OPTIMIZATION OF HPLC METHODS FOR SIMULTANEOUS ANALYSIS OF FOOD COLORS

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Abstract– Optimization of HPLC methods in reverse-phase and reverse-phase ion-pair mode for simultaneous determination of 10 synthetic food dyes with similar chemical structure has been studied. The separation of food dyes were performed on stationary phases: LiChrosorb RP-18, Purospher STAR RP-18e and Chromolith RPe.

Two different types of mobile phases were used, the first one with presence of tetra-bytilammonium salt as ion-pair reagent and another without its presence. A gradient method was choosing as a better solution for separation of food dyes with similar chemical structure so four gradient programs were studied for that purpose. With selection of optimal extreme gradient conditions, simple and fast reverse-phase chromatographic method was obtained for simultaneously determination of dyes.

There are more benefits when reverse-phase mode is used, such as shorter equilibration time for stationary phase and more stable retention time of food synthetic dyes.

Keywords: food dyes; gradient elution, tetra-bytilammonium salt

1. INTRODUCTION

Food pigments have been used for centuries to keep or to improve food or drink colour. They can be natural or synthetic. However during the manufacturing and storage synthetic food colours are more stable than the natural ones, thus they are commonly used in different foodstuffs. From the other side the used of synthetic pigments in food have adverse toxicological effects for human health, and for that reason they are considered as unhealthy substances for consumption [1].

Consequently, accurate and reliable methods for determination of synthetic colorants are required for food safety assurance. Many analytical techniques have been developed for identification and quantification of various synthetic colorants such as thin-layer chromatography, derivate spectrometry and adsorptive voltammetry but unfortunately they required time-consuming pretreatment or cannot be used for complex colorant mixtures. Reverse-phase and reverse-phase ion-pair liquid chromatography [1-8] with isocratic or gradient elution are the most preferred methods for that purpose.

However, there is still interest to develop simple and cheap method for simultaneous determination of synthetic colors. The purpose of this paper is development of reverse-phase HPLC method for simultaneous determination of azo compounds (E102, E110, E122, E123, E124, E129, E151), xanthene dye (E127) and indigo colorant (E131, E133) in a short time.

2. EXPERIMENTAL

2.1. Reagents and solutions

All solutions were prepared with deionized water and all chemicals were of analytical grade reagent. Tetra -butylammonium hydrogensulfate as an ion pair reagent and HPLC grade methanol were obtained from Aldrich. Potassium dihydrogen phosphate for mobile phase preparation was supplied from Merck. The colorants: Tartrazine E102, Sunset Yellow FCF E110, Azorubin S E122, Amaranth E123, Poncho 4 P E124, Eritrozin P127, Allura Red AC E129, Brilliant Black BN E151, Patent Blue V E131 and Brilliant Blue E133 were obtained from Etol, Slovenia. Purities of colorants were determined according to the spectrophotometric

method [4]. Specific absorption coefficient $A_{1cm}^{1\%}$ is reported in the regulation 95/45/EC [9].

2.2. Instrumentation

Chromatographic analysis was carried out with a Shimadzu liquid chromatography equipped with Diode Array Detector model SPD – M20A. Spectrophotometer Perkin Elmer UV/VIS Lambda 12 was used for all spectrophotometric measurements. A pH – meter 730 WTW was employed for pH measurements.

2.3. Chromatographic condition

For development of the chromatographic methods LiChrosorb RP-18 (250 mm x 4 mm, 5μm), Purospher STAR RP-18e (125 mm x 4 mm, 5μm) and Chromolith RPe (50-4,6 mm) columns supplied from Merck were used as stationary phase.

Absorption spectra of each dyes was selected from the maximum absorbance in visible range at 427 nm for the yellow color E102, 484 nm for the orange E110, 518nm red colors E122, E123, E124, E127, E129, 570 nm for E151 and 635 nm for the blue color E131 and E133.

Two different mobile phases for elution, with and without ion-pair reagent was used. There was difference in presence of ion-pair reagent, only.

2.4.Mobile phase

In this paper efficiency of separation of food colors with presence of ion pair-reagent and without its presence is described. For that purpose two different puffers were used for preparation of mobile phase: puffer 1 and puffer 2. The puffer 1 with ion-pair reagent is prepared by weighing 6.8 g tetra-butylammonium hydrogensulfate as a ion pair reagent, 3.12 g potassium dihydrogen phosphate and dissolving the mixture in 1 L water. The puffer 2 was prepared without ion-pair reagent by weighing only 3.12 g potassium di-hydrogen phosphate and dissolving in 1 L water.

