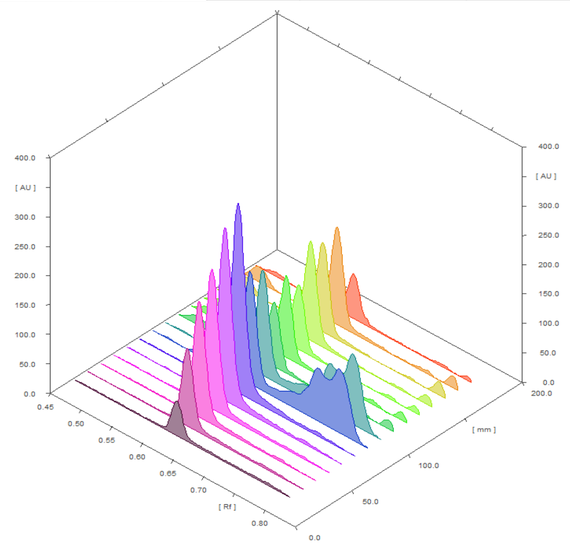
No.	Mobile phase solvent ratio
1	Tol : EtOAc : FA 7.0 : 3.0 : 0.1
2	Tol : EtOAc : FA 5.0 : 3.0 : 1.0
3	Tol : acetone : FA 5.0 : 4.0 : 1.0
4	Tol : EtOAc : HOAc 4.0 : 5.0 : 2.0
5	Tol : EtOAc : FA : H_2O 3.0 : 4.0 : 1.0 : 0.4
6	Tol : EtOAc : HOAc : MeOH : H_2O 2.0 : 6.0 : 2.0 : 2.0 : 0.3

Quantification of RA in herbal extracts



No.	Samples	Content RA (mg/kg)
7	*Salvia officinalis*	157.6
8	*Salvia officinalis*	149.1
9	*Salvia* M 2	99.1
10	*Salvia* M 6	126.5
11	*Salvia* M 18	97.1
12	*Salvia* M 18+St	145.3
13	*Salvia* M 38	130.7
14	*Salvia* M 39	145.6
15	*Salvia* M 202	68.1

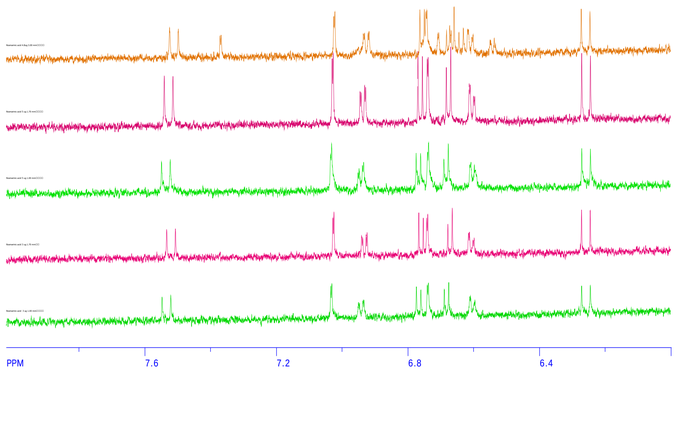


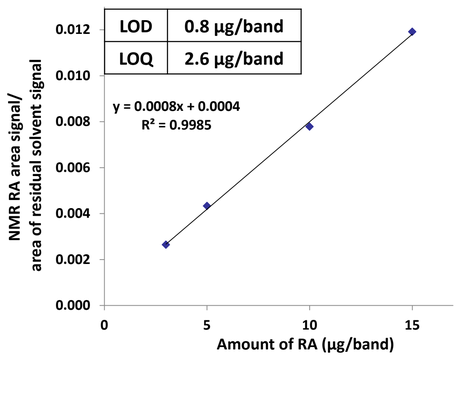


B) NMR method

NMR parameters were optimized via RA in deuterated solvents

- **Deuterated solvents**
  among CD_3OD and D_2O, CD_3OD due to less capillarity
- **Filling height and volume of solution in different NMR tubes**
  height ≥ 3.50 cm to be covered by NMR coil and probe
  3.0 mm tube 150 µL, 1.7 mm tube 50 µL, and 1.0 mm tube 10 µL
- **Size of NMR microtubes**
  among 3.0 mm , 1.7 mm (best), and 1.0 mm NMR microtube
- **Effect of solvent suppression**
  substantially improved signal to noise ratio → crucial for low concentration NMRs





