determination of caffeine by mass spectrometry in different samples

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Abstract

The caffeine content of sugar-free beverages (colas, coffee, energy drinks and herbal teas) was determined by gas chromatography-mass spectroscopy (GC-MS) without using any pre-separation or background correction techniques. This paper described determination caffeine in the relative saliva, plasma, urine, breast milk and coffee.

Introduction

The notion of atomic and molecular weights is one of the first concepts taught in a general chemistry class. Hence, what could be a better time to provide students with hands-on experience with mass spectrometry (MS)[1].

MS imaging has evolved as a valuable technique to localize the distribution of organic molecules on solid samples, such as human or animal tissues, plant materials, and historic objects like paintings. for example, in the field of forensic science, MS Imaging was applied to investigate trace evidence like textile fibres, in which synthetic dyes were mapped in single nylon fibres and single acetate yarns using TOF-SIMS[4].

demonstrate the capabilities of mass spectrometry in identifying the size, composition, and charge states of nanometer-sized metal clusters, as well

as the size evolution during the synthesis. All these studies have shown that MS has now become an indispensable tool in metal nanocluster research. During the last decade, various MS techniques have been used in the studies on gold, silver, copper, noble metals and their doped or alloyed nanoclusters[5]. Coffee is one of the most popular beverages in the world, being appreciated for its characteristic taste and aroma and, more recently, for its potential beneficial effects on human health. Typical compounds in coffee that are relevant for flavour and/or bioactivity are caffeine, trigonelline, nicotinic acid and sucrose[6,7].

2. Method and material

the method is described as in the paper [8]

2.1. Chemicals

1,3,7-Trimethylxantine (caffeine), 3,7-dimethylxantine (theobromine), 1,7-dimethylxanthine (paraxanthine), and 1,3-dimethylxanthine (theophylline) were all purchased from

Double-distilled water , HPLC-grade acetonitrile (Sigma–Aldrich) formic acid (Fisher-Scientific, Fair Lawn, NJ, USA) for mobile phase preparations. A 624_mol L−1 stock solution of 13C3 caffeine from Isotec (99 atom% 13C, Miamisburg, OH, USA) was used as an internal standard (IS) to improve quantitation

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3. Results and discussion

3.1. **Quantification of theobromine and caffeine in saliva, plasma and urine**

fig 1 shows Analyte peaks of separation mixture included (1, theobromine; 2, paraxanthine; 3, theophylline; and 4, caffeine)[8].

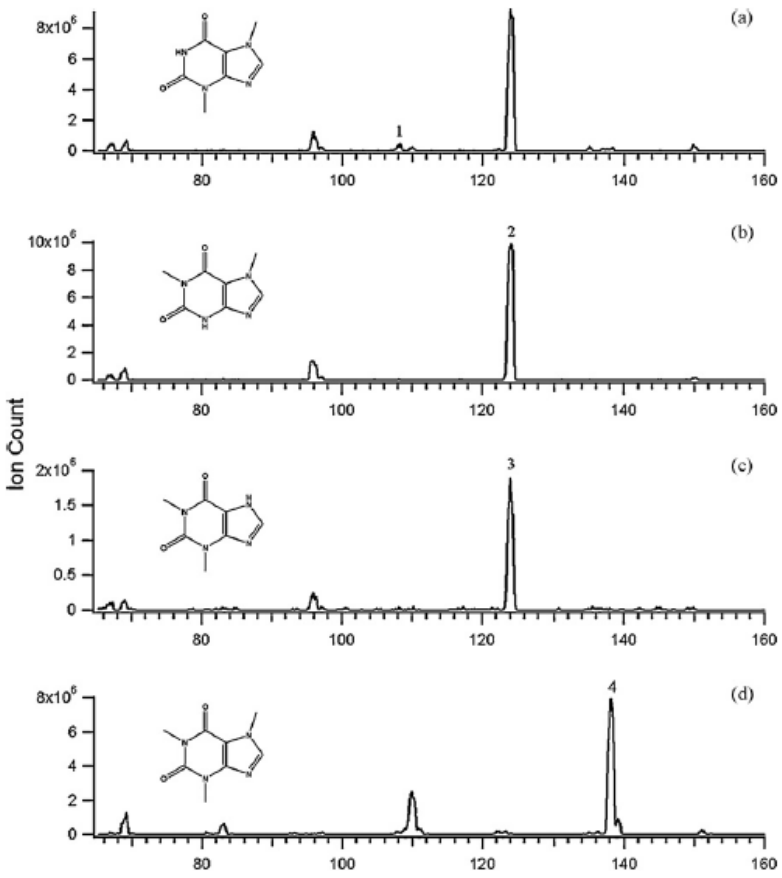

Fig. 1. Product ion scans of theobromine (a), paraxanthine (b), theophylline (c), and caffeine (d).

