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Comparison of GC and HPLC for the Quantification of Organic Acids in Coffee

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A GC and an HPLC method for the quantification of organic acids OAs in coffee have been compared. The GC procedure, employing trimethylsilyl derivatives, was found to be very tedious. The HPLC method, which employed an ion exchange column using a flow gradient of water containing 1% phosphoric acid and UV detection (210 nm), was found to be much simpler for the quantification of eight organic acids (oxalic, succinic, fumaric, malic, tartaric, citric, quinic and fumaric acids) in four representative coffee samples. The HPLC procedure was more convenient than that described in the literature since no pre-purification was required for quantification of the OAs. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: GC; HPLC; quantitative determination; organic acids; coffee.

INTRODUCTION

Various types of organic acids (OAs), such as oxalic, succinic, fumaric, malic, tartaric, citric and quinic acids, etc., are considered to play important roles in coffee flavour (Illy and Viani, 1995), but have been little studied. As a part of our research programme in this area, a rapid method for the quantification of OAs was required. The literature procedures described for the analysis of these compounds using GC and HPLC were not satisfactory. The GC method using trimethylsilyl derivatives (Hughes and Thorpe, 1987) could be applied to the analysis of only a few OAs, whilst the HPLC methods previously described have been employed mainly for the chlorogenic acids (Clifford and Wight, 1976; Van der Stegen and Van Duijn, 1980; Ohiokpehai *et al.*, 1982; Trugo *et al.*, 1991; Balyaya and Clifford, 1995). An HPLC method that has been used for the analysis of OAs in coffee (Van der Stegen and Van Duijn, 1987) was not satisfactory since it involved purification of the compounds on two columns prior to quantification, and significant losses of some OAs were reported to occur. The GC method for the analysis of OAs in coffee would appear to offer significant advantages compared to HPLC since the former could be readily interfaced with MS permitting unequivocal identification of known acids and the detection of new compounds. Hence, in the present study both GC and HPLC methods have been re-investigated for the quantification of eight organic acids (oxalic, succinic, fumaric, malic, tartaric, citric, quinic and fumaric acids) in four representative coffee samples.

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EXPERIMENTAL

Coffee samples. About 10 kg of coffee beans (Catuaí Vermelho; *Coffea arabica* L) were harvested by the "derriça" method (beans were hand-picked from the tree and allowed to drop onto the cloth-covered floor). From this sample of beans, ca. 5 kg were removed randomly, and the remainder were used to separate immature beans and cherry beans by placing the coffee sample in a tank containing 50 L of water. The dry beans floated immediately and were separated, whilst the immature and cherry beans remained at the bottom of the tank. After draining the tank, the immature beans and the cherry beans were hand-separated and allowed to dry in the air for 20 h. The cherry bean sample was then divided into two parts and one part was dried outside under ambient conditions on a cement patio for 10–12 days to a humidity of about 14% (sample *4*). The other three samples (the initial random mixture of coffee beans, the immature beans and the remaining cherry coffee beans) were dried separately in a conventional dryer at 40°C for 40 h to a humidity of about 12% to yield samples *1–3*, respectively. All four samples were ground (to 20 mesh) and the OAs quantified by GC and HPLC as described below.

Reagents. All reagents [organic acids, ion exchange resins and bis(trimethylsilyl)trifluoroacetamide (BSTFA)] were obtained from Sigma (St Louis, MO, USA). The water used in this study was doubly distilled.

GC analysis. The method described by Cambraia *et al.* (1983) was used, with slight modification, in order to extract the OAs from coffee beans. Ground coffee (2 g) was mixed with 80% ethanol, an internal standard (*trans*-aconitic acid) added and the whole agitated with a magnetic stirrer for 30 min. The extract was purified on two ion exchange columns, and the trimethylsilyl (TMS)

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Table 1. Conditions for the GC analysis of trimethylsilyl derivatives of organic acids in coffee bean samples

