APPLICATION OF HPSE CHROMATOGRAPHY WITH A REFRACTIVE INDEX DETECTOR TO GREEN COFFEE ANALYSIS

Carlos A. B. de Maria
Department of Physiological Sciences - Biomedical Institute - Universidade do Rio de Janeiro (UniRio)
Luiz C. Trugo* e Gustavo Corá
Department of Biochemistry - Institute of Chemistry - Universidade Federal do Rio de Janeiro - C. T. Bloco A - Cidade Universitária - 21949-900 - Rio de Janeiro - RJ

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A procedure for the simultaneous analysis of total chlorogenic acid (CGA), sucrose, trigonelline and caffeine in green coffee samples is described. Refractive index monitored - high performance size exclusion chromatography (RI-HPSEC) showed both good linearities and recoveries for CGA, sucrose, trigonelline and caffeine with correlation coefficients of 0.9995; 0.9993; 0.9998; 0.9997 and with recoveries of 95%, 97%, 98%, 97%, respectively. The proposed method was compared statistically with an ultra violet - HPSEC procedure applied to CGA, trigonelline and caffeine and with an amino-phase HPLC method for sucrose determination. RI-HPSEC is an interesting approach for simultaneous analysis of total CGA, sucrose, trigonelline and caffeine which is of importance for quality control in the coffee industry.

Keywords: green coffee; HPSEC; RI monitor.

INTRODUCTION

The final quality of coffee products is of paramount importance for the coffee industry and may be related to the levels of chlorogenic acid (CGA), sucrose, trigonelline and caffeine in green coffee beans.

Caffeine is associated with pronounced pharmacological effects of the coffee beverage[6] and CGA (quinic acid esters), trigonelline and sucrose are related to flavour formation during coffee roasting[2,4]. The chemical structures of the four compounds is shown in figure 1.

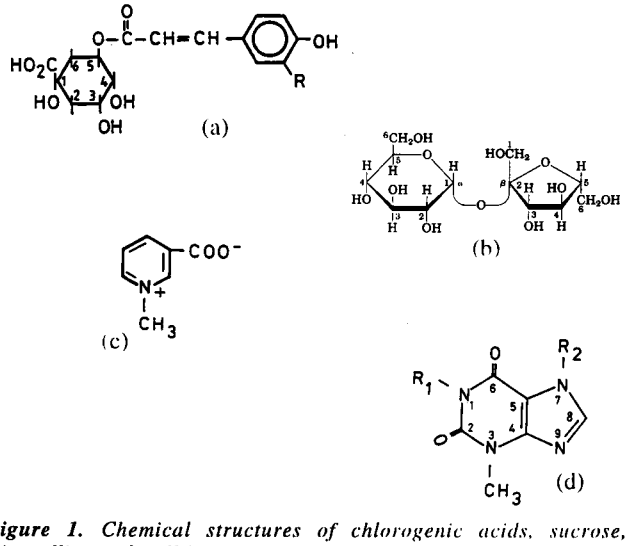

Figure 1. Chemical structures of chlorogenic acids, sucrose, trigonelline and caffeine.
a - Chlorogenic acids:
- R=H, 5-p-coumaroylquinic acid
- R=OH, 5-caffeoylquinic acid
- R=OCH$_3$, 5-feruloylquinic acid
b - Sucrose
c - Trigonelline
d - Caffeine

Reversed phase (RP) high performance liquid chromatography (HPLC) has been the method of choice for the determination of caffeine and trigonelline[16] and CGA[13,14] in coffee samples but since different chromatographic conditions are required, it is necessary to carry out separate analyses. On the other hand, assessment of sucrose generally has been carried out with an amino-phase HPLC method using acetonitrile/water as mobile phase[12,15]. Thus, neither RP nor amino-phase HPLC methods can be adopted for the simultaneous analysis of CGA, sucrose, trigonelline and caffeine in coffee.

High performance size exclusion (HPSE) chromatography has been used for the analyses of total CGA, trigonelline and caffeine in coffee samples[5,11] and correlated statistically with RP chromatography. The greatest advantages of the HPSEC are the simultaneous analysis of the three components and the use of pure water as mobile phase.

In this paper, the simultaneous determination of total CGA, sucrose, trigonelline and caffeine in green coffee samples by HPSEC using a refractive index (RI) monitor is described.

MATERIAL AND METHODS

Samples

Green coffee samples were supplied by Illycaffè (Italy). Samples were represented by letters (A-L). Samples were ground through a 0.75mm sieve before analysis.

Ground defatted green coffee (4g) was extracted with 80 ml hot distilled water (80°C) in a water bath with shaking for 30 min, followed by filtration and adjustment to 100 mL with distilled water in a volumetric flask. An aliquot was passed through a Millipore filter (0.45μm) and used for HPSEC.