Complex matrices of pooled unprocessed saliva (N= 12), plasma (N= 8) and urine (N= 8) samples were created to challenge the validity of the optimized assay. Each biofluid pool was then prepared using the protocol

outlined in Section 2.2. The isolated low MW filtrate was diluted 20-fold with distilled water prior to injection as described. Each respective validation parameter was assessed through spiking the appropriate concentration of caffeine, theobromine and 13C3-caffeine (IS) into the pooled sample. A blank sample (no analyte spike) for each biofluid was subtracted from the spiked samples to account for the presence of any

3.2. **determination of nicotine and metabolites, caffeine and arecoline in breast milk[2]**

Table 2 and fig 2 shows the results obtained for 20 breast milk samples analyzed during assay development. The selfreported smoking status was confirmed in nursing mothers.CAF was found in 75% of the breast milk samples of both smoking and non-smoking mothers, showing the widespread consumption of this substance during pregnancy.

Table 2. Analytes concentration in breast milk samples from nursing mothers

Sample	NIC (µg/L)	COT (µg/L)	TRANS-3-OH-COT (µg/L)	COT-N-OX (µg/L)	CAF (µg/L)	ARECA (µg/L)
1 (non-smoking mother)	neg*	neg	neg	neg	315.4	neg
2 (non-smoking mother)	neg	neg	neg	neg	2827.8	neg
3 (smoking mother)	neg	20.5	neg	neg.	47.1	neg
4 (non-smoking mother)	neg	neg	neg	neg	602.6	neg
5 (non-smoking mother)	neg	neg	neg	neg	602.0	neg
6 (smoking mother)	40.0	173.9	14.4	18.4	1166.2	neg
7 (non-smoking mother)	neg	neg	neg	neg	391.5	neg
8 (non-smoking mother)	neg	neg	neg	neg	1380.1	neg
9 (smoking mother)	neg	27.1	neg	neg	162.8	neg.
10 (smoking mother)	neg	128.5	neg	neg	neg	neg
11 (non-smoking mother)	neg	neg	neg	neg	neg	neg
12 (smoking mother)	240.5	36.1	neg	neg	110.0	neg
13 (smoking mother)	142.3	81.6	17.3	5.0	neg	neg
14 (non-smoking mother)	neg	neg	neg	neg	609.8	neg
15 (non-smoking mother)	neg	neg	neg	neg	554.9	neg
16 (smoking mother)	44.1	3.7**	neg	neg	1343.0	neg
17 (betel quid consumer)	100.1	344.8	neg	neg	263.4	18**
18 (betel quid consumer)	55.6	2.2**	neg	neg	498.5	22**
19 (betel quid consumer)	69.2	12.7	3.8	neg	neg	50.7
20 (betel quid consumer)	513.5	172.3	15.2	neg	neg	152.9

*Negative sample, no substance detection.

