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Research Article

Simultaneous Determination of Synthetic Food Dyes Using a Single Cartridge for Preconcentration and Separation Followed by Photometric Detection

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A novel preconcentration/separation method for simultaneous sorption-spectrophotometric determination of anionic food dyes Sunset Yellow and Tartrazine is proposed. The method is based on preconcentration of the dyes using solid phase extraction on a cartridge filled with silica chemically modified with C16 groups from aqueous solution at pH 1 followed by elution with water/acetonitrile mixture containing 2 mmol·L^{-1} KH_2PO_4 adjusted to pH 3 with a step gradient of acetonitrile content. This elution allows quantitative separation of the dyes which makes their individual spectrophotometric determination possible. The detection limits for Tartrazine and Sunset Yellow are 0.15 and 0.11 μg·mL^{-1} and the linearity range is 2–20 μg·mL^{-1}. The method is applied for analysis of beverages. The recovery of dyes is higher than 97% at the relative standard deviation not exceeding 10%.

1. Introduction

Recently, more and more attention has been paid to the determination of synthetic organic dyes, which are widely used to color food or medicines [1]. The need to control the content of synthetic dyes is associated with their negative impact on the humans. Almost all of them are not harmless and have varying degrees of toxicity associated with allergic, carcinogenic, and mutagenic effects. Maximum permitted levels of food dyes are steadily decreasing and now have reached 0.01 g of a dye per 100 g of a foodstuff [2]. Therefore, their content in food products is strictly regulated and requires permanent monitoring.

High resistance of synthetic food dyes to photo- and biodegradation in the environment leads to their accumulation in natural waters. The negative impact of synthetic food dyes on living organisms, particularly humans, sets the task of developing simple and express methods for their determination. To date, various methods for food dyes determination have been reported including spectrophotometry [3, 4], high performance liquid chromatography [5], mass-spectrometry [6], and digital image analysis [7].

A key stage preceding determination of dyes is sample preparation, which is usually required for preconcentration of target analytes and removal of the matrix components. Solid phase extraction (SPE) is one of the most efficient methods of dye extraction from complex matrices. To extract anionic dyes, the application of various adsorbents such as wool [8], polyamide [9], alumina [10], nonpolar organo-polymer sorbents [11, 12], magnetic adsorbents [13, 14], and amino-functionalised nanosilica [15] has been reported. Previously, we had developed a method for determination of Sunset Yellow dye (SY), based on its sorption preconcentration using hydrophobic adsorbent hexadecylsilica (HDS) with direct detection of the dye in the phase of adsorbent by diffuse reflectance spectroscopy [16]. However, due to the similarity of absorbance spectra of Sunset Yellow (SY, food additive E110) and other food dyes, this method is not selective. This resulted in problems for analysis of samples containing a mixture of dyes, especially of Sunset Yellow and

Tartrazine (TAR, food additive E102) having absorption maxima at 427 and 484 nm, respectively.

Multicomponent mixtures of dyes can be analyzed by reversed-phase HPLC [9, 11, 17]. Although there is limited practical need in simultaneous determination of many synthetic dyes, the separation up to 40 dyes is possible by HPLC with gradient elution [18]. Obviously, this method requires complex analytical instrumentation and skilled operators. Another disadvantage of this method is associated with sample preparation, which increases analysis time and may result in incomplete recovery of analytes and possible errors in their determination.

In practice many beverages contain a mixture of two dyes, for example, Sunset Yellow and Tartrazine. Sunset Yellow contains two sulfo-groups and is less hydrophilic ($\log P = -1.18$ [19]) as compared with Tartrazine ($\log P = -7.18$ [19]) having two sulfo-groups and one carboxylic group in the molecule. Therefore, an application of a single SPE cartridge packed with hydrophobic adsorbent is possible for both preconcentration and separation of two synthetic dyes having different properties. The aim of this work was to develop simple sorption-spectrophotometric method for the determination of Sunset Yellow and Tartrazine using commercially available SPE cartridge packed with hexadecylsilica.

