

## DETERMINATION OF CAFFEINE FROM DIFFERENT BRANDS OF COFFEE

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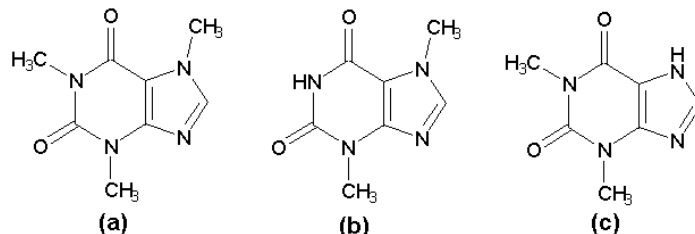
**DETERMINATION OF CAFFEINE FROM DIFFERENT BRANDS OF COFFEE (Abstract):** A high performance liquid chromatography coupled with mass spectrometry method (LC/MS) for quantification of caffeine from coffee was elaborated. It was utilised a Zorbax SB-C18, 100 mm x 3.0 mm i.d., 3.5  $\mu$ m column with a mobile phase containing methanol/solution 0.2% formic acid in water. Detection and quantification of xanthines was based on monitoring the protonated molecular ion abundance ( $m/z$  194.9 for caffeine). The quantification was made using the external standard method. The calibration curve were made on range 0.26-26  $\mu$ g/ml for caffeine with a correlation coefficient greater than 0.995. Due the detection and quantification parameters obtained, this analytical method is rapid, simple and specific. **Key words:** CAFFEINE, LC/MS

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. Caffeine is encountered in coffee beans (~1%), in tea leaves (up to 5%), in Cola nuts (~3%) and in some tropical plants. Besides caffeine, in natural products there are other methylxanthines like theobromine (3,5-dimethylxanthine) and theophylline (1,3-dimethylxanthine).

Coffee is known and cultivated for about 600.000-700.000 years. In the early years

it was consumed as a paste or even as beans. Later, it was discovered that by introducing the beans in hot water, a drink that produces behavior changes is obtained. There is a legend that tells that a goatherd discovered stimulant effects of coffee when his goats ate from coffee bushes and they became more bustling.

Caffeine and its metabolites do not accumulate in organism, but they are demethylated and excreted as methyluric acid. Coffee determines in human organism vasodilatation, increase of speed of respiration,



**Fig. 1.** Chemical structure of xanthines : caffeine (a), theobromine (b), theophylline (c)

of pulse and of blood pressure, stimulates gastric secretion and the synthesis of catecholamines, stimulates the diuresis and the intellectual activity.

In high doses, caffeine determines lack of appetite, hand trembling, gastric affections such as irritations and ulcer, increase of blood pressure, cardiac and renal manifestations, favors the breast tumors (1).

In this paper it was elaborated a very simple and rapid method of quantification of caffeine from different types of coffee from Romanian market.

## 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 caffeine was 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

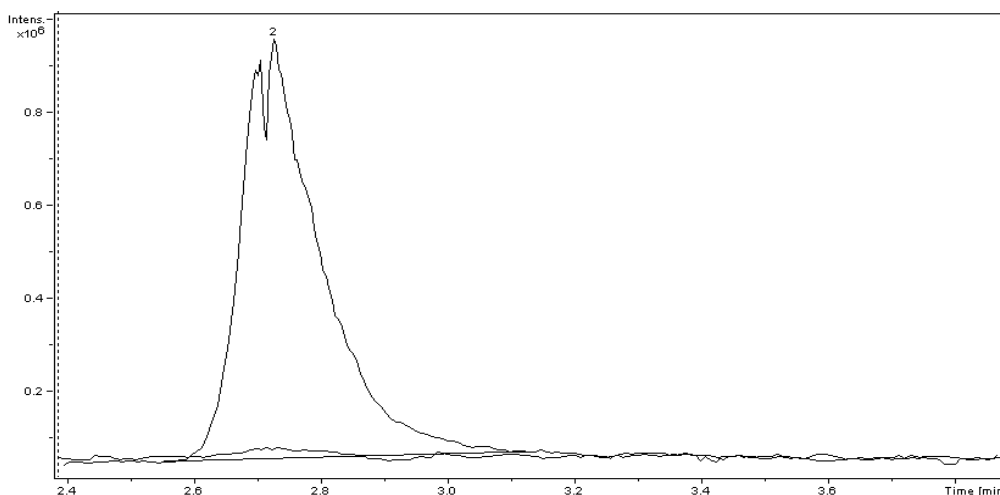
detector (Agilent, SUA). Analytical column: Zorbax SB-C18, 100 mm x 3.0 mm i.d., 3.5  $\mu$ m, (Agilent, USA) with an on-line filter (0.2  $\mu$ m). The mobile phase was a mixture 24:76 (v/v) of methanol : 0.2% formic acid in water. The flow was 0.6 ml/min and the injection volume of 5  $\mu$ l. The mass analyser operated with an ESI source, positive ion mode. For caffeine, measuring the protonated molecular ion 194.9 made the quantification.

### Standard solutions

Stock solution of caffeine (1-2 mg/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

Because of detection specificity due to MS detector, the sample preparation was reduced to a simple extraction of this solution. About 150 mg product were extracted for 10 min with 15 ml boiled water, the volume was adjusted to 25 ml and then 0.1 ml from this solution diluted to 5 ml. 5  $\mu$ l from



**Fig. 2.** The chromatograms of coffee and decaffeinated samples using methanol : 0.2% formic acid in water mobile phase

# Determination on Caffeine from Different Brands of Coffe

TABEL I  
The concentration of caffeine

Nr.	Product Name	Caffeine (g/100 g)
1	Ackerman - Auslest	1.517
2	Ackerman - Mocca rosu	2.096
3	Alka - Gold Mocca	2.047
4	Alka Mocca	1.059
5	Alka - Sultan - coffe ith cardamon	1.769
6	Amaroy Extra	1.339
7	Arabia - Julis Meinl	1.892
8	Celmar Caffee de Columbia	1.717
9	Celmar Viva clasical coffe	2.929
10	Eduscho - Dupla	2.363
11	Elite Elita red	2.751
12	Elite Fort	2.516
13	Elite Selected green	1.494
14	Fortuna Rendezvous	1.500
15	Hofer Extra	1.418
16	Jacobs Alintaroma (green)	1.564
17	Jacobs Aroma (red)	2.211
18	Lavazza - espresso	1.612
19	Metropolitan cafee-premium	1.966
20	Nova Brasilia	2.748
21	Nova Brasilia Caffee moulu	2.914
22	Paul Dequidt nr.1	1.481
23	Rafael - Italia	2.389
24	Segafredo	2.482
25	Tchibo -Exclusive	1.736
26	Tchibo Family	2.785
27	Inka Biogran - decaffeinated	0.000
28	Inka Extra Biogran - decaffeinated	0.039

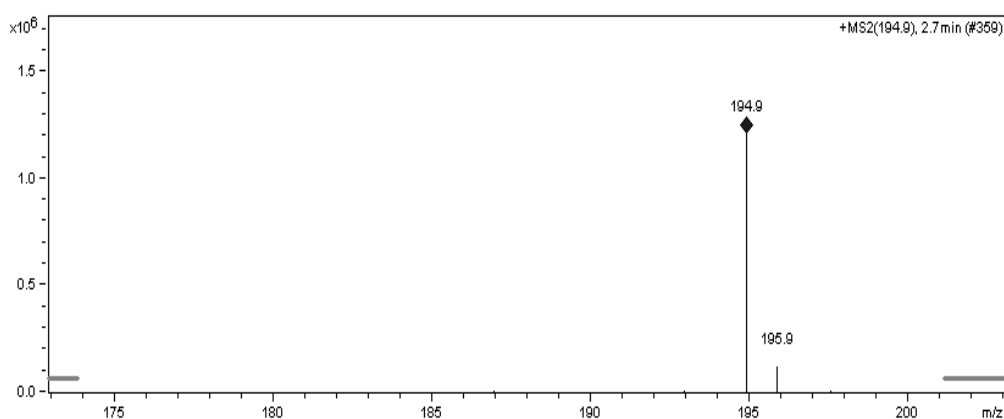
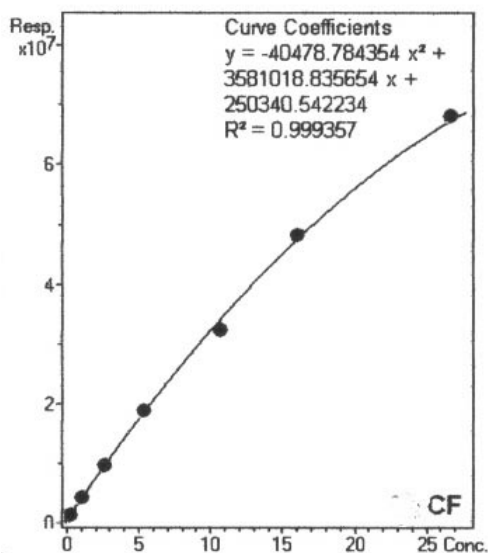


Fig. 3. Electrospray positive ion spectrum of caffeine with protonated molecular ion at m/z 194.9



**Fig. 4.** The calibration curve for caffeine final solution was injected into LC/MS system.

## RESULTS AND DISCUSSIONS

The identification of caffeine in coffee extracts was made by the retention time (2.75 min) in the chromatograms. Figure 2 presents a coffee sample chromatogram and

two decaffeinated sample chromatograms. Quantitative determination was made by monitoring the protonated molecular ion at  $m/z$  194.9, as shown in the full scan spectrum (fig. 3). The quantification of caffeine in coffee samples was performed using the calibration curve made in range of 0.26-26  $\mu\text{g/ml}$  (fig. 4).

The developed HPLC-MS method was then used for quantification of caffeine in 28 coffee samples that are frequently consumed by Romanian people. From the total of 28 samples, two were decaffeinated. The results in Table I show the concentration of caffeine between 1.059 and 2.929 g/100g coffee.

## 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 coffee, especially because of the performance of detection technique, which provides a very short time of analyze.

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