Ground defatted green coffee (4g) was submitted to same procedure described here, but the filtrate was cleared by the use of Carrez solutions[9] and centrifuged at 3000 x g for 10 min. An aliquot was passed through a Millipore filter and then used for amino-phase chromatography.

Chromatography

Chromatography was carried out using a Knauer (FRG) chromatograph [a pump, an UV detector set at 272nm (0.64

AUFS) and Rheodyne injection valve with a 20µl fixed loop] and a Waters (USA) RI monitor (a sensivity of 64 and a scale factor of 40). A TSK G3000 SW column (300 x 8mm, i.d.) and the respective guard column (Supelco) were used for the HPSEC. Mobile phase was bidistilled water at a flow rate of 0.5ml/min.

Quantification was achieved by comparison of peak height of the samples with external standards of caffeine (C.ROTH, FRG), 5-caffeoylquinic acid (5-CQA) (C.ROTH, FRG), trigonelline (Sigma, USA) and sucrose (Merck, USA). Calibration graphs were plotted using concentration ranges of 0.5-5mg/ml for caffeine or trigonelline and 1-10mg/ml for 5-CQA or sucrose. 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.

RI-HPSEC was compared with an UV-HPSEC method[5] for caffeine, total CGA and trigonelline analysis. Sucrose data was compared with data obtained by amino-phase chromatography[15].

RESULTS AND DISCUSSION

The RI-HPSEC method showed both good linearities and recoveries, for CGA, sucrose, trigonelline and caffeine, with correlation coefficients of 0.9995; 0.9993; 0.9998; 0.9997 and with recoveries of 95%, 97%, 98%, 97%, respectively. Standard deviations and coefficients of variation are shown in table 1. Analysis of 12 replicates from the same sample indicated that overall precision of the RI-HPSEC method was similar for those obtained by UV-HPSEC and amino-phase chromatography. The proposed method was compared with an UV-HPSEC applied to total CGA, trigonelline, caffeine and with amino-phase chromatography used for sucrose determination. Correlations between methods 1 and 2 (chlorogenic acid); 1 and 2 (trigonelline); 1 and 2 (caffeine); 1 and 3 (sucrose) were 0.9936, 0.8716, 0.9657 and 0.9854, respectively. These correlations were calculated for the data of table 1. No significant differences ($p < 0.01$) were observed between methods 1 and 2; or 1 and 3 when Student's t-test was applied to the data. The RI-HPSEC method was then used for the simultaneous analysis of total CGA, sucrose, trigonelline, and caffeine in different green coffee samples (Table 2). These figures fall within the wide range reported in the literature[1,10].

The separation of total CGA, sucrose, trigonelline and caffeine obtained with the RI-HPSEC method was perfectly acceptable. A chromatogram of the four components studied is shown in figure 2. Since a TSK G3000 SW phase has a separation range from 1000 to 300000 of molecular mass it was not expected to obtain such a good separation between components of low molecular masses (<500mw). However, other high performance size exclusion phases (i.e. TSK HW-40(s)) have also been used for low molecular mass compound analysis (i.e. acesulfame-K, aspartame, caffeine)[7,8]. Therefore, not only size exclusion is occuring but probably hydrophobic interactions are also involved in the separation of CGA, sucrose, trigonelline and caffeine obtained by HPSEC method. CGA isomers elute together as a single peak with the HPSEC method (Fig. 2, Peak 1). Then, 5-CQA was used as standard for quantitation of the total CGA because it is the principal CGA isomer found in green coffee[3]. There are some advantages for the use of the RI-HPSEC method in the analysis of total CGA, sucrose, trigonelline and caffeine in green coffee samples. While amino-phase and RP chromatography use acetonitrile/water and methanol/water, respectively, as chromatographic solvents[12,16] HPSEC uses only water as mobile phase.

Table 2. Results of chlorogenic acid, sucrose, trigonelline and caffeine in different green coffee samples determined by different methods.

Samples	Methods*							
	Chlorogenic acid		Trigonelline		Caffeine		Sucrose	
	1	2	1	2	1	2	1	3
A	7.3	7.0	1.1	1.1	1.0	1.1	8.4	8.2
B	7.0	6.8	1.4	1.3	1.1	1.2	8.3	8.1
C	6.1	6.0	1.1	1.0	1.9	1.9	7.6	7.8
D	12.0	13.0	1.4	1.4	1.1	1.1	8.0	8.2
E	9.7	10.2	1.3	1.3	1.2	1.2	9.4	9.3
F	7.3	6.7	1.0	1.1	1.1	1.0	7.3	7.4
G	7.6	7.7	1.3	1.3	1.4	1.4	7.6	7.2
H	8.0	8.0	1.1	1.0	1.0	1.0	6.6	6.5
I	8.7	9.0	1.0	1.1	1.1	1.0	7.6	7.6
J	7.9	8.0	1.3	1.3	0.7	0.8	6.9	6.8
K	6.9	7.0	1.1	1.0	1.1	1.1	7.3	7.3
L	7.3	7.4	1.1	1.0	1.1	1.0	5.0	5.2

Results are average of duplicate determinations, g% dry basis.
* 1 to 3 as in Table 1.

