Simultaneous determination of citrinin and lovastatin in lactone- and hydroxy acid-form with validated HPTLC-UV/FLD method

I. Klingelhöfer, G. Morlock

Justus Liebig University Giessen, Germany

Introduction

Lovastatin (also known as mevinolin, monacolin-K) is synthesized in the fermentation of rice with *Monascus* fungi strains and decreases the amount of cholesterol by inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [1]. Lovastatin may be present both, in the hydroxy acid-form and dehydrolyzed lactone-form. Both forms differ considerably in their solubility. The hydroxy acid is readily soluble in water, whereas the lactone form is slightly fat-soluble through the closed lactone ring. Depending on the fermentation process both forms of lovastatin are formed in a ratio of 3:2 to 2:1. During fermentation, the nephrotoxic mycotoxin citrinin [2] is also formed, which acts carcinogenic and mutagenic, but also antibiotic [3].

Results and discussion

For simultaneous detection of the three components of interest a normal phase HPTLC method was developed. Separation was carried out on HPTLC plates silica gel 60 using a mixture of *n*-hexane, acetone and acetic acid. Densitometry was carried out using the multi-wavelength scan. Lovastatin was present in the lactone- (LL, hR_F 35) and hydroxy acid-form (LH, hR_F 23), both detected by absorbance measurement at 238 nm. Citrinin (hR_F 7) was recorded via fluorescence measurement at 313/>400 nm (Fig. 1).

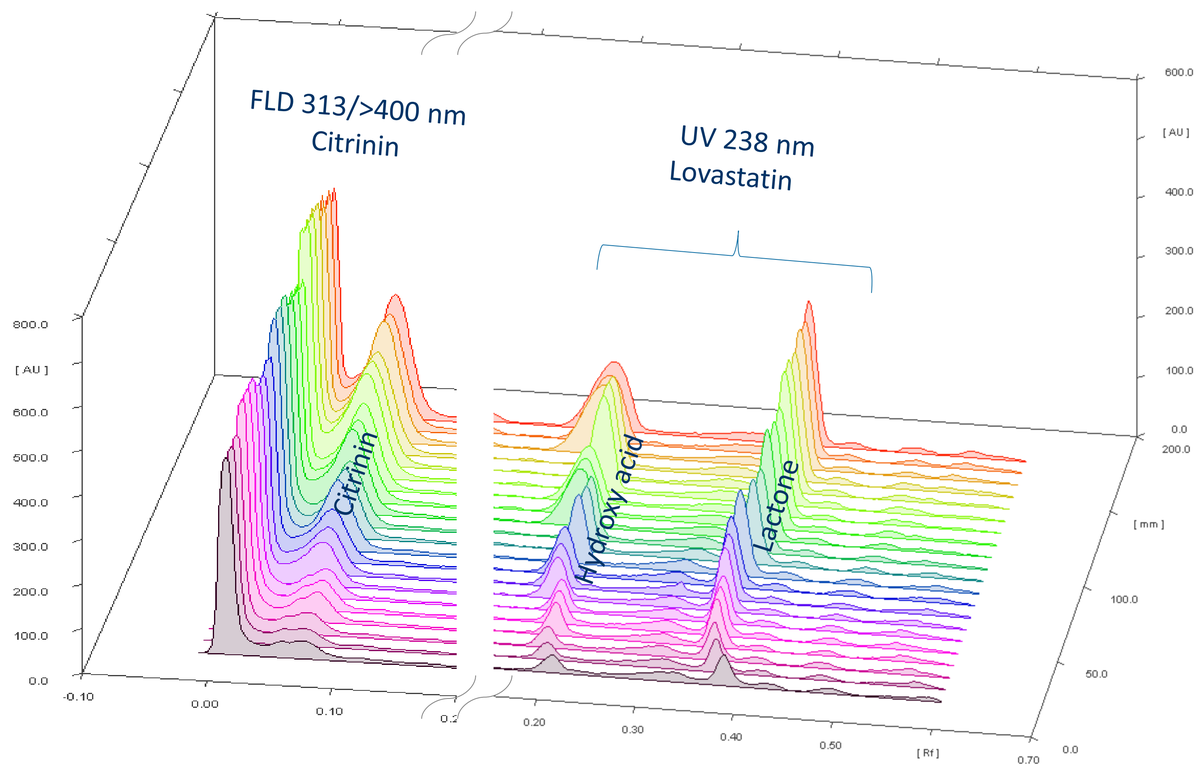

Fig. 1 Densitometric evaluation via absorption and fluorescence measurements in the multi-wavelength scan

