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Analytical Methods

Simultaneous determination of caffeine, caramel and riboflavin in cola-type and energy drinks by synchronous fluorescence technique coupled with partial least squares

L'udovít Žiak, Pavel Májek, Katarína Hroboňová, František Čacho, Jana Sádecká *

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovak Republic

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ABSTRACT

The aim of this work was to develop a multivariate method for the rapid determination of caffeine and Class IV caramel in cola-type soft drinks and of caffeine, Class III caramel and riboflavin in energy drinks using synchronous fluorescence spectra. The synchronous fluorescence spectra were recorded at constant wavelength difference 90 nm from 200 to 500 nm. Reference values of analyte concentrations by high performance liquid chromatography (HPLC) with fluorescence detection combined with the standard addition method were used to create the partial least squares (PLS) models. High coefficients of determination (>0.99) were obtained in 0.2–4.2, 0.25–5.25, 0.4–10.0 and 0.007–0.054 mg L^{-1} range for caffeine, Class III caramel, Class IV caramel and riboflavin, respectively. The PLS models were used to determine the concentration of analytes in different drink samples. The method provided comparable results with those found using the HPLC method.

1. Introduction

Caffeinated cola-type and energy drinks have become a modern day phenomenon. In US and Canada, caffeine is allowed at levels up to 200 mg L^{-1} in cola-type beverages. In the European Union, there is no upper limit for caffeine. As there are increasing reports of caffeine intoxication from energy drinks, Health Canada (2011) supports the establishment of an initial maximum limit for total caffeine of 400 mg L^{-1}. High-performance liquid chromatography (HPLC) (Rostagno et al., 2010) and ultraviolet spectroscopy (Pieszko, Baranowska, & Flores, 2010) are commonly used for the determination of caffeine in beverages.

Class IV caramel (E150d) has been widely used in cola-type soft drinks. Energy drink can contain Class III caramel (E150c). In the legislation, there is no maximum concentration value for caramels in soft or energy drinks. However, the presence of a minor Class III and IV caramel component (4-(5-)methylimidazole) in most beverages can be hazardous to humans (Cunha, Barrado, Faria, & Fernandes, 2011). The established method for determination of caramels is a spectrophotometric measurement at 610 nm. Recently, the quantification of Class IV caramel and caffeine simultaneously, together with other compounds, in carbonated cola beverages has become possible with 400 MHz ^{1}H NMR spectroscopy (Maes, Monakhova, & Kuballa, 2012).

Riboflavin (vitamin B_2) is often included in energy drinks because it may contribute to the maintenance of mental function. Spectrofluorimetric determination, either directly (AOAC, 2011) or coupled to HPLC (Andres-Lacueva, Mattivi, & Tonon, 1998) is one of the most commonly recommended methods for the determination of this vitamin.

Synchronous fluorescence spectroscopy (SFS) has become particularly popular because of its simplified spectra, reduced scattering light interference, and improved selectivity. Selectivity of SFS can be further improved in combination with techniques such as derivative plot or multivariate methods (Andrade-Eiroa, de-Armas, Estela, & Cerda, 2010). Recently, the potential of SFS has been demonstrated in the determination of Class IV caramel in non-aged mixed wine spirits (Rostagno et al., 2010). So far, no references are available for the simultaneous determination of caffeine, caramel and riboflavin by fluorescence spectroscopy.

In this paper, a multivariate method based on PLS calibration is proposed for the simultaneous determination of caffeine, caramel and riboflavin in caffeinated drinks, using the synchronous fluorescence properties of these compounds and the standard addition method.

* Corresponding author. Tel.: +421 2 59325722; fax: +421 2 52926043.
E-mail address: jana.sadecka@stuba.sk (J. Sádecká).

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2. Materials and methods

2.1. Drink samples

Twenty cola soft drink samples of six brands as well as sixteen energy drinks of five brands available were obtained from local market and stored at room temperature until analysis. Sixteen samples from each set were used in PLS.

2.2. Standards and solutions

All experiments were performed with analytical reagent grade chemicals and doubly distilled water. Caffeine and riboflavin were obtained from Sigma–Aldrich (Steinheim, Germany), and stock solutions (10 mg L^{-1}) prepared by dissolving 10.00 mg of caffeine or riboflavin in water. Working solutions were prepared by dilution with water before use. The Class III caramel (E150c, colour No. 252) and the Class IV caramel (E150d, colour No. 055) were the commercial caramels produced by D.D. Williamson (Manchester, UK). The stock solutions (1.000 g L^{-1}) of caramel products were daily prepared by dissolving 1.000 g of Class III caramel or Class IV caramel in water.

Methanol (HPLC, gradient grade), cetyltrimethylammonium bromide, orthophosphoric acid and sulphuric acid were purchased from Merck (Darmstadt, Germany).

