Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 68 (2006) 516–521
Talanta
www.elsevier.com/locate/talanta

Development of a green chromatographic method for determination of colorants in food samples

Eliane C. Vidotti, Willian F. Costa, Cláudio C. Oliveira *

Departamento de Química, Universidade Estadual de Maringá, Avenida Colombo, 5790 DQI, Maringá PR 87020-990, Brazil

Received 27 October 2004; received in revised form 6 January 2005; accepted 6 January 2005
Available online 13 June 2005

Abstract

A green chromatographic analytical method for determination of Tartrazine, Brilliant Blue and Sunset Yellow in food samples is proposed. The method is based on the modification of a C18 column with a 0.25% (v/v) Triton X-100 aqueous solution at pH 7 and in the usage of the same surfactant solution as mobile phase without the presence of any organic solvent modifier. After the separation process on the chromatographic column, the colorants are detected at 430, 630 and 480 nm, respectively. The chromatographic procedure yielded precise results and is able to run one sample in only 8 min, consuming 15.0 mg of Triton X-100 and 38.8 mg of phosphate. When the flow rate of the mobile phase is 1 ml min^{-1} the retention times are 2.1, 3.6 and 7.0 min for Tartrazine, Brilliant Blue and Sunset Yellow, respectively; and all peak resolutions are ca. 2. The analytical curves present the following linear equations: area = 7.44 10^5 + 2.71 10^5 [Tartrazine] (R = 0.998, n = 7); area = 1.09 10^5 + 3.75 10^5 [Brilliant] (R = 0.9995, n = 7) and area = −7.34 10^4 + 2.33 10^5 [Sunset] (R = 0.998), n = 7) and, the limits of detection for Tartrazine, Brilliant Blue and Sunset Yellow were estimated as 0.125, 0.080 and 0.143 mg l^{-1}. When the proposed method is applied to food samples analysis, precise results are obtained (R.S.D. < 5%, n = 3) and in agreement with those obtained by using the classical spectrophotometric method. The traditional usage of organic solvent as mobile phase in HPLC is not used here, which permits to classify the present method as green.

Keywords: HPLC; Green analytical method; Food colorants

1. Introduction

There are several definitions to analytical chemistry and to what the analytical chemists do. In general, “Analytical Chemistry seeks ever improved means of measuring the chemical composition of natural or artificial materials” and the Analytical Chemists is the person that works to “improve the reliability of existing techniques to meet the demands for better chemical measurements” [1]. Nowadays, the society claim for the development of new analytical methods where the later good characteristics as selectivity and sensitivity are not sufficient, the modern analytical methods needs to be Green [2,3]. In this way, the chemists should develop analytical methods that not use hazardous reagents and that the amount of chemical waste that is generated during the analysis should be minimal. Nowadays we see the advent of the methodologies that eliminate or minimize the utilization of organic solvent and toxic reagents. Among them, we can emphasize solid-phase extraction methodologies [4] that eliminate and minimize the usage of liquid–liquid extraction; the solid-phase spectrofotometry [5] that minimizes the usage of toxic reagents and the organic solvents; flow analysis [6] that minimizes the amount of the waste generated and the bead injection methods for metallic ions determinations [7] that combine the better characteristics of the flow analysis, solid-phase spectrophotometry and solid-phase extraction.

Associated with the tendency for Green Analytical Methods is the necessity for better control of the quality of the foods as people are worried about their heath and, it is known that some food additives, when are present in high concentration, can cause several diseases [8]. It should be stressed that

* Corresponding author. Tel.: +55 442614334; fax: +55 442635784.
E-mail address: ccoliveira@uem.br (C.C. Oliveira).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.01.059

with the free commerce, countries that want to export foods should give guarantees of their qualities.

Colorants are added to foods to make them more attractive [9], replacing their natural color that can be lost during the industrial process or to avoids variations in the color of the final product [10]. The trouble is that some synthetic azo dyes can be noxious to the human health and when in contact with some drugs can cause allergic and asthmatic reactions to some people [11], induced the development of cancer [12] and others diseases [13]. In this way, in the last years efforts have been made to control and to limit the amount of synthetic colorants that are added in foods [14], whereas the more toxic dyes have been banned [15]. In this way, it is necessary to have efficient methodologies to control the amount of colorants in foods.

There are a lot of analytical procedures to carry out the determination of dyes in foods, such as spectrophotometry [16], solid-phase spectrophotometry [8], voltametry [17] and chromatography [15]. As normally, the final color of the food is composed by a mixture of two or more colorants dyes, it is necessary to have a method that is able to determines multi-components, thus a preference is given to the chromatographic method as the spectrophotometric ones, sometimes suffer spectral interferences that cannot be solved by the derivative ratio spectra methods.

As the chromatographic method is elected to determine dyes in foods, and normally this method cannot be considered Green due to the utilization of several organic solvents as mobile phase, the present work proposes a new Green Chromatographic Method to determine Tartrazine, Brilliant Blue and Sunset Yellow in foods. Here, to avoid the common usage of organic solvent as mobile phase, a surfactant is utilized to vary the polarity of the water and to change the characteristics of the C18 chromatographic column. It is known that surfactant in aqueous solutions can be organized in micelles that are able to interact with organic compounds and thus, increase the solubility of non-polar molecules [18] in aqueous medium. Here, the strategy is utilized to determine dyes in food samples using as mobile phase only surfactant in aqueous medium becoming the chromatographic methods more green.

2. Experimental

2.1. Reagent and solutions

The colorants standard stock solution, 1000 mg l^{-1}, was prepared by dissolving 0.1000 g of Tartrazine (Aldrich), Brilliant Blue (Aldrich) and Sunset Yellow (Sigma) dye stuffs in 100 ml of HPLC-grade water, and the working standard solutions (0.0–50.0 mg l^{-1}) were prepared by proper dilution of the stock solution.

The mobile phase was prepared dissolving 0.25 ml of Triton X-100 (Sigma) up to 100 ml with 50 mmol l^{-1} phosphate buffer solution at pH 7 and, the solution was passed, before use, through a 0.45 μm membrane filter. The critical mecellar concentration (CMC) of Triton X-100 is 0.18% (v/v).

2.2. Samples

The artificial juice and gelatin samples were homogenized; ca. 2.0 g was weighed and dissolved in hot water (60 °C). After cooling, the sample was transferred to a volumetric flask and the volume was adjusted to 50 ml with water. Finally, the samples were filtered through a 0.45 μm membrane filter.

2.3. Liquid chromatography and separation conditions

Chromatographic separation was carried out using a Varian ProStar System with Star Chromatography Workstation and LC control software (Varian Analytical Instruments) comprising a ProStar 240 solvent delivery modules, a Model ProStar 410 AutoSampler with a sample loop of 20 μl, a Model ProStar 330 photodiode array detector with the Polyview 2000^{TM} program and a microsorb C18 250 mm × 4.6 mm column with particles of 5 μm equipped with a microsorb C18 10 mm × 4 mm guard column. All the experiments were conducted at room temperature. Before analysis, the column was conditioned making the mobile phase flows through the system for 20 min at 1.0 ml min^{-1}. During the chromatographic separation the mobile phase was kept isocratic, at 1.0 ml min^{-1} and the acquisition of the data were done at 426, 480 and 630 nm to Tartrazine, Sunset Yellow and Brilliant Blue, respectively.

For quantitative determination external calibration was used plotting peak area (y) versus injected amount (x, mg l^{-1}). The LODs ($n = 3$) were calculated by using a signal-to-noise ratio of 3.

2.4. System optimization

For system optimization the main important parameters, such as buffer concentration, surfactant concentration, pH and eluant flow rate were investigated. It should be stressed that for pH optimization acetate and phosphate buffer solutions were used and that the colorants pK_as were determined by using potentiometric titration and the modified Gran function [19].

3. Results and discussion

In the development of the green chromatographic method for determination of Brilliant Blue, Tartrazine and Sunset Yellow it was decided that all reagents should be as green as possible and that the selected stationary phase should be familiar to all HPLC laboratories. In this way, a C18 column was elected as stationary phase and the mobile phase should be an aqueous solution. Then, all the subsequent experiments were planned with the above mentioned strategy in mind.

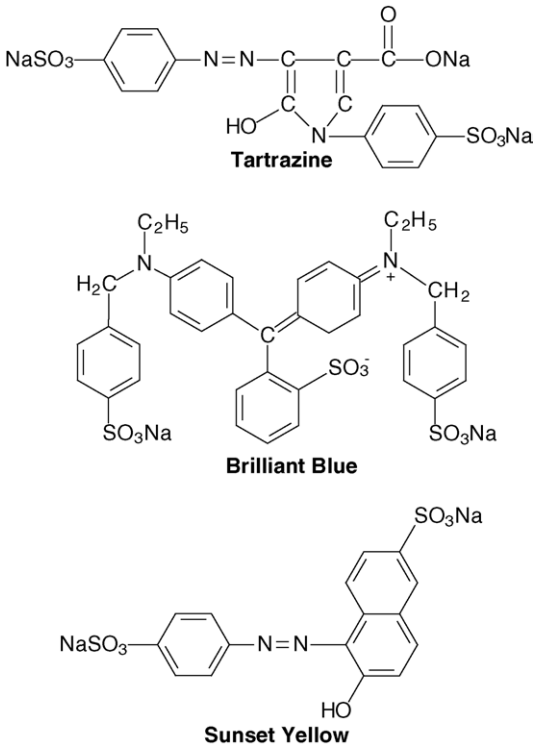

Fig. 1. Structure of Tartrazine, Brilliant Blue and Sunset Yellow.

Brilliant Blue is a triarylmethane compound whereas Tartrazine and Sunset Yellow are azo dye colorants (Fig. 1). As the hydrophobicity of non-azo colorants is weaker than those of azo colorants, this difference can be exploited to carried out their chromatographic separation. Other characteristic that should take into account is that the hydrophobicity of colorants with naphthalene ring is stronger than those with benzene ring. Thus, the hydrophobicity of Sunset Yellow that contains in its molecular structure a naphthalene ring is higher than the Tartrazine hydrophocity. Thus, hydrophobic sequence for the selected colorants should be: Sunset Yellow > Tartrazine > Brilliant Blue and, when a C18 column is used to carried out the separation of the colorants and aqueous solution is used as mobile phase the elution sequence should be Brilliant Blue, Tartrazine and Sunset Yellow. As normally, the chromatographic determination of the colorants is done in pH around of 7 the acid and alkaline groups present in the molecules can change the elution sequence. Tartrazine is the smaller molecule and presents two strong sulfonic acid groups (pK_a 2) and one acetate weak acid group (pK_a 5) that are dissociated in pH 7; furthermore, the pK_a of its azo group is 10.86, and is protonated at pH 7 becoming the molecule high hydrophilic. Brilliant Blue has three sulfonic acid groups and two amino groups with pK_a of 9.22 that are protonated at pH 7, but the molecule is big and less hydrophilic than the Tartrazine. Sunset Yellow has only two sulfonic acid groups, the azo group is protoneted at pH 7 (pK_a = 9.20) and the structure presents a naphthalene ring, for these reasons it is the less hydrophilic. Thus, an expected elution sequence for the three compounds when a C18 column and aqueous solutions is used as mobile phase is: Tartrazine, Brilliant Blue and Sunset Yellow.

Tartrazine, Brilliant Blue and Sunset Yellow are very soluble in water as they are charged in pH 7 and present low affinity by the stationary phase (C18) due to the non-polar characteristics of the C18 group. Thus, it is not possible to get the separation of the three colorants using only water as mobile phase, as any interaction with the column occurs and the colorants will be eluted together. The usage of a non-ionic surfactant as mobile phase is proposed here to circumvent this problem. With this strategy, the mechanism of the separation is changed as the surfactant is absorbed by the stationary phase increasing its polarity and, at the same time, the solvent characteristics are changed too, as the presence of the surfactant induces the formation of micelles. In this way, the C18 stationary phase in the presence of Triton X-100 becomes more polar, whereas the charges of the colorant molecules are stabilized due to the presence of the micelles, becoming possible to get the separation of Tartrazine, Sunset Yellow and Brilliant Blue using a C18 column as stationary phase and an aqueous Triton X-100 surfactant solution as mobile phase.

During the separation process the concentration of the Triton X-100 aqueous solution was fixed as 0.25% (v/v) as the better compromise among chromatographic resolution, analytical frequency, sensitivity and less consumption of surfactant and consequently less waste generation (Fig. 2). It was observed that the resolution is inversely proportional to the Triton X-100 concentration in the range of 0.25–1.0% (v/v). When the surfactant concentration in the mobile phase is lower than 0.25% (v/v) any separation occurs, demonstrating that the mobile phase did not interact with the stationary phase. On the other hand, when the concentration of Triton X-100 is high than 1.0% (v/v) the resolution become poor and a quantitative analysis cannot be done. The Triton X-100 concentration can be varied between 0.25 and 1.0% (v/v), but

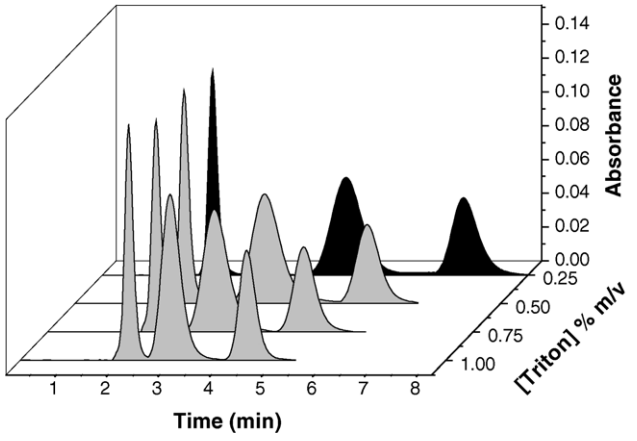

Fig. 2. Effect of the Triton X-100 concentration in the chromatographic separation. Data were obtained with the mobile phase flow rate at 1 ml min^{-1} in phosphate buffer solution 50 mmol l^{-1} at pH 7 and for 30 mg l^{-1} of Tartrazine, Brilliant Blue and Sunset Yellow. The black chromatogram corresponds to the selected condition (0.25%, v/v).

Table 1
Chromatographic resolutions

pH	Rs for Tartrazine/ Brilliant Blue	Rs for Brilliant Blue/ Sunset Yellow
4.5	0.56	0.65
5.0	0.68	1.30
5.5	0.75	1.49
6.0	1.21	2.56
6.5	1.31	2.34
7.0	1.60	2.28
7.2	1.83	2.09
7.3	1.62	2.13
7.4	1.73	2.18
7.5	1.63	2.16
7.6	1.42	2.16

The values were calculated for Triton X-100 concentration of 1% (v/v), mobile phase flow rate at 1 ml min^{-1}, phosphate buffer or acetate buffer solution 50 mmol l^{-1} and for 30 mg l^{-1} of Tartrazine, Brilliant Blue and Sunset Yellow.

the 0.25% (v/v) solution was selected because of the minor waste generation; but in cases where the analytical frequency is critical, the Triton X-100 concentration can be increased to 1% (v/v) (Fig. 2).

The pH plays an important role in the separation of the colorants. It was verified that at pH values lower than 6.5 the chromatographic resolution was poor (Table 1) and the data cannot be used to quantitative analysis (Fig. 3). For pH 4.5 it was observed only two peaks with high degree of superposition, indicating that the separation was poor. When the pH value is increased it is noted an improvement in the resolution and the third peak begin to appear. To pH values high than 6.5 the three colorants are separated, the sensitivity is practically constant and the resolution is adequate to quantitative

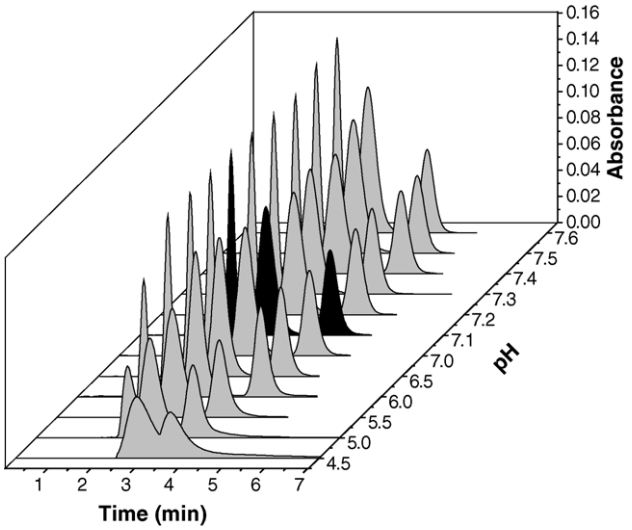

Fig. 3. Effect of the pH in the chromatographic separation. Data were obtained with the Triton X-100 concentration of 0.50% (v/v), mobile phase flow rate at 1 ml min^{-1} in phosphate buffer or acetate buffer solution 50 mmol l^{-1} and for 30 mg l^{-1} of Tartrazine, Brilliant Blue and Sunset Yellow. The black chromatogram corresponds to the selected condition (pH 7).

