SEPARATION OF SYNTHETIC FOOD DYES BY ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Introduction

Because of the increasing use of synthetic dyes in food, pharmaceutical, and chemical industry, their estimation in various products is becoming common in analytical laboratories.

In the past, paper [1], column [2], and thin-layer chromatography (TLC) [3], electrophoresis [4], and spectrophotometry [5] have been employed in dyes analysis. So far, the separation of dyes by means of column chromatography and subsequent spectrophotometric and TLC identification has been officially registered [6]. In recent years, high-performance liquid chromatography (HPLC), due to its high resolution power and good detection ability, has surpassed the other techniques. Several papers have been published where HPLC was used for dye analyses [7 - 13]. Most of the synthetic dyes can be successfully chromatographed by HPLC with ion-exchange stationary phases [7-9], but as shown the gradient elution with the high content of counter ion in the mobile phase may provide their satisfactorily separation on the reversed-phase column [10,13].

The aim of the present study was to investigate the ability of use a simple isocratic ion-paired reversed-phase HPLC method to separate nine commonly used dyes.

Experimental

The used dyes were E102 - Tartrazine, E110 - Sunset yellow FCF, E122 - Azorubin, E123 - Amaranth, E124 - Ponceau 4R, E127 - Erythrosine, E131 - Patent blue V, E132 - Indigocarmine, E151 - Black PN.

The HPLC system consisted of a Model SP8810 pump, a Spectra200 detector (both from Spectra-physics), a Rheodyne 7125 injector fitted with a 20 µl sample loop, and Varian Star 4.5 Chromatography workstation.

The sample handling involves dissolution of the dye mixtures with the mobile phase (MF) followed by a filtration step and subsequent analysis of the filtrate by HPLC.

The food dyes were separated from each other as well as from ingredients in the sample matrix on a reversed phase octadecyl silica column (Supelcosil® LC-18, 100 Å, 5 µm, 250 x 4.6 mm ID), proceeded with the same type guard column. Two

separated runs, (a), and (b) with different MF compositions: (a) 40 vol. % CH_3OH, 0.001 M tetrabuthyl ammonium hydroxide(TBA), 0.001M NaH_2PO_4, H_3PO_4, pH = 6.0, and (b) 60 vol. % CH_3OH, 0.001 M TBA, 0.001M NaH_2PO_4, H_3PO_4, pH = 6.0, were used for isocratic elution (1.0 ml/min flow-rate) of four dyes per run, and E123 dye was extra chromatographed.

Table 1. The λ, t_R , and S of analysed dyes (see the text).

Dyes	λ	t_R	S
E102	426	4.06	40.2
E110	482	8.46	5.0
E122	525	2.86	28.5
E123	522	2.34	19.9
E124	510	15.04	20.0
E127	530	11.94	22.4
E131	636	4.22	30.1
E132	620	2.43	27.1
E151	576	31.05	3.6

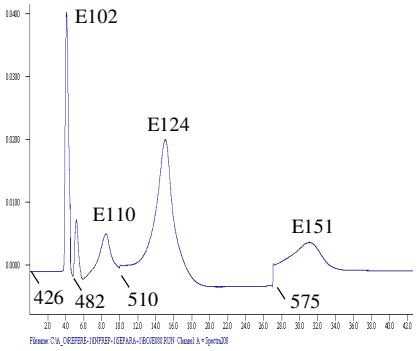

Fig. 1. A chromatogram of four food dyes. Wavelength changes were made as indicated. MF (a).

Results and discussion

Table 1 presents the E-numbers of the analysed dyes, their wavelengths at maximum absorption, λ (nm), retention times, t_R (min), and detector sensitivity, S ($\mu V g^{-1} m^3$).

E102, E110, E124, E151, were chromatographed with MF (a), for E122, E127, E131, E132, and E123, MF (b) was used. However, due to co-elution of E132 and E123, E123 was injected separately. The chromatograms are shown in Figs. 1. and 2.

