CAMAG BIBLIOGRAPHY SERVICE PLANAR CHROMATOGRAPHY CBS 103 • September 2009

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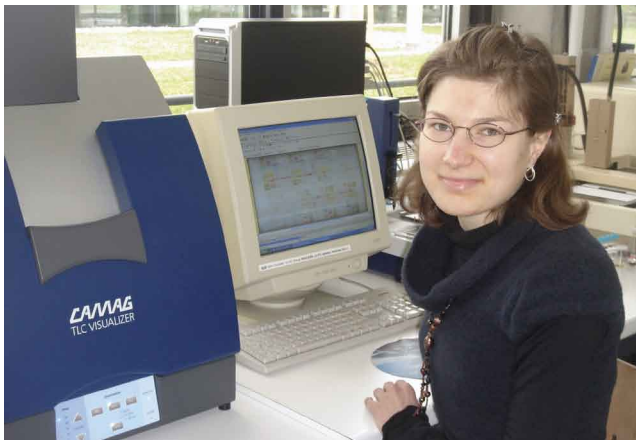
Topic: HPTLC for investigation of food for unauthorized dye additions

CAMAG 103

Analysis of water-soluble food dyes



7

Claudia Oellig

At the Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany planar chromatography is used for food analysis due to its advantages. For her diploma thesis Claudia Oellig employed the technique for the analysis of water-soluble food dyes.

Introduction

In the endeavour to enhance food safety the number of approved food colorings has been dramatically reduced in recent years. The approximately 40 food colorings remaining are being regulated through EG94/36 which specifies their use and the maximum permissible amounts. Rapid quantitative methods are necessary to ensure strict adherence to these regulations, since some food colorings are considered to be carcinogenic. Previous TLC/HPTLC methods were generally aimed at and somewhat limited to separating 9 to 12 food dyes. However, this study focused on the much more daunting task of developing a method for the separation of the most frequently used water-soluble food dyes, not 9–12 but up to 25.

Compared to existing methods for analysis of food dyes, the new HPTLC method is a reliable, rapid and cost-effective quantitative alternative [1–3]. It allows a high sample throughput of 1000 runs/day at moderate costs with an overall time per run of 1.5 min and a solvent consumption of 200 µL. As the analytical objective becomes clear, the basis for interpretation can be chosen from visual inspection of the plate to spectral correlation of the absorbance spectra to HPTLC-ESI/MS spectra. The analyst can proceed as far as necessary.

Sample preparation

Commercially available food samples were just diluted with methanol – ammonium acetate buffer (pH 6.8) 1:1 and, if necessary, degassed.

Standard solutions

The dyes were dissolved in methanol – ammonium acetate buffer (pH 6.8) 1:1 in the following concentrations:

Mix 1			Mix 2			Mix 3		
Dye	Concentr. [ng/µL]	hR_F	Dye	Concentr. [ng/µL]	hR_F	Dye	Concentr. [ng/µL]	hR_F
E 100	30	93	E 103	50	86	E101	30	72
E 101b	45	5	E 104	100	55	E102	20	19
E 110	20	57	E 120	70	0	E 105	25	53
E 122	20	71	E 121	125	93	E 129	15	60
E 124	15	27	E 123	8	25	E 133	8	26
E 126	30	10	E 125	60	72	E 141Na	860	86
E 127	10	93	E 151	15	15	E 141Cu	200	97
E 131	10	40				E 163	300	0
E 132	200	0						
E 142	8	23						

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 x 10 cm, prewashed by chromatography with methanol – water 4:1

Sample application

Bandwise with Automatic TLC Sampler 4, 18 tracks, band length 7.5 mm, track distance 9 mm, distance from the side 24 mm, distance from lower edge 8 mm (5 mm for development from both sides), application volume 2 µL (samples) and 1-4 µL (standard mixture solutions)

Chromatography

In the twin-trough chamber with 8 mL ethyl acetate – methanol – water – acetic acid 65:23:11:1, migration distance max. 50 mm, migration time 12 min; alternatively the ADC2 or, especially for a high sample throughput, the HDC was used.

