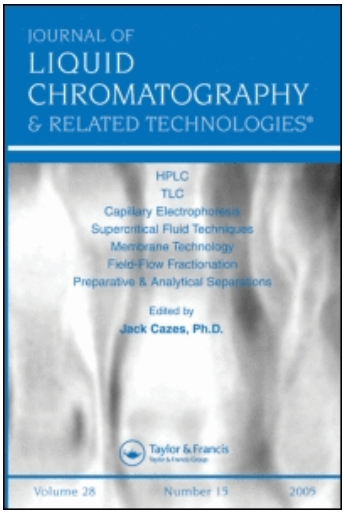

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Development of an HPLC/Diode-Array Detector Method for Simultaneous Determination of 5-HMF, Furfural, 5-*O*-Caffeoylquinic Acid and Caffeine in Coffee

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DEVELOPMENT OF AN HPLC/DIODE-ARRAY DETECTOR METHOD FOR SIMULTANEOUS DETERMINATION OF 5-HMF, FURFURAL, 5-*O*-CAFFEOYLQUINIC ACID AND CAFFEINE IN COFFEE

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ABSTRACT

This paper describes an interference-free reversed-phase HPLC procedure for the simultaneous quantification of 5-HMF, furfural, 5-*O*-caffeoylquinic acid and caffeine in green and roasted coffee samples. The sample preparation was simple, only involving a boiled water extraction followed by filtration. The chromatographic separation was achieved using a reversed-phase column Spherisorb ODS2 (5μm; 25.0 x 0.46 cm). Gradient elution was carried out using water+acetic acid (0.2%) (A) and methanol (B). The effluent was monitored by a diode-array detector and chromatograms were recorded at 280 nm. The determinations were performed in the linear ranges 0.25-10 μg/mL, 0.1-10 μg/mL, 2.5-100 μg/mL, 0.25-200 μg/mL for 5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine,

respectively. Extensive quality assurance of the method was performed by the standard additions method in both coffee matrices (green and roasted coffee). For roasted coffee, the precision obtained (n=10) was better than CV% 0.52, 0.62, 0.14, and 0.50 for 5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine, respectively; for green coffee, it was better than 0.76 for 5-*O*-caffeoylquinic acid and 0.89 for caffeine.

For roasted coffee samples, recovery values were between 85 and 104%, 87 and 103%, 88 and 94%, and 74 and 77% for 5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine, respectively. For green coffee sample, recovery values were between 73 and 74% for 5-*O*-caffeoylquinic acid and between 94 and 98% for caffeine.

INTRODUCTION

Green and roasted coffee are matrices of the same food, although with very diversified chemical composition, aromatic and textural properties. Mechanisms of roasting reactions have been largely studied, yet too much remains to be understood.[1] Therefore, in spite of the enormous effort undertaken until now to chemically discriminate between *Coffea arabica* and *Coffea canephora* var. *robusta*, once roasted, no unquestionable marker has been found.[2,3] Simultaneous monitorization of furanic aldehydes, such as furfural (2-furaldehyde) and hydroxymethylfurfural (5-hydroxy-2-furaldehyde, 5-HMF), 5-*O*-caffeoylquinic acid and caffeine in coffee, among others, can be particularly helpful, namely, as a guarantee of authenticity of coffee varieties, in the characterization of its geographic origins, to ensure the quality of roasted coffee and indirectly the quality control of the roasting process itself. Individual methodologies to quantify these four compounds are quite spread in literature,[1,4] although multiparametric techniques are quite scarce.[5-10] Furthermore, these latter methodologies do not involve all the above mentioned compounds.

To fulfil this gap and reach the proposed aims of contributing to help the quality control of coffee in all steps, viz. from geographic origin until sailing to the final consumer, this simple, expeditious, and economic methodology herein presented was developed and its validation, accuracy, and reproducibility were assessed in both green and roasted coffee matrices.

MATERIALS AND METHODS

Coffee Samples and Standards

Green Robusta (India) coffee beans were supplied by the coffee industry, and one portion was roasted at 205°C, during 15min. in a stove. The green or roasted beans were ground in a hammer mill to pass 0.8 mm.

5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine were obtained from Sigma Chemical Co.

Extraction of Compounds from Coffee

A 1 g portion of powdered coffee bean samples, was blended with 150 mL of water and boiled during 2 min. This solution was transferred to a 200 mL volume flask and immediately diluted to the volume mark. The mixture was filtered and 20 μl were analysed by HPLC.

HPLC Analysis

Separation of compounds was achieved with an analytical HPLC unit (Gilson), using a reversed-phase Spherisorb ODS2 (5μm, particle size; 25.0 x 0.46 cm) column. The solvent system used was a gradient of water+acetic acid (0.2%) (A) and methanol (B). The gradient was as follows: 0'-7.5% B, 10'-20% B, 12'-30 % B, 20'-35% B, 28'-40% B. Elution was performed at a solvent flow rate of 1 mL/min. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 280 nm.