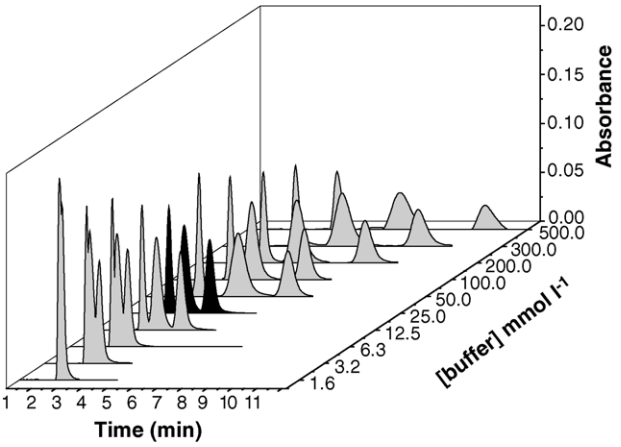

Fig. 4. Effect of the buffer concentration in the chromatographic separation. Data were obtained with the Triton X-100 concentration of 0.50% (v/v), mobile phase flow rate at 1 ml min^{-1} in phosphate buffer at pH 7 and for 30 mg l^{-1} of Tartrazine, Brilliant Blue and Sunset Yellow. The black chromatogram corresponds to the selected condition (buffer at 50 mmol l^{-1}).

analysis (Fig. 3). Thus, the pH 7 was elected as the better condition and all chemical analysis were carried out at this pH value because the separation is quantitative (Fig. 3), the low toxic phosphate can be used as buffer solution and the chemical residues are neutral, characteristics that becoming the method more green.

It was noted that the buffer concentration has influence in the chromatographic separation (Fig. 4); probably the effect is associated with the charges of the buffer components that can stabilize the charged colorant molecules. When the phosphate buffer concentration was 1.5 mmol l^{-1} and the pH was 7 the chromatographic separation was very poor as only one peak with a small shoulder was observed (Fig. 4). It was observed an improvement in the resolution when the concentration of the phosphate buffer solution was increased and to concentration values higher than 50 mmol l^{-1} the separation was adequate and the obtained date could be used to quantitative analysis (Fig. 4). It was observed that for higher buffer solution concentration the resolution continues to be adequate but peak broadening was observed as well as an increase in the retention time, which could decrease the sensitivity and the analytical frequency (Fig. 4). Thus, the concentration of the buffer solution was fixed as 50 mmol l^{-1} as the better compromise among sensitivity, analytical frequency and the minor amount of waste generation; this last factor depend on the buffer concentration.

The flow rate of the mobile phase is an important parameter in the separation of Tartrazine, Sunset Yellow and Brilliant Blue. When the flow rate is very high (2 ml min^{-1}) it was noted a decrease in the chromatographic resolution and in the sensitivity (Fig. 5). On the other hand, for low flow rate (0.25 ml min^{-1}) the sensitivity increase, but the analytical frequency is poor (Fig. 5). Thus, in the present work the mobile phase flow rate was fixed as 1.0 ml min^{-1} as the better compromise between sensitivity and analytical frequency. It should be emphasized that if the sensitivity was critical and

Table 2
Determination of Tartrazine (T), Brilliant Blue (BB) and Sunset Yellow (SY) in food samples by the proposed chromatographic method (HPLC) and by the spectrophotometric one (SPEC)

Sample	T (HPLC)	BB (HPLC)	SY (HPLC)	T (SPEC)	BB (SPEC)	SY (SPEC)
1	1.44 ± 0.07	–	1.16 ± 0.06	1.43 ± 0.03	–	1.24 ± 0.03
2	0.179 ± 0.009	0.020 ± 0.002	0.088 ± 0.005	0.193 ± 0.007	0.096 ± 0.004	0.020 ± 0.001
3	0.033 ± 0.003	–	0.020 ± 0.002	0.026 ± 0.002	–	0.019 ± 0.001
4[a]	0.94 ± 0.04	–	0.80 ± 0.04	0.97 ± 0.02	–	0.81 ± 0.02

The results are expressed in mg/g ± the standard deviation.
[a] Liquid sample and the results are expressed in mg l^{-1}.

the analytical frequency was a secondary factor, it is possible to decrease the flow rate (Fig. 5).

