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Simultaneous determination of total chlorogenic acid, trigonelline and caffeine in green coffee samples by high performance gel filtration chromatography

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A method is proposed for the simultaneous determination of total chlorogenic acid (CGA), trigonelline and caffeine in green coffee using high performance gel filtration chromatography. The method presents good linearities (0·9996, 0·9999 and 0·9998) and recoveries (97%, 96% and 96%) with high correlations (0·9158, 0·9715 and 0·9445) for the CGA, trigonelline and caffeine, respectively when compared to reverse phase HPLC techniques. The proposed method appears to be an adequate method for quality control in the coffee industry.

INTRODUCTION

Assessment of caffeine, trigonelline and chlorogenic acid (CGA) (quinic acid esters) levels in green coffee is very important for the coffee industry, since they have a large effect on the final quality of the coffee products.

Caffeine has been related to the pharmacological effects of coffee (Würzner, 1988), and both CGA and trigonelline have been associated with flavour formation and aroma production during coffee roasting (Viani & Horman, 1974; Clifford, 1985).

There are many analytical methods available for the determination of these components in coffee. However, high performance liquid chromatography (HPLC) has been the usual technique due to its accuracy, precision and rapidity. Different reversed phase (RP) HPLC methods have been developed for the simultaneous determination of caffeine and trigonelline in coffee (Trugo *et al.*, 1983) and for CGA analysis (van der Stegen & Duijn, 1980; Trugo & Macrae, 1984), but not for the simultaneous analysis of all three components.

A procedure for the simultaneous determination of total CGA and caffeine in coffee by high performance gel filtration (HPGF) chromatography has been described previously (Trugo *et al.*, 1991), and correlated very well with RP chromatography.

In the present work, the simultaneous determination of total CGA, trigonelline and caffeine in green coffee samples by HPGF chromatography is reported.

MATERIALS AND METHODS

Samples

Green coffee samples were provided by ILLYCAFFÈ (Italy). Samples were milled to pass through a 0·75 mm sieve prior to analysis. Ground defatted green coffee (0·5 g) was extracted with 30 ml hot distilled water (80°C) in a water bath, with shaking for 15 min, followed by filtration and adjustment to 50 ml with distilled water in a volumetric flask. An aliquot was refiltered using a Millipore filter (0·45 μm). The Millipore filtrates were used directly for HPGF chromatography.

Chromatography

A Shimadzu (Japan) chromatograph with a pump, a UV detector (0·16 AUFS and at 272 nm) and a Rheodyne

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injection valve with a 20 μl fixed loop was used. A TSK G-3000 SW HPGF chromatographic column (300 × 8 mm, i.d.) and the respective guard column (Supelco, USA) were used. Mobile phase was bi-distilled water at a flow rate of 0·5 ml/min.

Quantitation was by peak height comparison with standards of caffeine, 5-caffeoilquinic (a quinic acid ester) (5-CQA) (C. Roth, FRG) and trigonelline (Sigma, USA). Calibration graphs were plotted using concentration ranges of 30–100 μg/ml for caffeine or trigonelline and 1·0–20 μg/ml for 5-CQA. Recoveries were checked by standard addition to the samples. Precision was estimated by calculation of coefficients of variation using 12 replicate extracts from the same sample.

The same samples were used for RP chromatography of CGA, trigonelline and caffeine. CGA analysis was based on the method of Trugo and Macrae (1984). Total CGA was expressed by the sum of the areas of individual isomers as compared to a 5-CQA standard. Caffeine and trigonelline analyses were based on previous methods described elsewhere (Trugo *et al.*, 1983; Trugo & Macrae, 1989).