C) Zone elution

Evaluation of the TLC-MS Interface efficiency

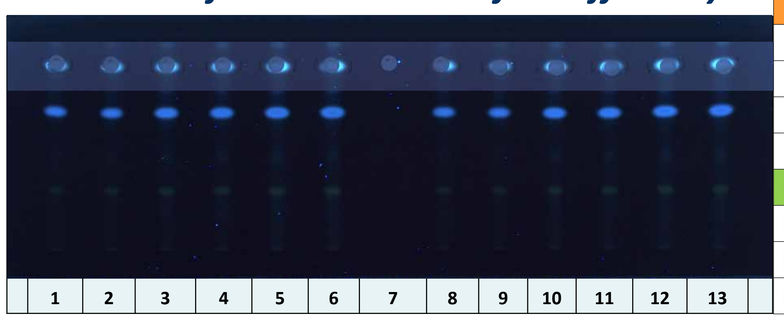
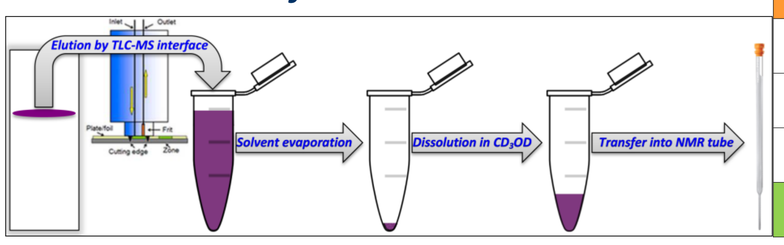


Plate	Band length (mm)	Flow rate (ml/min)	Efficiency (%)
PLC	3	0.1	33
PLC	3	0.2	28
PLC	3.5	0.2	28
PLC	3.5	0.1	27
HPTLC	3	0.1	40
HPTLC	3	0.2	37
HPTLC	3.5	0.2	37
HPTLC	3.5	0.1	38

HPTLC-NMR workflow



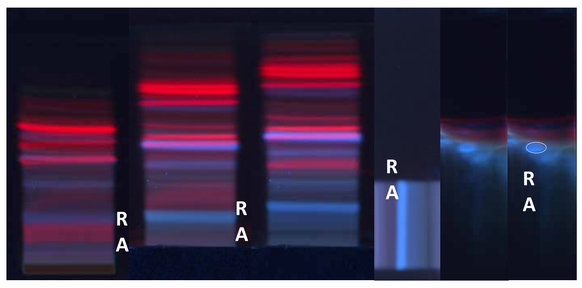
Elution solvent	Peak area	Improvement (%)
MeOH	1592	
MeOH + 2% FA	1699	7
EtOH	1666	5
EtOH + 2% FA	1817	14

D) Matrix interference

To reach an adequate amount of RA for NMR detection, high volumes of the herbal extract had to be applied. To avoid matrix interferences, a combination of area application and 2D-TLC was used.

Workflow:

- **100 µL extract as 30 x 3 mm area (15 µg/band RA)**
- **Development with Tol-EtOAc-FA, 7:3:1 (*V/V/V*)**
- **Plate cut below 1.5 cm**
- **Development with Tol-EtOAc, 7:3 (*V/V*)**
- **Development with Tol-EtOAc-FA , 7:3:0.2 (*V/V/V*)**
- **Cut upper part (above RA)**
- **Development in second dimension with Tol-EtOAc-FA-water, 3:4:1:0.4 (*V/V/V/V*)**



E) Conclusions

- Set up a fast and reliable hyphenation between HPTLC with NMR
- Benefits from flexibility and matrix-robustness of HPTLC that avoided any further sample preparation steps for isolation, fractionation and purification, such as column chromatography, dialysis and solid phase extraction
- Optimized parameters (solvent, flow rate and band width) of online zone elution via TLC-MS Interface to collect most of the band
- Analyzing RA by HPTLC-NMR hyphenation as example for a fast and accurate quantification as well as identification of unknown bioactive compounds in herbal extracts.

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References

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Food Sciences, Justus Liebig University Giessen, Institute of Nutritional Science, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany