STUDIA UNIVERSITATIS BABEŞ-BOLYAI, PHYSICA, L, 4b, 2005

DETERMINATION OF COFFEINE BY LC/MS FROM BEVERAGES

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ABSTRACT

A high performance liquid chromatography coupled with mass spectrometry method (LC/MS) for quantification of caffeine from beverages was elaborated. Detection and quantification of caffeine was based on monitoring the protonated molecular ion abundance (m/z 194.9). The quantification was made using the external standard method. The calibration curve was made on range 0.26-26 μg/ml for caffeine with a correlation coefficient greater than 0.995. there were analyzed 17 samples of different brands of beverages and the content of caffeine found was between 0 and 14.39 mg/100 ml. Due the detection and quantification parameters obtained, this analytical method is rapid, simple and specific.

INTRODUCTION

Nowadays, beverages do not miss almost from any meal and they are a part of the daily fare. Many beverages are prepared of fruit juice and others are made of a mixture of substances that confers them a pleasant cooling taste. The first beverage containing caffeine was prepared in 1886 in Georgia and it's name is Coca-Cola. The pharmacist John S Pemberton prepared, by chance, a delightful and cooling drink from a combination of water, sugar, cola nuts, coca leaves natural flavors. This stimulating and tonic beverage had not the effect of a universal cure-all, but it becomes one of the most popular beverage in the world. Starting with this formula and keeping the caffeine content, that determines the specific properties and taste, there is produced a great variety of beverages which are consumated in high quantities, even by children.

Caffeine (1,3,7-trimethylxanthine) is a methyl derivative of xanthine, a natural product with pharmacological actions in the human organism and with toxic effects in high doses [1].

In this paper it was elaborated a very simple and rapid method of quantification of caffeine from different types of beverages from Romanian market [2,3,4].

MATERIALS AND METHOD

Chemicals

Caffeine was purchased from Sigma-Aldrich Germany. Methanol and formic acid 98% were from Merck KGaA (Darmstadt, Germany).

Apparatus and chromatographic conditions

The concentrations of coffeine were determined using a LC/MS analytical method [2]. Briefly, an Agilent 1100 Series (Agilent, USA) chromatographic system was used coupled with an 1100 LC/MSD Ion Trap detector (Agilent, SUA). Analytical column: Zorbax SB-C18, 100 mm x 3.0 mm i.d., 3.5 µm, (Agilent, USA) with an on-line filter (0.2µm). The mobile phase was a mixture 24:76 (v/v) of methanol: 0.2% formic acid in water. The flow was 0.6 ml/min and the injection volume of 5 µl. The mass analyzer operated with an ESI source, positive ion mode. For caffeine, the quantification was made by measuring the intensity of protonated molecular ion of coffeine at m/z 194.9.

Standard solutions

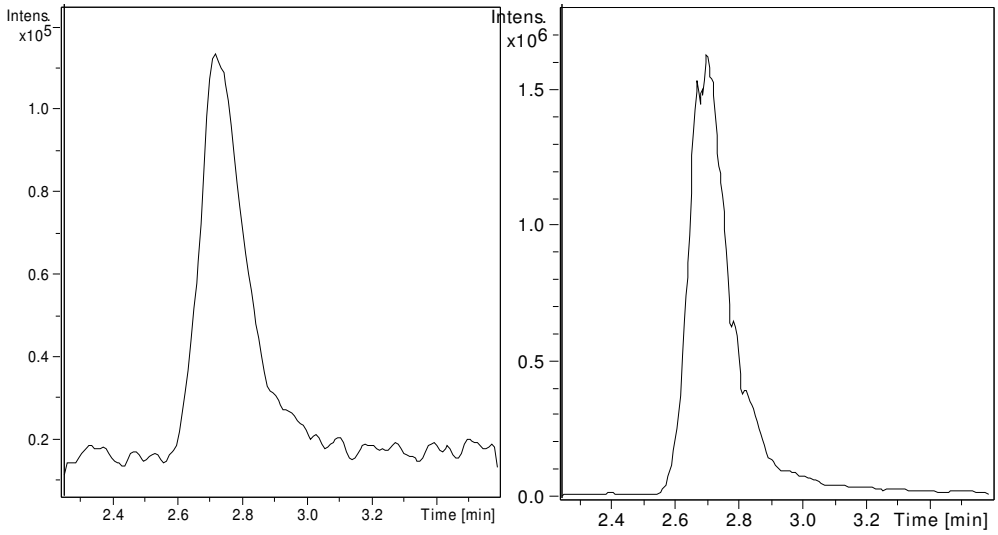
Stock solution of caffeine (1-2mg/ml) was obtained by dissolving corresponding compounds in methanol. Standard solutions (7 concentrations) were obtained by diluting appropriate volumes of stock solution with bidistilled water.