**2 mL milk were analyzed to quantify these samples where analytes showed concentrations between LOD and LOQ.

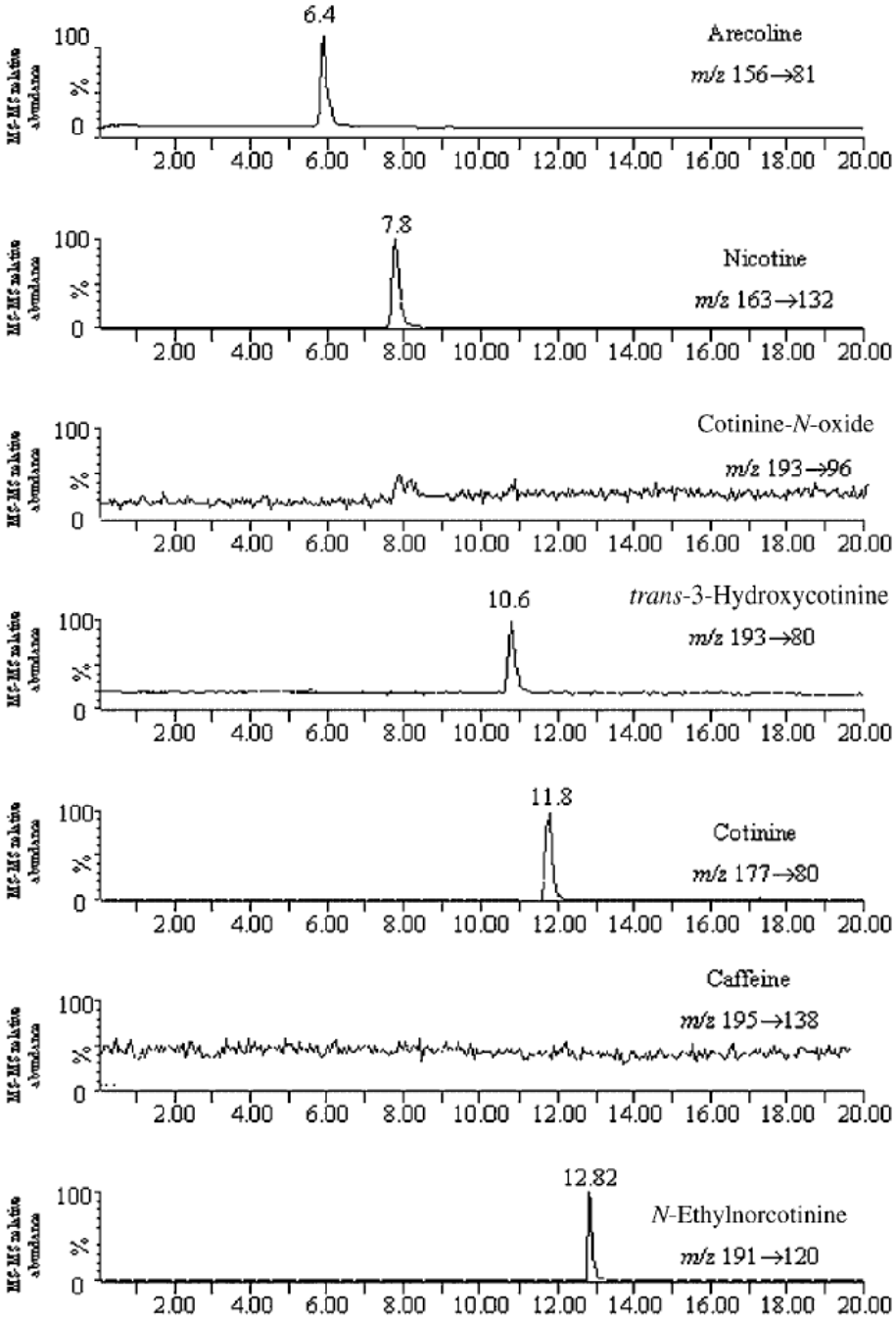

Figure 2. LC/MS/MS chromatogram of an extract of sample 20 containing 513.5 mg/L NIC, 172.3 mg/L COT, 15.2mg/L TRANS-3-OH-COT and 152.9 mg/L ARECA.

3.3. analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee[9].

In order to evaluate the applicability of the method, green and commercial samples of regular and decaffeinated roasted and instant coffees, roasted to different degrees (samples A–K), were analyzed. Fig. 3 shows typical chromatograms for green and roasted regular coffee samples. The contents of caffeine, trigonelline, nicotinic acid and sucrose in these samples are shown in Table 3.

