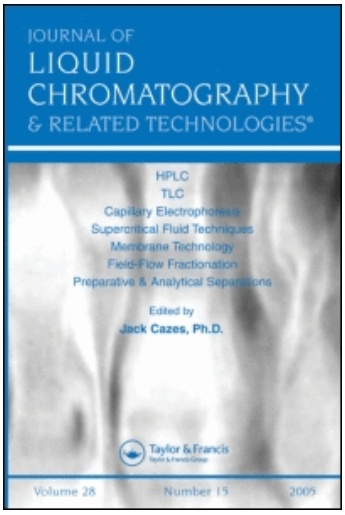

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S. Casal[a]; M. B. Oliveira[a]; M. A. Ferreira[a]
[a] CEQUP/Lab. de Bromatologia Faculdade de Farmácia Universidade do Porto Aníbal Cunha, Porto, Portugal

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DEVELOPMENT OF AN HPLC/DIODE-ARRAY DETECTOR METHOD FOR SIMULTANEOUS DETERMINATION OF TRIGONELLINE, NICOTINIC ACID, AND CAFFEINE IN COFFEE

S. Casal, M. B. Oliveira, M. A. Ferreira*

CEQUP/Lab. de Bromatologia
Faculdade de Farmácia
Universidade do Porto
Aníbal Cunha
4050 Porto, Portugal

ABSTRACT

This paper describes an adequate procedure of reversed-phase HPLC/diode-array detector to be used in quality control to simultaneously quantify three nitrogen compounds: trigonelline, nicotinic acid, and caffeine, in coffee samples either in the green or roasted states. The chromatographic separation was achieved using a reversed-phase column (Spherisorb ODS2) with gradient elution of 0.01M phosphate buffer pH 4.0 (A) and methanol (B). The effluent was monitored by a diode-array detector and the chromatograms were recorded at 265 nm. The sample preparation was quite simple involving only boiling water extraction and filtration.

A linear relationship was found between peak area and concentration range of 0.15-450 μg/mL, 0.10-500 μg/mL, and 0.05-500 μg/mL for trigonelline (at 268 nm), nicotinic acid (at 264 nm), and caffeine (at 276 nm), respectively.

Extensive quality assurance of the proposed method was performed by the standard addition method in both green and roasted coffee.

The precision in green coffee samples was better than 1.3, 5.8, and 1.1% and, for roasted coffee, better than 0.5, 2.4, and 1.2% for trigonelline, nicotinic acid, and caffeine, respectively. Also, for green coffee, the mean recovery values were 98 ± 1%, 84 ± 5%, and 99 ± 1% and for roasted coffee, these were 101 ± 1%, 98 ± 1%, and 99 ± 1 % for trigonelline, nicotinic acid, and caffeine, respectively.

The proposed method appears to be an adequate method for quality control in the coffee industry.

INTRODUCTION

Flavours, nutritional and toxicological properties of coffee, are affected by roasting as a consequence of thermal degradation of several endogenous compounds. Trigonelline (N-methylbetaine of pyridine-3-carboxylic acid) (I), nicotinic acid (pyridine-3-carboxylic acid) (II), and caffeine (1,3,7-trimethylxanthine) (III) are natural nitrogen compounds present in coffee beans described[1,2] as suffering modifications during the roasting procedure.

Due to the variations in contents of trigonelline, nicotinic acid, and caffeine in commercial coffee brought about by botanical, ecological, and roasting reasons, the simultaneous quantification of these three nitrogenous components should be useful both for quality control of raw materials and roasted coffee and to follow the technology of the thermal procedure used for roasting.

Several chromatographic methods are found in the literature[3-7] for the individual quantification of these compounds and for biparametric determinations of trigonelline and caffeine in coffee.[8-10] Nicotinic acid is usually determined by microbiological assays.

Several chromatographic attempts to analyse it in coffee by reversed-phase have been tried, however complete peak resolution due to certain interfering components has not yet been achieved.[1]

As far as we know, this paper represents the first attempt to simultaneously quantify trigonelline, nicotinic acid, and caffeine free from interferences by a reversed-phase HPLC/diode-array method.

