Quantitation of coumarin in food, confirmed by mass spectrometry

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Introduction

Coumarin is a naturally occurring phytochemical, *e.g.* in tonka beans, cinnamon and woodruff [1, 2]. According to plant and origin there are different types of cinnamon. Ceylon Cinnamon (*Cinnamomum verum*, *J. Presl*) is from Sri Lanka and Cassia Cinnamon can originate from either China, Vietnam (*Cinnamomum cassia* or *Cinnamomum aromaticum*) or Indonesia (Padang Cassia) [2]. Cassia cinnamon, mostly used as spice, contains high amounts of coumarin [3]. Coumarin potentially causes liver damage, thus the tolerable daily intake was set to 0.1 mg/kg body weight [4]. Existing quantitative methods for cinnamon and its products mainly use HPLC, necessitating complex sample preparation, whereas HPTLC offers a simplification of the analysis. Since cinnamon and cinnamon containing foods are not only consumed around Christmas time, various other products were also analyzed with the newly developed HPTLC method.

Results and discussion

Method development

Ten different commercially available cinnamon samples (Fig. 1) and 16 different cinnamon containing foods like tea, breakfast cereals, jam, milk rice, cinnamon stars and cinnamon buns were, if necessary, crushed and dissolved in methanol. The separation was carried out on HPTLC plates silica gel 60 with *n*-hexane, ethyl acetate and ammonia. The method was optimized to allow coumarin identification and quantification in every sample. However, for some matrix-rich samples like milk rice, it was difficult to get satisfying results concerning repeatability and intermediate precision. For such a protein-rich matrix, an additional front elution with tetrahydrofuran was necessary. After derivatization with alcoholic potassium hydroxide, documentation was performed at UV 366 nm illumination (Fig. 1). The turquoise fluorescence was recorded at 365/>400 nm. The coumarin solutions remained stable for at least nine days at 8 °C.

Method validation

The limit of detection of coumarin was determined to be 200 pg/band and the limit of quantitation was 400 pg/band. The determination coefficient R^2 of the polynomial calibration was 0.9999. For all the different food samples containing cinnamon, repeatabilities and intermediate precisions were determined. These precision values (*%RSD, n = 2*) were mostly ≤ 6 %, with some exceptions (Table 1).
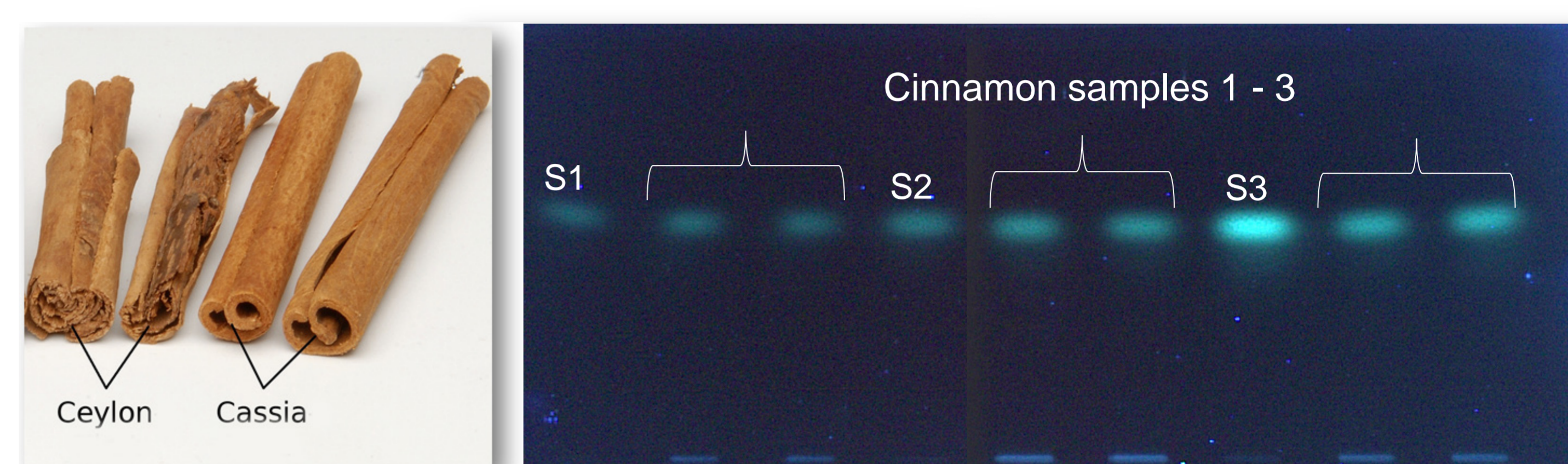

