

Method development for the determination of manganese, cobalt and copper in green coffee comparing direct solid sampling electrothermal atomic absorption spectrometry and inductively coupled plasma optical emission spectrometry

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Abstract

A method has been developed for the determination of cobalt, copper and manganese in green coffee using direct solid sampling electrothermal atomic absorption spectrometry (SS-ET AAS). The motivation for the study was that only a few elements might be suitable to determine the origin of green coffee so that the multi-element techniques usually applied for this purpose might not be necessary. The three elements have been chosen as test elements as they were found to be significant in previous investigations. A number of botanical certified reference materials (CRM) and pre-analyzed samples of green coffee have been used for method validation, and inductively coupled plasma optical emission spectrometry (ICP OES) after microwave-assisted acid digestion of the samples as reference method. Calibration against aqueous standards could be used for the determination of Mn and Co by SS-ET AAS, but calibration against solid CRM was necessary for the determination of Cu. No significant difference was found between the results obtained with the proposed method and certified or independently determined values. The limits of detection for Mn, Cu and Co were 0.012, 0.006 and 0.004 $\mu\text{g g}^{-1}$ using SS-ET AAS and 0.015, 0.13 and 0.10 $\mu\text{g g}^{-1}$ using ICP OES. Seven samples of Brazilian green coffee have been analyzed, and there was no significant difference between the values obtained with SS-ET AAS and ICP OES for Mn and Cu. ICP OES could not be used as a reference method for Co, as essentially all values were below the limit of quantification of this technique. © 2007 Elsevier B.V. All rights reserved.

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1. Introduction

The vast majority of trace element determinations in food and beverage are carried out because of their nutritive importance and toxic effects [1,2]. The determination of trace elements in order to identify the geographic origin of food products is a relatively new, but increasingly active research area, driven by increasing demands on the agrifood industry from free-trade, globalization and changing technology. Financial incentives continue to drive retailers/resellers to misidentify the geographic

origin of food products. Coffee is currently exported by more than 50 countries and on the international trade market ranks second only to petroleum, providing livelihood for over 100 million people worldwide [3]. With over 50 billion in US dollars in coffee retail sales [4], import/export, legal implications and financial concerns make determining country of origin for coffee important.

It is recognized that mineral and trace metal compositions of fruits and vegetables are a distorted reflection of the trace mineral composition of the soil and environment in which the plant grows [5]. Several authors have tried to differentiate geographic growing origins of coffee using elemental analysis – typically inductively coupled plasma optical emission spectrometry (ICP OES) – and statistical pattern recognition methods, such as

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principal component analysis (PCA), cluster analysis, discriminant function analysis and neural network modeling [6–10]. Fernandes et al. [9] determined 15 elements but did not succeed to correlate the metal content with the geographical origin of coffee. Martin et al. [6,7] determined 11 elements and found that Cu, Mn and P were the most discriminating variables. Anderson and Smith [8] investigated 18 elements and found that several geographic areas could be identified by their content of Al, Mn and Na, and better distinction could be obtained by adding the content of Cu and Fe. The most comprehensive study about the correlation between the trace element content of coffee and its origin was carried out by Krivan et al. [10] who determined 20 elements in coffee samples from 8 different countries using instrumental neutron activation analysis (INAA), electrothermal atomic absorption spectrometry (ET AAS), flame atomic absorption spectrometry (F AAS) and combustion elemental analysis. These authors found that among the investigated elements manganese was best suited as an indicator for the origin of coffee, but elements like Co, Cs, Na and Rb proved to be of interest too.

Inductively coupled plasma OES has been used in most of the above studies as the analytical technique because of its multi-element capability, its good sensitivity, wide linear dynamic range, relatively high freedom from non-spectral interferences and its precision [11]. Hence, ICP OES could offer considerable advantages for the quantitative analysis of food and has been applied repeatedly for the analysis of coffee [12–15]. Although instant coffee might be analyzed directly after appropriate dilution [15] or after solubilization with tetra methylammonium hydroxide [14], green or roasted coffee beans require an acid digestion before their analysis by ICP OES [9,12,13]. Microwave-assisted digestion in closed vessels using a combination of mineral acids is the technique of choice for sample preparation nowadays. A mixture of HNO₃ and H₂O₂ is preferred in many instances for the digestion of food samples since they are strong oxidizing agents and produce minimum matrix effects in ICP OES [16].

However, microwave-assisted acid digestion in closed vessels requires high-purity reagents in order to avoid sample contamination, and it is rather time consuming when a large number of samples have to be analyzed. Digestion inevitably also results in significant dilution, so that the concentration of trace elements in the final solution might be below the limit of quantification of ICP OES. In addition, in the studies carried out until now, it turned out that only a few elements are really useful in characterizing the geographic origin of coffee, hence a multi-element technique might not be necessary for this task. It could therefore be of interest to investigate alternate techniques for that purpose, and solid sampling ET AAS (SS-ET AAS) in a graphite tube furnace was considered a good approach, as it offers maximum sensitivity due to the absence of any dilution, a minimum risk of contamination or loss of analyte as no reagents and only a minimum of sample preparation is required, and calibration can often be carried out against aqueous standards, as has been shown in a recent review article [17]. Obviously, direct SS analysis also has some limitations, the most important one being the inferior precision, compared to solution analysis, due to the natural

inhomogeneity of solid samples. However, a relative standard deviation (R.S.D.) of some 10% of an accurate result appears to be much more acceptable than a R.S.D. of 1–2% of a result that is affected by errors due to contamination or analyte loss in the sample preparation stage. It has been shown in several recent publications that SS-ET AAS could be applied successfully for the determination of elements such as cobalt [18], lead [19] and mercury [20] in various biological materials. However, SS-ET AAS has never been described for the direct determination of trace elements in green coffee.

The goal of this work was to develop a method for the determination of a few key elements in green coffee using direct SS-ET AAS and compare its performance with that of ICP OES after conventional acid digestion. Manganese was first choice, because it was found to be best suited to identify the origin of coffee in all related publications [6–8,10], followed by copper [6–8], and cobalt was added because of the results obtained by Krivan et al. [10]. The relative advantages and limitations of both techniques have been evaluated, including speed of analysis, sensitivity and accuracy of the results. As no certified reference material (CRM) for coffee is available, several coffee samples that have been pre-analyzed by several techniques [10] were used in this work in addition to a few botanical CRM. No attempt has been made to correlate the content of the above metals found in the samples of Brazilian coffee investigated here with their origin, as only one sample from each region has been analyzed, which does not allow any statistical evaluation. The goal has been to develop a fast and reliable routine method that could be used in future work to analyze a large number of samples for a few elements in order to establish a data basis for the determination of the origin of green coffee.

2. Experimental

2.1. Instrumentation

All SS-ET AAS measurements were carried out using an AAS 5EA atomic absorption spectrometer (Analytik Jena AG, Germany), equipped with a transversely heated graphite tube atomizer and deuterium background correction. Hollow cathode lamps (Narwa, Berlin, Germany) were used as the radiation source with a lamp current of 4.5, 3.0 and 3.5 mA for Co, Cu and Mn, respectively. The analytical lines at 240.7, 324.8 and 403.1 nm with a spectral bandpass of 0.2, 0.5 and 0.5 nm were used for Co, Cu and Mn, respectively. The graphite furnace temperature program is given in Table 1. The spectrometer was interfaced to an IBM PC/AT compatible computer. All experiments were carried out using pyrolytically coated SS graphite tubes without a dosing hole (Analytik Jena, Part No. 07-8130325) and SS graphite platforms (Analytik Jena, Part No. 407-A81.312). An M2P microbalance (Sartorius, Göttingen, Germany) was used for weighing the samples directly into the SS platforms. The sample weight was automatically transmitted to the instrument computer to calculate the normalized absorbance (integrated absorbance per milligram of sample) after each measurement. A pre-adjusted pair of tweezers, which is part of the SSA 5 manual solid sampling accessory (Analytik Jena) was

Table 1
Graphite furnace temperature program for the determination of manganese, cobalt and copper in green coffee

Stage	Temperature (°C)	Ramp (°C s ⁻¹)	Hold time (s)	Gas flow rate (L min ⁻¹)
Drying 1	90	10	10	2.0
Drying 2	120	10	10	2.0
Drying 3	150	10	10	2.0
Pyrolysis	900 ^a ; 1100 ^b ; 1400 ^c	400	30	2.0
Atomization	1900 ^a ; 2100 ^b ; 2400 ^c	1500	10	0.1 ^a ; 0 ^{b,c}
Cleaning	2500	1000	4	2.0

^a Conditions for Mn.

^b Conditions for Cu.