Quantitative data were obtained injecting a sample volume of 20 μl and monitoring Tartrazine, Brilliant Blue and Sunset Yellow at 430, 630 and 480 nm, respectively. It should be emphasised that it is possible to do the measurements of the three colorants at the same wavelength, 430 nm, with a lost in sensitivity to Sunset Yellow and Brilliant Blue but, this

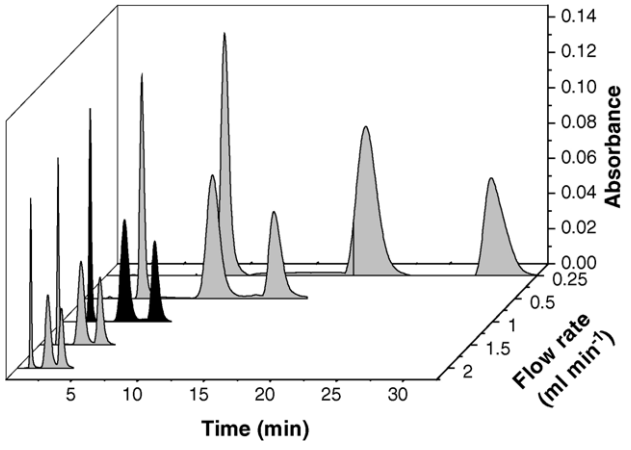

Fig. 5. Effect of the flow rate in the chromatographic separation. Data were obtained with the Triton X-100 concentration of 0.25% (v/v), 50 mmol l^{-1} phosphate buffer at pH 7 and for 30 mg l^{-1} of Tartrazine, Brilliant Blue and Sunset Yellow. The black chromatogram corresponds to the selected condition (1.0 ml min^{-1}).

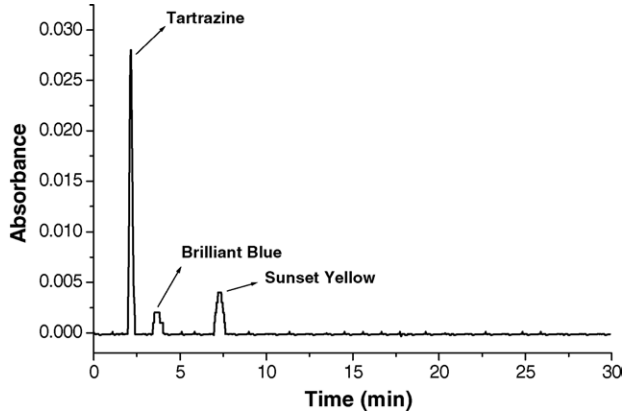

Fig. 6. Chromatogram of the sample 2. The chromatogram was obtained using as mobile phase a solution containing Triton X-100 0.25% (v/v) plus 50 mmol l^{-1} phosphate buffer at pH 7 and a flow rate of 1 ml min^{-1}.

procedure is recommended to laboratories that did not have spectrophotometer with photodiode array detector facilities. The elution process is done in isocratic manner, thus a simple HPLC can be used to carry out the chemical analysis.

