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Analytical Methods

Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography–mass spectrometry

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ABSTRACT

A rapid liquid chromatography–mass spectrometry method for the simultaneous quantification of caffeine, trigonelline, nicotinic acid and sucrose in coffee was developed and validated. The method involved extraction with hot water, clarification with basic lead acetate and membrane filtration, followed by chromatographic separation using a Spherisorb® S5 ODS2, 5 μm chromatographic column and gradient elution with 0.3% aqueous formic acid/methanol at a flow rate of 0.2 mL/min. The electrospray ionization source was operated in the negative mode to generate sucrose ions and in the positive mode to generate caffeine, trigonelline and nicotinic acid ions. Ionization suppression of all analytes was found due to matrix effect. Calibrations curves prepared in green and roasted coffee extracts were linear with $r^2 > 0.999$. Roasted coffee was spiked and recoveries ranged from 93.0% to 105.1% for caffeine, from 85.2% to 116.2% for trigonelline, from 89.6% to 113.5% for nicotinic acid and from 94.1% to 109.7% for sucrose. Good repeatibilities (RSD < 5%) were found for all analytes in the matrix. The limit of detection (LOD), calculated on the basis of signal-to-noise ratios of 3:1, was 11.9, 36.4, 18.5 and 5.0 ng/mL for caffeine, trigonelline, nicotinic acid and sucrose, respectively. Analysis of 11 coffee samples (regular or decaffeinated green, ground roasted and instant) gave results in agreement with the literature. The method showed to be suitable for different types of coffee available in the market thus appearing as a fast and reliable alternative method to be used for routine coffee analysis.

1. Introduction

Coffee is one of the most popular beverages in the world, being appreciated for its characteristic taste and aroma and, more recently, for its potential beneficial effects on human health. Typical compounds in coffee that are relevant for flavor and/or bioactivity are caffeine, trigonelline, nicotinic acid and sucrose (Trugo, 2003).

Caffeine is a xantine derivative known to stimulate of the nervous central system and is generally associated with improvements in alertness, learning capacity and exercise performance when moderately consumed (Farah, de Paulis, Moreira, Trugo, & Martin, 2006). Its characteristic bitter taste is an important determinant for flavor formation (Farah, Monteiro, Calado, Franca, & Trugo, 2006).

Trigonelline is a pyridine derivative known to contribute indirectly to the formation of appreciated flavor products including furans, pyrazine, alkyl-pyridines and pyrroles during coffee roasting (Ky, Dussert, Guyot, Hamon, & Noirot, 2001). Demethylation of trigonelline during coffee roasting generates nicotinic acid, a water-soluble B vitamin also known as niacin. Since nicotinic acid produced during coffee processing is highly bioavailable in the beverage, in contrast to natural sources where it is present in bound form (Trugo, 2003), coffee is a significant source of this vitamin in the diet (Trugo, Macrae, & Trugo, 1985). Moreover, trigonelline appears to have anti-invasive activity against cancer cells (Hirakawa, Okauchi, Miura, & Yagasaki, 2005) and may regenerate dendrites and axons, in addition to memory improvement in animal models (Tohda, Kuboyama, & Komatsu, 2005).

Sucrose, which is the most abundant simple carbohydrate present in green coffee, acts as an aroma precursor during roasting, generating several classes of compounds such as furans, aldehydes and carboxylic acids that will affect the flavor of the beverage.

The contents of caffeine, trigonelline, nicotinic acid and sucrose in commercial coffee may be highly influenced by coffee species, variety, geographical origin and roasting conditions (Casal, Oliveira, & Ferreira, 1998, 2000; Knopp, Bytof, & Selmar, 2006; Ky et al., 2001; Macrae, 1985; Monteiro & Trugo, 2005; Oosterveld, Voragen, & Schols, 2003; Trugo, 1985). In addition to their impact in coffee quality and flavor, the content of sucrose as well as the trigonelline/nicotinic acid ratio can also be used as an indicator of roasting degree by the coffee industry (Stennert & Maier, 1996). Therefore, the simultaneous determination of these components both before and after coffee processing should be a useful

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tool for quality control of raw materials and for monitoring of coffee roasting conditions.

There are several reported chromatographic methods for the individual quantification of caffeine, trigonelline, nicotinic acid and sucrose in coffee (Trugo, 2003; Trugo, de Maria, & Werneck, 1991; Trugo, Macrae, & Dick, 1983). Simultaneous methods using HPLC/UV have also been reported for the combined analysis of caffeine, trigonelline and nicotinic acid (Alves, Dias, Benassi, & Scholz, 2006; Casal et al., 1998). However, there are no reports on simultaneous methods for coffee analysis including sucrose.

The analysis of sucrose and other carbohydrates in green coffee is usually performed by HPLC with the use of refractive index detection (de Maria, Trugo, & Corá, 1996). Recently, Alcázar, Jurado, Martín, Pablos, and González (2005) proposed an enzymatic-spectrophotometric method for the analysis of sucrose in coffee beans. However, sucrose analysis of ground roasted and instant coffees – which have much lower sucrose contents as a consequence of its rapid degradation during roasting – may be difficult with the use of refractive index detector or by the enzymatic-spectrophotometric assay due to their low sensitivity. Alternatively, pulsed amperometric (Bruggink, Maurer, Herrmann, Cavalli, & Hoefler, 2005; Knopp et al., 2006; Murkovic & Derler, 2006), evaporative light scattering (Nogueira, Silva, Ferreira, & Trugo, 2005; Trugo & Macrae, 1982) and mass spectrometric (Nielsen, Granby, Hedegaard, & Skibsted, 2006) detectors have been used for the analysis of small amounts of simple carbohydrates in food samples, due to their high sensitivity. Therefore, mass spectrometry could be a detector of choice in order to incorporate sucrose analysis to HPLC methods designed for caffeine, trigonelline and nicotinic acid analysis, especially in ground roasted and instant coffee samples.

The aim of this work was to develop and validate a rapid LC–MS method for the simultaneous quantification of caffeine, trigonelline, nicotinic acid and sucrose that could be applied routinely to different types of coffee available in the market.

2. Materials and methods

2.1. Standards and chemicals

Trigonelline and sucrose were purchased from Sigma (St. Louis, MO, USA); caffeine from Vetec Química Fina (Duque de Caxias, RJ, Brazil); nicotinic acid from Calbiochem (Los Angeles, CA, USA) and basic lead acetate from Cromato Produtos Químicos (Diadema, SP, Brazil). All solvents were HPLC grade from Tedia (Fairfield, OH, USA). LC grade water was used throughout the experiments (Milli-Q system, Millipore, Bedford, MA, USA).

2.2. Coffee samples

Thirteen different coffee samples were used in this study. A decaffeinated green *Coffea canephora*, cv. Conillon sample was obtained from the coffee industry and roasted in a commercial spouted bed roaster (i-Roast® Model No. 40009, Hearthware Home Products, USA) at 188 °C for 12 min. Both green and roasted Conillon samples were used to validate the method. Eleven coffee samples were analyzed using the validated method to demonstrate its applicability towards different kinds of coffee available in the market. These samples included two good cup quality green samples (*C. arabica*, cv. Mundo Novo and *C. canephora*, cv. Conillon), obtained directly from producers in Guaxupé, Minas Gerais, Brazil; seven ground roasted (six regular and one decaffeinated) and two instant coffee samples, obtained from retail outlets. Roasting degrees of ground roasted and instant coffee samples were determined by comparison with color disks from the "Roasting Color Classification System" (Agtron-SCAA, Reno, NV, USA, 1995), following the standards used by the Brazilian Coffee Industries Association (ABIC).

2.3. Water content

To express the amount of trigonelline, caffeine, nicotinic acid and sucrose on a dry weight basis (dwb), the water content of all ground coffee samples was determined according to the AOAC method (AOAC, 2000).