For method validation, red rice samples were spiked with LH, LL and citrinin at three concentration levels and extracted with a mixture of acetone and water. The validation showed very good performance characteristics (Table 1). Both, for LH and LL, the mean LOD and LOQ (S/N 3 and 10; n = 3) were 10 and 50 ng/band, respectively. For citrinin, the mean LOD and LOQ were found to be 1 and 4 ng/band.

Table 1 Validation parameters

	Mean coefficient of determination R^2 (n = 5) (calibration range; ng/band)	Amount (ng/band)	Recovery (%, l = 3, j = 5)	Repeatability (%*RSD*, l = 3)	Laboratory precision (%*RSD*, j = 5)
LH	0.9998 (25– 500)	80	109.3 ± 2.8	0.7	2.6
		200	105.7 ± 5.8	3.0	5.5
		400	102.9 ± 3.8	2.4	3.7
LL	0.9999 (25– 350)	80	114.7 ± 5.2	1.6	4.5
		200	111.3 ± 7.7	2.7	6.9
		400	110.6 ± 4.9	3.3	4.4
Citrinin	0.9989 (2.5– 50)	8	113.5 ± 11.1	2.2	9.8
		20	120.0 ± 6.8	3.3	5.7
		40	101.4 ± 2.9	2.2	2.9

References [1] A. Seenivasan *et al.* Indian J Pharm Sci 70 (2008) 701-709. [2] J.P. Blanc *et al.* Int J Food Microbio 27 (1995) 201-213. [3] V. Betina, J Chromatogr 477 (1989) 187-233.

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The developed and validated HPTLC method was used for quantitation of 17 red rice samples, including Zhibituo and Xuezhikang (Fig. 2).

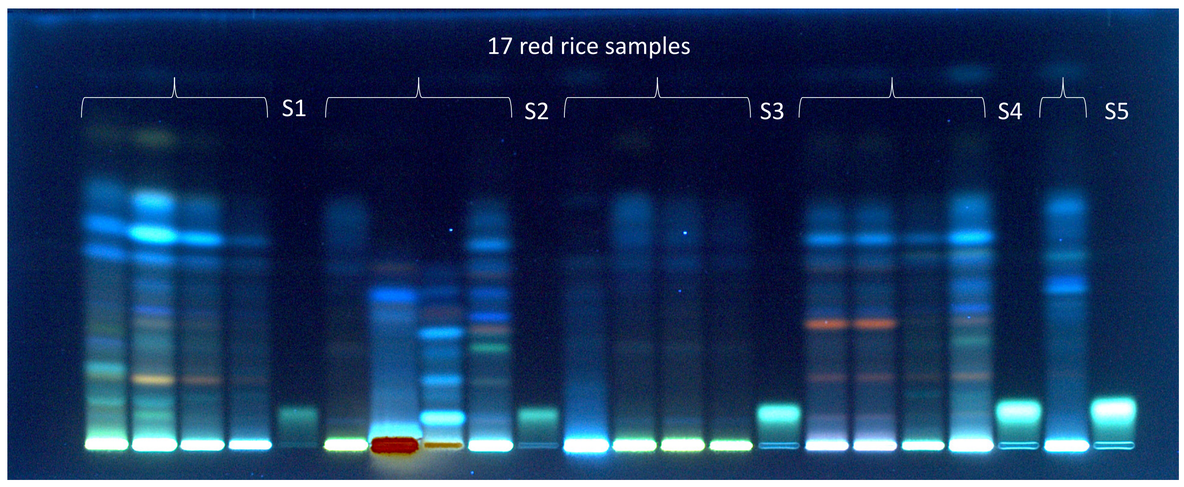

Fig. 2 HPTLC chromatogram of 17 red rice samples (extracted with acetone-water) and standard mixture (S1-S5: 30-600 ng/band, both for LL and LH; 3-60 ng/band for citrinin)