The pH of the puffer 1 and puffer 2 was then adjusted to 6.5 by addition of 2 M sodium hydroxide solution. The mobile phase for reverse-phase ion-pair liquid chromatography is consisted of Solvent A1 and Solvent B1. Solvent A1 was prepared by mixing one volume of the puffer 1 and three volumes of water (buffer 1/water (1:3, V/V). Solvent B1 is organic phase and was prepared by mixing one volume of the puffer1 and three volumes of methanol (buffer 1/methanol (1:3, V/V).

The mobile phase for reverse-phase liquid chromatography is consisted of Solvent A2 and Solvent B2. Solvent A2 was prepared by mixing one volume of the puffer 2 and three volumes of water (buffer 2/water (1:3, V/V), Solvent B2 was prepared by mixing one volume of the puffer2 and three volumes of methanol (buffer2/methanol (1:3, V/V). The flow rate of the eluent was kept constant at 1 ml min-1. Injection volume was set at 10 μL.

2.5. Gradient programs

A gradient method for separation of food dyes with a similar chemical structure was used as a better solution for determination of food dyes. In this paper four gradient programs are presented.

Gradient elution 1, 42:58 % (V/V) solvent A1 : solvent B1 to 15:85 % (V/V) solvent A1 : solvent B1, 15 min.

Gradient elution 2, 65:35 % (V/V) solvent A2 : solvent B2 isocratic for 5 min, to 10:90 % (V/V) solvent A2 : solvent B2, 15 min.

Gradient elution 3, 90:10 % (V/V) solvent A2 : solvent B2 isocratic for 5 min, to 10:90 % (V/V) solvent A : solvent B, 15 min.

Gradient elution 4, 95:5 % (V/V) solvent A2 : solvent B2 to 5:95 % (V/V) solvent A2 : solvent B2, 10 min.

3. RESULTS AND DISCUSSION

In this paper many experiments were performed to obtain an efficient separation by means of reverse-phase mode. As a target colors were selected ten colors E102, E110, E122, E123, E124, E127, E129, E151, E133 and E131. From the preliminary investigation in the Macedonian markets E129 and E133 are the most frequent colors used in food industry.

3.1. Reversed-phase ion-pair liquid chromatography

The method with a presence of an ion-pair reagent in mobile phase was used as a referent method [7]. Mobile phase is consisted of Solvent A1 (puffer1/water (1:3, V/V)) and as an organic phase Solvent B1 (puffer1/methanol (1:3, V/V)). Gradient elution 1 was performed. Methanol is used as the organic component in the mobile phase because of its lower cost, even though the literature data found that acetonitril or added acetonitril to methanol significantly improves the symmetry of the peaks.

Under gradient conditions mention above, separation of a mixture of dyes can be achieved but with unstable retention of the colors and without E127 determination. There is no possibility to determine the color E127 under these conditions since it has no tendency to form ion-pair. One of the features for reverse phase ion-pair chromatography when RP column is used is unstable retention. Inter-day reproducibility of retention was unsatisfactory, also. In this case the utilization of diode-array detector is fundamental in identification of synthetic dyes by means of their spectra. However aim of this examination is receiving stable and different retention time for each color that is basic identification parameter in liquid chromatography. This is particularly important in colors with similar structure and UV/Vis spectra as some red colors as E122, E123, E124 and E129 (Figure 1).

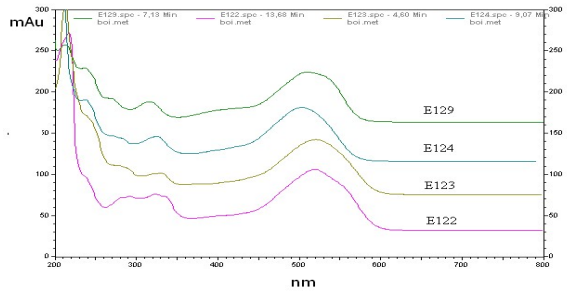

Figure 1 UV-VIS Spectra of the red colours E122, E123, E124 and E129

In order to obtain more stable retention time endcapped column is preferred for ion-pair reverse-phase chromatography, so in further examination Purospher STAR RP-18e (125 mm x 4 mm, 5 μm) is used.

The retention time on Purospher STAR RP-18e, 125 mm was less for insignificant two minutes than those on LiChrosorb RP-18, 250 mm. Elution order for E131 and E127 is different for Purospher and LiChrosorb column.