2.4. Sample preparation

Samples were extracted in triplicate according to a modification of the method of de Maria, Trugo, Moreira, and Petracco (1995). Briefly, 0.2 g of ground coffee was suspended in 60 mL of boiling water and shaken at room temperature for 15 min at 300 rpm. The mixture was filtered through filter paper (Whatman No. 1) and washed with approximately 30 mL of water. In the case of instant coffees, 0.1 g of the sample was directly diluted in 60 mL of water at room temperature. For clarification of the extract, 2 mL of saturated aqueous basic lead acetate solution was added, and the final volume was made up with water to 100 mL. The colloidal dispersion was then filtered through both filter paper (Whatman No. 1) and 0.22 μm cellulose ester membrane (Millipore, Brazil). The final extract was diluted with water (1:1) prior to LC–MS analysis.

2.5. LC–MS analysis

The LC equipment (Shimadzu, Kyoto, Japan) comprised a LC-10AD*vp* quaternary pump, a CTO-10AS*vp* column oven and an 8125 manual injector (Rheodyne) with a 5 μL loop. This LC system was interfaced with a LC–MS 2010 mass spectrometer (Shimadzu, Kyoto, Japan) fitted with an electrospray ion source.

Chromatographic separations were achieved using a Spherisorb® S5 ODS2 Microbore HPLC column (150 × 2.0 mm, 5 μm, Waters, Milford, MA, USA) maintained at a constant temperature of 40 °C. The mobile phase consisted of 0.3% aqueous formic acid (eluent A) and methanol (eluent B), delivered at a flow rate of 0.2 mL/min. Before injection, the column was equilibrated with 25% B. Immediately after injection, this proportion was changed to 60% B until the end of the run at 6 min. In between injections, 5 min intervals were used for column re-equilibration with 25% B.

The electrospray ionization source was operated in the negative mode from 0.0 to 2.2 min to generate the formic acid adduct of sucrose $(M+HCOO)^-$ and in the positive mode from 2.2 to 6.0 min to generate caffeine, trigonelline and nicotinic acid $(M+H)^+$ ions. Nebulizer gas (N_2) flow was set to 3.0 L/min and desolvation temperature was adjusted to 300 °C. The mass spectrometer was operated in the single ion monitoring (SIM) mode to detect caffeine, trigonelline, nicotinic acid and sucrose pseudomolecular ions. Identification of compounds of interest was performed by comparison with retention time and molecular weight of the respective standard. Data were acquired by LCMSsolution software (Shimadzu Corp., version 2.00, 2000) for the mass spectrometer.

2.6. Validation scheme

The method was validated for the determination of trigonelline, caffeine, nicotinic acid and sucrose in green and roasted coffee samples.

2.7. Calibration curves and matrix effect

Extracts of green and roasted decaffeinated *C. canephora*, cv. Conillon were used to investigate the presence of matrix effect.

The matrix effect was assessed "to ensure that precision, selectivity, and sensivity of the proposed method would not be compromised" (FDA Food & Department of Health, 2001; Matuszewski, Constanzer, & Chavez-Eng, 2003). This effect was evaluated by comparing the slopes of the standard calibration curves prepared in water with those prepared in green and roasted coffee extracts. Calibration curves in the coffee extracts were prepared by diluting the standard solutions prepared in water with the same volume of the coffee extracts. Standard peak areas of calibration curves prepared in green and roasted coffee extracts were corrected by subtracting the peak areas of the compounds already present in the corresponding blank extract. The means of two 4-point calibration curves in water and of two 6-point calibration curves prepared in the coffee extracts were obtained. The concentrations of the standards were selected in a way that they could cover a wide range of concentrations detected. All calibration curves were forced through the origin and linearly fitted.

2.8. Recovery

Ground roasted decaffeinated *C. canephora*, cv. Conillon was used as blank matrix for the recovery experiments. The repeatibility was calculated using multiple ($n = 4$) determinations of the recovery assay. Accuracy was determined in a recovery experiment where the ground roasted decaffeinated Conillon coffee matrix was spiked at three different levels – low, medium and high – for each compound of interest. Those levels were chosen in order to mimic the typical reported minimum, medium and maximum amounts of these components in green or roasted coffee (Macrae, 1985; Trugo, 1985, 2003). For coffee matrix spiking, the appropriate amounts of solid standards and of ground coffee were mixed and extensively homogenized in a mortar. The extraction and LC–MS analysis of four replicates of each spike level were carried out as mentioned above. The quantification of trigonelline, caffeine, nicotinic acid and sucrose in each spiked sample was based on the external standard method using the curves prepared in green and roasted coffee extracts.

2.9. Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ for each analyte were determined as the concentrations equivalents to three times and 10 times, respectively, the signal-to-noise ratio of the compounds of interest in the lowest concentration of the calibration curve prepared using the roasted coffee extract. Signal-to-noise ratios were calculated by LCMSsolutions software, using the S/N calculation tool.

2.10. Statistical analyses

Data are presented as mean ± SD. Recovery results obtained using the calibration curves prepared in green and roasted coffee extracts were compared using the Student's *t*-test. Sample results were statistically treated by analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Statistical analyses were performed using Prism 4 for Windows, version 4.00 (GraphPad Software Inc.). Differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Chromatographic separation

The optimization of the chromatographic conditions was focused in enhancing the ionization of the analytes as well as minimizing the analysis time. Instrumental parameters affecting ionization were nebulizer gas flow and desolvation temperature. Maximum ionization was achieved with 3.0 L/min flow of N_2 and a desolvation temperature of 300 °C. Gradient elution was used to reduce caffeine retention time, without affecting the separation of the other analytes. Overall analysis time was reduced to 6 min, comparably lower than in other published methods. Fig. 1 shows a typical chromatogram for separation of caffeine, trigonelline, nicotinic acid and sucrose standards.

3.2. Method validation

3.2.1. Calibration and matrix effect

The slopes of the calibration curves prepared in water were higher (from 1.5 times for caffeine to 8.5 times for trigonelline) than those of calibration curves prepared in coffee extracts for all of the analytes, indicating the presence of matrix effect by ionization suppression (Table 1). Suppression of the analyte response due to matrix effect has been extensively reported, especially when analyzing complex matrices, such as biofluids, environmental and food samples (Dams, Huestis, Lambert, & Murphy, 2003; King, Bonfiglio, Fernandez-Metzler, Miller-Stein, & Olah, 2002; Matuszewski et al., 2003). The slopes of calibration curves prepared in green and roasted coffee extracts were approximately the same for caffeine, trigonelline and nicotinic acid. For sucrose, the slope of the calibration curve prepared in the roasted coffee extract was approximately 25% lower than that of the calibration curve prepared in the green coffee extract, indicating that ionization suppression of sucrose was influenced by changes in the coffee matrix as a consequence of roasting of green coffee. Similarly, during the LC–MS analysis of sucrose in bread, Nielsen et al. (2006) found that this compound suffered approximately 60% of ionization suppression due to the food matrix. Therefore, calibration curves for sucrose analysis should be prepared in green or roasted coffee extracts depending on the kind of coffees that one wish to analyze.

3.3. Accuracy

The mean recoveries for the four analytes in each spike level are shown in Table 2. Using the calibration curves prepared in the roasted coffee extract, recoveries within 15% from unity were found for the compounds of interest in the three spike levels, with the exception of trigonelline low spike level (0.01 g/100 g coffee dwb), which presented recovery over 200%. When using green extracts, recovery results were consistently lower than those obtained when using roasted extracts, but they were still within 15% from unity, except for sucrose high spike level (5 g/100 g coffee dwb), which presented a recovery of only 73%. Comparing recoveries using the curves prepared in the green and roasted coffee extracts, significant differences ($p < 0.05$) were found only for