Remarkably stability and robustness were observed when the proposed method was applied to colorants analysis in food samples. The chromatographic procedure yielded precise results and baseline drift was not observed (Fig. 6). The proposed method was able to run one sample in only 8 min, consuming 15.0 mg of Triton X-100 and 38.8 mg of phosphate. Under the selected conditions the retention times are 2.1, 3.6 and 7.0 min for Tartrazine, Brilliant Blue and Sunset Yellow, respectively (Figs. 2 and 5) and all peak resolutions are ca. 2. It should be stressed that the C18 column should be conditioned with the mobile phase during 20 min before analysis to eliminate any trace of organic solvents which can change the chromatographic resolution. The analytical curves present the following linear equations (data in mg l^{-1}): area = 7.44 10^5 + 2.71 10^5 [Tartrazine] (R = 0.998, n = 7); area = 1.09 10^5 + 3.75 10^5 [Brilliant] (R = 0.9995, n = 7) and area = −7.34 10^4 + 2.33 10^5 [Sunset] (R = 0.998, n = 7) for Tartrazine, Brilliant Blue and Sunset Yellow, respectively. For the above conditions the limit of detections for Tartrazine, Brilliant Blue and Sunset Yellow were estimated as 0.125, 0.080 and 0.143 mg l^{-1}, respectively. When the proposed method is applied to food samples analysis, precise results are obtained (R.S.D. 5%, n = 3) (Table 2 and Fig. 6) and comparable to those obtained by using the classical spectrophotometric method. It should be stressed that the limit of detections for the spectrophotometric method were estimated as 0.2, 0.18 and 0.16 mg l^{-1} for Tartrazine, Brilliant Blue and Sunset Yellow, respectively; but the method is not easily applicable when the proportion among the food dyes is very different due to the spectral interference.

4. Conclusion

It is possible to do the chromatographic determination of Tartrazine, Brilliant Blue and Sunset Yellow in food samples using a C18 column as stationary phase and an aqueous Triton X-100 solution as mobile phase. The procedure is simple, robust, precise, present high analytical frequency and can be considered Green as it is demonstrated that HPLC can be utilized without the usage of organic solvent. It should be noted

that there are others articles exploiting micellar medium but always organic solvents are used as modifier and the analytical frequency is lower when compared to that obtained in the present work. Here, a sample can be analysed in 8 min (or less increasing the flow rate and the Triton X-100 concentration) and any organic solvent is used as the presence of the chemical modifier is dispensable.

Acknowledgement

The support of Fundação Araucária, CAPES and CNPq are greatly appreciated.

References

[1] G.D. Christian, Analytical Chemistry, sixth ed., Wiley, 2004.
[2] R.K. Sharma, S. Mittal, M. Koel, Crit. Rev. Anal. Chem. 33 (2003) 183.
[3] E.C. Vidotti, V.C. Almeida, C.C. Oliveira, Talanta 64 (2004) 993.
[4] C. Arthur, L. Killiam, K. Buchholz, J. Pawliszn, Anal. Chem. 64 (1992) 1960.
[5] C.M. Hill, K.W. Street, S.P. Tanner, W.H. Philipp, Anal. Lett. 33 (2000) 2779.
[6] J. Ruzicka, E.H. Hansen, Flow Injection Analysis, second ed., Wiley, New York, 1988.
[7] C.C. Oliveira, E.A.G. Zagatto, J. Ruzicka, G.D. Christian, Anal. Lett. 33 (2000) 929.
[8] L.F. Capitán-Vallvey, M.D. Fernández, I. de Orbe, R. Avidad, Talanta 47 (1998) 861.
[9] Q. Chen, S. Mou, X. Hou, J.M. Riviello, Z. Ni, J. Chrom. A 827 (1998) 73.
[10] J.J. Berzas, J.R. Flores, M.J.V. Llerena, N.R. Farinas, Anal. Chim. Acta 391 (1999) 353.
[11] J.J.B. Nevado, C.G. Cabanillas, A.M. Contento Salcedo, Talanta 42 (1995) 2043.
[12] M.R. Fuh, K.J. Chia, Talanta 56 (2002) 663.
[13] G.M. Greenway, N. Kometa, R. Macrae, Food Chem. 43 (1992) 137.
[14] L.D. Giovine, A.P. Bocca, Food Control 14 (2003) 131.
[15] M.C. Genaro, E. Gioannini, S. Angelino, R. Aigotti, D. Giacosa, J. Chrom. 767 (1997) 87.
[16] J.J.B. Nevado, J.R. Flores, C.G. Cabanillas, M.J.V. Llerena, A.C. Salcedo, Talanta 46 (1998) 933.
[17] A.G. Fogg, A.M. Summan, Analyst (1983) 891.
[18] M.E. Diaz-Garcia, A. Sanz-Medel, Talanta 33 (1986) 255.
[19] L.M. Aleixo, O.E.S. Godinho, W.F. Costa, Anal. Chim. Acta 257 (1992) 35.