Documentation

With TLC Visualizer at UV 254, UV 366 nm and white light illumination

Densitometry

- Digital image evaluation by VideoScan software (Savitsky Golay filter width of mostly 7 or 9, lowest slope as baseline correction mode and different electronic filters for evaluation) or
- Evaluation by TLC scanner 3 and winCATS Software, absorbance measurement via multi wavelength scan at 11 different wavelengths [1]

Spectra recording (Vis, MS)

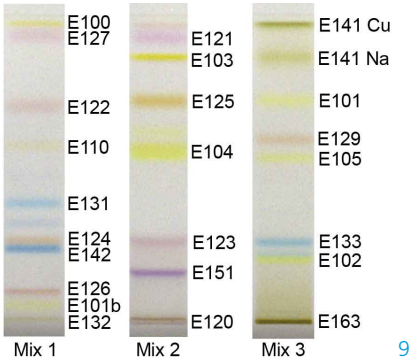
- Recording of Vis-spectra (400–800 nm) and calculation of the spectra correlation (sample versus standard) with TLC Scanner 3 and winCATS software or/and
- Recording of HPTLC/ESI mass spectra using a prototype of the TLC-MS interface (extraction solvent methanol, flow rate 0.2 mL/min)

Results and discussion

Separation on silica gel plates was best using ethyl acetate – methanol – water – acetic acid 65:23:11:1 as mobile phase. The 1%-acid concentration was crucial to obtain sharp zones. Referred to other literature, the food dyes were divided into dye mixtures for improved quantification.

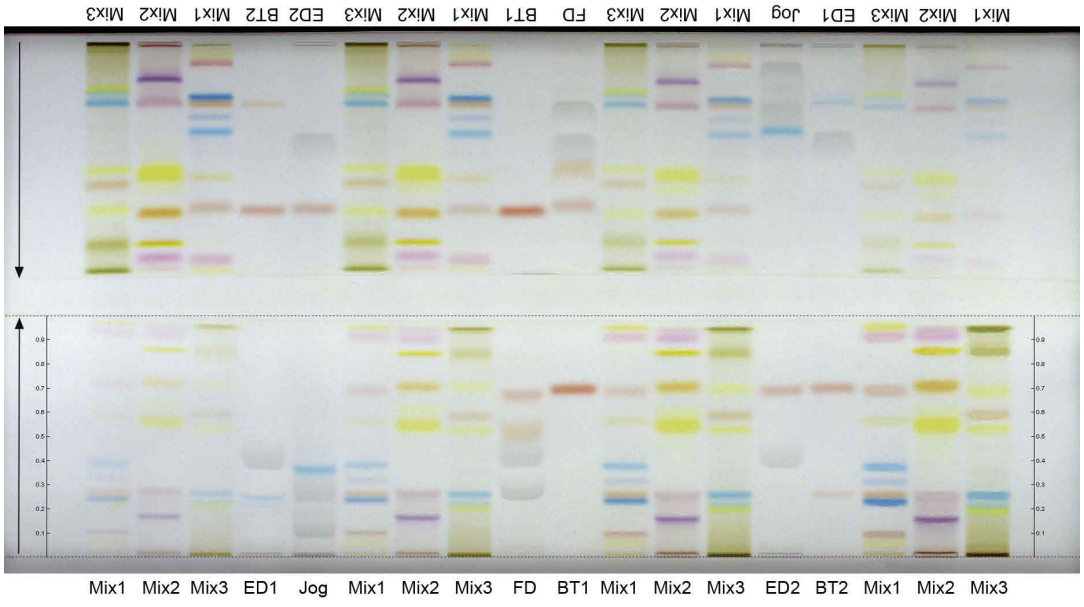
For a migration distance of max. 50 mm, the required separation time was 12 min. From both sides, 36 runs were developed isochronally under identical conditions. If calculated per sample, the development time was 20 s and the solvent consumption 220 µL; disposal costs were significantly below 0.01 Cent. The analysis time (incl. sample preparation, sample application and digital image evaluation) was 1.5 min which is exceptional even in this age of ultra-rapid chromatographic methods.