Column	Supelco (College Park, PA, USA) DB 1 (30 m × 0.25 mm i.d.)
Initial column temperature	100°C
Temperature program	100–150°C at 5°C/min and 150–280°C at 3°C/min
Detector temperature	280°C
Purge rate	3 mL/min
Column pressure	130 pK_a
Column flow (at 60°C)	1.5 mL/min
Linear velocity	37.7 cm/s
Total flow	20 mL
Split ratio	10:1
Time of analysis	35 min

derivatives of the OAs prepared using a slightly modified version of the method described by Cambraia *et al.* (1983). BSTFA (200 μL) and pyridine (100 μL) were added to the coffee extract (or to 10 mg of a standard mixture of OAs), the mixture heated for 30 min at 50°C and analysed immediately by GC. TMS derivatives of the OAs were also prepared by heating the reagents with standard mixtures (concentrations as above) for 70, 110 and 150 min at 50°C. The stability of the TMS-OAs was determined following GC analysis of the above samples by comparing peak areas. The relative stability was defined to be the ratio of peak area obtained after a given reaction time (70, 110 and 150 min) to that obtained after 30 min reaction time (maximum response obtained), multiplied by 100.

GC analysis was carried out on a Shimadzu (Tokyo, Japan) model 17A gas chromatograph equipped with a flame ionisation detector, an auto sampler and a computer-based system to accumulate data; the analytical conditions are described in Table 1. The individual TMS-OAs in coffee samples were identified by comparing the retention times and relative (to the internal standard) retention times of the peaks with those of standard TMS-OAs. Quantification was carried out by injecting known amounts of TMS-OAs and the internal standard (Cambraia *et al.*, 1983). Calibration curves were generated with the system software; only linear regions of the curves were utilised for quantification. All samples were analysed three times.

In order to determine the recoveries of OAs following extraction, a known amount of standard OA mixture was passed through the two ion exchange columns as described by Cambraia *et al.* (1983), derivatised (30 min reaction time), and immediately analysed by GC. The percentage recovery of each OA was determined by dividing (OA peak area/internal standard peak area) obtained after passing through the column by (OA peak area/internal standard peak area) obtained directly, and multiplying by 100.

HPLC analysis. OAs were extracted from coffee beans using the method described by Van der Stegen and Van Duijn (1987) with slight modification. Ground coffee (2 g) was mixed with water, an internal standard (glutaric acid; 15 mg) added and the whole agitated with a magnetic stirrer for 30 min. The solution was diluted to 10 mL, filtered and a 20 μL aliquot taken for analysis.

HPLC analysis was performed using a GBC system (Victoria, Australia) model 1150 fitted with a Rheodyne injector, a Shimadzu variable UV detector, and a computer-based system to accumulate data. The analytical conditions are described in Table 2. The individual OAs in coffee samples were identified by comparing the retention times and relative (to the internal standard) retention times of the peaks with those of the standard OAs. The detection limits of the OAs (defined as two times baseline noise) were determined visually from a coffee sample. Quantification of OAs was carried out by the internal standard method by injecting known amounts of OAs and glutaric acid. Calibration curves were generated with the system software. Only linear regions of the curves were utilised for quantification. All samples were analysed three times.

In order to determine the percentage recoveries of the OAs, previously quantified coffee samples were spiked with known amounts of OA standards and the samples

Table 2. Conditions for the HPLC analysis of organic acids in coffee bean samples

Column	Supelco Supelcogel-C610H (250 × 7.8 mm i.d.)	
Pre-column	Supelco Supelcogel-C610H (50 × 7.8 mm i.d.)	
Column temperature	40°C	
Detector	UV operating at 210 nm	
Solvent	Water containing 1% phosphoric acid	
Flow rate program	Time (min)	Flow rate (mL/min)
	0	0.5
	13	0.3
	16	0.5
	24	0.8

Table 3. Relative responses (in terms of GC peak areas) of trimethylsilyl derivatives of organic acids as a function of the reaction time of silylation

Trimethylsilyl derivative	Relative response[a] following silylation reaction			
	30 min	70 min	110 min	150 min
Oxalic acid	100	87	88.8	108.9
Succinic acid	100	85	84.3	102.7
Fumaric acid	100	84.5	84.0	101.8
Glutaric acid	100	84.3	84.0	101.5
Malic acid	100	83.7	84.2	94.7
Tartaric acid	100	85.4	86.0	99.2
trans-Aconitic acid	100	52.7	40.0	33.7
Citric acid	100	87.9	90.0	97.7
Quinic acid	100	88.3	90.0	98.3

[a] Percentage response normalised with respect to the response obtained after 30 min silylation.

quantified again. The ratio of the increase in OA obtained after spiking coffee samples to the OA standard added, when multiplied by 100, gave the percentage recovery.