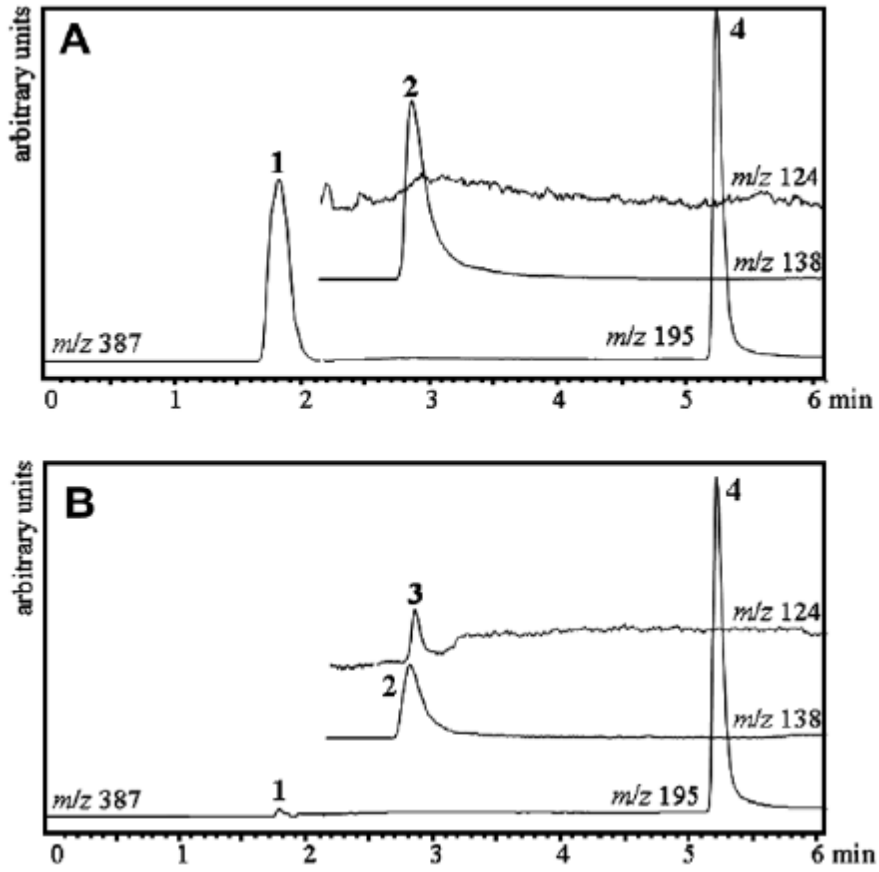

Fig. 3. Typical chromatographic separation of (A): green coffee, represented by C. arabica from Brazil (sample A); (B): ground roasted coffee, represented by C. Arabica from Colombia

Table 3

Caffeine, trigonelline, nicotinic acid and sucrose contents in green and commercial coffee samples using the proposed analytical method

Coffee sample	Species	Origin	Color[b]	Caffeine	Trigonelline	Nicotinic acid	Sucrose
Green coffee							
A	Arabica	Brazil	n.d.[c]	919.0 ± 12.4	1029.8 ± 26.0	ND[d]	8346.9 ± 118.8
B	Robusta	Brazil	n.d.	1701.3 ± 83.7	900.6 ± 2.6	ND	6401.6 ± 143.3
Commercial ground roasted coffee							
Regular							
C	Arabica	Hawaii	35	851.6 ± 10.0	279.7 ± 9.4	29.5 ± 0.4	15.9 ± 0.7
D	Arabica	Brazil	45	908.6 ± 30.4	526.8 ± 21.9	30.4 ± 1.3	52.0 ± 0.7
E	Arabica	Brazil	55	843.3 ± 28.9	641.3 ± 23.0	20.3 ± 0.8	79.2 ± 2.0
F	Blend	Africa	65	1456.0 ± 8.6	592.2 ± 18.5	13.7 ± 0.1	87.8 ± 3.2
G	Arabica	Colombia	65	848.1 ± 35.2	682.5 ± 7.5	9.6 ± 0.3	173.3 ± 0.1
H	Arabica	Brazil	65	930.9 ± 34.9	955.7 ± 33.5	10.3 ± 0.2	183.8 ± 8.3
Decaffeinated							
I	Blend	Brazil	55	20.4 ± 0.3	663.1 ± 14.1	10.4 ± 0.5	31.1 ± 1.1
Commercial instant coffee							
J	Blend	Brazil	65	2054.7 ± 14.0	917.4 ± 4.1	36.3 ± 1.1	129.9 ± 3.6
K	Blend	Brazil	65	2163.4 ± 10.6	832.8 ± 21.0	34.2 ± 1.2	128.1 ± 3.4

[a] Results are shown as the means of extraction in three replicates ± standard deviation, expressed as mg/100 g of coffee dry weight.
[b] Roasting Color Classification System (Agtron – SCAA, 1995) where 65 = light medium, 55 = medium, 45 = moderately dark, 35 = dark.
[c] Not determined.
[d] Not detected, below limit of detection.

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