EXPERIMENTAL

Apparatus

The chromatographic analysis was carried out in a analytical HPLC unit (Jasco) equipped with two PU-980 pumps, a MD-910 diode-array detector (DAD) and a AS-950 auto-sampler.

Reagents and Standards

Trigonelline, nicotinic acid, and caffeine were purchased from Sigma. Potassium dihydrogen phosphate (p.a.) and methanol (LiChrosolv - gradient grade) were purchased from Merck.

Coffee Samples

Two samples from Brazil and Ivory Coast, before and after roasting were provided by a local coffee industry. The beans were ground and powdered to pass through a 0.75mm sieve. Sample moisture was determined by drying at 103 ± 2°C until constant weight.

Extraction of Compounds from Coffee

A 2 g portion of each powdered coffee bean sample was blended with several portions of 20 mL of water in a Erlenmeyer flask and boiled during 5 min on a heated magnetic stirrer. The extracts were combined and transferred to a 100 mL volume flask and diluted to the volume mark. The mixture was filtered through 0.2 μm filter paper and 20 μL was analysed by HPLC.

HPLC Analysis

The chromatographic separation of compounds was achieved with a reversed-phase Spherisorb ODS2 (5 μm, particle size; 25.0 x 0.46 cm) column. The solvent system used was a gradient of phosphate buffer (pH 4.0) (A), prepared with 5% of potassium dihydrogen phosphate 0.2 M, and methanol (B). The gradient was as follows: 0'-7% B, 4'-9% B, 6'-25% B, 13'-29% B, and 21'-50% B with a flow rate of 1.0 mL/min. Detection was accomplished with a diode-array detector at 268 nm for trigonelline, 264 nm for nicotinic acid, and 276 nm for caffeine.

The chromatograms were recorded at 265 nm. The compounds under study were identified by chromatographic comparisons with authentic standards, by coelution and by their UV spectra. Quantification was based on the external standard method.

RESULTS AND DISCUSSION

Analytical Curve and Detection Limit

The above mentioned extraction procedure has proved to be the best of several attempts, involving time and temperature variations. The use of acid or basic conditions was also tested.

In order to study the precision of the chosen extraction method one sample was extracted three times. The coefficients of variation were 1.6, 1.0, and 2.3% for trigonelline, nicotinic acid, and caffeine, respectively.

Under the assay conditions, a linear relationship between the concentration of the compounds in study and the UV absorbance was obtained. This linearity was maintained over the concentration range 0.15-450 μg/mL, 0.10-500 μg/mL, and 0.05-500 μg/mL for trigonelline, nicotinic acid, and caffeine, respectively. The correlation coefficient for each standard curve invariably exceeded 0.999.

The calibration curves were obtained by triplicate determinations of each calibration standard with the peak area values (arbitrary units) plotted as average values. The relative average deviations of triplicates were less than 2% in all cases. The average regression equations found were: $y = 60.26362x - 1.57708$, $y = 73.55656x + 0.25439$, and $y = 111.53150x + 1.36925$ for trigonelline, nicotinic acid, and caffeine, respectively.

The detection limit values were calculated as the concentration corresponding to three times the standard deviation of the background noise and were 0.15 μg/mL for trigonelline, 0.10 μg/mL for nicotinic acid, and 0.05 μg/mL for caffeine.

Validation of the Method

The chromatograms obtained for green and roasted coffee samples from Ivory Coast (*Coffea canephora* var. *robusta*) are shown in Figures 1 and 2. The retention times (RT) obtained were: RT 3m 34s for trigonelline, RT 4m 36s for