RESULTS AND DISCUSSION

GC analysis

The literature method described for the analysis of the OAs (as TMS derivatives) without purification of the extract has been applied to the quantification of only a few acids such as quinic and chlorogenic acids (Hughes and Thorpe, 1987). Our preliminary studies indicated that analysis of TMS-OAs in coffee without purification did not give acceptable results and that some kind of purification was needed. This obviously would be a great drawback of using GC, as purification procedures are very time-consuming and significant losses of some OAs have been reported (Van der Stegen and Van Duijn, 1987).

Two potential strategies have been considered for the purification of coffee OAs before quantification: an electrophoretic/ultrafiltration method has been described (Bähre and Maier, 1996) but no quantitative data presented, and the use of ion exchangers has been reported to be unsatisfactory (Engelhardt 1994). Since a clean-up step was necessary before the formation of the TMS derivatives, it was decided to employ a method previously described by Cambraia *et al.* (1983) for sorghum. When a standard mixture of OAs was reacted with BSTFA and pyridine, however, a precipitate formed after some time, although such a precipitation had not been described by Cambraia *et al.* (1983). Thus, in order to evaluate the efficiency of the GC procedure, it was necessary to consider not only the recovery of the OAs from ion exchange columns, but also the stability of the TMS derivatives of the OAs.

To verify the stability of TMS-OAs, derivatives of standard OAs were prepared and their GC response (in terms of peak areas) determined after reaction times of 30, 70, 110 and 150 min. The initial highest response was obtained following a reaction time of 30 min for all the OAs (Table 3), and in most cases, the response decreased by about 15% at 70 min reaction time but returned almost to its original value when the reaction time was 150 min. The response of *trans*-aconitic acid decreased drastically with the longer reaction times, and fell to 33.7% of its maximum value when the reaction time was 150 min. It was obvious that in order to achieve good quantification it was important to prepare the TMS-OAs and inject them immediately, hence all analyses were carried out in this manner. This constitutes, of course, a great disadvantage of the method as it would not be possible to prepare several samples at one time for later quantification. A decrease in response of TMS-OAs has not been reported in the literature and is difficult to explain.

Determination of the recoveries of OAs from two ion exchange columns was achieved by quantifying known quantities of standard OAs following elution, formation of TMS derivative and immediate injection onto the GC. Reasonable recoveries were obtained for several OAs (Table 4), however, the procedure was found to be extremely tedious and would not be applicable to a large number of samples. Nevertheless, quantification of four coffee samples was carried using this method (Table 5), and typical chromatograms of a standard mixture and a coffee sample are presented in Figs 1 and 2, respectively.

The amounts of coffee OAs obtained by GC (Table 5) were about 500–1000 times lower than those reported by Van der Stegen and Van Duijn (1987), from which it was apparent that the solvent used for the extraction was not efficient. Other extraction solvents such as dimethyl-

Table 4. Percentage recoveries of standard organic acids after passing through two ion exchange columns followed by formation of trimethylsilyl derivatives and immediate analysis by GC

Organic acid (determined as TMS derivative)	Percentage recovered[a]
Oxalic acid	89.0 ± 4.1
Succinic acid	85.0 ± 3.0
Fumaric acid	90.0 ± 2.1
Glutaric acid	96.2 ± 4.1
Malic acid	95.0 ± 5.0
Tartaric acid	87.0 ± 4.1
trans-Aconitic acid	99.3 ± 2.1
Citric acid	98.0 ± 6.1
Quinic acid	87.0 ± 8.2

[a] Mean ± standard deviation; $n = 3$.

Table 5. Concentrations of organic acids (measured as trimethylsilyl derivatives by GC) in four coffee samples

Organic acid (measured as TMS derivative)	Concentrations[a] (mg/g) of OAs			
	Sample 1 (random mixture of coffee beans)	Sample 2 (immature coffee beans)	Sample 3 (cherry coffee beans)	Sample 4 (cherry coffee beans dried on a cement patio)
Citric acid	0.0091 ± 0.0019	0.0103 ± 0.0017	0.013 ± 0.002	0.0112 ± 0.0018
Quinic acid	0.0149 ± 0.0027	0.022 ± 0.0018	0.023 ± 0.003	0.00187 ± 0.0025
Malic acid	0.0091 ± 0.0018	0.0049 ± 0.0005	0.012.8 ± 0.0015	0.0149 ± 0.002
Succinic acid	nd[b]	trace	trace	nd
Tartaric acid	nd	nd	nd	nd
Oxalic acid	nd	nd	nd	nd
Fumaric acid	nd	nd	nd	nd

[a] Mean ± standard deviation; $n = 3$.
[b] nd = none detected.

sulphoxide (Hughes and Thorpe, 1987) and water (Van der Stegen and Van Duijn, 1987) were not evaluated since the GC procedure was found to be very tedious and not appropriate for further studies.