3.2. Reversed-phase liquid chromatography

At the next stage of the examination separation of dyes was performed without presence of the ion-pair reagent. Solvent A2 (puffer2 /water (1/3, V/V), Solvent B2 (puffer2/methanol (1/3, V/V) and the same gradient condition was used for separation of target group of food colors. Under these conditions most of the colors E102, E110, E123, E124, E122 and E129 are eluted close to the third minute. Under the same condition, successful separation for E127 and E131 was achieved. In order to get better separation for the colors that eluted until third minute, gradient program was changed by increasing the share of the water phase on the initial conditions up to 65 %. Elution was isocratic in the first five minutes since colors exhibited different hydrophobicity. The amount of methanol in the mobile phase strongly affected retention time of the hydrophobic colors, so share of methanol is increasing up to 90 %, in order to receive shorter retention time for more hydrophobic colors. This change did not achieve a significant improvement for separation of the color that eluted until third minute. The result showed the retention of colors E127 and E131 are increased up to 17 min (Figure 2)

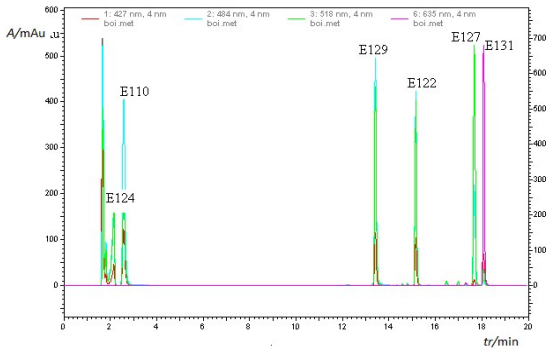

Figure 2 Chromatogram of colours on stationary phase Chromolith RPe (50-4.6 mm)

Main features of the colors that should be considered for effective separation are their hydrophobicity and the presence of acidic and basic groups. Using Reversed-phase liquid chromatography some colors has a short retention time due to presence of sulfonic groups and in some cases carboxyl groups which increases water solubility. Affinity of the colors towards water increases, with increase in atomic masses of elements that form these groups.

To provide better separation of the less hydrophobic dyes there was conducted change again towards extremely gradient conditions by increasing the share of the water phase in the initial conditions up to 95 %. The amount of methanol in the mobile phase strongly affected retention time on the hydrophobic dyes, so the extremely gradient with the minimal share of organic phase in the beginning and maximal share in the end of the gradient will prevent early elution of the less hydrophobic dyes and long retention of more hydrophobic dyes. On the other hand many polar compounds are separate better with high percentage of an aqueous mobile phase and can be retained only with a minimal concentration of organic modifier. With gradient conditions 95:5 % (V/V) solvent A2: solvent B2 to 5:95 % (V/V) solvent A2: solvent B2 optimal separation was obtain for ten food colorants. Shorter retention time was obtained when Chromolith RPe (50-4.6 mm) is employed as a stationary phase. Optimal separation was achieved with flow on 3 mL/min. Calculated separation factors a for Chromolith column when flow of 3 mL/min is employed are: 1.73; 1.56; 1.24; 1.12; 1.15; 1.35; 1.12; 1.11 and 1.06. Determination of 10 food colours the most used in the food industry for a very short time from 10 minutes is shown in the Figure 3.

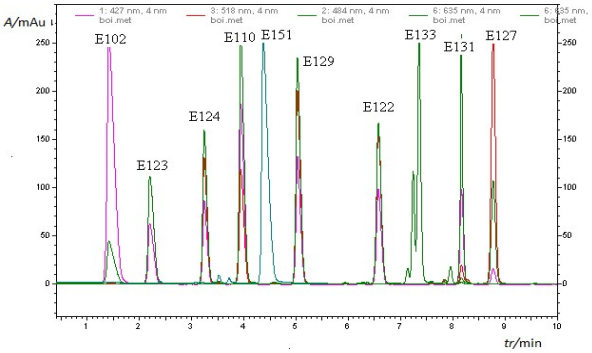

Figure 3 Chromatogram of 10 colours on stationary phase Chromolith RPe (50-4.6 mm)

Method with reversed-phase mode is optimized for separation of ten food colors by selection of optimal gradient conditions.

4. CONCLUSIONS

Benefit of working without ion-pair reagent is obtaining more stable retention time. On the other hand reverse-phase is optimal separation mode for determination of E127 with feature of the neutral molecule. In this paper is shown that retained of such kind of molecules is possible by reverse-phase mode when optimal gradient condition is used.

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