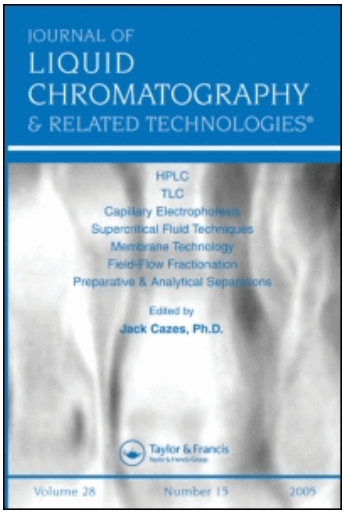

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DEVELOPMENT OF AN HPLC/DIODE-ARRAY DETECTOR METHOD FOR SIMULTANEOUS DETERMINATION OF SEVEN HYDROXY-CINNAMIC ACIDS IN GREEN COFFEE

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ABSTRACT

A simple, rapid, sensitive, reproducible, and accurate reverse phase HPLC procedure is proposed for the determination of seven phenolic acids in green coffee samples. The sample preparation was simple, involving extraction, alkaline hydrolysis, and liquid/liquid extraction. The chromatographic separation was achieved using a reverse phase column Spherisorb ODS2 (5μm; 25.0 x 0.46 cm). Gradient elution was carried out using water-formic acid (19:1) (A) and methanol (B). The effluent was monitored by a diode-array detector and chromatograms were recorded at 320 nm.

The detection limit value of the method was 0.09 μg/mL and the method was precise(CV%= 0.5%; n=10). Recovery values of caffeic acid from spiked green coffee samples were between 88.3 and 93.2%.

INTRODUCTION

Chlorogenic acids (CGA) are a group of compounds well represented in coffee beans (5-10%).[1,2] Chemically, CGAs comprise a group of esters of quinic acid with some specific phenols, mainly caffeic, ferulic, and p-coumaric acids known collectively as hydroxycinnamic acids (HCAs). The real contribution of these compounds to flavor in roasted coffee and the real importance in coffee quality remains not well understood.[1,3] By enzymatic or alkaline hydrolysis, HCAs can be obtained from endogenous CGAs in green coffee and can be of importance in its quality control.[4,5]

Monitoring hydroxycinnamic acids in coffee can be useful, particularly for the coffee industry, both for assessing their levels in raw materials before and after roasting and also to help the definition of authenticity of the commercial coffee varieties and for a possible characterisation of their geographical origins.[4-6] Due to their significative absorption and characteristic shape under UV, spectrophotometric methods are the most frequently used for global determinations of CGAs. Discrimination and specification of CGAs and their isomers are achieved by specific colorimetric reactions[1] and by HPLC techniques, respectively.[7,8,9] However, the methodologies for individual HCAs are quite scarce in literature; thus an adequate methodology is required. The aim of this paper, herein, is to describe an adequate, simple, reproducible, and accurate technique for simultaneous determination of seven phenolic acids in green coffee samples: caffeic, p-coumaric, ferulic, o-coumaric, 3,4-dimethoxycinnamic, 3,4,5-trimethoxycinnamic and 4-methoxycinnamic.

MATERIALS AND METHODS

Coffee Samples and Standards

Green Arabica (Honduras) and green Robusta (Uganda) coffee beans were supplied by the coffee industry. Green coffee beans were ground in a hammer mill to pass 0.8 mm.

Caffeic acid, p-coumaric acid, ferulic acid, o-coumaric acid, 3,4-dimethoxycinnamic acid, 3,4,5-trimethoxycinnamic acid, 4-methoxycinnamic acid were obtained from Sigma Chemical Co.

Extraction of Phenolic Acids from Coffee

A 5 g portion of green coffee bean samples, finely powdered, was blended with 60 mL of methanol/water (40/60),[9] during 24h. The mixture was filtered and the filtrate concentrated under vacuum (40°C) to a volume of 5 mL. This solution was hydrolysed with 5 mL of 2N NaOH for 240 min. The pH of the mixture was adjusted to pH 7.00 with 2N HCl and the phenolic acids were extracted by liquid/liquid extraction with ethyl acetate (20mLx3). The extracts were then combined and the ethyl acetate removed under reduced pressure. The residue was dissolved in 7 mL of methanol and 20 μl were analysed by HPLC.

HPLC Analysis of Phenolic Acids

Separation of phenolic acids was achieved with an analytical HPLC unit (Gilson), using a reverse phase Spherisorb ODS2 (5μm, particle size; 25.0 x 0.46 cm) column. The solvent system used was a gradient of water-formic acid (19:1) (A) and methanol (B). The gradient was as follows: 0'- 15% B, 10'-25% B, 25'-30 % B, 30'-35% B, 34'-50% B, 41'-70% B, 43'-75% B, 47'-80% B. Elution was performed at a solvent flow rate of 0.9 mL/min. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 320 nm.

