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Simultaneous Determination of Total Chlorogenic Acid and Caffeine in Coffee by High Performance Gel Filtration Chromatography

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ABSTRACT

A method based on high performance gel filtration chromatography, using a TSK 3000-SW column, was developed for the simultaneous determination of total chlorogenic acid and caffeine in coffee samples. The method presented good linearities and recoveries for both compounds. Comparison with results obtained by reversed phase chromatography for caffeine and by the AOAC procedure and an adapted reversed phase chromatographic method for chlorogenic acid showed good correlations between the proposed method and the reversed phase procedures (0·997 for caffeine and 0·998 for chlorogenic acid). The AOAC results for chlorogenic acid showed systematically higher values than the proposed method. The proposed method gave a CV(%) of 6·5 for caffeine and 5·0 for chlorogenic acid and is adequate when applied to the analysis of different samples of green, roasted decaffeinated and instant coffees.

INTRODUCTION

Monitoring of caffeine in coffee is of importance particularly for the coffee industry, not only for assessing caffeine level in green coffee beans before processing, but also to determine the final content in coffee products. Several methods have been used for the determination of caffeine mainly based on spectrophotometry (Newton, 1979), gas chromatography (Schilling & Gal, 1973; Strahl *et al.*, 1977; Hughes & Thorpe, 1987; Rauch *et al.*, 1989) or high

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performance liquid chromatography (HPLC) (Murgia *et al.*, 1973; Kreiser & Martin Jr, 1978; Trugo *et al.*, 1983). The use of HPLC for caffeine analysis has grown rapidly and reversed phase chromatography has been the method of choice in most cases.

Chlorogenic acids comprise some groups of compounds mainly formed by quinic acid esterification with either caffeic, ferulic or *p*-coumaric acids (Clifford & Wight, 1976). Their importance for coffee quality or for coffee flavour formation during roasting is not well understood. It is generally accepted that Arabica coffee is superior in quality to Robusta and it has been established that the latter presents higher chlorogenic acid levels than the former (Clifford & Wight, 1976). However, there is little evidence which could allow the establishment of any inverse correlation between chlorogenic acid content and specific coffee sensory attributes. During roasting, chlorogenic acid is progressively degraded, contributing largely to aroma formation and its final content in coffee products may be an indication of the degree of roasting (Trugo & Macrae, 1984).

The analysis of total chlorogenic acid may be carried out by the AOAC official procedure which is based on the differential UV measurement of coffee extracts before and after chlorogenic acid precipitation with lead acetate (AOAC, 1984). This method, although simple, may produce inflated data and this is particularly critical when applied to roasted and instant coffees (Clifford, 1975). Discrimination of chlorogenic acid groups may be achieved by specific colorimetric reactions followed by photometry (Clifford & Wight, 1976). However, speciation of chlorogenic acid isomers has been accomplished by the use of HPLC techniques with reversed phase chromatography (Stegen & Duijn, 1980; Trugo & Macrae, 1984).

In this work a method is described for the simultaneous determination of total chlorogenic acid and caffeine which was then applied to the analysis of green, roasted and instant coffee samples. The term 'chlorogenic acid' will be used throughout this work, meaning the sum of the quinic acid esters normally found in coffee.

MATERIALS AND METHODS

Samples

Samples of green Arabica and the respective soluble coffee were obtained from a local industry. Other coffee samples were purchased in the market. Green and roasted coffees were milled to pass a 0·75 mm sieve prior to analysis.

Green and roasted coffee samples (0·5 g) were extracted with 30 ml

hot distilled water (80°C) in a water bath with shaking for 15 min. The contents were then filtered into a 50 ml volumetric flask and cleared by the use of Carrez solutions (Pearson, 1976). The mixture was made up to volume, centrifuged at $3000 \times g$ for 10 min and the supernatant filtered using a Millipore membrane (0·45 μm). The filtrate was then used for chromatography.

Instant coffee samples (0·5 g) were just dissolved in distilled water and cleared as described for green and roasted coffee.

The same samples were used for method comparison with the official AOAC procedure (AOAC, 1984) or with a reversed phase chromatographic method for chlorogenic acid analysis. Caffeine data were compared with data obtained by reversed phase chromatography (Trugo *et al.*, 1983).

Chromatography

Chromatography was carried out using a Knauer (FRG) chromatograph consisting of a pump, a UV detector set at 280 nm (0·16 AUFS) and a Rheodyne injection valve with a 20 μl fixed loop. A TSK-G 3000-SW (300 × 8 mm, i.d.) and the respective guard column (LKB, Sweden) were used. Mobile phase was bidistilled water containing 0·05% of sodium azide with a flow rate of 0·5 ml/min.

Quantification was achieved by comparison of peak height of the samples with external standards of caffeine and 5-caffeoylquinic acid (5-CQA) (C. Roth, FRG). Calibration graphs were plotted using caffeine and 5-CQA aqueous solutions at concentration ranges of 30–100 μg/ml for caffeine and 1·0–20 μg/ml for 5-CQA, respectively. Recoveries were checked by the method of standard addition to the samples. Precision was estimated by calculation of coefficients of variation for caffeine and chlorogenic acid using 12 replicate extracts from the same sample.

The same equipment was used for reversed phase chromatography of chlorogenic acid isomers using a previously described method (Trugo & Macrae, 1984). This was modified to be used in the isocratic mode. The mobile phase consisted of trisodium citrate (0·01 M) adjusted to pH 2·5 with dilute hydrochloric acid and containing 40% methanol (v/v). In this case total chlorogenic acid was expressed by the sum of the areas of individual isomers as compared to a 5-CQA standard.