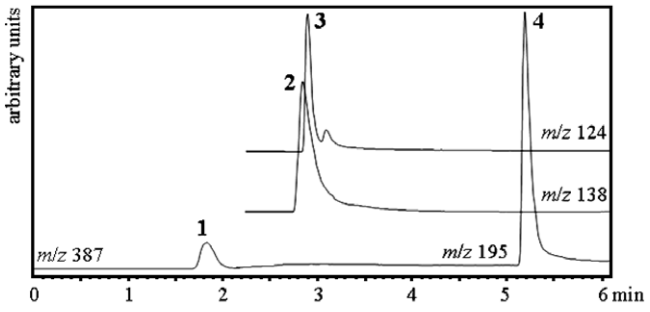

Fig. 1. Typical chromatographic separation of a mixture of sucrose (1; $m/z = 387$), trigonelline (2; $m/z = 138$), nicotinic acid (3; $m/z = 124$) and caffeine (4; $m/z = 195$) standards.

Table 1
Calibration parameters for concentration curves prepared in water and coffee extracts[a]

Compound	Preparation media	Concentration range (μg/mL)	n[b]	Slope (area/concentration)	R^2[c]
Caffeine	Water	0.10–10.00	4	1,141,872	0.9999
	Green coffee	0.05–25.00	6	794,542	0.9995
	Roasted coffee	0.05–25.00	6	769,700	0.9997
Trigonelline	Water	0.08–8.00	4	2,798,193	0.9987
	Green coffee	0.04–20.00	6	329,658	0.9991
	Roasted coffee	0.04–20.00	6	328,274	0.9993
Nicotinic acid	Water	0.10–10.00	4	1,756,402	0.9979
	Green coffee	0.05–27.50	6	213,208	0.9994
	Roasted coffee	0.05–27.50	6	208,601	0.9998
Sucrose	Water	0.10–10.00	4	268,529	0.9890
	Green coffee	0.05–25.00	6	113,375	0.9990
	Roasted coffee	0.05–25.00	6	85,643	0.9994

[a] Coffee extracts were prepared from green and roasted decaffeinated *C. canephora* cv. Conillon.
[b] Number of concentration points considered for the linear regression. Each point represents the average of two injections.
[c] Correlation coefficient.

Table 2
Recovery and repeatability of the proposed analytical method at three different spike levels of standard compounds in ground coffee[a]

Compound	Spike level (g/100 g)	n	Recovery (%)[b]		Repeatability (%)[c]
			Roasted extract	Green extract	
Caffeine	0.01	4	102.0 ± 4.0	98.8 ± 3.9	3.9
	1.0	4	105.1 ± 5.0	101.8 ± 4.8	4.7
	10.0	4	93.0 ± 1.9	90.1 ± 1.8	2.1
Trigonelline	0.01	4	220.0 ± 41.0	219.0 ± 39.8	n.d.[d]
	0.1	4	116.2 ± 4.1	115.7 ± 4.1	3.5
	1.0	4	85.2 ± 2.7	84.9 ± 2.7	3.2
Nicotinic acid	0.001	4	89.6 ± 4.4	87.6 ± 4.3	4.9
	0.01	4	99.4 ± 1.5	97.2 ± 1.5	1.6
	0.1	4	113.5 ± 3.6	111.0 ± 3.5	3.2
Sucrose	0.05	4	109.7 ± 5.3	85.1 ± 4.1	4.9
	0.5	4	104.2 ± 4.6	80.9 ± 3.5	4.4
	5.0	4	94.1 ± 3.0	73.0 ± 2.4	3.2

[a] Coffee extracts were prepared from green and roasted decaffeinated *C. canephora* cv. Conillon.
[b] Mean ± standard deviation.
[c] Repeatability is given as the relative standard deviation (RSD).
[d] Not determined.

sucrose, in all three spike levels. This result was already expected since green coffee had a lower matrix effect over sucrose than roasted coffee (Table 2).

The above results, however, do not compromise the proposed method, since trigonelline medium spike level (0.1 g/100 g coffee dwb) is still lower than the minimum reported amount for this compound in coffee (0.2 g/100 g coffee dwb). For sucrose analysis, the same type of coffee that is being analyzed may be used as media for calibration curves preparation.