At a high matrix load, area application and/or dilution of the sample as well as a powerful digital image evaluation was precondition. In the infrequent case of dyes found in samples at hR_F-value 0 (E120, E132, E163), the plate was developed again in a mobile phase of a slightly higher elution power, e.g. in the ratio 45:35:18:2 for a twofold development over 1 cm (6 s each). Quantification was performed by absorbance measurement using the multi wavelength scan in the UV/Vis-range or by digital quantification of the plate image.



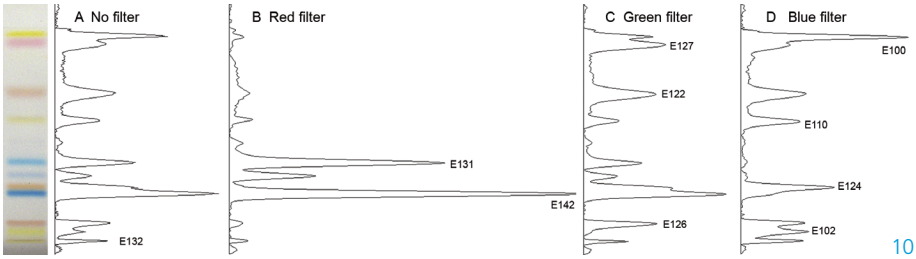
9

Separation of 25 water-soluble food dyes divided into 3 mixtures (partially dyes were just 50 or 85 % pure)

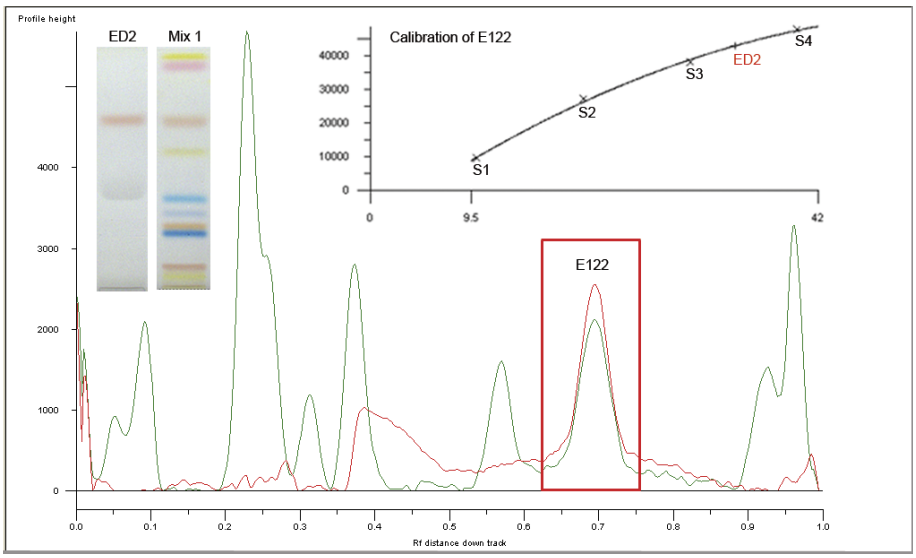


8

Analysis of 12 food samples (energy drink (ED), yoghurt (Jog), fruit drink (FD), bakery ink formulation (BT)) for 25 water-soluble food dyes by anti-parallel development in 12 min



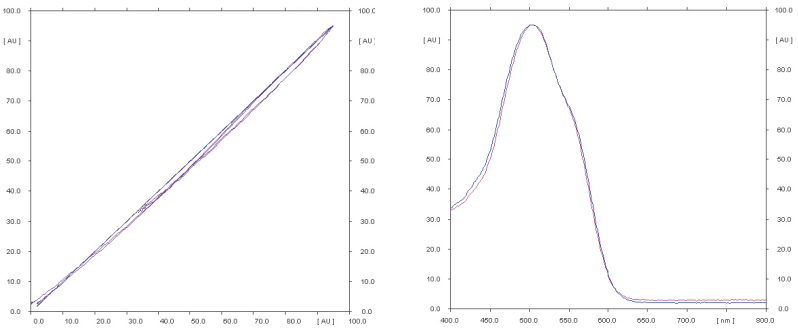
10 *Digital image evaluation using electronic filters (B - D) for improved post-chromatographic resolution of dyes (Mix 1)*



11 *Digital image evaluation (without filter) of the diluted energy drink sample 2 (ED2), which contained the red dye E122 (Mix 1); overlay of the sample curve (red) and Mix 1 (green) as well as polynomial calibration function (peak area)*

Depending on the task at hand, analysis procedures can be selected as required, from visual inspection of the plate, to review of recorded absorbance spectra to MS. Hence, the offline system is optimal in that it provides cost savings, and has the flexibility of handling a large sample throughput.