2. Materials and Methods

2.1. Reagents and Instruments. Tartrazine (Sigma-Aldrich, ≥85%) and Sunset Yellow FCF (Sigma-Aldrich, ≥90%) (Acros Organic, ≥95%) are used in this work. Stock solutions of the dyes (1.0 $g \cdot L^{-1}$) were prepared according to National Standard method (GOST R 52470-2005). Working solutions (0.1 $g \cdot L^{-1}$) were prepared by dilution of the stock solutions with deionized water.

Hydrochloric acid (chemically pure), sodium hydroxide solution (analytical grade), nitric acid (chemically pure), phosphoric acid (analytical grade), potassium hydrophosphate (analytical grade), potassium dihydrophosphate (analytical grade), sodium chloride (chemically pure), ethyl alcohol (chemically pure), ammonia solution (analytical grade), and acetonitrile (chemically pure) were also used.

Absorbance of the solutions and molecular absorption spectra of the dyes was recorded using an SF-103 spectrophotometer (Akvilon, Moscow, Russia) in an 1 cm glass cuvette. A pH meter Expert pH (Ekoniks Ekspert, Moscow, Russia) with a glass electrode was used to measure pH of solutions. When carrying out sorption in a dynamic mode, a Multiperpex peristaltic pump 2115 (LKB Bromma, Sweden) was used. Analytical scales, Voyager (OHAUS, Switzerland), with a weighing accuracy of ±0.0001 were used.

Polypropylene cartridges (40 × 5 mm ID) packed with hexadecylsilica Diasorb-130-C16 (BioKhimMak ST, Moscow, Russia) having specific surface area 250 $m^2 \cdot g^{-1}$, particle size 40–160 μm, and pore diameter 13 nm were used.

2.2. Separation of Dyes on a Cartridge. Sorption of dyes was carried out in a dynamic mode from an aqueous solution under optimal extraction conditions (pH 1) using a cartridge containing 0.5 g of the HDS sorbent. Before use, the sorbent in the cartridge was conditioned with 0.5 mL of ethanol. For preconcentration, 10 mL of standard solution ($c = 7.5\ \mu g \cdot mL^{-1}$) was passed through the cartridge using a peristaltic pump at the rate of 4-5 $mL \cdot min^{-1}$. Then, the eluent was passed at the rate of 1 $mL \cdot min^{-1}$ with collecting fractions and measuring optical density of the solution at the outlet from the cartridge at the adsorption maxima of dyes ($\lambda = 427$ nm for TAR and $\lambda = 484$ nm for SY). After desorption, the cartridge was repeatedly washed with distilled water.

3. Results and Discussion

The aim of this work was to develop the simple and inexpensive analytical procedure combining preconcentration, separation, and determination of anionic of two popular synthetic dyes. For this purpose, it was proposed to preconcentrate and separate the analytes on a single cartridge followed by spectrophotometric measurement of absorbance of the effluent as schematically shown in Figure 1. A peristaltic pump was used for sample loading and elution of preconcentrated dyes.

3.1. Preconcentration of TAR and SY on a Cartridge. Due to the presence of chemically bonded hexadecyl groups at the silica surface HDS has a pronounced ability to collect hydrophobic molecules [20] including dyes having several highly polar functional groups. In this case, an important factor for quantitative adsorption is the lack of charge of analytes. SY and TAR molecules contain two sulfo- and two sulfo- and one carboxylic groups, respectively, so dissociation of these polar groups should be suppressed for effective adsorption onto hydrophobic adsorbent. In order to suppress dissociation of sulfo-groups, the adsorption was carried out from 0.1 $mol \cdot L^{-1}$ HCl solutions, which was sufficient for complete retention of these dyes [20].