3.4. Precision

Repeatability is given as the relative standard deviation (RSD) of four replicates recovery experiments in Table 2. For caffeine, nicotinic acid and sucrose, good repeatability (RSD < 5%) in all three spike levels were obtained, whereas for trigonelline good repeatability was found only in the medium (RSD = 3.5%) and high (RSD = 3.2%) spike levels, with poor repeatability in the low spike level. Nevertheless, trigonelline analysis would not be compromised since minimum trigonelline reported levels in coffee are still higher than the medium spike level of this compound tested in our study.

3.5. Limits of detection and quantification

Table 3 shows the calculated LOD and LOQ of the analytes of interest. Caffeine, trigonelline and nicotinic acid LOD were in the range of 11.9–36.4 ng/mL, approximately 5 times lower than those found by Casal et al. (1998) using a diode-array detector. Caffeine LOD was comparable to that reported by Zhu et al. (2004) using the MS detector. Sucrose LOD (5.0 ng/mL) was considerably lower than values reported elsewhere, using enzymatic-spectrophotometric assays (LOD = 20.3 μg/mL) (Alcázar et al., 2005) or HPLC with refractive index (LOD = 0.1 mg/mL) (de Maria et al., 1996), evaporative light scattering (LOD = 5.0 μg/mL) (Nogueira et al., 2005), mass spectrometric (LOD = 2.3 μg/mL) (Nielsen et al., 2006) and pulsed amperometric (LOD = 1.1 μg/mL) detection (Murkovic & Derler, 2006). The LODs in our study means that the minimum detectable amount of caffeine, trigonelline, nicotinic acid and sucrose in coffee are 1.2, 3.6, 1.8 and 0.5 mg/100g coffee (dwb), respectively. These levels are much lower than the minimum

Table 3
Limits of detection (LOD) and quantification (LOQ) of the analyzed compounds in coffee for instrument and analytical method[a]

Compound	LOD (ng/mL)	LOQ (ng/mL)	LOD in coffee (mg/100 g)	LOQ in coffee (mg/100 g)
Caffeine	11.9	39.6	1.2	4.0
Trigonelline	36.4	121.5	3.6	12.1
Nicotinic acid	18.5	61.7	1.8	6.2
Sucrose	5.0	16.5	0.5	1.6

[a] Determined as the concentrations equivalents to three times (LOD) and 10 times (LOQ) the signal-to-noise ratio of the analytes in the blank roasted decaffeinated coffee matrix.

amounts typically found in coffee samples for caffeine, trigonelline and sucrose (Macrae, 1985; Trugo, 1985, 2003) and are similar to the minimum reported amount in coffee for nicotinic acid (1.6 mg/100 g coffee dry weight) (Macrae, 1985; Trugo, 1985, 2003).

3.6. Analysis of commercial coffee samples

In order to evaluate the applicability of the method, green and commercial samples of regular and decaffeinated roasted and instant coffees, roasted to different degrees (samples A–K), were analyzed. Fig. 2 shows typical chromatograms for green and roasted regular coffee samples. The contents of caffeine, trigonelline, nicotinic acid and sucrose in these samples are shown in Table 4.