The mentioned compounds were identified by chromatographic comparisons with authentic standards and by their specific UV spectra. Quantification was based on the external standard method.

RESULTS AND DISCUSSION

Analytical Curve and Detection Limit

For the sample preparation, we observed that previous clarification with Carrez solutions[8] or a passage through a Sep-pack C-18 cartridge[11] were not

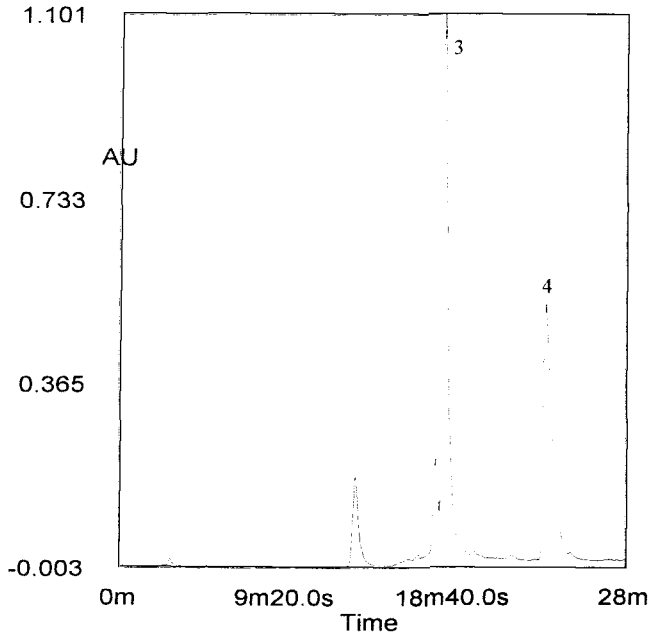

Figure 1. HPLC profile of a green coffee sample from India. Detection at 280 nm. (3) 5-*O*-caffeoylquinic acid; (4) Caffeine.

necessary and caused losses of 5-HMF and furfural. Under the assay conditions described, a linear relationship between the concentration and the UV absorbance at 280 nm was obtained. This linearity was maintained over the concentration range 0.25-10 μg/mL, 0.1-10 μg/mL, 2.5-100 μg/mL, 0.25-200 μg/mL for 5-HMF, furfural, 5-*O*-caffeoylquinic acid and caffeine, respectively. The correlation coefficient for each standard curve invariably exceeded 0.99 for all compounds under study.

The calibration curves were obtained by triplicate determinations of each of the calibration standards, the peak area values (arbitrary units) were plotted as average values. The relative percent average deviations of triplicates were less than 2% in all cases. The average regression equation for 5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine were $y = 69433x + 2883.8$, $y = 66225x + 2721.0$, $y = 12751x + 4301.7$, $y = 21124x + 23423.0$, respectively. The detection limit values for 5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine were calculated as the concentration corresponding to three times the standard deviation of the background noise and were 0.25 μg/mL, 0.10 μg/mL, 2.50 μg/mL, and 0.25 μg/mL, respectively.

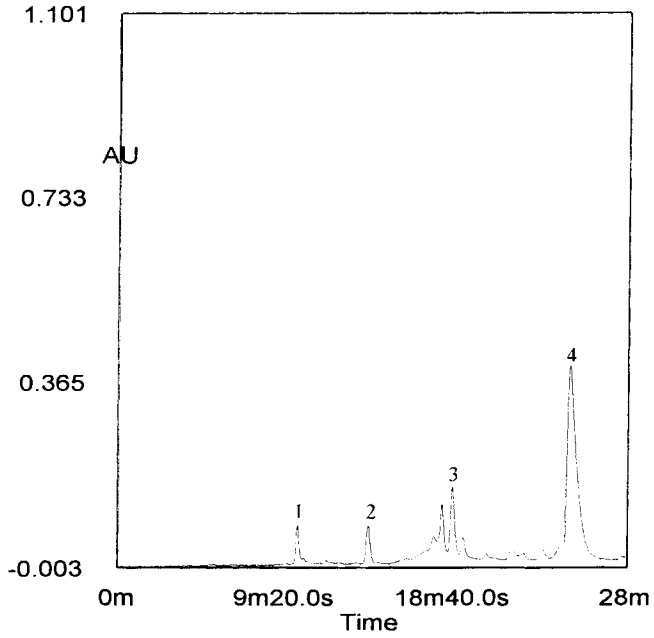

Figure 2. HPLC profile of a roasted coffee sample from India. Detection at 280 nm. (1) 5-HMF; (2) Furfural; (3) 5-*O*-caffeoylquinic acid; (4) Caffeine.

