Cost-Effective and Rapid Quantification of 25 Water-Soluble Dyes in Food by HPTLC

C. Oellig and G. Morlock

Institute of Food Chemistry, University of Hohenheim

Scope

In recent years the number of food colourings approved has been dramatically reduced for reasons of food safety. The approximately 40 food colourings are being regulated through EG94/36 which specifies their use and the maximum quantities allowed. Rapid quantitative methods are necessary to ensure correct food additions, which is especially important as some food colorings are thought to be carcinogenic. Previous TLC/HPTLC methods were generally aimed at separating 9 to 12 food dyes. Thus, this study focused the development of a rapid planar chromatographic method for the separation of as many water-soluble food dyes as possible, especially frequently used ones. Important aspects were high sample throughput, time efficiency and cost effective quantitative analysis.

Results and discussion

The best chromatographic separation of 25 water-soluble dyes (Fig. 1) was performed on HPTLC plates silica gel 60 F_{254} using ethyl acetate – methanol – water – acetic acid 65/23/11/1 (v/v/v/v) as mobile phase and a migration time of **12 min**. The sample throughput could be doubled by anti-parallel chromatography in the horizontal development chamber (up to 36 runs at one go).

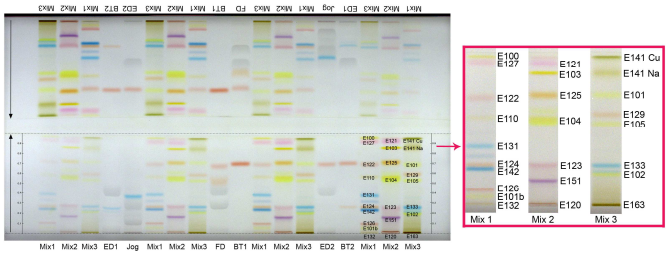

Fig. 1 HPTLC analysis of energy drinks (ED), yoghurt (Jog), fruit juice drink (FD) and bakery ink formulations (BT) for 25 water-soluble dyes grouped into 3 mixtures (Mix 1-3)

Quantification was performed by either digital image evaluation (Fig. 2) or absorption measurement using the multi-wavelength scan at eleven different wavelengths in the UV/Vis range (Table 1). Correlation coefficients ($R \geq 0.9987$) and relative standard deviations (*%RSD* ≤ 4.2 %) of the 4-point calibration curves were highly satisfying. Repeatabilities (*%RSD)* at the first or second calibration level were mostly ≤ 2.7 %, ranging between 0.2 and 5.2 %.

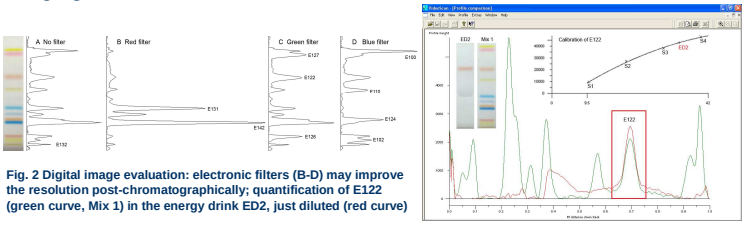

Fig. 2 Digital image evaluation: electronic filters (B-D) may improve the resolution post-chromatographically; quantification of E122 (green curve, Mix 1) in the energy drink ED2, just diluted (red curve)

Table 1 Data of classical quantification by TLC-Scanner 3 (CAMAG)

Dye	Concentration [ng/µL]	hR_F-value	Wavelength [nm]	S/N*	Calibration range [ng/band]	4-Point calibration function	Linearity R	Linearity *RSD*, %
Mixture 1								
E100	31	87	293	181	61 - 427	$y = 79.12 + 0.4816x - 2.194*10^{-4}x^2$	0.99937	2.0
E101b	46	74	262	32	91 - 640	$y' = 519.6 + 6.822x - 2.347*10^{-3}x^2$	0.99995	0.8
E110	18	60	468	11	36 - 249	$y' = 100.5 + 13.39x - 0.1574\ x^2$	0.99943	3.0
E122	21	68	485	28	42 - 293	$y = 7.61 + 1.173x - 0.001x^2$	0.99910	3.9
E124	16	21	485	21	32 - 225	$y = -0.66 + 1.09x - 0.001x^2$	0.99997	0.8
E126	32	6	485	28	64 - 446	$y = 7.67 + 0.9196x - 7.155*10^{-4}x^2$	0.99909	3.8
E127	9	81	354	93	18 - 123	$y = 24.86 + 4.77x - 0.0138x^2$	0.99871	4.2
E131	8	35	626	12	17 - 118	$y' = 300.5 + 92.13x - 0.1548x^2$	0.99967	2.5
E132	105	0	597	12	210-1470	$y = -785.45 + 6.032x - 2.0*10^{-3}x^2$	0.99928	4.2
E142	9	20	597	20	18 - 123	$y' = 168 + 31.95x - 0.0717x^2$	0.99921	3.5
Mixture 2								
E103	50	78	397	89	100-700	$y = 181.3 + 13.23x - 1.9*10^{-3}x^2$	0.99997	0.5
E104	342	48	397	58	684 - 4787	$y = -28.55 + 0.176x - 1.87*10^{-5}x^2$	0.99948	2.3
E120	67	0	293	81	134 - 939	$y' = -345.5 + 10.83x - 4.57*10^{-3}x^2$	0.99999	0.4
E121	124	88	485	14	248 - 1734	$y' = -22.33 + 3.11x - 3.335*10^{-4}x^2$	0.99999	0.5
E123	8	23	533	15	16-112	$y = 9.89 + 0.235x - 3.8*10^{-4}x^2$	0.99952	1.8
E125	73	59	485	65	146 - 1021	$y' = 359.2 + 10.85x - 3.69*10^{-3}x^2$	0.99966	2.2
E151	15	14	533	33	30 - 208	$y' = 147.9 + 29.68x - 0.0448x^2$	0.99891	4.2
Mixture 3								
E101	28	63	262	13	56 - 392	$y' = 18.094 + 8.845x - 0.001x^2$	0.99997	0.7
E102	17	15	397	35	34 - 240	$y = 10.83 + 1.568x - 2.264*10^{-3}x^2$	0.99992	1.1
E105	50	46	397	15	100-700	$y = 103.6 + 1.356x - 0.001x^2$	0.99994	0.5
E129	15	53	485	29	30 - 209	$y' = 592.4 + 27.93x - 5.48*10^{-3}x^2$	0.99998	0.6
E133	8	23	626	24	16 - 112	$y = 14.46 + 3.44x - 0.011x^2$	1	0.2
E141Na	870	75	417	20	1741 -12184	$y' = 366 + 3.114x - 2.91*10^{-5}x^2$	0.99989	1.6
E141Cu	217	90	417	32	434 - 3035	$y = 16.7404 + 36.66x - 0.898x^2$	0.99999	0.1
E163	440	0	417	62	880 - 6160	$y = 45.731 + 0.123x - 8*10^{-6}x^2$	0.99991	1.0