Sample preparation

Due to MS detection specificity, a preliminary extraction was not necessary and the samples were prepared by only dilution of 0.2 ml product to a 10 ml final volume. A 5 µl volume of final solution was injected in the HPLC/MS system.

RESULTS AND DISCUSSIONS

The identification of caffeine in beverages was made by the retention time (2.75 min) of ion with m/z 194.9. In Figure 1a and b are presented sample chromatograms with coffeine at quantification limit (0.26 µg/ml) and extracted from beverages.



(a) (b)

Fig. 1. Typical chromatograms of coffeine (a) at quantification limit, 0.26 µg/ml, (b) extracted from beverages (Coca-Cola)

Quantitative determination was made by monitoring the protonated molecular ion of coffeine at m/z 194.9, as shown in the full scan spectrum in Fig. 2.

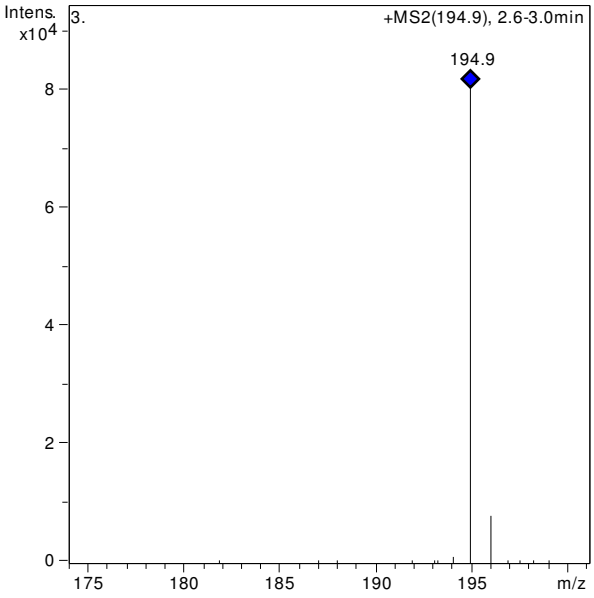


Fig. 2. Full-scan MS spectrum of coffeine

The quantification of caffeine in beverage samples was performed using the calibration curve made in range of 0.26-26 μg/ml.

There were analyzed 17 beverages and the coffeine content was found between 0.00 and 14.39/100ml. The results are presented in Table 1

CONCLUSIONS

A rapid and simple LC/MS method was elaborated, that proved to be suitable for quantification of caffeine in different brands of coffee. The developed method can be successfully used for fast and accurate determinations of caffeine in beverages, especially because of the performance of detection technique, which provides a very short time of analyze and minimal need for sample preparation.

The content of coffeine found in beverages was between 0 and 14.39 mg/100 ml product, which is in concordance with the content declared of producer.

Table 1

The caffeine content in different brands of beverages

No.	Product Name	Producer	Coffeine content, mg/100 ml
1	Coca Cola	Coca Cola Company- Bucuresti	**9.40**
2	Coca Cola light	Coca Cola Company- Bucuresti	**11.38**
3	Coca Cola Vanilla	Coca Cola Company- Bucuresti	**9.12**
4	Pepsi	Quadrant Amrog Beverages-Bucuresti	**9.05**
5	Pepsi Max	Quadrant Amrog Beverages-Bucuresti	**9.48**
6	Pepsi Twist	Quadrant Amrog Beverages-Bucuresti	**10.00**
7	Pepsi light	Quadrant Amrog Beverages-Bucuresti	**9.97**
8	Pepsi Twist light	Quadrant Amrog Beverages-Bucuresti	**9.75**
9	American Cola	European Drinks -Stei BH	**7.98**
10	Adria Cola	European Drinks -Stei BH	**8.04**
11	Mara Cool Cola	Mara Agroind Morariu Dej	**7.80**
12	Cola&Cola-Giusto	Romaqua Group Borsec	**8.39**
13	Fanta portocale	Coca Cola Company- Bucurest	**0.00**
14	Schweppes-Kinley Tonic	Coca Cola Company- Bucurest	**0.00**
15	7 up	Quadrant Amrog Beverages-Bucuresti	**0.00**
16	Mountain Dew	Quadrant Amrog Beverages-Bucuresti	**14.39**
17	Biborteni- Cola	Bibco Biborteni	**1.70**

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