Fig. 1 Difference between Ceylon and Cassia Cinnamon [5] and HPTLC chromatogram of 3 cinnamon samples two-fold applied and coumarin standards S1 to S3

Bioassay

As coumarin potentially causes liver damage, its limit of detection was also determined using the *Aliivibrio fischeri* bioassay to be 100 ng/band, which was more than 500 times higher if compared to the very sensitive fluorescence detection.

HPTLC-MS

Mass spectra were recorded in positive electrospray ionization mode by direct elution of the zones of interest into the expresssion CMS mass spectrometer using the TLC-MS Interface equipped with the oval elution head. The coumarin standard zone showed a mass signal at *m/z* 147 [M+H]⁺ and *m/z* 309 [2M-H+NH₄]⁺ (Fig. 2), which confirmed the zones' identity in the food samples.

Table 1 Coumarin content of various food products inclusive of repeatability and intermediate precision (all by peak area)

Product	Producer/brand	Sort	Coumarin (mg/kg)	Repeatability (%RSD, n=2) Plate 1 (n=2)	Repeatability (%RSD, n=2) Plate 2 (n=2)	Intermediate precision (%RSD, n=2)
Cinnamon	Fuchs	ground	1327	5.3	1.1	3.3
	tegut (1)	ground	2625	5.1	2.3	4.7
	tegut (2)	ground	3647	1.9	3.7	1.4
	Lucullus	ground	3631	2.5	0.0	2.2
	Lidl	ground	1615	0.4	0.3	2.4
	Sonnentor	ground	3732	0.4	1.2	1.0
	Ceylon	ground	2434	2.9	1.3	5.1
	Ostmann	stick	202	5.1	1.6	3.6
	Fuchs	stick	150	1.0	0.6	3.0
	Bio Cassia	chopped	5017	0.2	3.0	1.4
Tea	Goldmännchen	Wintertee	137	1.3	4.8	0.7
	Milford	Wintergenuss – Winter Delight	62	0.8	1.0	3.3
	Milford	Eismärchen	45	0.7	4.2	3.7
	Meßmer	Himmelszauber	39	*	1.2	0.7
	Meßmer	Persischer Bazar	103	*	0.8	0.9
	Yogi Tea	Frauen Power	19	1.4	3.2	4.5
	Teekanne	Orientalischer Gewürztee	92	1.5	0.9	5.9
	TeeGschwendner	Zimtapfel	59	1.3	4.2	6.1
	TeeGschwendner	Kaminfeuer	22	3.3	2.0	0.5
Cereals	Nestlé	Cini Minis	0.7	1.4	28.6	12.3
	Knusperone	Zimt Chips	1.3	3.8	5.9	0.4
	Gut und Günstig	Zimtos	0.7	9.1	4.7	11.3
Milk rice	Müller	Zimt	0.3	6.8	20.2	2.3
Cinnamon bun	IKEA	Kanelbullar	16	1.4	4.5	6.6
Cinnamon star	selfmade	Luisa	16	0.1	7.6	0.9
Jam	Grafschafter Krautfabrik	Winterzauber Apfel-Sauerkirsch	3.5	1.6	1.6	1.6