Validation of the Method

The chromatograms obtained for a green and a roasted coffee samples from India are shown in Figures 1 and 2. The retention times (RT) obtained for compounds under study were: RT 9m53s for 5-HMF, RT 13m49s for furfural, RT 18m27s for 5-*O*-caffeoylquinic acid, and RT 24m49s for caffeine. The use of a diode-array detector proved to be very helpful to observe the peak purity and chemical nature of each peak. The unidentified compounds 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.

Results of quantification applied to one sample are shown in Table 1. During the roasting process, compositional changes occurred, with the decomposition of naturally 5-*O*-caffeoylquinic acid and appearance of 5-HMF and furfural, which agrees with previous works.[1,11,12]

Table 1

5-HMF, Furfural, 5-*O*-Caffeoylquinic Acid and Caffeine Content in Green and Roasted Coffee Sample[a] From India (*Coffea Canephora* var. *Robusta)*

	Green Coffee mg/kg ± sd	Roasted Coffee mg/kg ± sd
5-HMF	-----	61.6 ± 0.776
Furfural	-----	19.9 ± 1.270
5-*O*-caffeoylquinic acid	$46.5x10^3$ ± 0.350	$7.3x10^3$ ± 0.115
Caffeine	$29.4x10^3$ ± 0.263	$27.5x10^3$ ± 0.251

[a] Values are expressed as mean ± sd of three determinations.

Table 2

Recovery of 5-*O*-Caffeoylquinic Acid and Caffeine from a Spiked Green Coffee Sample[a] From India (*Coffea Canephora* var. *Robusta)*

	Added (mg/kg)	Found (mg/kg)	Standard Deviation	CV%	Recovery %
Chlorogenic acid*	10.0	7.28	0.438	6.02	72.8
	25.0	18.53	0.919	4.96	74.1
	40.0	29.00	0.177	0.61	72.5
Caffeine	10.0	9.43	0.148	1.58	94.3
	25.0	24.53	0.156	0.63	98.1
	50.0	48.90	0.007	0.01	97.8

[a] Mean value found for 3 assays for each studied concentration.
* Corresponds to 5-*O*-caffeoylquinic acid.

The precision of the analytical method was evaluated by measuring the peak chromatographic area of the compounds 10 times on the same sample. In the roasted coffee sample the standard deviation was 0.697, 0.768, 0.189, 0.622, and the coefficient of variation was 0.52%, 0.62%, 0.14%, and 0.5%, for 5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine, respectively. For the green coffee sample, the standard deviation was 0.351, 0.263, and the

Table 3

Recovery of 5-HMF, Furfural, 5-*O*-Caffeoylquinic Acid and Caffeine from a Spiked Green Coffee Sample[a] from India (*Coffea Canephora* var. *Robusta)*

	Added (mg/kg)	Found (mg/kg)	Standard Deviation	CV%	Recovery %
5-HMF	0.5	0.43	0.007	1.7	85.0
	1.0	0.92	0.21	2.3	91.0
	2.5	2.61	0.141	5.4	104.4
Furfural	0.5	0.44	0.078	7.8	87.0
	1.0	1.03	0.156	5.0	103.0
	2.0	2.04	0.092	4.5	101.8
Chlorogenic acid	10.0	9.40	0.325	3.5	94.0
	25.0	22.43	0.198	0.9	89.7
	40.0	35.24	0.170	0.5	88.1
Caffeine	10.0	7.71	0.120	1.6	77.1
	25.0	17.09	0.255	1.5	68.4
	50.0	37.11	0.834	2.3	74.2

[a] Mean value found for 3 assays for each studied concentration.

* Corresponds to 5-*O*-caffeoylquinic acid.

coefficient of variation was 0.76%, 0.89%, for 5-*O*-caffeoylquinic acid and caffeine, respectively. In order to study the recovery of the procedure, one roasted coffee sample was added to known quantities of 5-HMF, furfural, 5-*O*-caffeoylquinic acid and caffeine, and one green coffee sample was added to known quantities of 5-*O*-caffeoylquinic acid and caffeine. The samples were analysed in triplicate before and after the addition of these compounds in order to demonstrate the effectiveness of the extraction and the accuracy of the proposed method.

The results are listed in Tables 2 and 3. For the roasted coffee sample, recovery values were between 85 and 104%, 87 and 103%, 88 and 94%, and 74 and 77% for 5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine, respectively. For the green coffee sample, recovery values were between 73 and 74% for 5-*O*-caffeoylquinic acid and between 94 and 98% for caffeine.

In conclusion, this study suggests that the technique proposed herein is quite useful for the simultaneous analysis of 5-HMF, furfural, 5-*O*-caffeoylquinic acid, and caffeine in coffee samples. Despite the complexity of the matrix, the sample pre-treatment is simple and this approach only requires an HPLC/diode-array detector. This technique could also be indirectly helpful in the roasting industry.

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