HPLC analyses

Few HPLC procedures for the analysis of coffee OAs have been described. The procedure described by Van der

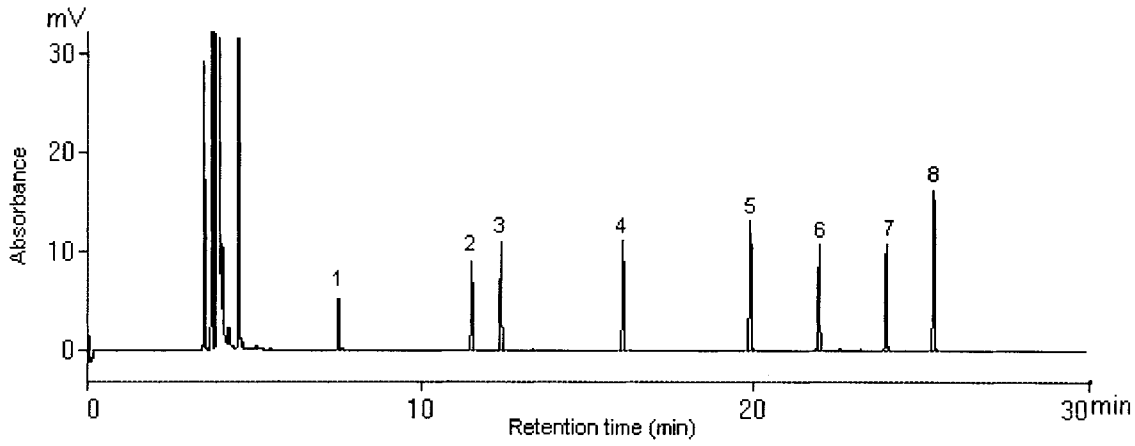

Figure 1. Typical gas chromatogram of a standard mixture of trimethylsilyl derivatives of organic acids. Key to peak identities: **1**, oxalic acid; **2**, succinic acid; **3**, fumaric acid; **4**, malic acid; **5**, tartaric acid; **6**, *trans*-aconitic acid; **7**, citric acid; and **8**, quinic acid. (For details of the chromatographic protocol see Experimental section.)

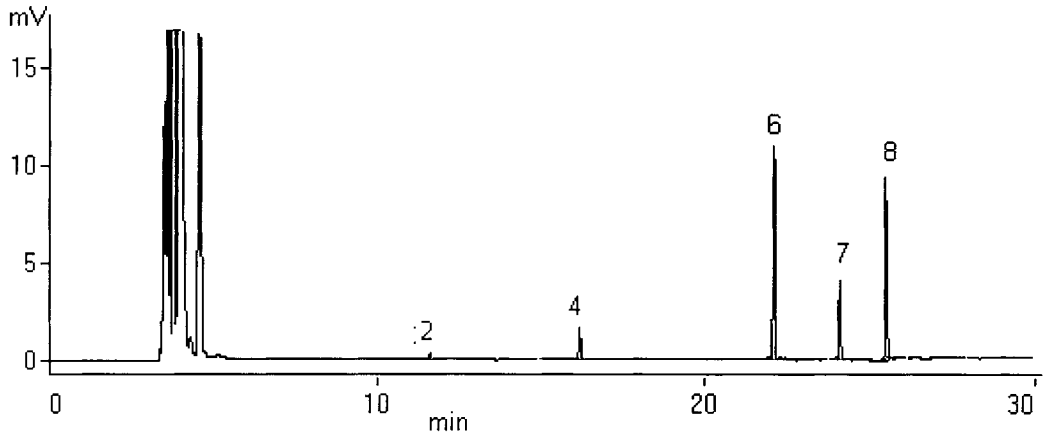

Figure 2. Typical gas chromatogram of the trimethylsilyl derivatives of organic acids found in a coffee sample (sample *3*). For key to peak identification see legend to Fig. 1.

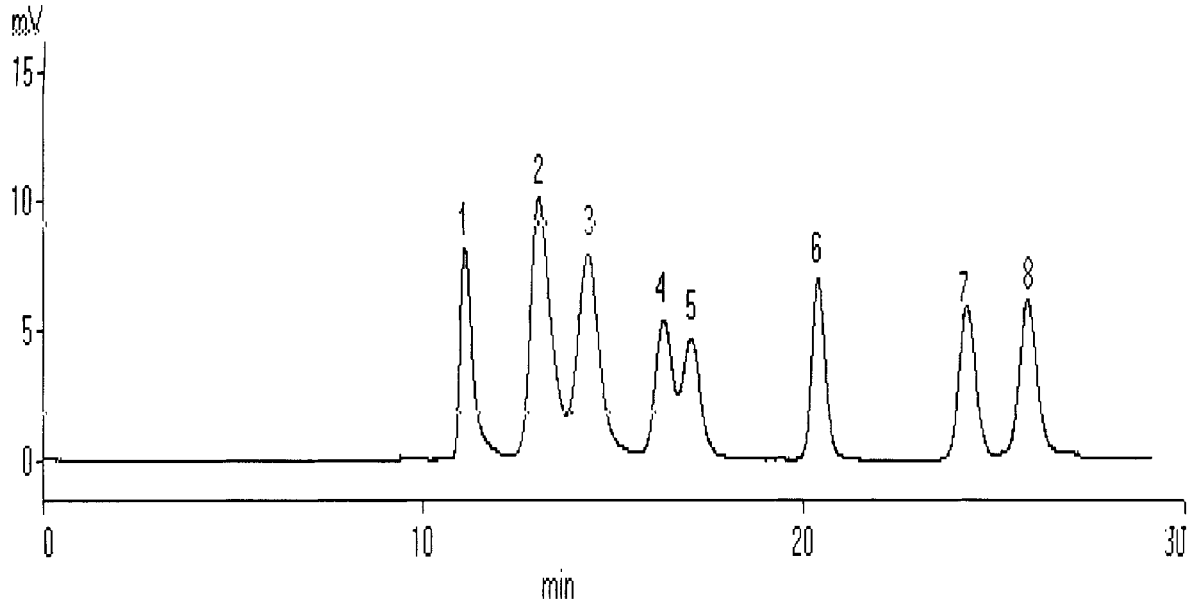

Figure 3. Typical HPLC chromatogram of a standard mixture of organic acids. Key to peak identities: **1**, oxalic acid; **2**, citric acid; **3**, tartaric acid; **4**, malic acid; **5**, quinic acid; **6**, succinic acid; **7**, glutaric acid (internal standard); and **8**, fumaric acid. (For details of the chromatographic protocol see Experimental section.). HPLC chromatogram of a water extract of coffee sample 3. For key to peak identification see legend to Fig. 3.

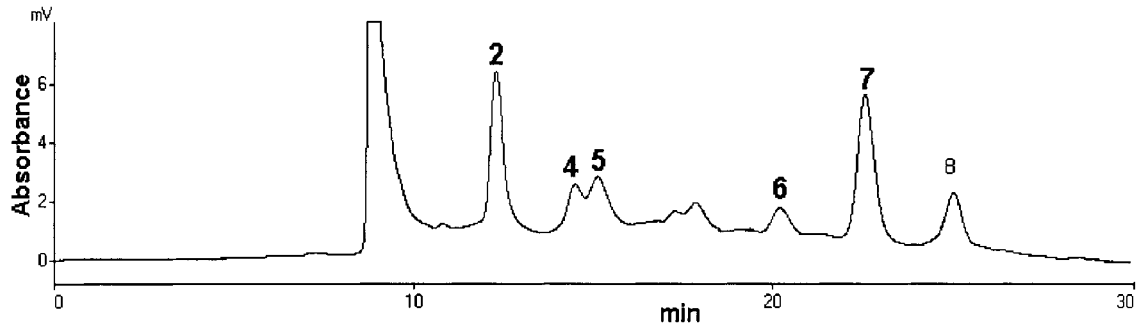

Figure 4. HPLC chromatogram of a water extract of coffee sample 3. For key to peak identification see legend to Fig. 3.