^c Conditions for Co.

used to transfer the SS platforms to the atomizer. Argon was used as the purge gas with a flow rate of 2.0 L min⁻¹ during all stages, except during atomization, when the flow was stopped. Integrated absorbance (peak area) was used exclusively for all measurements. Standard reference materials were used to establish the optimum parameters for the graphite furnace temperature program in Table 1.

An inductively coupled plasma optical emission spectrometer with axially viewed configuration (VISTA PRO, Varian, Mulgrave, Australia) equipped with solid state detector, cyclonic spray chamber, and concentric nebulizer was employed for ICP OES determinations of Co, Cu and Mn. The operational parameters are described in Table 2.

A microwave oven, model Ethos EZ (Milestone, Sorisole, Italy), equipped with 100 mL Teflon PFA vessels and pressure sensor, was used for digestion of coffee samples for ICP OES analysis.

The samples for SS-ET AAS were ground in a Vibratory Micro-Mill “pulviresette 0” (Fritsch GmbH, Idar-Oberstein, Germany). During the grinding stage liquid nitrogen was used to facilitate the operation and to reach the desired granulometry. Different mesh-size polyester sieves were used in order to obtain four sets of particle sizes: (a) 250–150 μm; (b) 150–85 μm; (c) 85–45 μm; and (d) <45 μm.

The particle size analysis was carried out in an MS-17 particle size analyzer (Malvern Instruments, Malvern, UK).

2.2. Reagents and solutions

Analytical grade reagents were used throughout. Distilled, deionized water (DDW) with a specific resistivity of 18 MΩ cm,

Table 2
Operational parameters for the determination of Co, Cu and Mn using axial-view ICP OES

Instrumental parameter	
RF generator (MHz)	40
Power (kW)	1.3
Plasma gas flow (L min ⁻¹)	15.0
Auxiliary gas flow (L min ⁻¹)	1.5
Nebulizer gas flow (L min ⁻¹)	0.70
Analytical wavelengths (nm)	
Co(II)	230.786
Cu(I)	327.397
Mn(II)	257.611

from a Millipore water purification system (Milli-Q, Millipore, Bedford, MA, USA), was used for the preparation of the samples and standards. The nitric acid (Merck, Germany) used to prepare the aqueous calibration standards was further purified by sub-boiling distillation in a quartz sub-boiling still (Kürner Analysentechnik, Rosenheim, Germany). All containers and glassware were soaked in 3.0 mol L⁻¹ nitric acid for at least 24 h and rinsed three times with deionized water before use. Reference solutions were prepared by diluting stock solutions containing 1000 mg L⁻¹ of each element (Tec-Lab, Hexis, São Paulo, SP, Brazil) with deionized water. The palladium nitrate and magnesium nitrate modifier solutions were from Merck, Germany, and Triton X-100 from Union Carbide.

2.3. Samples and reference materials

The description of the investigated samples of green coffee obtained from the Research and Test Unit of ICO (London, Great Britain), which have been pre-analyzed by Krivan et al. [10] and the Brazilian coffees obtained from the Coffee Market (Porto Alegre, Rio Grande do Sul, Brazil) are shown in Table 3. The samples were coffees of commercial types available on the international markets. All coffee samples, with one exception, were of the *Arabica* type.

The following certified reference materials (CRM) have been used in this work: NIST SRM 8433 Corn Bran, NIST SRM

Table 3
Investigated samples of green coffee

Country of origin	Type	Crop year
Colombia	Medellin excelso	1987/1988
Costa Rica	Strictly hard bean	1987/1988
Cuba	Crystal mountain	1987/1988
Mexico	Strictly hard bean	1987/1988
Nicaragua	A	1987/1988
Panamá	Chiriqui	1987/1988
Brazil	XX	2003/2004
Brazil (BA)	Planalto Cerrado	2004/2005
Brazil (ES) ^a	Robusta	2004/2005
Brazil (MG)	Cerrado Mineiro	2004/2005
Brazil (MG)	Sul de Minas	2004/2005
Brazil (PR)	Apucarana	2004/2005
Brazil (SP)	Mogiana	2004/2005

All samples except one were of *Arabica* species.

^a *Robusta* species.

1568a Rice Flour, NIST SRM 1572 Citrus Leaves and NIST SRM 1515 Apples Leaves (all from National Institute of Standards and Technology, Gaithersburg, MD, USA) and NIES CRM 10a Rice Flour (National Institute for Environmental Studies, Japan Environment Agency, Yatabe-machi, Tsukuba, Ibaraki, Japan).