RESULTS AND DISCUSSION

The proposed method showed good linearity, both for caffeine and for chlorogenic acid, with correlation coefficients of 0·9998 and 0·9992, respectively. Recoveries were perfectly accepted with an average of 105%

TABLE 1
Average of Results for Caffeine and Chlorogenic Acid in Instant Coffee Determined by Different Methods

	Caffeine		*Chlorogenic acid*		
	1	*2*	*3*	*4*	*5*
Average	2·8	2·9	2·7	2·6	10·0
SD	0·18	0·16	0·14	0·14	0·53
CV%	6·5	5·4	5·0	5·4	5·3

[a] Results obtained from 12 replicates of the same instant coffee sample, in g% dry basis.
1 and 3— Proposed method.
2— Reversed phase method for caffeine (Trugo *et al.*, 1983).
4— Reversed phase method for chlorogenic acid (Trugo & Macrae, 1984).
5— AOAC method for chlorogenic acid (AOAC, 1984).

for caffeine and 95% for chlorogenic acid. The method was compared with a reversed phase chromatography procedure usually applied to caffeine and chlorogenic acid, independently. The average results and variation obtained with replicates from the same sample are shown in Table 1. The correlations found were 0·997 between methods 1 and 2, 0·998 for methods 3 and 4, 0·766 for methods 3 and 5 and 0·756 between methods 4 and 5. Application of Student's *t*-test to the data showed significant differences ($p < 0{\cdot}01$) between methods 3 and 5 and 4 and 5. The method was then applied to the analysis of samples of green, roasted decaffeinated and instant coffees. The results were then compared to those obtained by reversed phase chromatography (for caffeine and for chlorogenic acid) and by the AOAC procedure (only for chlorogenic acid) (Table 2). An example of the chromatographic separation achieved is shown in Fig. 1.

Reversed phase chromatography has been applied for the analysis of caffeine (Murgia *et al.*, 1973; Kreiser & Martin Jr, 1978; Trugo *et al.*, 1983) and also for the determination of individual chlorogenic acids (Stegen & Duijn, 1980; Trugo & Macrae, 1984). Total chlorogenic acid content may be obtained by the sum of individual isomers as determined by HPLC. The AOAC method, which is based on the differential UV measurement before and after chlorogenic acid precipitation, may be of use when only the total amount is needed.

However, for caffeine and chlorogenic acid determination by reversed phase HPLC, the chromatographic conditions are considerably different, it being necessary to carry out independent determinations. A different

TABLE 2
Results of Caffeine and Chlorogenic Acid in Different Coffee Samples Obtained by Different Methods[a]

Samples	*Methods*[b]				
	Caffeine		*Chlorogenic acid*		
	1	*2*	*3*	*4*	*5*
Green coffee	1·1	1·1	7·2	7·8	9·9
Roasted decaffeinated	0·10	0·07	1·2	1·2	4·3
Instant coffees					
A	3·4	3·5	5·9	6·4	12·7
B	2·8	2·9	2·7	2·6	10·0
C	2·1	2·0	1·1	0·9	6·8
D	2·3	2·2	2·1	2·3	7·7
E	2·2	2·1	0·9	0·8	7·4
F	2·2	2·2	2·5	2·3	8·0

[a] Results are average of duplicate determinations, g% dry basis.
[b] Methods 1 to 5 as in Table 1.

chromatographic approach has been pursued in this work for the determination of these compounds based on the use of a gel filtration stationary phase. A TSK-Gel 3000 SW phase, which is a porous, spherical and rigid gel, was used. It was unexpected to achieve separation between chlorogenic acid and caffeine using this kind of column since it has a separation range from 1000 to 300 000 of molecular weight and these compounds have molecular weights of only 194·20 and 354·31 (5-CQA),

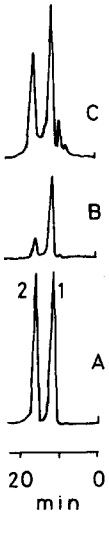

Fig. 1. Chromatograms of (A) standards, (B) green coffee and (C) instant coffee, using the TSK G 3000-SW gel filtration column. (1) chlorogenic acid; (2) caffeine. Mobile phase was bidistilled water containing 0·05% (w/v) of sodium azide at flow rate of 0·5 ml/min. Detection at 280 nm.

respectively. However, complete separation was obtained indicating that, not only gel filtration occurred, but probably hydrophobic interactions are also involved, making caffeine more easily retained than chlorogenic acid (Fig. 1). Mixtures of caffeoylquinic acids, obtained by isomerisation of the 5-CQA standard, and of di-caffeoylquinic acids obtained from Roth (FRG) have the same retention times as the peak assigned to chlorogenic acid, showing that it represented, in fact, the main chlorogenic acid isomers found in coffee. Although the maximum absorbance for caffeine is 272 nm (Newton, 1979) and for chlorogenic acid is 325 nm (Clifford & Wight, 1976), 280 nm was selected for the simultaneous detection because it gave adequate response for both compounds and also because it is a wavelength easily obtainable if only a fixed wavelength monitor is available.

The method showed good linearity and recoveries both for caffeine and chlorogenic acid. Also, the results obtained with the samples showed high correlation with the reversed phase HPLC methods used for comparison. Roasted decaffeinated coffee showed a minor peak corresponding to caffeine, supporting peak assigment. In this case the caffeine content was also in agreement with the reversed phase method (Table 2). The results obtained for chlorogenic acid showed always lower values when compared to the AOAC results (50–90% lower for instant coffees). However, this difference was much smaller when green coffee was analysed (21% lower) and this is due to a higher amount of interfering material which is formed during roasting and which dramatically inflated the AOAC results (Clifford, 1985).

The proposed method is applicable to the analyses of different coffee products and it may be useful for quality control. In addition to adequate precision, it uses water as mobile phase which is attractive in terms of cost.

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