Stegen and Van Duijn (1987), which employed an ion exchange column eluted with 0.009M sulphuric acid, involved extensive pre-purification on two ion exchange columns prior to HPLC quantification. In addition, the recoveries reported for the OAs varied between 1 and 100%. Lues *et al.*, (1998) reported the successful use of a

Table 6. Concentrations of organic acids (determined by HPLC) in four coffee samples

Organic acid	Concentrations[a] (mg/g) of OAs				
	Sample 1 (random mixture of coffee beans)	Sample 2 (immature coffee beans)	Sample 3 (cherry coffee beans)	Sample 4 (cherry coffee beans dried on a cement patio)	Literature values[b]
Citric acid	11.0 ± 0.4	13.0 ± 1.3	11.5 ± 1.2	11.2 ± 1.9	5–14.9
Quinic acid	6.7 ± 0.3	8.5 ± 0.7	6.7 ± 0.8	7.0 ± 0.9	3.3–6.1
Malic acid	4.6 ± 0.3	3.1 ± 0.2	4.7 ± 0.6	4.4 ± 0.4	2.6–6.7
Succinic acid	nd[c]	trace	3.3 ± 0.3	nd	trace-1.5
Acetic acid	nd	nd	nd	nd	trace
Formic acid	nd	nd	nd	nd	trace-0.7
Oxalic acid	nd	0.9 ± 0.2	nd	nd	nd
Fumaric acid	0.03 ± 0.005	0.010 ± 0.002	0.021 ± 0.001	0.030 ± 0.004	nd

[a] Mean ± standard deviation; $n = 3$.
[b] According to Van der Stegen and Van Duijn (1987).
[c] nd = none detected.

Table 7. Percentage recoveries of standard organic acids by HPLC

Organic acid	Percentage recovered[a]
Oxalic acid	97 ± 2.1
Succinic acid	100 ± 3.1
Fumaric acid	100 ± 2.0
Glutaric acid	100 ± 1.2
Malic acid	100 ± 2.1
Tartaric acid	100 ± 5.1
Citric acid	100 ± 3.0
Quinic acid	98 ± 2.0

[a] Mean ± standard deviation; $n = 3$.

Supelcogel C-610H ion-exchange column (sulphonated divinylbenzene-styrene co-polymer) for the analysis of OAs in cheese without pre-purification of the extract, but they did not provide data on the recovery of OAs. This method has been re-evaluated for the analysis of coffee OAs, addressing the question of recovery of OAs. In Figs 3 and 4, typical chromatograms obtained with a brand new column are presented for a standard mixture of OAs and for a coffee sample, respectively. Excellent reproducibility was possible, and the chromatograms were clean, with good resolution for most OAs, although malic and quinic acids were only ca. 50% resolved. Several different flow gradients were tested, but the chromatograms could not be substantially improved from those shown in Figs 3 and 4. The minimum quantities detectable for succinic, malic, citric and quinic acids were about the same, namely ca. 1 μg, while that for fumaric acid was ca. 0.01 μg. These values were much higher than those with GC, but this would not be a limitation for the analysis of coffee OAs. When the obtained results (Table 6) were compared with those in the literature (Van der Stegen and Van Duijn, 1987), they were found to be similar for the major acids (citric, quinic, malic and succinic acids). Obviously, a direct comparison of both procedures was not possible since different samples were used. In addition, the reproducibility and the errors involved in the study of Van der Stegen and Van Duijin (1987) are not available. A distinct advantage of our method over that described in the literature is that we have used the internal standard method for quantification as compared to the external standard method employed previously.

In order to verify the quantitative recovery of OAs using the new procedure, previously analysed coffee samples were spiked with known amounts of standard OAs, and the mixtures were re-quantified. The results are presented in Table 7, which shows that excellent recoveries were obtained. When the GC results (Table 5) were compared to those obtained by HPLC (Table 6) significantly lower values (about 500–1000 times) were obtained with the former procedure. Hence, it was clear that the problem with the GC method was the solvent used for extraction (see above).

In conclusion, it may be stated that the GC method for the quantification of coffee OAs was found to be unsuitable since the procedure was very tedious. The HPLC method was found to be very simple and reliable for the quantification of OAs in coffee and superior to that previously described in the literature since it does not involve purification of the crude extract on two ion exchange columns (where significant losses of some OAs were reported).

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