For the evaluation of preconcentration efficiency of HDS for the extraction of SY, an aliquot of 10 mL of standard solution containing 7.5 $\mu g \cdot mL^{-1}$ of dye was passed on a cartridge containing 0.5 g of HDS at flow rate 4-5 $mL \cdot min^{-1}$. The preconcentration factor was calculated as ratio of dye concentration in the total volume of the effluent required for complete elution of dye from the cartridge to the concentration of standard solution. The calculated preconcentration factor was equal to 20. It was also established that a quantitative extraction of SY is possible from 200 mL of an aqueous solution with concentration 0.4 $\mu g \cdot mL^{-1}$ by cartridge containing 0.2 g of the sorbent. In this case, the complete elution of SY was achieved by passing 4 mL of ethanol. Correspondingly, preconcentration factor of 50 was achieved in this case. These data indicated a high efficiency of HDS adsorbent for preconcentration of these dyes.

3.2. Optimization of Separation Conditions for TAR and SY on a Cartridge. The adsorption of analytes on HDS is defined by hydrophobic interactions with immobilized hexadecyl-moieties onto silica surface and it depends on organic

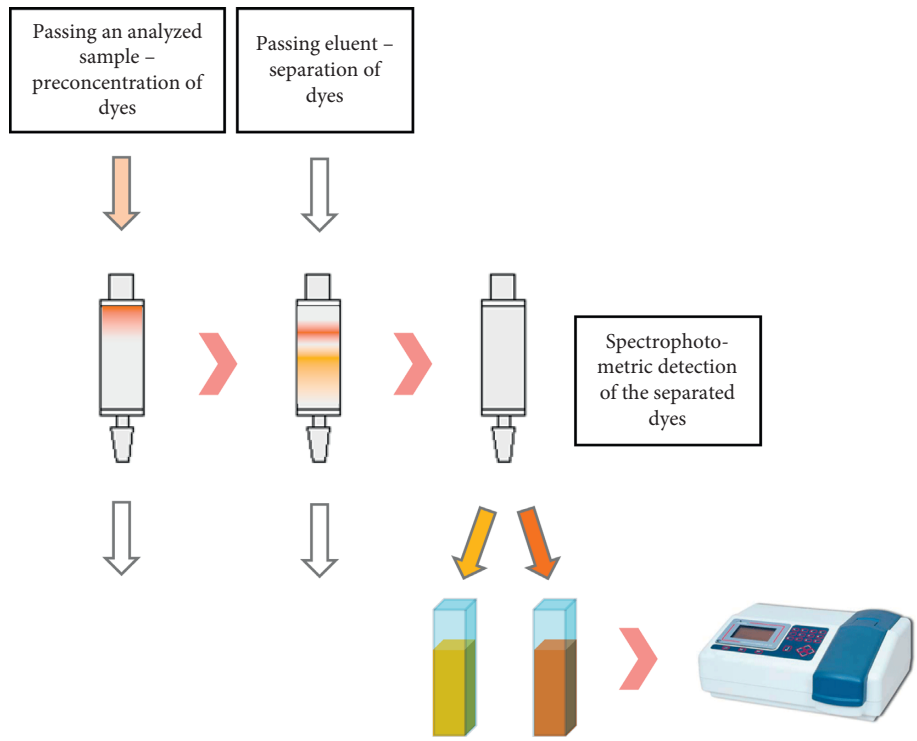

FIGURE 1: Scheme of the proposed method for sorption-spectroscopic determination of dyes with the preconcentration and separation on a single cartridge.

solvent content, concentration of an electrolyte or buffer concentration, and pH of the sample [21].

3.2.1. Effect of Eluent Acidity. The adsorption of dyes by hydrophobic adsorbents depends strongly on the charge of the molecules, which defines polarity and hydrophobicity of analytes. SY molecule has four ionizable groups including two sulfo-, diazo- ($pK_1 = 2.9$), and phenol ($pK_2 = 10.6$) groups [22]. TAR molecule has five ionizable groups including two sulfo-, diazo- ($pK_1 = 1.51$), carboxylic ($pK_2 = 3.95$), and one phenol ($pK_3 = 9.62$) groups [23]. The increase of solution acidity from pH 6.0 to 3.0 causes partial protonation of diazo-group in the molecule of SY and carboxylic group in the molecule of TAR. Correspondingly, acidification of the sample results in decreased hydrophobicity of SY and increased hydrophobicity of TAR.

