Studii şi Cercetări Ştiinţifice 2013, **14** (2), pp. 071 – 078
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

OPTIMIZATION OF A UV-VIS SPECTROMETRIC METHOD FOR CAFFEINE ANALYSIS IN TEA, COFFEE AND OTHER BEVERAGES

S. Dobrinas, A. Soceanu*, V. Popescu, G. Stanciu, S. Smalberger

Ovidius University, Chemistry and Chemical Engineering Department, 124, Mamaia Blvd., 900524, Constanta, Romania

*Corresponding author: asoceanu@univ-ovidius.ro

Received: November, 14, 2012
Accepted: March, 29, 2013

Abstract: A method has been developed and validated for the determination of caffeine in tea, coffee and other beverages by UV-VIS spectrometry. A linear calibration curve was generated with caffeine concentration ranging from 3 $mg \cdot L^{-1}$ to 18 $mg \cdot L^{-1}$. The procedure developed provides a 0.85 $mg \cdot L^{-1}$ detection limit of caffeine, respectively 1.52 $mg \cdot L^{-1}$ quantification limit and the relative standard deviation (RSD) was less than 0.05 % for independent measurement. The developed method was sensitive/specific and robust. Caffeine in tea infusions was found to be dependent on infusion time, the longer of the infusion time and the higher of the caffeine concentrations in tea infusions.

Keywords: *caffeine, tea, coffee, beverages, UV/VIS spectrometry*

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine), a naturally occurring alkaloid found in tea leaves, coffee beans, kola nuts, cocoa beans and other plants, is used as a flavoring agent in a variety of beverages, including some soft drinks and energy drinks.
Caffeine causes various physiological effects such as relaxation of bronchial muscle, stimulation of the central nervous system, gastric acid secretion and dieresis. On the other hand, chemical analysis of caffeine in coffee beans and tea leaves are also used as an additional tool for evaluating coffee, respectively tea quality [1].
Therefore, caffeine analysis is requested to ensure proper caffeine levels in beverages and to meet regulatory standards.
Several methods developed for the determination of caffeine in tea, coffee and other beverages are available in the literatures: UV–VIS spectrometry [2], gas chromatography/mass spectrometry [3], high-performance liquid chromatography [4], micellar electrokinetic chromatography [5], voltammetry [6], near-infrared spectroscopy [7], FT-Raman spectroscopy [8] and planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry [9].
The aim of this study has been the development of a UV-VIS spectrometry procedure for routine caffeine determination in tea, coffee and other beverages.

MATERIALS AND METHODS

Materials

Black tea, green tea, coffee, ness and various brand soft drinks were collected from local market.
All reagents were of analytical-reagent grade (Merck and Fluka) and all solutions were prepared using distilled-deionized water.
For the spectrophotometric method the following reagents were prepared: $Zn(CH_3COO)_2$ solution of 1M (Merck), the $K_4[Fe(CN)_6]$ solution of 0.25M (Fluka), 0.1N H_2SO_4 (Merck).

Standard solutions

0.0100 g of caffeine (anhydrous) (Merck) were weighted in a 100 mL volumetric flask and filled up to 100 mL with ultra-pure water. Then the solution was kept at 4^0C. Working standard solutions (3, 6, 9, 12, 15 and 18 $mg \cdot L^{-1}$) were freshly prepared. Absorbance of caffeine was measured at 273.5 nm with an UV/VIS spectrophotometer with double beam optical system (Jasco 550). A calibration curve was constructed each day before analysis of the samples. Deionized water was used as the blank.

Determination of caffeine

1 g solid sample (or 200 mL liquid sample) was taken in 750 mL Erlenmeyer beaker and subsequently distilled water (450 mL) was added to it. To remove tannins 50 mL H_2SO_4 0.1N were added to the sample. The mixture was boiled for 30 min and filtered

through filter paper. Then 7 mL $Zn(CH_3COO)_2$ 1M and 6 mL $K_4[Fe(CN)_6]$ 0.25 M was added to 50 mL of the filtrate in a 100 mL volumetric flask. After the volume was adjusted to the mark, the solution containing zinc precipitate was filtered. Absorbance of this filtrate was measured at 273.5 nm.