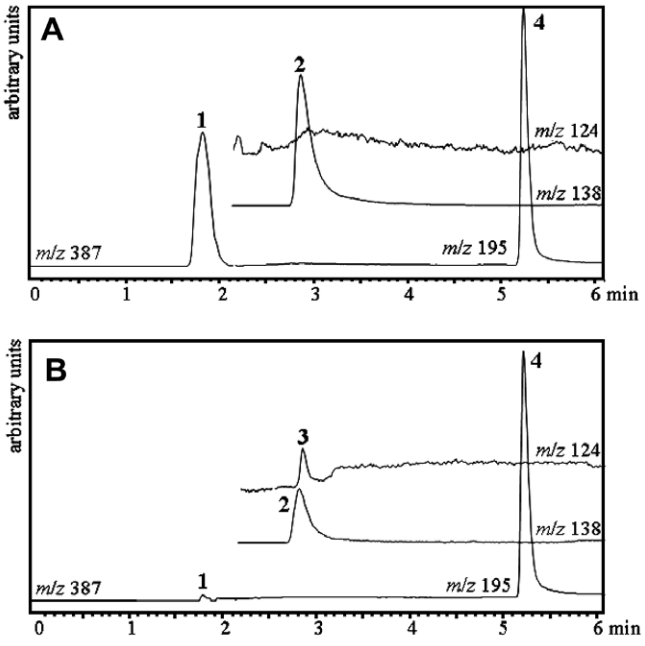

Fig. 2. Typical chromatographic separation of (A): green coffee, represented by *C. arabica* from Brazil (sample A); (B): ground roasted coffee, represented by *C. arabica* from Colombia (sample G). 1 = sucrose (m/z = 387), 2 = trigonelline (m/z = 138), 3 - = nicotinic acid (m/z = 124), 4 = caffeine (m/z = 195).

Caffeine values ranged from 843.3 to 930.9 mg/100 g coffee (dwb) in green (A) and roasted (C, D, E, G, H) Arabica coffee samples. Caffeine content in Green Robusta coffee (B) was 1701.3 mg/100 g coffee (dwb), while in the ground roasted decaffeinated coffee sample (I) was only 20.4 mg/100 g coffee (dwb). Caffeine amounts were similar in instant coffee samples (J and K), with an average of 2106.0 mg/100 g coffee (dwb). All of these results are in agreement with caffeine contents reported elsewhere (Macrae, 1985; Trugo, 2003; Trugo et al., 1983, 1991).

Trigonelline contents in green Arabica (A) and Robusta (B) were 1029.8 and 900.6 mg/100 g coffee (dwb), respectively. The higher content of trigonelline in green Arabica coffee in comparison to green Robusta coffee ($p < 0.001$) was already expected, since it has been extensively reported (Ky et al., 2001; Macrae, 1985; Trugo, 2003). In commercial roasted coffee samples (C–I), trigonelline values ranged from 279.7 to 955.9 mg/100 g coffee (dwb). Considering only Arabica commercial coffee samples (C–H), trigonelline content was inversely related to color intensity. This behavior, which has previously been reported (Casal et al., 2000; Macrae, 1985; Trugo, 2003), is expected since trigonelline is thermally labile and degrades during the roasting process. Additionally, when comparing samples of different coffee species with the same color, we found less trigonelline in sample F (blend of Arabica and Robusta) then in samples G and H (Arabica coffees) ($p < 0.001$), which can be explained by the lower content of trigonelline in Robusta species. The content of trigonelline in instant coffee samples (J and K) was also in accordance with previous studies (Trugo, 2003; Trugo et al., 1983).

Nicotinic acid was not found in the analyzed green coffee samples (A and B), as expected since it is produced as a consequence of trigonelline thermal degradation during the roasting process. Among commercial coffee samples (C–I), nicotinic acid values ranged from 9.6 to 30.4 mg/100 g coffee (dwb), with the higher levels of nicotinic acid found in the darker coffee samples (C and D). These levels are in agreement with those reported elsewhere (Macrae, 1985; Trugo, 2003). Nicotinic acid contents were similar in instant coffee samples (J and K), with an average of 35.2 mg/100 g of coffee (dwb). This result agrees with the values reported by Trugo, et al. (1985) when analyzing thirteen instant coffee samples, which presented a range of 21.5–46.8 mg of nicotinic acid per 100 g of coffee dwb.