2.4. Microwave-assisted sample digestion

A mass of about 500 mg of coffee was directly weighed in a PTFE digestion vessel, 7 mL of HNO₃ conc. and 1 mL of H₂O₂ conc. were added, and the vessels were placed in a fume hood for 1 h for pre-digestion before they were placed on the turntable of the microwave system. The coffee samples were digested by a four-step temperature program. In the first step the temperature was linearly increased to 90 °C in 4 min with maximum power of the rotating magnetron of 1000 W. In the second step the temperature was kept at 90 °C for 2 min. In the third step the temperature was linearly increased to 180 °C in 4 min and in the fourth step the temperature was kept at 180 °C for 15 min. After digestion and cooling, which took about 3 h, each solution was diluted to 25 mL with deionized water in a volumetric flask and the analytes were determined by ICP OES. Matrix effect studies were carried out by spiking some of the original non-digested samples (accurately weighed different amounts) with variable amounts of standard solution of the metals. The spiked samples were then mineralized using the same digestion procedures as were applied to the non-spiked samples. All digestions were performed in triplicate.

2.5. Direct SS-ET AAS

The green coffee samples were dried to constant weight and kept in a closed plastic vial until they were analyzed. The sample mass weighed directly onto the SS platforms and introduced into the graphite furnace for SS-ET AAS was between 0.03 and 0.17 mg for the determination of Cu and Mn and between 0.3 and 0.5 mg for the determination of Co. As the actual sample mass was obviously different for each measurement the integrated absorbance obtained in each measurement was 'normalized' for

a sample mass of 0.1 mg in the case of Cu and Mn, and 1.0 mg in the case of Co for better comparison.

3. Results and discussion

3.1. Digestion procedure for ICP OES analysis

As ICP OES was intended to be used as reference method it was necessary to verify first the accuracy of this technique, particularly with respect of the digestion procedure. Initially results obtained for digested samples using aqueous standards for calibration were biased low. It was suspected that there was some influence from the high nitric acid concentration in the digested samples on the analyte signal that had to be taken into consideration. Previous investigations have demonstrated that inorganic acids cause a decrease in signal intensity and plasma thermal characteristics are deteriorated compared to aqueous standards. Stewart and Olesik [21] studied the effect of nitric acid on the aerosol generation and transport processes and concluded that the higher the acid concentration the lower the aerosol liquid mass transported to the plasma. Hence, the slopes of the regression lines by plotting the emission intensities of aqueous standards versus the emission intensities of standard addition solutions were calculated and used for correction. Based on these results, calibration using the analyte addition technique is recommended for determination of trace elements in digested coffee samples to overcome or to eliminate most of the errors due to acid interference that causes low sensitivity.

The values that have been obtained for the three analytes in one CRM and in six pre-analyzed samples of green coffee are summarized in Table 4. There is no significant difference between the certified or reference values and those that have been obtained in this work according to a Student's *t*-test on a 95% confidence level for Mn and Cu. However, the results for Co were all below the limit of quantification (LOQ) of this technique, so that their accuracy is limited. Nevertheless, ICP OES after microwave-assisted acid digestion of the samples could be considered a reliable reference technique, at least for the determination of Mn and Cu in green coffee.

Table 4

Determination of manganese, copper and cobalt in NIST SRM 1569a rice flour and six pre-analyzed samples of green coffee using ICP OES after microwave-assisted acid digestion

Sample	Mn ($\mu\text{g g}^{-1}$)		Cu ($\mu\text{g g}^{-1}$)		Co ($\mu\text{g g}^{-1}$)	
	Reference value	Found	Reference value	Found	Reference value	Found
NIST SRM 1568a	20.0 ± 1.6 ^a	19.5 ± 0.2	2.4 ± 0.3 ^a	1.6 ± 0.01	0.018 ^b	<LOD
Colombia	46.5	50.5 ± 0.6	13.7 ± 1.0	13.0 ± 0.04	0.13 ± 0.02	0.1 ^c
Costa Rica	33.3 ± 6.5	29.4 ± 0.3	15.3 ± 1.1	14.5 ± 0.2	0.15 ± 0.02	0.2 ^c
Cuba	42.4 ± 3.0	37.2 ± 0.4	14.0 ± 0.8	14.5 ± 0.2	0.095 ± 0.020	0.1 ^c
Mexico	57.7 ± 4.0	44.0 ± 0.6	12.1 ± 0.9	10.7 ± 0.06	0.18 ± 0.02	0.1 ^c
Nicaragua	18.0 ± 1.0	17.2 ± 0.4	19.8 ± 0.4	18.6 ± 0.2	0.10 ± 0.01	0.1 ^c
Panama	19.4 ± 1.1	19.7 ± 0.1	15.0 ± 0.5	12.6 ± 0.2	0.064 ± 0.008	0.1 ^c

Reference values are from Ref. [10].