Analytical validation

In order to validate the spectrometric method for routine caffeine determination in tea, coffee and other beverages the following performance parameters were verified: selectivity/specificity, linearity, working range, detection and quantification limits, precision and robustness.

Selectivity/specificity

To demonstrate selectivity in this study, the molecular absorption spectrum in visible and ultra-violet for standard solution of caffeine and for caffeine extracts of studied samples were plotted. Then absorption spectra recorded were overlapped for all analyzed solutions.

Linearity

Linearity of response for standards was tested by assaying in triplicate using six levels of concentrations, ranging from 3 to 18 $mg \cdot L^{-1}$ caffeine, covering in this way all the expected values.

Working range

To determine the concentrations first should be done the homogeneity test to verify the significant differences between the limits of working area.
To determine homogeneity of variances were performed identical measurements on 10 samples of caffeine to the lowest and highest concentration, achieving 10-values information. Further a test for homogeneity of variances was performed, calculating the dispersions on the two levels of concentrations.

Detection limit (LOD) and quantification limit (LOQ)

LOD and LOQ were calculated according to equations Eq. (1) and (2), where b is the slope of the calibration curve and s_a is the standard deviation of intercept of regression equation.

$$\mathrm{LOD} = \frac{3 \cdot s_a - a}{b} \qquad (1)$$

$$\mathrm{LOQ} = \frac{10 \cdot s_a - a}{b} \qquad (2)$$

Precision

Intra-day precision was tested to check the constancy of instrumental response to a given analyte and the repetitiveness of concentrations. For this purpose, the assay was performed with ten solutions of standard (6 $mg \cdot L^{-1}$) and ten of coffee, ness, soft drinks, black and green tea samples.

Robustness

Robustness of a method is tested by deliberately introducing small changes to the proposed method and then by the examination of the consequences. Robustness evaluation of the proposed method has to demonstrate the confidence in analysis. Thus, it seeks stability solutions and change of the entrance slit in the monochromator.

RESULTS AND DISCUSSION

Selectivity/specificity

Figure 1 presents the UV–VIS overlapped absorption spectra of the caffeine standard solution and extracts in the range 200–350 nm. This overlapped spectrum presents three absorption bands with $\lambda_{max,1}$= 273 nm, $\lambda_{max,2}$= 238.5 nm and $\lambda_{max,1}$= 216.5 nm.

The UV–VIS absorption spectrum of caffeine in water is found to be in the region of 243–302 nm at room temperature. This region can be associated with n → π* electronic transition of caffeine, chlorogenic acids and trigonelline molecules [2]. In particular, the band around 275 nm is related to the C=O chromophore absorption of caffeine [10].

It is clearly shown in Figure 1 that the spectral intensity of caffeine drops to zero at wavelength greater than 302 nm. On the other hand a new absorbance peak is noticed at 216.5 nm. This new spectrum is expected to be the peak absorbance due to the solvent.

The maximum absorbance peak observed for caffeine in this experiment was quite similar to those reported in the literature [1, 11].

Overlapping spectra reveals that there is no interference peaks of impurities in the sample with the analytes of interest.

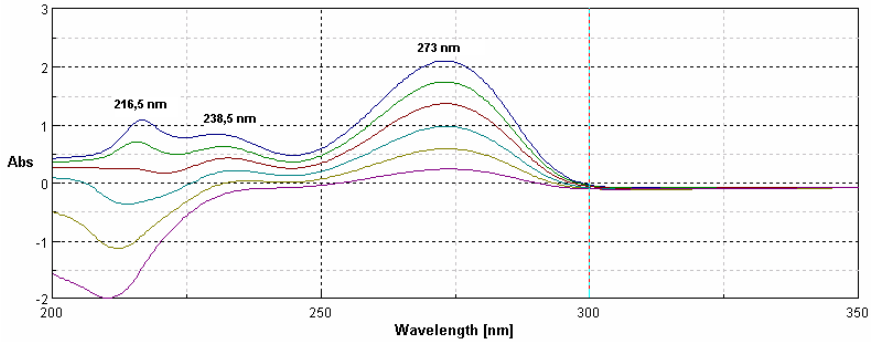

Figure 1. *Molecular absorption spectra in visible and ultraviolet caffeine standard solution and caffeine extracts of studied samples*

Linearity

The concentration range was found to be 3-18 mg·L^{-1}. The PG test was calculated and compared with tabulated values distribution F (Fischer-Snedecor). The results showed that PG value (4.45) was below F value (5.35) for this working range [12]. This means that dispersions deviation wasn't significant, so the working range was chosen correctly.