*Signal-to-noise ratio via peak height at first level of calibration
y: Quantification via peak height, y': quantification via peak area

The developed HPTLC method was exemplarily applied for energy drinks, fruit juice drinks and bakery ink formulations. In the samples, the dyes E122, E124 and E133 were found and analyzed (Fig. 3, Table 2).

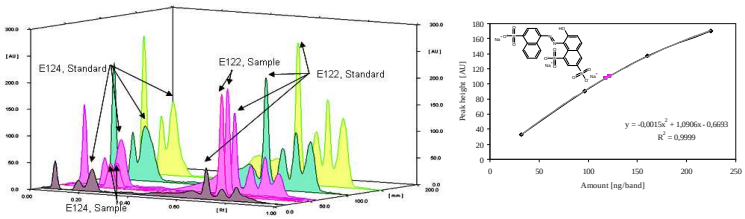

Fig. 3 Absorbance scans for determination of E122 and E124 found in the red bakery ink formulation; calibration function for E124, exemplarily shown

Table 2 Results of sample analysis

Sample	Dye found	Amount found	*% RSD* (n = 2)	Identity by: Spectral correlation of standard and sample spectra (400 - 800 nm)	Identity by: MS spectra (full scan, *m/z* 100-900, negative ESI mode)
Bakery ink formulation	122 124	66.4 g/L 13.3 g/L	0.0 2.1	≥ 0.99996 ≥ 0.99957	228 $[M-2Na]^{2-}$ 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 $[M-2Na]^{2-}$

Identification was confirmed by recording of absorbance spectra (400 - 800 nm) or HPTLC-ESI/MS spectra (Fig. 4).

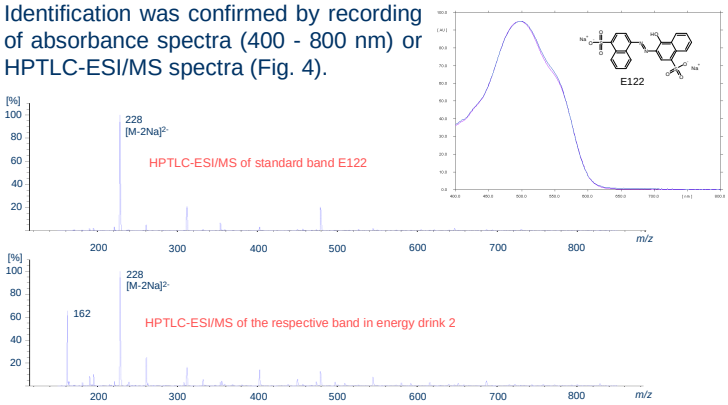

Fig. 4 Spectral comparison in the visible range (r = 0.9999) of the red-colored band in energy drink sample 2 and the standard band E122 as well as mass spectra (full-scan *m/z* 100-900, negative mode) showing *m/z* 228 $[M-2Na]^{2-}$ (mobile phase without acetic acid, *m/z* 162 is a matrix signal)

Conclusion

Compared to existing methods for analysis of food dyes, the new HPTLC method is a cost-effective, reliable and rapid quantitative alternative. It allows a sample throughput of **1000 runs/day** at low e costs with an overall time per run of **1.5 min** at a solvent consumption of **200 µL**. The analytical effort can gradually be chosen according to necessity from visual inspection to spectral correlation of the absorbance spectra or mass spectra.

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Literature: G. Morlock, C. Oellig, *J. AOAC Int.* 92 (2009) 745-756; G. Morlock, W. Schwack, *Die Aktuelle Wochenschau der Gesellschaft Deutscher Chemiker (GDCh),* week 26 (2009); http://www.aktuelle-wochenschau.de/index09.htm