Preliminary experiments demonstrated no adsorption of SY and TAR on HDS cartridge from 5.7 mmol·L^{-1} phosphate buffer with pH 6.0 containing 10 v/v% of acetonitrile. At these conditions, SY and TAR molecules have effective negative charge of −2 and −3, correspondingly. It was found that adsorption of dyes onto HDS increases drastically from acidified solutions with 75–85% adsorption at pH 1 [20]. However, strong adsorption of dyes at this pH substantially increases their retention time in HDS cartridge and requires more eluent for desorption of dyes. Based on these consideration, pH 3 was chosen for the eluent providing a reasonable difference between effective charges of SY and TAR molecules.

3.2.2. Effect of Buffer Concentration. The effect of KH_2PO_4 concentration on the retention of TAR on HDS cartridge is shown in Figure 2. The increase of the concentration of potassium dihydrophosphate in the eluent increases the retention of TAR and influences the shape of peaks. This is probably due to the "salting out effect," when an increase in the electrolyte concentration results in increased adsorption of organic molecules by hydrophobic adsorbents. The sharp narrow peaks were observed in the absence of KH_2PO_4 but under these conditions the retention was weak and insufficient for complete separation of TAR and SY which was not quantitative. For this reason, 2 mmol·L^{-1} KH_2PO_4 was used in the following experiments. TAR and SY can be separated with 2 mmol·L^{-1} KH_2PO_4 (pH 3) containing 2.5% acetonitrile, but SY was eluted as a too broad peak at these conditions. To overcome this problem, application of a step gradient of acetonitrile was applied.

3.2.3. Effect of Acetonitrile Content. Firstly, no separation of TAR and SY was achieved with eluent containing 10% of acetonitrile due to low retention of dyes by cartridge. It may be due to the high eluting ability of used eluent due to the presence of 10% acetonitrile. The decrease in acetonitrile content down to 5% makes it possible to separate TAR and SY using only 40 mm long cartridge. Both dyes eluted as broad peaks, so to optimize the separation conditions, effects of buffer (KH_2PO_4) concentration and acetonitrile gradient profile on the retention and separation of dyes were additionally studied.

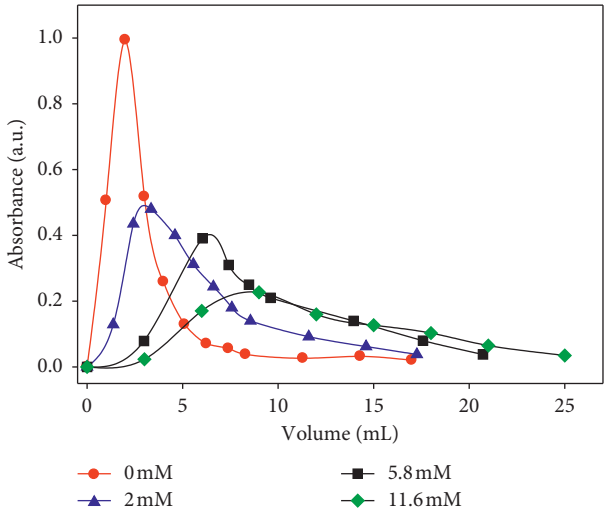

Figure 2: TAR elution profiles with eluent containing 2.5% acetonitrile at pH 3 with varied concentration of KH_2PO_4. TAR amount is 75 μg.

3.2.4. Step Gradient of Acetonitrile. The use of sharp increase in the concentration of acetonitrile reduces the separation time and improves peak shape of SY. Figure 3 presents the elution profiles of SY obtained with different elution gradients. The gradient included isocratic elution with 17 mL of eluent containing 2.5% acetonitrile followed by step increase in concentration of acetonitrile to 2.5%, 5%, or 10%. The best results were obtained for gradient elution with a step change in concentration of acetonitrile from 2.5% to 10% after 17 mL passed through the cartridge was used to separate the mixture of TAR and SY.