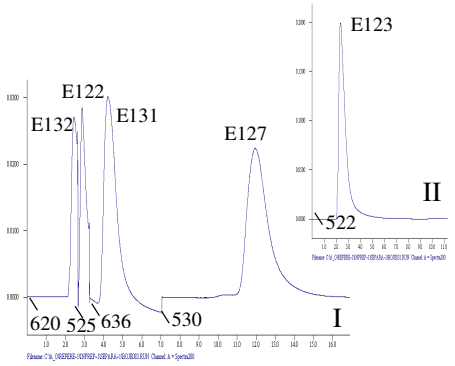

Fig. 2. A chromatogram of four food dyes (I), and of E123 dye (II), obtained from separate runs. Wavelength changes were made as indicated. MF (b)

It should be pointed out that the programmable wavelength detection provides a better sensitivity of the method. Wavelengths in the visible part of spectra eliminate many of the UV absorbing ingredients present in the dyes formulations.

During the experiment optimization, the influence of MF compositions on the separation was thoroughly examined. The content of metanol in MF of 80 vol. % results in no retention of all dyes on the column used. Otherwise, with 20 vol. % of metanol, the elution of most dyes was disabled. A change in pH of MF causes hogher dye retentions at lower pH (pH~3), except for E122, and E131, with the

highest t_R at intermediate pH (pH~4.5). Better separation was obtained when higher content of TBA in MF was used. Twice as larger average capacity factors were obtained for 0.0015 M TBA compared to 0.001 M TBA. However, the difficulties with the column clean-up forced us to keep the TBA amount in MF as low as possible.

Conclusion

In general, nine food dyes can be assayed using the isocratic elution on common 5 μm C18 column, but the separated injections, together with wavelength programming are needed. The described HPLC method is faster and less tedious than the TLC-spectrophotometric technique, and more simple than gradient elution, but the column with reduced diameter and particles size is needed in order to eliminate multiple runs.

Reference

[1] The Analytical Chemistry of Synthetic Dyes, K. Venkataraman, ed., Wiley, New York, 1977.
[2] R. Patel, M. Patel, A.A. Patel, A. Shah, A.G. Patel, Analyst, 111 (1986) 577.
[3] N.T. Crosby, IARC Sci. Publ., 40 (1981) 337.
[4] D.-B. Yeh, J. Chromatogr., 132 (1977) 566.
[5] C. Graichen, J. Assoc. Off. Anal. Chem., 58 (1975) 609.
[6] AOAC 988.13, FD&C Color Additives in Foods, Official method of analysis of AOAC Int., 16th ed., Arlington, USA, 1995.
[7] J. Chady, N.T. Crosby, I. Patel, J. Chromatogr. 154 (1978) 306.
[8] M.C Gennaro, C. Abrigo, G. Cipolla, J. Chromatogr. 674 (1994) 281.
[9] Q. Chen, S. Mou, X. Hou, J. Riviello, Z. Ni, J. Chromatogr., 827 (1998) 73.
[10] A. Hüesgen, R. Schuster, Hewlett Packard Appl. Note, 5964-3559E, 1995.
[11] N. Boley, N. Bunton, N. Crosby, A. Johnson, P. Roper, L. Somers, Analyst, 105 (1980) 589.
[12] J. Lawrence, F. Lancester, H. Conacher, J. Chromatogr., 210 (1981) 168.
[13] J. Berzas-Nevado, C. Guiberteau-Cabanillas, A. Contento-Salcedo, Anal. Lett., 31 (1998) 2513.

Apstrakt

Rađena je analiza devet veštačkih prehrambenih boja (E102, E110, E122, E123, E124, E127, E131, E132, E151), metodom reverzno-fazne tečne hromatografije, sa reagensom u funkciji jonskog para (tetrabutil amonijum hidroksid), u mobilnoj fazi. Razdvajanje boja je izvođeno na oktadecil silika koloni, sa detekcijom uz programiranje talasnih dužina, u oblasti λ= 426 - 636 nm. Dva izokratska eluiranja sa različitim sastavom mobilne faze, omogućuju razdvajanje po četiri različite boje po jednom hromatogramu. Boja 123 je posebno hromatografisana zbog koeluiranja sa E132.