It was calculated also the dispersion coefficient *b* (slope deviation s_b), the dispersion of the entire population of *y* values (standard deviation for the entire population of *y* values s_0), the dispersion coefficient *a* (intersection with ordinate deviation s_a), coefficient of determination (R^2). The linear regression equation applied to the results gave an

intercept significantly different from zero. The slope was also different from zero and the correlation coefficient was 0.9988.
The obtained LOD and LOQ values were: 0.85 $mg \cdot L^{-1}$, respectively 1.52 $mg \cdot L^{-1}$. These limits were lower than the expected values in studied samples.

Precision
Intra-day precision of the method for samples gave R.S.D. ranging 0.02–0.05 % considering both standard and samples (coffee, ness, soft drink, energy drink, black and green tea extracts). These values were much lower than those in Horwitz equation: $RSD < 2^{(1-0,5 \lg c)}$, for the working range (3-18 $mg \cdot L^{-1}$), for which RSD % should be between 8.0 and 11.3 %.

Robustness
Slot size depends on several factors such as time and wear of deuterium lamp, the wavelength at which it works sensitivity of photomultiplier tube. This study was on four values of the monochromator width entrance slit (0.5 nm; 1.0 nm; 2.0 nm; 5.0 nm).
To demonstrate the influence of the width monochromator entrance slit must establish the linear calibration function and must calculate the dispersion coefficient *b* (slope deviation s_b), the dispersion coefficient a (intersection with ordinate deviation s_a) and determination coefficient for each case (R^2). From the obtained results (presented in Table 1) it was observed that the best linearity was obtained for a width slit of 0.5 nm. Also, can be considered that the size of monochromator slit has a significant influence on the measurement. In order to verify this hypothesis RSD % value was calculated for each concentration level (3, 6, 9, 12, 15 and 18 $mg \cdot L^{-1}$). The results presented in Table 2 confirm this hypothesis. In conclusion according with the obtained results can be considered that the proposed method is robust.

Table1. *The influence of the width monochromator entrance slit*

Parameter	**Series 1 (0.5 nm)**	**Series 2 (1.0 nm)**	**Series 3 (2.0 nm)**	**Series 4 (5.0 nm)**
Linear equation	y = 0.1246x – 0.0643	y = 0.1248x – 0.0629	y = 0.1245x – 0.0575	y = 0.1231x– 0.0575
dispersion coefficient, *b*	0.1246	0.1248	0.1245	0.1231
dispersion coefficient, *a*	-0.0643	- 0.0629	- 0.0575	- 0.0575
slope deviation, s_b	0.00180	0.00059	0.00211	0.00231
intersection with ordinate deviation, s_a	0.021141	0.006991	0.024652	0.027097
Correlation coefficient, *R*	0.99964	0.99959	0.99954	0.99954
Determination coefficient, R^2	0.9993	0.9992	0.9991	0.9991

Table 2. *The values of statistical parameters obtained from experimental results*

Concentration levels / Statistical parameters	**Concentration levels, mg·L^{-1}**					
	Level 1 (3 mg·L^{-1})	Level 2 (6 mg·L^{-1})	Level 3 (9 mg·L^{-1})	Level 4 (12 mg·L^{-1})	Level 5 (15 mg·L^{-1})	Level 6 (18 mg·L^{-1})
	0.332	0.653	1.050	1.443	1.807	2.177
	0.337	0.654	1.052	1.444	1.814	2.183
	0.344	0.656	1.054	1.445	1.815	2.185
	0.341	0.646	1.042	1.427	1.795	2.160
The average, x	0.3385	0.65225	1.0495	1.43975	1.80775	2.17625
Standard deviation, *s*	0.005196	0.004349	0.0052599	0.008539	0.009215	0.0113541
Standard deviation of the average, s_x	0.002598	0.0021745	0.00262995	0.0042695	0.0046075	0.00567705
RSD	0.0153	0.0066	0.005	0.0059	0.0051	0.0052
RSD %	1.53	0.66	0.50	0.59	0.51	0.52

Solution stability

Literature studies on the stability of standard solutions show that standard solutions with concentration of 1000 mg·mL^{-1} which are kept in polyethylene bottles are valid for one year, those with concentrations between 10-100 mg·mL^{-1}, are valid for a month and standard solutions with concentrations between 1 and 10 mg·mL^{-1}, about a week.