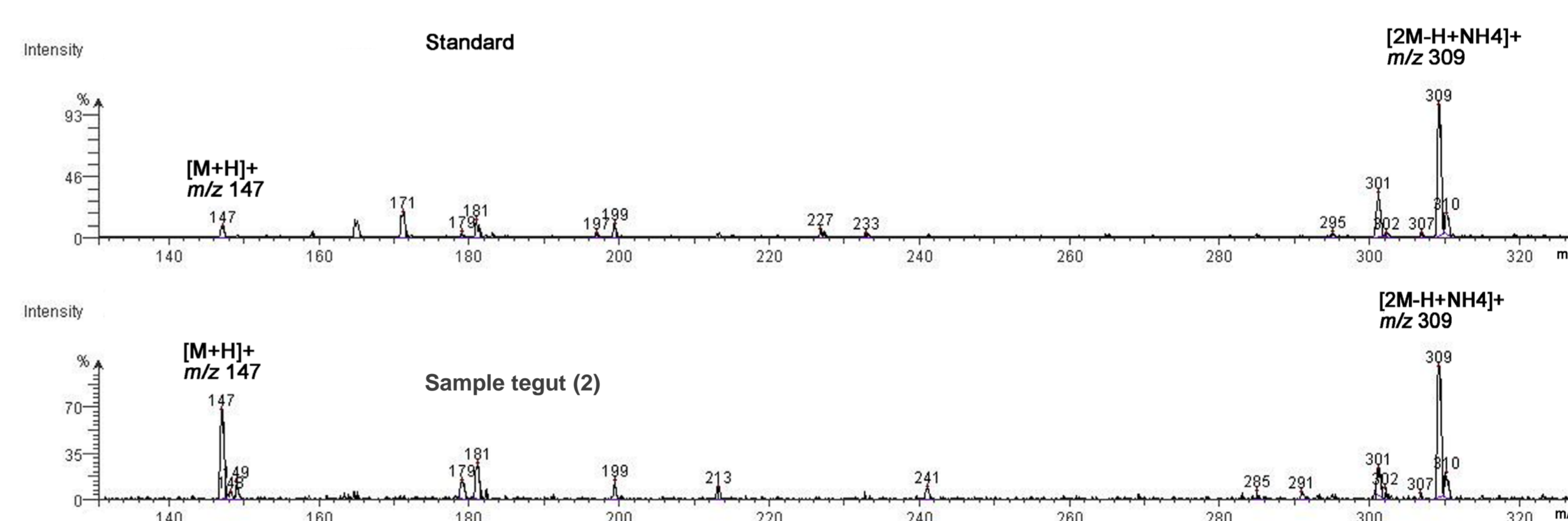*Fluorescent lint on HPTLC plate influenced the result.

Fig. 2 Mass spectra comparison of the coumarin standard and the cinnamon sample tegut (2)

Conclusions

A fast and efficient HPTLC method was newly developed, allowing parallel analysis of up to 15 raw extract samples on one plate. A further advantage was that a single mobile phase was suitable for all samples. It allowed the quantitation of coumarin in all analyzed food products. There are big differences in the coumarin content between the different products. The highest amounts of coumarin were found in cinnamon (up to 5 g/kg). Tea samples contained up to 140 mg/L coumarin. Only one sample exceeded the maximum level from the European flavor regulation VO (EG) 1334/2008, but a lot of products do not have a restriction at all. In further research the separation of some zones and the stabilization of the fluorescence could be optimized.

References [1] S.P. Felter *et al*. Food Chem Toxicol 44 (2006) 462-475. [2] C. Sproll *et al*. Food Chem 109 (2008) 462-469. [3] F. Woehrlin *et al*. J Agric Food Chem 58 (2010) 10568-10575. [4] EFSA, The EFSA Journal 104 (2004) 1-36. [5] BfR, www.bfr.bund.de/de/a-z_index/zimt-8403.html, 2012.

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