RESULTS AND DISCUSSION

Good linearity and recovery were obtained for the proposed method with correlation coefficients of 0·9996, 0·9999 and 0·9998 and recoveries of 97%, 96% and 96% for CGA, trigonelline and caffeine, respectively. The proposed method was then applied to the simultaneous analysis of total CGA, trigonelline and caffeine in different green coffee samples and compared with data obtained by using the RP chromatography (Table 1). Coefficients of variation obtained with 12 replicates from the same sample were perfectly acceptable in terms of the overall precision of the method (Table 1). Correlations between methods 1 and 2; 1 and 3; 1 and 4 were 0·9158, 0·9715 and 0·9445, respectively. Student's *t*-test did not show significant differences ($P < 0·01$) between methods 1 and 2; 1 and 3; 1 and 4. The results obtained with different green coffee samples were in accordance with results from literature (Smith, 1985).

Table 1. Average of results of CGA, trigonelline and caffeine in different green coffee samples obtained by different methods

Samples	CGA		Trigonelline		Caffeine	
	Methods					
	1	2	1	3	1	4
A	8·0	9·0	1·1	1·1	1·1	1·2
SD	0·32	0·31	0·05	0·04	0·03	0·04
CV%	4·0	3·4	4·5	3·6	2·7	3·3
B	6·7	7·7	1·2	1·2	1·1	1·3
C	7·4	8·1	1·0	1·1	1·1	1·1
D	7·2	8·0	1·6	1·6	0·7	0·7
E	6·8	7·1	1·5	1·4	1·1	1·3
F	6·8	7·0	1·2	1·2	1·2	1·3

A – Results are average of 12 replicates originating the respective standard deviation (SD) and coefficient of variation (CV%); B–F – Results are average of duplicate determinations.
Results in dry basis:
1 – proposed method
2 – RP method for CGA (Trugo & Macrae, 1984)
3 – RP method for trigonelline (Trugo & Macrae, 1989)
4 – RP method for caffeine (Trugo *et al.*, 1983).

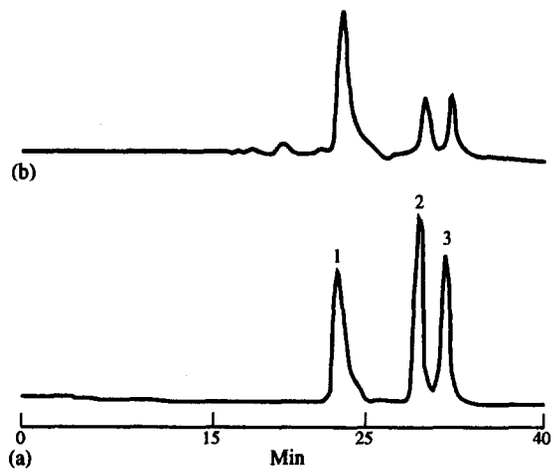

Fig. 1. Chromatograms of (a) standards and (b) green coffee, using the TSK G-3000 SW HPGF chromatographic column. (1) CGA; (2) trigonelline; (3) caffeine. Mobile phase was bi-distilled water at flow rate of 0·5 ml/min. Detection at 272 nm.

The separation of the three components studied is showed in Fig. 1. Although the maximum absorbance for CGA is 325 nm (Clifford & Wight, 1976), 272 nm was adopted for the simultaneous detection because it gave adequate response for both components when fixed wavelength monitor was used. Furthermore, 272 nm is the maximum absorbance for both caffeine and trigonelline (Newton, 1979). Although different groups of CGA isomers are found in coffee (Clifford, 1985) they come all together in a single peak with the HPGF method (Fig. 1, peak 1).

Determination of CGA, trigonelline and caffeine based on the use of HPGF, represented an interesting alternative to RP chromatography. The greatest advantages of the method are the simultaneous determination of the three compounds and also the use of pure water as mobile phase, which is attractive in terms of cost. TSK-gel 3000 SW phase has a separation range from 1000 to 300000 of the molecular weight. Then, it was unexpected to obtain good resolution between three components of close molecular weights. The good resolution obtained between CGA, trigonelline and caffeine may be explained by the fact that not only conventional steric exclusion mechanism occurred, but also hydrophobic interactions. This explanation is supported by the relative polarities of these components and their order of elution.

In conclusion, the method proposed for the simultaneous determination of CGA, trigonelline and caffeine in green coffee appears to be very useful for fundamental investigation and also for quality control in the industry.

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