3.3. Analytical Characteristics of the Developed Method. Both preconcentration and separation of TAR and SY can be achieved on a single cartridge under optimized conditions and used for their spectrophotometric determination. It was shown that the dyes are quantitatively separated at their ratio in the sample from 1 : 1 to 1 : 5 as shown in Figure 4.

Calibration plots of the peak area (S) versus the concentration (c, $\mu g \cdot mL^{-1}$) of TAR and SY are described by the following dependences $S = 2.24c$ ($r = 0.999$) and $S = 3.04c$ ($r = 0.998$), for TAR and SY, respectively. The calibration plots are linear in concentration range of 2–20 $\mu g \cdot mL^{-1}$. Detection limits for TAR and SY are 0.15 and 0.11 $\mu g \cdot mL^{-1}$, respectively.

It has been established that under the same conditions it is possible to separate the pairs TAR–Ponceau 4R and TAR–Quinoline Yellow WS, but it is not possible to separate SY and Ponceau 4R or Quinoline Yellow WS.

3.4. The Determination of Synthetic Dyes in Soft Beverages. The developed method was used for determination of dyes in soft beverages "Fresh," "Orange," and "Festival." They contain mixtures of TAR (E102) and SY (E104) at different ratios.

An aliquot of a sample to be analyzed (4.5 mL) was placed in a test tube, and 0.5 mL of 1 $mol \cdot L^{-1}$ HCl was added. The sample was passed through the cartridge at the flow rate 1 $mL \cdot min^{-1}$ using a peristaltic pump. Then, 2 $mmol \cdot L^{-1}$ KH_2PO_4 (pH 3.0) was passed through the cartridge at the rate of 1 $mL \cdot min^{-1}$ using a peristaltic pump with a step gradient of acetonitrile as described earlier. The obtained results are represented in Figure 5. The concentrations of dyes were determined by the calibration curves. Accuracy of the proposed method during analyzing "Fresh" and "Orange" beverages was verified by using official method of the Russian National Standard Agency [24] with a chemometric algorithm for data processing by the principal component analysis method using Unscramble 9.8 (Table 1).

The developed method was also applied for determination of the dyes in a beverage "Festival." In this case, the accuracy was verified by the standard addition method (Table 2).

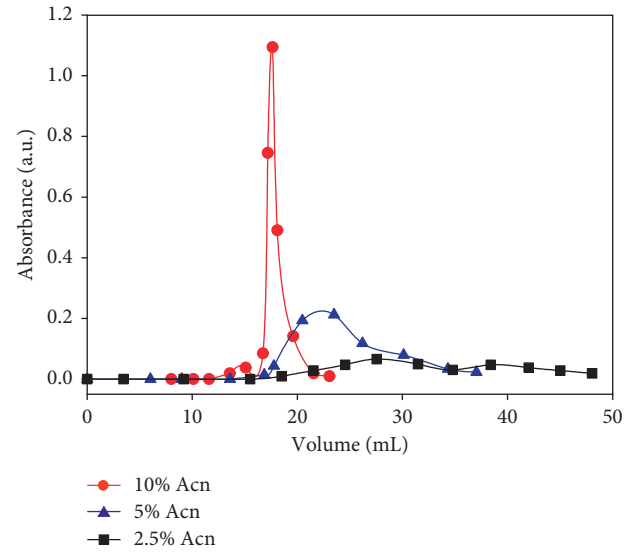

Figure 3: SY profiles obtained with step gradient elution. Eluent: 2 $mmol \cdot L^{-1}$ KH_2PO_4 (pH 3) containing 2.5% acetonitrile with step increase to 5% or 10% after 10 mL. SY amount is 75 μg.