^a Certified value.

^b Not certified.

^c No S.D. reported; value below LOQ.

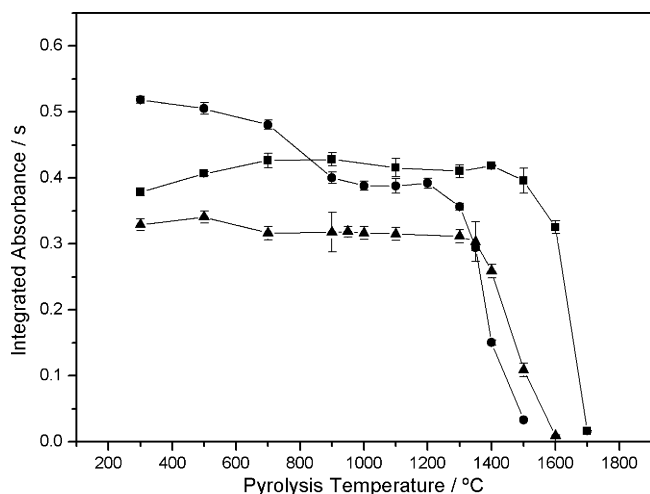


Fig. 1. Pyrolysis curves for manganese with and without modifier; atomization temperature 1900 °C; (●) 2.0 ng Mn aqueous standard without modifier; (■) 2.0 ng Mn aqueous standard with 5 μg Pd, 3 μg Mg and 5 μg Triton X-100 as modifier; (▲) Café Brasil, integrated absorbance normalized for 0.1 mg sample.

3.2. Method development for direct SS-ET AAS

Pyrolysis and atomization curves have been established for all three elements using both, aqueous standards and a sample of green coffee (Café Brasil). For cobalt and copper the pyrolysis and atomization curves established with aqueous standards and the coffee sample were essentially identical and are therefore not shown here. Pyrolysis temperatures of 1400 and 1100 °C could be used for Co and Cu, respectively, without adding a modifier, and the optimum atomization temperatures were 2400 and 2100 °C, respectively.

The only analyte that required some additional optimization was manganese, mostly because of the relatively high content of this element in coffee, which made it necessary to use the secondary analytical line at 403.1 nm. Although this line is about one order of magnitude less sensitive than the main resonance line at 279.5 nm, the sensitivity had to be further reduced by using a gas flow of 0.1 L min⁻¹ during atomization in order to allow the introduction of reasonably high sample mass into the furnace—a pre-requirement for an acceptable precision. Another problem in the case of manganese was that the pyrolysis curves established with aqueous standards and the coffee sample were significantly different, as can be seen in Fig. 1. While manganese in the coffee sample was thermally stable up to 1300 °C, analyte losses from aqueous standards were observed already above 700 °C. Hence, the addition of a modifier was considered mandatory, and a mixture of 5 μg Pd, 3 μg Mg, both as the nitrates [22], and 5 μg Triton X-100 was found optimum, as it could stabilize the analyte in aqueous standards to a pyrolysis temperature of 1400 °C (see Fig. 1). The addition of a modifier to the solid samples was found to be not necessary, which simplified the procedure significantly. The optimum atomization temperature was determined to be 1900 °C.

Another very important issue in direct SS-ET AAS is particle size, as only small sample mass of the order of 1 mg can

Table 5

Influence of particle size of green coffee (Mogiana) on the normalized (for 0.1 mg of sample) integrated absorbance measured for manganese without and with correction for humidity

Particle size (μm)	Normalized integrated absorbance (s)	
	Without drying	Corrected for humidity
>150	0.590 ± 0.030	0.414 ± 0.037
85–150	0.537 ± 0.025	0.411 ± 0.017
45–85	0.386 ± 0.031	0.415 ± 0.032
<45	0.334 ± 0.024	0.435 ± 0.018

Average and standard deviation of $n=5$ measurements.