The stability of stock caffeine solution of 0.1 mg·mL^{-1} concentration was examined during the day, executing 12 readings at intervals of 1/2 hour. Stability of the same stock caffeine solution was rechecked after a month (at this time the solution was stored in a refrigerator). During measurement (during the 12 readings) solutions were kept at room temperature in 50 mL volumetric flasks. As can be seen from the obtained data (0.0016 and 0.030 % values for RSD), the solution is stable during the day and after one month of storage.

Stability of the samples: Coca Cola, Red Bull, coffee, Jacobs Ness, green tea and Tomurcuk Turkish black tea examined during the day was verified executing 11 readings at intervals of 1/2 hour. All this time solutions were stored at room temperature, in the light in glass bottles. As can be seen from the obtained data (RSD %: 0.32 - 3.75), the solutions are stable throughout the day, giving favorable results and a RSD % which is within the range provided by Horwitz equation. It is noted that the measurements were performed one month after solutions preparation (in this time the solutions were kept in a refrigerator).

Caffeine analysis

Caffeine concentrations in Coca Cola is 132 mg·L^{-1}, in Red Bull is 130 mg·L^{-1}, in coffee is 926 mg·L^{-1} and in ness is 1584 mg·L^{-1}. Caffeine concentrations in green and black tea are presented in Figures 2 and 3.

From the literature data, caffeine dissolution and diffusion in aqueous solutions increases with temperature increasing. Thus, comparing caffeine content at room temperature and at the boiling point (from literature data) was observed that the percentage of caffeine at boiling temperature is much higher.

To determine the caffeine concentration in tea infusion was chosen an infusion time of 5, 10 or 15 minutes. From literature data it is known that for a time of 0.5 to 15 minute infusion the caffeine concentration increases rapidly while for 15-60 minutes the concentration of caffeine increases very slowly. When boiling water is added over the tea leaves, caffeine diffusion occurs in a rapid manner because there is no caffeine in water. When leaves are infused over a long period of time, caffeine concentration becomes higher and therefore closer to the final concentration and caffeine delivering to a lower percentage.
In conclusion (see Figures 2-3), the caffeine concentration in the tea samples was influenced by the infusion time. It was observed an increase of caffeine concentration directly proportion with infusion time.

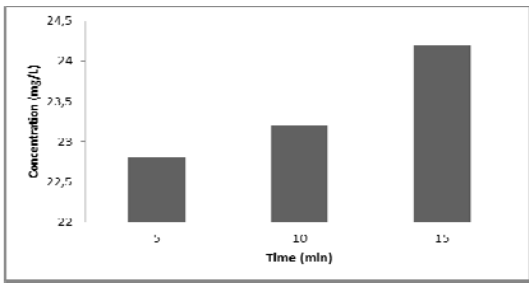

Figure 2. *Evolution of caffeine concentration in black tea infusions*

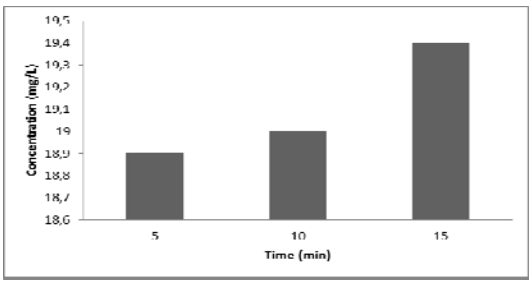

Figure 3. *Evolution of caffeine concentration in green tea infusions*

From experimental data can be observed the similarity of caffeine concentration in Coca Cola and Red Bull and also much higher caffeine concentration in ness comparatively with instant coffee, green tea and black tea.
Caffeine concentration determined in beverages analyzed (coca-cola and red bull) is similar to concentrations found by other researchers and reported in the literature [13].
In some energy drinks, caffeine concentration was found between 106.4 and 308.7 $mg \cdot L^{-1}$ [8], while in four types of coffee (natural, blended, torrefacto and decaffeinated) and three types of tea, caffeine concentration levels were 1.91-9.91 $g \cdot Kg^{-1}$ [14].