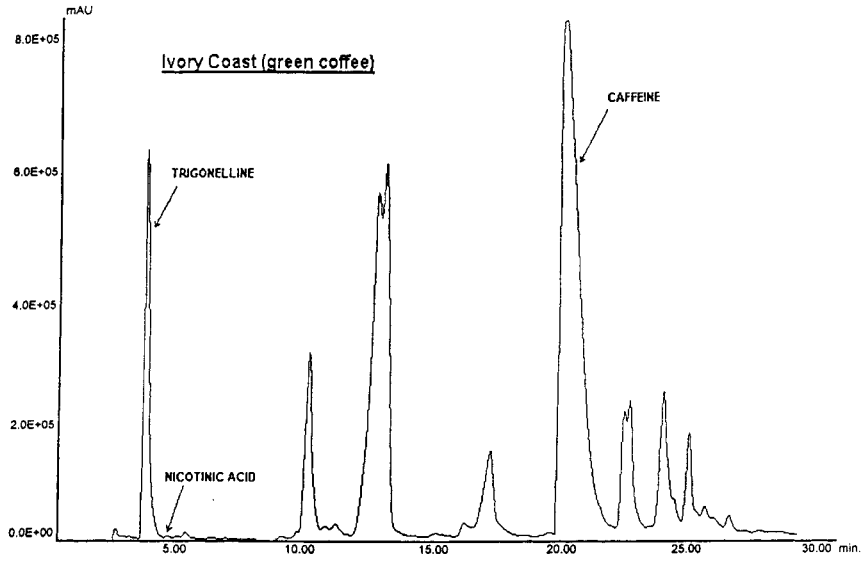

Figure 1. HPLC profile of a green coffee sample from Ivory Coast. Recorded at 265 nm.

nicotinic acid, and RT 20m 10s for caffeine. The unidentified peaks had identical UV spectra when recorded with a diode-array detector, with identical shape and maximum at 320 nm, which suggested that they could be hydroxycinnamic acids, which were not identified because they were not in the scope of this research work.

Results from the quantification applied to one sample of *Coffea arabica* (Brazil) and one sample of *Coffea canephora* var. *robusta* (Ivory Coast) are shown in Table 1. All determinations were referred to dry base as the average of triplicate determinations.

The values in both coffee species are in good agreement with those reported in the literature where they were measured by other different methodologies except for M.J. Martín[3] and Mazzafera[7] who found higher contents of trigonelline and M. Daglia[9] who found higher contents of nicotinic acid. The precision of the analytical method was evaluated by measuring the peak chromatographic area of the three compounds 10 times on the same sample. The relative standard deviations for trigonelline, nicotinic acid, and caffeine were, respectively, 1.3, 5.8 and 1.1 % in the green and 0.5, 2.4 and 1.2% in the roasted coffee.

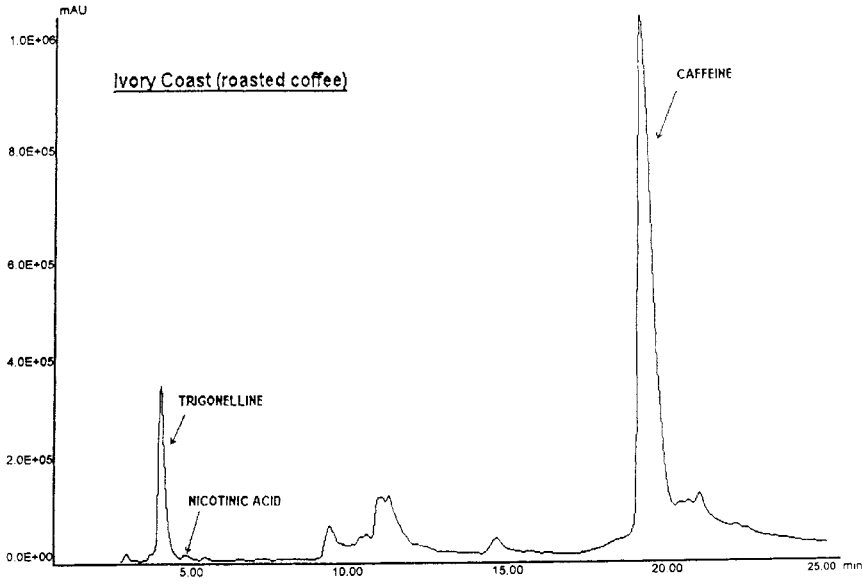

Figure 2. HPLC profile of a roasted coffee sample from Ivory Coast. Recorded at 265 nm.