The different phenolic acids were identified by chromatographic comparisons with authentic standards and by their UV spectra. Quantification was based on the external standard method.

RESULTS AND DISCUSSION

Analytical Curve and Detection Limit

Under the assay conditions described, a linear relationship between the concentration of phenolic acids and the UV absorbance at 320 nm was obtained. This linearity was maintained over the concentration range 4-400 μg/mL. The correlation coefficient for each standard curve invariably exceeded

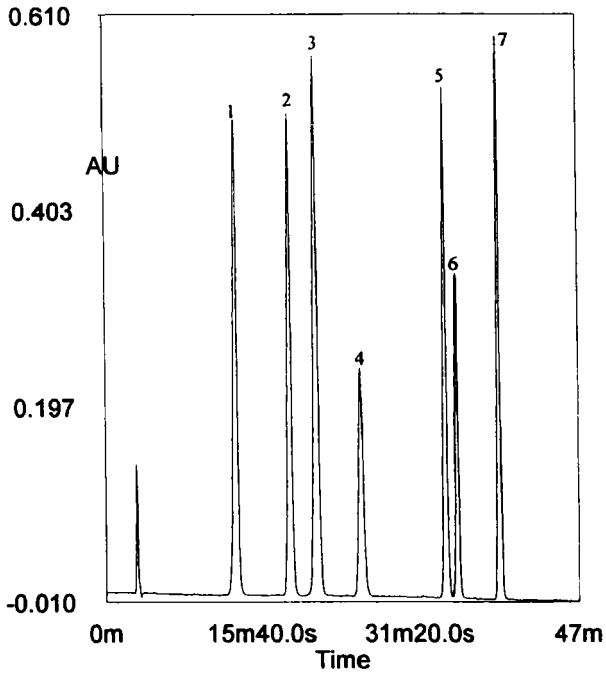

Figure 1. HPLC profile of a standard solution of phenolic acids. Detection at 320 nm. (1) Caffeic acid; (2) *p*-coumaric acid; (3)Ferulic acid; (4)*o*-coumaric acid; (5)3.4-dimethoxycinnamic acid; (6)3.4.5-trimethoxycinnamic acid; (7) 4-methoxycinnamic acid.

0.99 for all phenolic acids. The calibration curves for phenolic acids 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 caffeic, p-coumaric, ferulic, o-coumaric, 3,4-dimethoxycinnamic, 3,4,5-trimethoxycinnamic, and 4-methoxycinnamic acids were: $y=3.62 \times 10^8x + 131114.3$, $y = 3.46 \times 10^8x + 13614836$, $y = 4.10 \times 10^8x + 151863.6$, $y = 2.23 \times 10^8x + 60385.32$, $y = 2.67 \times 10^8 + 172406.5$, $y = 1.87 \times 10^8x + 68167.15$, and $y = 2.37 \times 10^8x + 140739.3$, respectively.

Given the similarity of the chemical structures of the acids analysed and, therefore their UV spectra and absortivity, the detection limit and precision of the method were determined only for caffeic acid. The detection limit value was calculated for caffeic acid as the concentration corresponding to three times the standard deviation of the background noise and was 0.09 μg/mL.

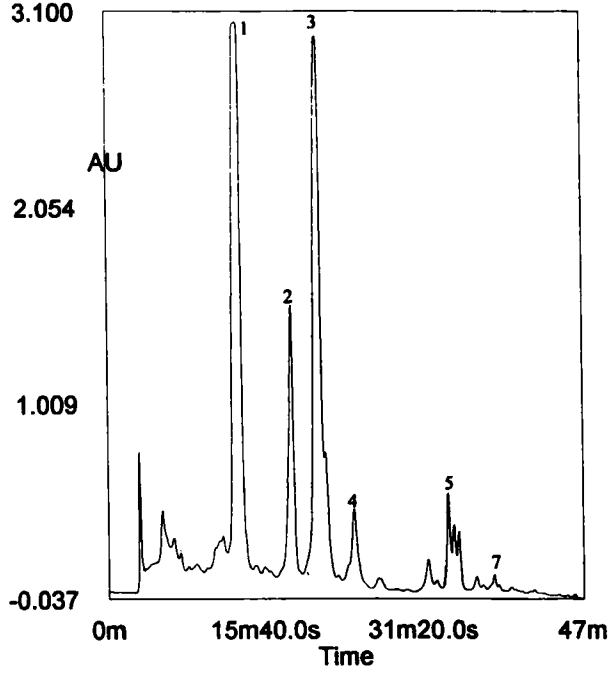

Figure 2. HPLC phenolic acids profile of a green Arabica coffee sample from Honduras. Detection at 320 nm. (1) Caffeic acid; (2) *p*-coumaric acid; (3)Ferulic acid; (4)*o*-coumaric acid; (5)3.4-dimethoxycinnamic acid; (7)4-methoxycinnamic acid.