CONCLUSIONS

The current method developed on UV/VIS spectrophotometer is relatively easy, precise, robust and sensitive for the determination of caffeine content in tea, coffee and other beverages. Moreover chemicals and equipment necessary to carry out the analysis by proposed method are those which are available in most common laboratories. Therefore, the method can be considered suitable for the intended purpose.
It was observed an increase of caffeine concentration directly proportion with infusion time.

REFERENCES

1. Belay, A., Ture, K., Redi, M., Asfaw, A.: Measurement of caffeine in coffee beans with UV/VIS spectrometer, *Food Chemistry*, **2008, 108**, 310–315;
2. Souto, U.T.C.P., Pontes, M.J.C., Silva, E.C., Galvão, R.K.H., Araújo, M.C.U., Sanches, F.A.C., Cunha, F.A.S., Oliveira, M.S.R.: UV–VIS spectrometric classification of coffees by SPA–LDA, *Food Chemistry*, **2010**, **119**, 368–371;
3. Shrivas, K., Wu, H.F.: Rapid determination of caffeine in one drop of beverages and foods using drop-to-drop solvent microextraction with gas chromatography/mass spectrometry, *Journal of Chromatography A*, **2007**, **1170**, 9–14;
4. Tzanavaras, P.D., Themelis, D.G.: Development and validation of a high-throughput high-performance liquid chromatographic assay for the determination of caffeine in food samples using a monolithic column, *Analytica Chimica Acta*, **2007**, **581**, 89–94;
5. Meinhart, A.D., Bizzotto, C.S., Ballus, C.A., Prado, M.A., Bruns, R.E., Filho, J.T., Godoy, H.T.: Optimisation of a CE method for caffeine analysis in decaffeinated coffee, *Food Chemistry*, **2010**, **120**, 1155–1161;
6. Alizadeh, T., Ganjali, M.R., Zar, M., Norouzi, P.: Development of a voltammetric sensor based on a molecularly imprinted polymer (MIP) for caffeine measurement, *Electrochimica Acta*, **2010**, **55**, 1568–1574;
7. Ito, M., Suzuki, T., Yada, S., Kusai, A., Nakagami, H., Yonemochib, E., Terada, K.: Development of a method for the determination of caffeine anhydrate in various designed intact tables by near-infrared spectroscopy: A comparison between reflectance and transmittance technique, *Journal of Pharmaceutical and Biomedical Analysis*, **2008**, **47**, 819–827;
8. Armenta, S., Garrigues, S., Guardia, M.: Solid-phase FT-Raman determination of caffeine in energy drinks, *Analytica Chimica Acta*, **2005**, **547**, 197–203;
9. Aranda, M., Morlock, G.: Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry, *Journal of Chromatography A*, **2006**, **1131**, 253–260;
10. López-Martínez, L., López-de-Alba, P.L., García-Campos, R., León-Rodríguez, L.M.D.: Simultaneous determination of methylxanthines in coffees and teas by UV–VIS spectrophotometry and partial least squares, *Analytica Chimica Acta*, **2003, 493(1),** 83–94;
11. Suteerapataranon, S., Butsoongnern, J., Punturat, P., Jorpalit, W., Thanomsilp, C.: Caffeine in Chiang Rai tea infusions: Effects of tea variety, type, leaf form, and infusion conditions, *Food Chemistry*, **2009, 114**, 1335–1338;
12. Tanase, I.G., Pana, Al., Radu, G.L., Buleandra, M.: *Validation of Analytical Methods, Theoretical Principles and Case Study*, Printech, Bucharest, Romania **2007**;
13. Jiankai, Z., Na, L.: Simple and environmental friendly procedure for the gas chromatographic–mass spectrometric determination of caffeine in beverages, *Journal of Chromatography A*, **2006**, **1136**, 106–110;
14. Zougagh, M., Rıos, A., Valcarcel, M.: Automatic selective determination of caffeine in coffee and tea samples by using a supported liquid membrane-modified piezoelectric flow sensor with molecularly imprinted polymer, *Analytica Chimica Acta*, **2005**, **539**, 117–124.