usually be introduced into the furnace, which has to be representative for the sample. One sample of green coffee (Mogiana Verde) was therefore ground and divided into four different granulometric fractions, >150 μm, 85–150 μm, 45–85 μm, and <45 μm, using sieves of different mesh size. The initial result was kind of surprising, because decreasing analyte content was found with decreasing particle size, as is shown in Table 5 for manganese. The source of the problem was found to be the hygroscopic nature of green coffee, which resulted in much more rapid adsorption of humidity from the environment for the fractions with small particle size, compared to the coarser fractions. This problem can be solved by drying the samples to constant weight just before analysis or to correct for humidity that has been determined in a separate sample aliquot, as can be seen in Table 5. The most important outcome of this experiment was that neither the analytical result nor the precision of the analysis appears to depend on the particle size, which means that the analyte must be distributed rather homogeneously in the coffee beans. This is important for two reasons, firstly, green coffee in contrast to roasted coffee is rather difficult to be ground to small particle size, and secondly, the problem of adsorption of humidity from the environment is minimized when the beans are not ground to small particle size.

3.3. Analysis of reference materials using SS-ET AAS

The next step was to analyze the six coffee reference materials and a number of botanical CRM using direct SS-ET AAS and aqueous standards for calibration. In order to test the validity of this approach we also established a linear correlation equation between the certified values of the CRM and the normalized integrated absorbance obtained by SS-ET AAS. The slope of this correlation curve was then used to calculate the analyte content in the coffee reference samples, and the results compared with those obtained by calibration against aqueous standards. For manganese and cobalt there was no significant difference between the two sets of data at a 95% confidence level according to a paired Student's t -test. Therefore only the values obtained by calibration against aqueous standards are shown in Table 6. The validity of using aqueous standards for calibration for these elements can also be deduced from the good agreement between the values obtained in this work and the certified or reference values, which showed no significant difference using the same test.

Table 6

Determination of manganese, copper and cobalt in certified reference materials and six pre-analyzed samples of green coffee using direct SS-ET AAS with calibration against aqueous standards, except for copper

Sample	Mn ($\mu\text{g g}^{-1}$)		Cu ($\mu\text{g g}^{-1}$)		Co ($\mu\text{g g}^{-1}$)	
	Reference value	Found	Reference value	Found ^a	Reference value	Found
NIST SRM 1568a	20.0 ± 1.6	19.3 ± 1.8	2.4 ± 0.3	n.d. ^b	0.018 ^c	0.011 ± 0.002
NIST SRM 8433	2.55 ± 0.29	2.28 ± 0.2	2.47 ± 0.40	1.6 ± 0.5	0.006 ± 0.006	n.d. ^b
NIST SRM 1515	54 ± 3	57.7 ± 1.6	5.64 ± 0.24	3.1 ± 0.2	0.09 ^c	0.071 ± 0.002
NIST SRM 1572	23 ± 2	n.d. ^b	16.5 ± 1.0	11.5 ± 1.4	0.02 ^c	n.d. ^b
NIES CRM 10a	34.7 ± 1.8	30.3 ± 1.2	3.5 ± 0.3	2.3 ± 0.2	0.02	n.d. ^b
Colombia	46.5	49.4 ± 1.3	13.7 ± 1.0	13.3 ± 0.9	0.13 ± 0.02	0.18 ± 0.005
Costa Rica	33.3 ± 6.5	32.4 ± 1.4	15.3 ± 1.1	14.5 ± 0.8	0.15 ± 0.02	0.19 ± 0.005
Cuba	42.4 ± 3.0	39.6 ± 3.0	14.0 ± 0.8	15.5 ± 1.7	0.095 ± 0.020	0.082 ± 0.001
Mexico	57.7 ± 4.0	53.8 ± 6.0	12.1 ± 0.9	11.8 ± 1.2	0.18 ± 0.02	0.13 ± 0.005
Nicaragua	18.0 ± 1.0	19.8 ± 0.5	19.8 ± 0.4	17.2 ± 1.5	0.10 ± 0.01	0.11 ± 0.002
Panama	19.4 ± 1.1	22.1 ± 1.8	15.0 ± 0.5	12.7 ± 0.8	0.064 ± 0.008	0.066 ± 0.001

Reference values are from Ref. [10]. For identification of the CRM see Section 2.

^a Correlation curve of the CRM was used for calibration of the coffee samples.

^b n.d. = not determined.

^c Non-certified value.