Validation of the Method

The chromatogram obtained for standard phenolic acids solution is shown in Figure 1. The retention times (RT) obtained for phenolic acids were: RT 12m33s for caffeic acid, RT 18m33s for p-coumaric acid, RT 21m7s for ferulic acid, RT 24m56s for o-coumaric acid, RT 33m55s for 3,4-dimethoxycinnamic acid, RT 34m49s for 3,4,5-trimethoxycinnamic acid, and RT 38m13s for 4-methoxycinnamic acid.

As an example, Figure 2 shows the HPLC phenolic acid profile of a green coffee sample from Honduras. 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 from quantification applied to two samples (from Honduras and Uganda) are shown in Table 1.

Table 1

Phenolic Acids Content in Green Coffee Samples[a]

	Honduras (Coffea Arabica) g/kg ± SD	Uganda (Coffea Robusta) g/kg ± SD
Caffeic	1.051 ± 0.0007	1.125 ± 0.01
p-coumaric	0.340 ± 0.03	0.302 ± 0.007
Ferulic	0.841 ± 0.0007	1.153 ± 0.05
o-coumaric	0.200 ± 0.005	0.165 ± 0.005
3,4-dimethoxycinamic	0.056 ± 0.0042	0.489 ± 0.02
3,4,5-trimethoxycinamic	------	0.734 ± 0.05
4-methoxycinamic	nq	------

*Values are expressed as mean ± SD of three determinations.
nq - Not quantified.

Table 2

Recovery of Caffeic Acid from a Spiked Green Coffee Sample

Present (g/kg)	Added (g/kg)	Found[a] (g/kg)	Standard Deviation	CV (%)	Recovery (%)
	0.2	1.047	0.05	7.6	93.2
0.924	0.3	1.141	0.02	1.7	93.2
	0.4	1.169	0.03	2.5	88.3

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

The precision of the analytical method was evaluated by measuring the peak chromatographic area of caffeic acid, 10 times on the same sample. The standard deviation was 0.006 and the coefficient of variation was 0.5%. In order to study the recovery of the procedure, and bearing in mind that the method includes an alkaline hydrolysis, one coffee sample was added to known quantities of 5-caffeoylquinic acid and recovery percentage calculated as caffeic acid. The sample was analysed in triplicate before and after the addition

of 5-caffeoylquinic acid. Thus, this procedure demonstrated the effectiveness of the extraction and hydrolysis step and the accuracy of the proposed method. The results are listed in table 2. Recovery values were between 88.3 and 93.2%.

In conclusion, this study suggests that the technique herein is quite useful for the analysis of phenolic acids in samples, allowing the separation and quantification of the main coffee phenolic acids. This technique can be useful to help in the differentiation of coffee varieties and, hypothetically, for the characterisation of their geographical origin.

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REFERENCES

1. M. N. Clifford, "Chlorogenic Acids", In **Coffee Vol I : Chemistry**, R. J. Clarke, R. Macrae, Eds., Elsevier Applied Science Publishers, Ltd., London 1985.

2. M. N. Clifford, T. Williams, D. Bridson, Phytochemistry, **28(3)**, 829-838 (1989).

3. C.W. Nagel, I. W. Herrick, W. R. Graber, J. Food Science, **52 (1)**, 213 (1987).

4. J. J. R. Rakotomalala, E. Cros, A. Charrier, ASIC, 15e Colloque, Montpellier, 47-53, (1993).

5. J. J. R. Rakotomalala, E. Cros, A. Charrier, F. Anthony, M. Noirot, ASIC, 15e Colloque, Montpellier, 637-643, (1993).

6. C. P. Bicchi, A. E. Binello, M. M. Legovich, G. M. Pellegrino, A. C. Vanni, J. Agric. Food Chem., **41**, 2324-2328 (1993).

7. L. C. Trugo, R. Macrae, Food Chemistry, **15**, 219-217 (1984).

8. L. C. Trugo, R. Macrae, Analyst, March, **109**, 263-266 (1984).

9. C. P. Bicchi, A. E. Binello, G. M. Pellegrino, A. C. Vanni, J. Agric. Food Chem., **43**, 1549-1555 (1995).

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