Table 4
Caffeine, trigonelline, nicotinic acid and sucrose contents in green and commercial coffee samples using the proposed analytical method[a]

Coffee sample	Species	Origin	Color[b]	Caffeine	Trigonelline	Nicotinic acid	Sucrose
Green coffee							
A	Arabica	Brazil	n.d.[c]	919.0 ± 12.4	1029.8 ± 26.0	ND[d]	8346.9 ± 118.8
B	Robusta	Brazil	n.d.	1701.3 ± 83.7	900.6 ± 2.6	ND	6401.6 ± 143.3
Commercial ground roasted coffee							
Regular							
C	Arabica	Hawaii	35	851.6 ± 10.0	279.7 ± 9.4	29.5 ± 0.4	15.9 ± 0.7
D	Arabica	Brazil	45	908.6 ± 30.4	526.8 ± 21.9	30.4 ± 1.3	52.0 ± 0.7
E	Arabica	Brazil	55	843.3 ± 28.9	641.3 ± 23.0	20.3 ± 0.8	79.2 ± 2.0
F	Blend	Africa	65	1456.0 ± 8.6	592.2 ± 18.5	13.7 ± 0.1	87.8 ± 3.2
G	Arabica	Colombia	65	848.1 ± 35.2	682.5 ± 7.5	9.6 ± 0.3	173.3 ± 0.1
H	Arabica	Brazil	65	930.9 ± 34.9	955.7 ± 33.5	10.3 ± 0.2	183.8 ± 8.3
Decaffeinated							
I	Blend	Brazil	55	20.4 ± 0.3	663.1 ± 14.1	10.4 ± 0.5	31.1 ± 1.1
Commercial instant coffee							
J	Blend	Brazil	65	2054.7 ± 14.0	917.4 ± 4.1	36.3 ± 1.1	129.9 ± 3.6
K	Blend	Brazil	65	2163.4 ± 10.6	832.8 ± 21.0	34.2 ± 1.2	128.1 ± 3.4

[a] Results are shown as the means of extraction in three replicates ± standard deviation, expressed as mg/100 g of coffee dry weight.
[b] Roasting Color Classification System (Agtron – SCAA, 1995) where 65 = light medium, 55 = medium, 45 = moderately dark, 35 = dark.
[c] Not determined.
[d] Not detected, below limit of detection.

Sucrose amounts found in green Arabica (A) and Robusta (B) coffee samples were 8346.9 and 6401.6 mg/100 g coffee (dwb), respectively. The higher content of sucrose in green Arabica coffee in comparison to green Robusta coffee ($p < 0.001$) was expected and has been extensively reported (Ky et al., 2001; Knopp et al., 2006; Murkovic & Derler, 2006; Trugo, 1985). Sucrose content in regular roasted coffee samples (C–H) was inversely related to color intensity and ranged between 15.9 and 183.8 mg/100 g coffee (dwb). Sucrose degradation during roasting and its relation to color development is well reported in the literature (Trugo, 1985). Instant coffee samples (J and K) presented very similar sucrose contents, with an average of 129.0 mg/100 g of coffee (dwb). The few studies in the literature dealing with sucrose contents of instant coffees reported levels that ranged from traces to 1300 mg/100 g of coffee (dwb) (Trugo, 1985), which are in agreement with the amounts observed in this work.

4. Conclusions

Caffeine, trigonelline, nicotinic acid and sucrose were simultaneously analyzed for the first time by LC–MS in a 6 min run. The developed method showed appropriate recoveries and repeatibilities. Caffeine, trigonelline, nicotinic acid and sucrose detection and quantification limits were lower or comparable to the minimum reported contents of these components in coffee. Analysis of various commercial coffee samples showed that this method may be applied to a wide range of coffees available in the market, offering a good alternative for routine analysis due to its fastness and sensitivity. Although ionization suppression was observed due to matrix effect, it was demonstrated that this effect can be overcome using calibration curves prepared in coffee extracts. While caffeine, trigonelline and nicotinic acid may be analyzed in green and roasted coffee independently of the coffee matrix used to prepare calibration curves, sucrose analysis should be performed using calibration curves prepared in the same type of coffee extract (green or roasted) which is being analyzed. Despite the apparent higher cost of LC–MS in comparison to other analytical techniques, the efficiency, applicability and fastness of the proposed LC–MS method counterbalances its overall cost thus appointing it as a reliable alternative method for routine coffee analysis.

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