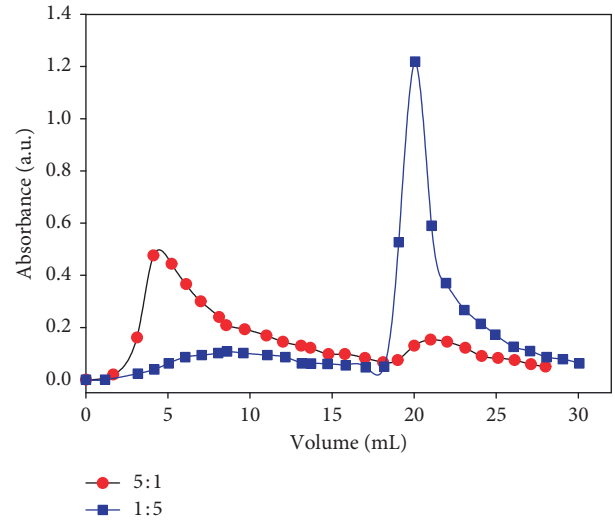

Figure 4: Elution curves of a mixture of TAR and SY in the ratio of 5 : 1 (20 and 100 μg) and 1 : 5. Eluent: 2 $mmol \cdot L^{-1}$ KH_2PO_4 (pH 3) containing 2.5% acetonitrile with step increase to 10% after 17 mL.

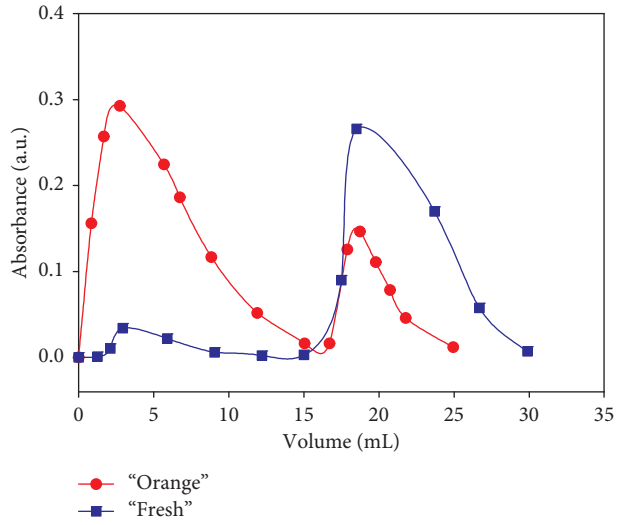

Figure 5: Elution profile obtained for analysis of "Fresh" and "Orange" beverages. Eluent: 2 mmol·L^{-1} KH_2PO_4 (pH 3) containing 2.5% acetonitrile with step increase to 10% after 17 mL. The rate of the eluent is 1 mL·min^{-1}. The detector wavelength is 427 nm and 484 nm for TAR and SY, respectively.

Table 1: Results for the determination of dyes in beverages "Fresh" and "Orange" ($n = 3$, $P = 0.95$).

Beverage	Official method (mg·L^{-1})		Proposed method (mg·L^{-1})	
	TAR	SY	TAR	SY
"Fresh"	4.2 ± 1.5	12.6 ± 1.9	3.5 ± 0.3	13.4 ± 1.3
"Orange"	14.8 ± 1.4	4.8 ± 1.7	15.3 ± 1.5	5.2 ± 0.5

Table 2: Results for the determination of dyes in the beverage "Festival" ($n = 3$, $P = 0.95$).

Food dye	Original content		Added (mg·L^{-1})	Found content		Recovery (%)
	mg·L^{-1}	RSD (%)		mg·L^{-1}	RSD (%)	
TAR	1.2 ± 0.2	10.0	4.0	5.1 ± 0.5	7.0	97.5
SY	4.4 ± 0.3	4.0	4.0	8.3 ± 0.5	4.0	97.5

4. Conclusion

Thus, the possibility of using a single SPE cartridge containing hydrophobic hexadecylsilica for preconcentration of TAR and SY from aqueous solutions and their subsequent separation has been shown. This procedure can be successfully combined with their further spectrophotometric determination. The developed method has been applied for the analysis of beverages. The key features of the method are simplicity of simultaneous determination of two food dyes and high throughput.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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