For copper, however, the above test exhibited a significant difference, which means that calibration with aqueous standards is not feasible for this element. This also becomes obvious from the results obtained for the CRM shown in Table 6, which are all biased low, indicating the existence of some interference. For this reason for copper only the results obtained by calibration using the correlation curve established with the CRM are shown for the coffee samples in Table 6. These values are in agreement with the reference values, as there is no significant difference between the two sets of data at a 95% confidence level according to a paired Student's *t*-test. This means that the matrix effect is the same not only for all the coffee samples, but actually for all the botanical CRM as well. The source of this interference could not be identified in this work, nor could it be eliminated.

3.4. Analysis of Brazilian green coffee samples comparing ICP OES and SS-ET AAS

Seven samples of green coffee from different growing areas of Brazil were analyzed comparing ICP OES after microwave-assisted acid digestion in closed vessels and direct SS-ET AAS. In the case of ICP OES calibration was against analytical curves

established with aqueous standards and the results were corrected for the nitric acid interference as described in Section 3.1. In the case of SS-ET AAS calibration for Mn and Co was against analytical curves established with aqueous standards; only Cu had to be calibrated using a regression curve established with solid CRM, as described in the previous section. All results are shown in Table 7. According to a Student's *t*-test there was no significant difference at a 95% confidence level between the data obtained by the two techniques for Mn and Cu. The results obtained for Co with ICP OES could not be used as reference values for a statistical comparison, as all of them were below or very close to the LOQ. Direct SS-ET AAS is obviously better suited for the determination of very low Co concentrations because of its higher sensitivity and the absence of any sample preparation except grinding, which is also necessary for ICP OES prior to digestion.

3.5. Figures of merit

The most important figures of merit for both techniques, ICP OES and SS-ET AAS are summarized in Table 8. The limits of detection (LOD) for ICP OES were obtained using the back-

Table 7

Determination of manganese, copper and cobalt in green coffee samples from different regions in Brazil comparing ICP OES after microwave-assisted acid digestion and direct SS-ET AAS

Sample	Mn ($\mu\text{g g}^{-1}$)		Cu ($\mu\text{g g}^{-1}$)		Co ($\mu\text{g g}^{-1}$)	
	ICP OES	SS-ET AAS ^a	ICP OES	SS-ET AAS ^b	ICP OES	SS-ET AAS ^a
Café Brasil	32.0 ± 0.2	36.2 ± 6.7	13.4 ± 0.2	13.8 ± 1.1	0.1 ^c	0.62 ± 0.02
Planalto Cerrado BA	15.7 ± 0.2	15.8 ± 0.5	10.1 ± 0.1	10.9 ± 0.2	0.1 ^c	0.019 ± 0.001
Robusta ES	16.9 ± 0.1	19.1 ± 0.7	16.2 ± 0.1	16.0 ± 0.8	0.2 ^c	0.36 ± 0.01
Cerrado Mineiro MG	18.7 ± 0.4	18.3 ± 0.3	13.4 ± 0.2	14.6 ± 0.1	0.1 ^c	0.020 ± 0.002
Sul de Minas MG	28.6 ± 0.5	28.1 ± 0.9	13.9 ± 0.2	13.6 ± 0.6	0.3 ± 0.03	0.51 ± 0.02
Apucarana PR	30.5 ± 0.4	34.0 ± 0.8	13.4 ± 0.1	17.2 ± 1.1	0.3 ± 0.04	0.23 ± 0.01
Mogiana SP	27.2 ± 0.5	31.8 ± 2.2	10.8 ± 0.2	11.4 ± 0.8	0.3 ± 0.03	0.24 ± 0.01

^a Calibration against aqueous standards.

^b Calibration against solid CRM.

^c No S.D. reported; value below LOQ.

Table 8
 Figures of merit for the determination of Mn, Cu and Co in coffee using ICP OES after microwave-assisted acid digestion and direct SS-ET AAS

Technique	Parameter	Mn	Cu	Co
ICP OES	LOD ($\mu\text{g L}^{-1}$)	0.31	2.5	2.0
ICP OES	LOD ^a ($\mu\text{g g}^{-1}$)	0.015	0.13	0.10
SS-ET AAS	LOD ^b ($\mu\text{g g}^{-1}$)	0.012	0.006	0.004
ICP OES	LOQ ^a ($\mu\text{g g}^{-1}$)	0.050	0.42	0.33
SS-ET AAS	LOQ ^b ($\mu\text{g g}^{-1}$)	0.040	0.021	0.012
SS-ET AAS	m_0 (pg)	19	5.8	5.6

^a Based on 500 mg sample in 25 mL of solution after digestion.