Table 1. Comparison of results for chlorogenic acid, trigonelline caffeine and sucrose in green coffee obtained by different methods.

Sample	Chlorogenic acid		Trigonelline		Caffeine		Sucrose	
	1	2	1	2	1	2	1	3
A_1	7.1	7.2	1.1	1.1	1.0	1.1	8.2	8.2
A_2	7.5	6.8	1.2	1.1	1.1	1.2	8.3	8.3
A_3	7.2	6.9	1.1	1.1	1.0	1.0	8.5	8.1
A_4	7.4	7.1	1.2	1.2	1.0	1.2	8.2	8.4
A_5	7.3	7.0	1.1	1.2	1.0	1.1	8.2	8.0
A_6	7.1	7.0	1.1	1.0	0.9	1.1	8.6	8.2
A_7	7.5	7.0	1.0	1.1	1.1	1.1	8.3	8.4
A_8	7.2	6.8	1.1	1.1	1.0	1.1	8.6	8.0
A_9	7.0	7.2	1.1	1.1	1.0	1.1	8.6	8.3
A_{10}	7.6	6.9	1.2	1.2	1.0	1.0	8.2	8.1
A_{11}	7.3	7.1	1.0	1.0	0.9	1.2	8.6	8.4
A_{12}	7.3	7.0	1.1	1.1	1.0	1.1	8.5	8.0
Average	7.3	7.0	1.1	1.1	1.0	1.1	8.4	8.2
SD	0.1754	0.1291	0.068	0.064	0.058	0.064	0.1732	0.1527
CV%	2.4	1.8	5.7	5.8	5.8	5.8	2.1	1.9

Results obtained from 12 replicates of the same green coffe sample, in g% dry basis.
1-Proposed method (RI-HPSEC)
2-UV-HPSEC method for chlorogenic acid, trigonelline

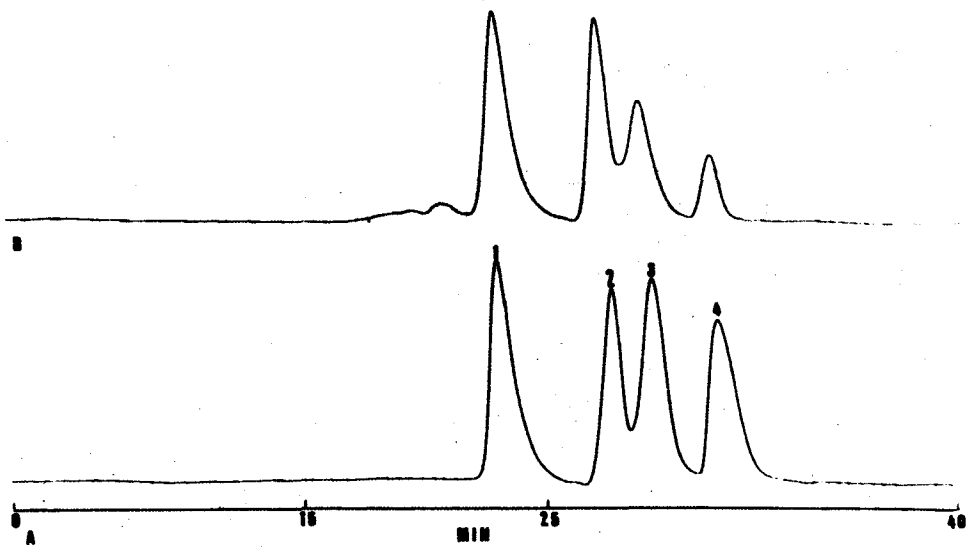

Figure 2. *Chromatograms of (a) standards and (b) green coffee using the TSK G-3000 SW HPGF chromatographic column. (1) CGA (5mg/ml), (2) sucrose(5mg/ml), (3) trigoneline(2.5mg/ml), (4) caffeine(2.5mg/ml). Mobile phase was bidistilled water at a flow rate of 0.5ml/min. Detector: R.I.*

Therefore, the HPSEC method is attractive both in terms of cost and for not using toxic solvents. Furthermore, the simultaneous determination of the four compounds promotes a decrease in the analysis time when compared to the RP16 and amino-phase[15] HPLC methods which need different chromatographic conditions. Finally, automatic control analysis, which is of great importance for routine determinations, is favoured by application of the HPSEC method.

In conclusion, RI-HPSEC is an interesting approach for the simultaneous analysis of total CGA, sucrose, trigonelline and caffeine in green coffee samples, being an useful tool for control quality in the coffee industry.

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