2.3. HPLC reference method

The method described by González, Gallego, and Valcárcel (2003) was followed. HPLC analysis was carried out on an Agilent 1260 Series HPLC system consisting of a quaternary pump equipped with an injection valve (Rheodyne), thermostat, and fluorescence detector. Chromatographic column was Platinum C18 (4.6 × 250 mm I.D., 5 μm). The mobile phase consisted of a mixture of 85% methanol and 15% water containing 0.07 g L^{-1} cetyltrimehtylammonium bromide adjusted to pH 6.0 with orthophosphoric acid. The flow rate was 1.0 mL min^{-1}. The column temperature was kept constant at 22 °C and the injection volume was 20 μL. The fluorescence detector was operated at λ_{ex} = 300 and λ_{em} = 360 nm (caffeine, caramel) or at λ_{ex} = 270 and λ_{em} = 520 - nm (riboflavin). The linear calibration ranges were 5–1000, 0.5–10000 and 0.001–5 mg L^{-1} for caffeine, caramel and riboflavin, respectively. Detection limits were calculated as three times the standard deviation of the background noise divided by the slope of each calibration graph and were 2 mg L^{-1} (caffeine), 0.2 mg L^{-1} (caramel) and 0.0003 mg L^{-1} (riboflavin). Analyses were performed in triplicate and the mean determined in all cases.

2.4. Fluorescence spectrometry

2.4.1. Apparatus

Fluorescence spectra were recorded using a Perkin-Elmer LS 50 Luminescence spectrometer equipped with a Xenon lamp. Excitation and emission slits were both set at 5 nm. Scan speed was 200 nm min^{-1}. Fluorescence emission and excitation spectra were recorded at the wavelength of excitation/emission maxima ($\lambda_{ex,max}/\lambda_{em,max}$): caffeine (303/370 nm), Class III caramel (292/390 nm), Class IV caramel (299/359 nm) and riboflavin (448/520 nm). Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromator in the excitation wavelength range 200–500 nm, with constant wavelength differences $\Delta\lambda$ (from 10 to 100 nm, in steps of 10 nm) between them. Fluorescence intensities were plotted as a function of the excitation wavelength. The right-angle geometry was used for samples in 10 × 10 × 45 mm quartz cell. The spectrometer was connected to a computer supplied with FL Data Manager Software (Perkin-Elmer).

2.4.2. One component calibration

The fluorescence spectra were recorded at $\Delta\lambda$ = 90 nm over the 200–500 nm spectral range. The synchronous fluorescence intensity measurements were made at the synchronous maxima λ_{max} of each component. In Table 1, the values of λ_{max}, intercepts (a), slopes (b), R^2 and linearity ranges for the calibration curves are presented. A satisfactory linear relationship ($R^2 > 0.998$) was obtained between the concentration and the fluorescence intensity for each component. Limit of detection (LOD) was the sample concentration that causes a peak three times as high as the baseline noise level and limit of quantitation (LOQ) was calculated as 10 times the baseline noise level.

2.4.3. PLS

Based on preliminary HPLC results for caffeine, caramel and riboflavin concentrations in the drink samples, two different sets (cola-type and energy drink) were prepared. In general terms, to cover a concentration range modeling the batch variability of the samples, the concentrations of analytes were adapted by adding appropriate volumes of the stock/working solutions plus 2.5–125 μL samples to 10 mL volumetric flasks, which were made up to volume with water. Amounts of standard addition for the analytes were determined so the final concentrations were within the linear range for the relevant fluorimetric methods.

The first step in the simultaneous determination of caffeine and Class IV caramel in cola-type drink involved preparing the two sample sets (i.e. calibration and prediction sets) using a four-level experimental design. Thus, 16 calibration samples were prepared, containing different amounts of cola-type drink (2.5–125 μL) and the two analytes within the previously established linear ranges (caffeine, 0.2–4.2 mg L^{-1}; Class IV caramel, 0.4–10 mg L^{-1}). The prediction set contained 16 samples with concentrations inside the limits of the calibration set (cola-type drink, 2.5–75 μL; caffeine, 0.4–3.5 mg L^{-1}; Class IV caramel, 1–8 mg L^{-1}). Calibration (16) and prediction samples (16) were prepared in a similar manner for the energy drinks, each containing different amounts of energy drink (calibration, 5–60 μL; prediction, 10–75 μL), caffeine (calibration, 0.2–4.2 mg L^{-1}; prediction, 0.5–3.0 mg L^{-1}), Class III caramel (calibration, 0.25–5.25 mg L^{-1}; prediction, 0.5–4.5 mg L^{-1}), and riboflavin (calibration, 0.007–0.054 mg L^{-1}; prediction, 0.008–0.048 mg L^{-1}). Besides eight drink samples were prepared by adding 50–100 μL drink into 10 mL volumetric flask which were made up to volume with water.

All the solutions were measured at $\Delta\lambda$ = 90 nm from 200 to 500 nm. The spectral regions 250–450 nm (cola-type drinks) and 250–500 nm (energy drinks) were selected for PLS because these intervals contain the maximum spectral information for the target components. This means working with 401 and 501 experimental

Table 1
Analytical parameters of the SFS method using $\Delta\lambda$ = 90 nm.

Parameter	Caffeine	Class III caramel	Class IV caramel	Riboflavin
λ_{max} (nm)	302	322	331	442
Intercept (a)	0.9	1.4	1.2	−0.4
Slope (b)	35.1	11.8	12.5	727
Coefficient of determination (R^2)	0.9990	0.9989	0.9987	0.9989
Linear range (mg L^{-1})	0.2–4.2	0.25–10	0.4–10	0.007–0.55
Limit of detection (mg L^{-1})	0.07	0.07	0.1	0.002
Limit of quantitation (mg L^{-1})	0.2	0.23	0.4	0.007