Sample	Dyes found	Concentration calculated	*%RSD* (n = 2)	Identity	
				Spectra correlation (400–800 nm) of standard and sample	Mass signal(s) (full scan, *m/z* 100 – 900)
Bakery ink formulation	122	66.4 g/L	0.0	≥ 0.99996	228 $[M-2Na]^{2-}$
	124	13.3 g/L	2.1	≥ 0.99957	279 $[M-2Na]^{2-}$
					178 $[M-3Na]^{3-}$
Energy drink 1	133	9.1 mg/L	0.1	≥ 0.99964	373 $[M-2Na]^{2-}$
Energy drink 2	122	76.2 mg/L	3.6	≥ 0.99958	228 $[M-2Na]^{2-}$



12 *Correlation of Vis-spectra of standard zone E122 and the respective zone in energy drink 2*

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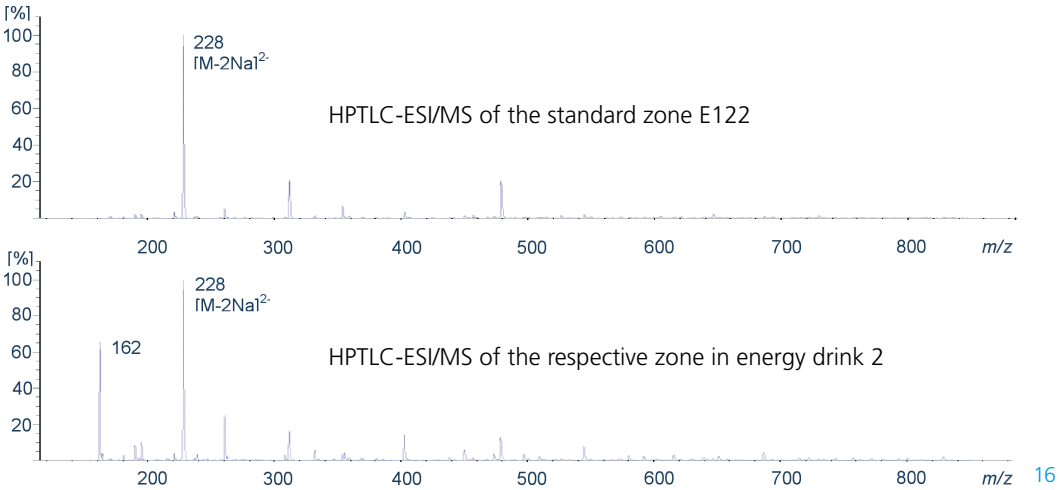

16

HPTLC-ESI/MS spectra of standard zone E122 (m/z 228 $[M-2Na]^{2-}$) and the respective zone in energy drink 2 (m/z 162 from matrix, chromatography without acetic acid)

	HPLC [4]	**HPTLC**
Mobile phase	0.58	0.003
Stationary phase	0.64	0.11
Disposal	0.04	0.0001
Operating costs/run (€)	**1.26**	**0.11**
		=>11 x lower
Application/Injection		0.50
Run time	43	0.20
Detection		0.10
Time/run (min)	**43**	**0.80**
		=> 54 x faster
thereof labor time/40 runs	none	5 min

Further information is available on request from the author.

[1] G. Morlock, C. Oellig, J AOAC Int 92 (2009) 745

[2] G. Morlock, W. Schwack, Die Aktuelle Wochenschau der GDCh (2009) week 21 and 26, www.aktuelle-wochenschau.de/index09.htm

[3] G. Morlock, W. Schwack, GIT 9 (2009) 489–492

[4] K. Minioti et al., Anal Chim Acta 583 (2007) 103

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