^b Based on the introduction of 1 mg of sample into the graphite tube.

ground equivalent concentration (BEC) of the analyte, which was calculated from the ratio of 10 measurements of the blank and the inclination of the analytical curve. The LOD were calculated as three times the standard deviation of the blank. The LOD in the solid samples were calculated based on a mass of 500 mg of sample in 25 mL of solution after digestion and dilution. The LOD in SS-ET AAS was obtained according to the 'zero mass response' as proposed by Kurfürst [23], i.e., by inserting an empty solid sampling platform that only contains the modifier (if used) 10 times into the graphite tube and run a full atomization cycle. The LOD is then calculated as three times the standard deviation of the blank readings. The limit of quantification (LOQ) is based on the same measurements, using 10 times the standard deviation of the blank readings.

For manganese the LOD and LOQ in the original sample are comparable for the two techniques, but it has to be kept in mind that the sensitivity for SS-ET AAS has been reduced deliberately by choosing a less sensitive analytical line and by maintaining a gas flow through the tube in order to bring the absorbance signals within the working range of the spectrometer. For the other two analytes, for which the primary analytical lines could be used, the LOD and LOQ of SS-ET AAS are more than a factor of 20 better than the values for ICP OES, mostly because of the absence of any dilution with the former technique. This is not a problem in the case of copper, as the concentration of this element in essentially all of the investigated samples is more than an order of magnitude above the LOQ, which means that quantitative determination is possible with good precision. However, this is not the case for the determination of cobalt, as essentially all values for this analyte in the investigated samples are lower than the LOQ, which means that no quantitative determination is possible and only approximate values can be reported. This fact also explains the significant differences that have been observed in the values found for cobalt between SS-ET AAS and ICP OES.

The precision obtained with SS-ET AAS in this work for real samples, based on five replicates, with one exception (Mn in Café Brasil with an R.S.D. of ~20%) was typically better than 10% R.S.D., which is normal for direct solid sampling analysis, but frequently even better than 5% R.S.D. for all three analytes, indicating good homogeneity of analyte distribution in the samples. The precision obtained with ICP OES for the determination of Mn and Cu was typically better than 5% R.S.D. and

often in the range of 2–3% R.S.D., which is within expectation for solution analysis. However, the question has to be raised if this improved precision is of importance in the present investigation. The natural heterogeneity in the trace element content of samples even from the same plantation area, and even more from the same region probably exceeds already 10%, so that an R.S.D. < 10% appears to be more than acceptable for the purpose. The only element that did not meet this criterion was cobalt when it was determined by ICP OES, as the imprecision at the LOD by definition is 33%.

The comparison of the time required for an analysis with ICP OES after microwave-assisted digestion and with SS-ET AAS could obviously be made only for the equipment and the experimental conditions used in this work. This includes that all digestions for ICP OES were carried out in triplicate, i.e., three samples could be digested per run, and that five independent sample portions were weighed and determined per element and sample for SS-ET AAS. The sample preparation for ICP OES including digestion and cooling takes about 270 min for three samples in triplicate; the measurement time with ICP OES is essentially negligible in this context. This means that the time required per element and per sample is about 30 min. The graphite furnace temperature program for SS-ET AAS takes about 90 s, followed by about 30 s of cooling and another 30 s for weighing and sample introduction, i.e., one measurement takes about 2.5 min and five repetitive measurements per element and sample take 12.5 min. This means that the sample throughput is more than a factor of 2 higher in the case of SS-ET AAS compared to the digestion approach.

4. Conclusion

Direct SS-ET AAS has been shown to be a powerful tool for the determination of selected trace elements in green coffee for the determination of its origin. The method has been validated by analyzing several botanical CRM and a number of pre-analyzed samples of green coffee, as well as by comparing the results with values obtained by ICP OES after microwave-assisted acid digestion as independent method. Manganese and cobalt could be determined using aqueous standards for calibration, but calibration with solid CRM was necessary for accurate determination of copper. The LOD for copper and cobalt were more than an order of magnitude better for SS-GF AAS due to the absence of any dilution, demonstrating the superiority of this technique for trace element determination. The multi-element capability of ICP OES appears to be of little advantage for this kind of application, as all papers on this subject agree that only a very few elements might be used to determine the origin of coffee, while the majority of the others does not contribute. Hence, a single-element technique that does not require any sample preparation besides grinding of the coffee beans appears to be an attractive alternative to the multi-element techniques that have been used up to now. The much better sensitivity if this technique is an additional advantage in the determination of trace elements such as cobalt and others that might be of importance.

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