During the fermentation process, a series of pigments are formed in addition to the secondary metabolites already mentioned. One of these pigments is rubropunctamin, which gave the fermented rice its name “red rice”. Rubropunctamin coeluted with LH generating a peak shoulder (Fig. 3). For improved resolution, this dye could be separated either with a varied extraction mixture of acetone and water (3:2) or optically removed with a spectral background correction (Fig. 4). For this purpose, the 2-wavelength scan was performed at 238 and 588 nm, which automatically subtracted the signal intensities obtained at 238 nm from those at 548 nm.

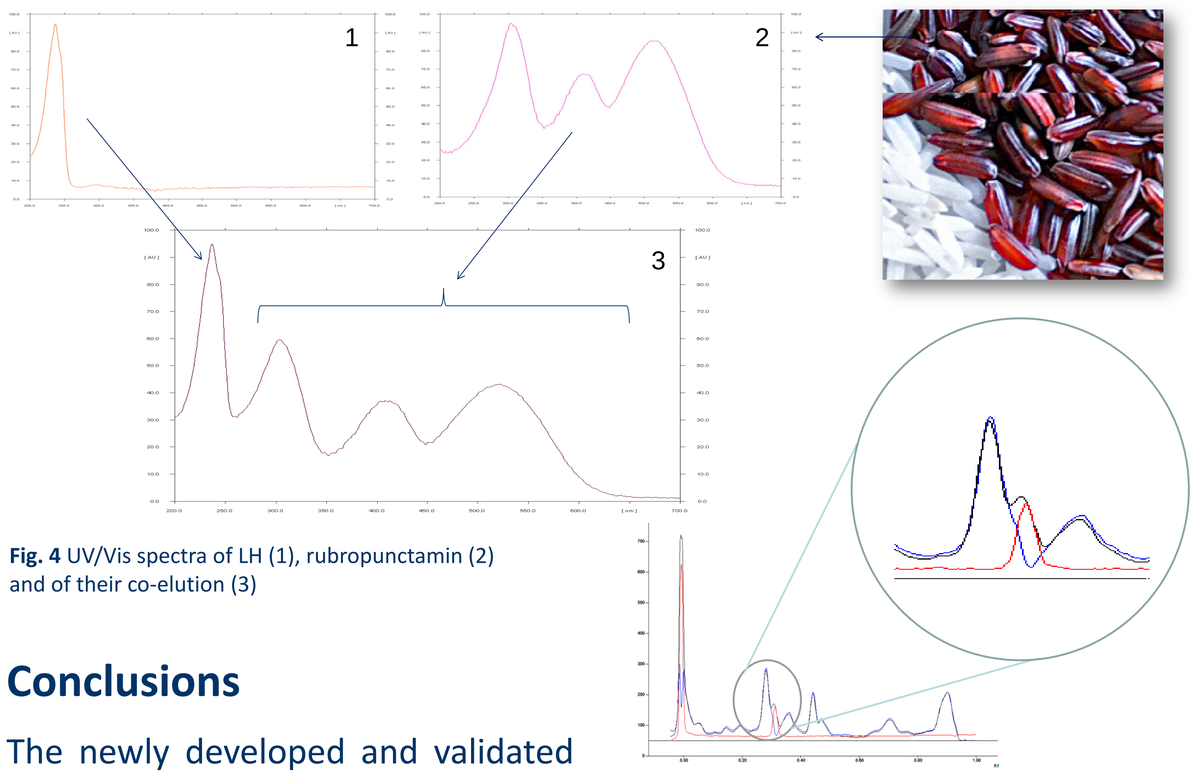

Fig. 4 UV/Vis spectra of LH (1), rubropunctamin (2) and of their co-elution (3)

Fig. 3 Two-wavelength scan for background correction:
Measurement wavelength (238 nm)
Background correction wavelength (588 nm)
→ subtracted result

Conclusions

The newly developed and validated HPTLC method enabled the simultaneous quantification of LH, LL and citrinin in red rice samples after a minimal sample preparation.

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