Table 1

Trigonelline, Nicotinic Acid, and Caffeine Content in Green and Roasted Coffee Samples from Brazil (*Coffee Arabica*) and Ivory Coast (*Coffee Canephora Var. Robusta*) (db)

	Brazil		Ivory Coast	
	Green g/kg ± sd	Roasted g/kg ± sd	Green g/kg ± sd	Roasted g/kg ± sd
Trigonelline	8.912±0.006	5.499±0.013	6.162±0.059	3.600±0.081
Nicotinic Acid	0.028±0.001	0.114±0.011	0.025±0.004	0.095±0.007
Caffeine	12.361±0.101	16.128±0.005	20.832±0.224	25.551±0.185

In order to demonstrate the effectiveness of the extraction procedure and the accuracy of the proposed method, one sample of green coffee was analysed in triplicate before and after the addition of three known quantities of trigonelline, nicotinic acid, and caffeine. The same procedure was applied to a

Table 2

Recovery of Trigonelline, Nicotinic Acid, and Caffeine From a Spiked Green Coffee Sample*

	Present (g/kg)	Added (g/Kg)	Found (g/Kg)	Standard Deviation	CV%	Recovery %
Trigonelline	7.063	0.981	7.936	0.100	1.3	98.7±1.2
		0.742	7.591	0.070	0.9	97.3±0.9
		0.534	7.440	0.019	0.3	97.9±0.3
Nicotinic acid	0.018	0.196	0.183	0.012	6.6	85.5±5.6
		0.148	0.136	0.006	4.4	81.9±3.6
		0.097	0.097	0.005	6.2	84.3±5.2
Caffeine	15.487	3.091	18.668	0.186	1.0	100.5±1.0
		1.682	16.771	0.114	0.7	97.7±0.7
		0.631	15.972	0.003	0.0	99.1±0.0

* Mean value found for 3 assays for each studied concentration

roasted coffee sample. The results are listed in Tables 2 and 3. The mean recovery values for trigonelline, nicotinic acid, and caffeine were, respectively, 98 ± 1%, 84 ± 5%, and 99 ± 1% in green coffee and 101 ± 1%, 98 ±1%, and 99 ± 1% in roasted coffee. The low recovery for nicotinic acid in the green coffee can be explained by its small concentration, near to the detection limit. These results confirm no interference effects due to matrix composition.

For the simultaneous determination of trigonelline and nicotinic acid it was very important to control the pH of the solvent used in the chromatographic separation, therefore, it was essential to work close to pH 4.0 (3.9-4.1). At higher pH the separation of compounds was compromised. With pH=6 an overlapping of both compounds was observed. The use of the diode-array detector was crucial to achieve a chromatographic gradient with no interfering components in the area of the nicotinic acid peak in both green and roasted coffee.

In conclusion, this study suggests that the technique herein proposed is a new useful approach for the simultaneous analysis of trigonelline, nicotinic acid, and caffeine in both green and roasted coffee samples.

Table 3

Recovery of Trigonelline, Nicotinic Acid and Caffeine from a Spiked Roasted Coffee Sample*

	Present (g/kg)	Added (g/Kg)	Found (g/Kg)	Standard Deviation	CV%	Recovery %
Trigonelline	3.393	1.143	4.490	0.016	0.36	99.0±0.4
		0.747	4.419	0.051	1.22	101.2±1.2
		0.549	3.996	0.102	2.55	101.4±2.6
Nicotinic acid	0.191	0.519	0.688	0.008	1.16	96.8±1.1
		0.449	0.643	0.005	0.78	100.5±0.8
		0.249	0.430	0.003	0.70	97.7±0.7
Caffeine	19.478	2.701	21.810	0.020	0.09	98.3±0.1
		1.644	20.891	0.158	0.76	98.6±0.7
		0.549	20.364	0.616	3.02	101.7±3.1

* Mean value found for 3 assays for each studied concentration.

Despite the complexity of the matrix, the sample pre-treatment applied is simple and this approach only requires an HPLC/diode-array detector. This method is suitable for rapid routine assays and could indirectly be helpful to study the evolution of these compounds with the roasting procedure or to help in the differentiation of coffee varieties.

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