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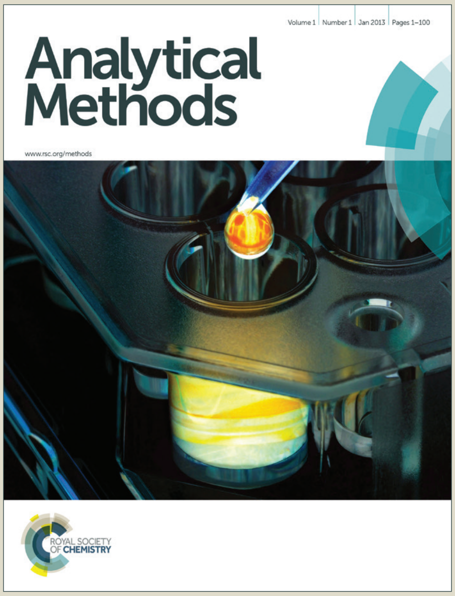

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Analytical Methods RSCPublishing

ARTICLE

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2014,
Accepted 00th January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/

Simultaneous determination of potassium, sodium, calcium, and magnesium in virgin olive oils by capillary electrophoresis with capacitively coupled contactless conductivity detection

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Analysis of trace elements in virgin olive oil (VOO) is important for nutritional information, geographical characterization, and adulteration detection. In this study, Na^+, K^+, Ca^{2+}, and Mg^{2+} in VOO were extracted to an aqueous solution with the aid of ultrasound energy, which was analyzed by capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D). The metal ions were separated in less than 3 min with good peak resolutions. The CE-C^4D method exhibited good linearity, with coefficients of determination (R^2) ranging from 0.9978 to 0.9995. The limits of quantification for Na^+, K^+, Ca^{2+}, and Mg^{2+} were 0.029, 0.029, 0.033, and 0.044 mg kg^{-1}, respectively. The results of the recovery tests at three concentration levels ranged from 80.5% to 119.6% with a relative standard deviation of 0.6 to 18.1%. The proposed CE-C^4D method was successfully applied for determination of the target analytes in five commercial samples of VOO.

Introduction

According to the definition of the International Olive Oil Council (IOOC),[1] virgin olive oil (VOO) is obtained from the fruit of the olive tree (*Olea europaea*) by physical processes under particular thermal conditions. After the extraction, the VOO can only undergo certain treatments, such as washing, decantation, centrifugation, and filtration, that do not lead to alterations of the oil.

The human consumption of VOO has been spread worldwide not only because of its delicious taste and aroma but also due to the health benefits associated with olive oil-rich diets. The lipid composition of the VOO is rich in unsaturated fatty acids, especially oleic and linoleic acids that are effective in preventing cardiovascular diseases.[2] Moreover, VOO is a source of certain micronutrients, such as phenolic compounds, vitamin E, and carotenes, that have important antioxidant activity in vivo.[3]

Chemical analysis of VOO is important to provide nutritional information, quality level, geographical origin, and detection of adulteration of this high-value product with cheaper edible oils.[4, 5] Thus, several analytical methods have been reported in the literature[6-10] for the determination of volatile compounds, triglycerides, free fatty acids, phenolic compounds, metals, and other macro and micro-constituents in VOO.

The metal ions Na^+, K^+, Ca^{2+}, and Mg^{2+} are among the mineral elements found in VOO and their concentrations can vary according to the olive fruit composition. The main sources of these metals are the soil, fertilizers, and irrigation water used in the olive plantation. So, the investigation of the concentration profiles of these elements in VOO may be used for the geographical classification. Moreover, because these metals are essential elements in human nutrition, the determination of their concentrations in VOO is important from the nutritional point of view.

Nevertheless, determination of metals in VOO poses a difficult challenge because this foodstuff has a complex organic matrix.[11] Thus, usually samples need to be pretreated before being introduced into analytical instruments in order to avoid systematic errors due to matrix interferences. Certainly, the most popular pretreatment for this kind of analysis is the total decomposition of the organic matrix by acid digestion or dry ashing and the extraction of the analytes with acids or by emulsification.[11]

Atomic spectrometric techniques are widely used for metal determinations in VOO. Several authors have reported the use of flame atomic absorption spectrometry (FAAS),[12-14] graphite furnace atomic absorption spectrometry (GFAAS),[12, 14-16] inductively coupled plasma optical emission spectrometry (ICP-OES),[15, 17-19] and inductively coupled plasma–mass spectrometry (ICP-MS)[20, 21] for determination of metals in VOO.

Although ion chromatography and capillary electrophoresis (CE) have already been used for determination of inorganic ions in VOO[22, 23] and biodiesel samples,[24, 25] no work was reported for the determination of Na^+, K^+, Ca^{2+}, and Mg^{2+} in VOO using separation techniques.

CE is a powerful analytical technique for VOO analysis because it can provide high separation efficiency for ionic and ionizable analytes, with short analysis time and consumption of low volumes of sample and reagents. Silva et al.[8] reviewed the application of CE for determination of phenolic compounds, fatty acids, chlorophylls, betaines, protein, and amino acids in VOO.

In this paper, CE was evaluated with capacitively coupled contactless conductivity detection (C^4D) for simultaneous determination of Na^+, K^+, Ca^{2+}, and Mg^{2+} extracted from the VOO using a simple liquid-liquid extraction procedure. This simple CE-C^4D method was applied for determination of these analytes in commercial samples of VOO. To the best of our knowledge, this work is the first to use CE or C^4D for determination of metals in VOO.

Experimental

Samples, Reagents, and Solutions

All the reagents were of analytical grade except methanol (Tedia, Brazil), which was of HPLC grade. The lactic acid and $CaCl_2$ were obtained from Merck (Darmstadt, Germany), and the KCl, NaCl, $MgCl_2.6H_2O$, and LiCl were purchased from Synth (São Paulo, Brazil). The L-histidine was obtained from Sigma-Aldrich (Steinhein, Germany). The ultra-pure water was obtained from Direct-Q 3 UV Water Purification System (Millipore, Molsheim, France).

The background electrolyte (BGE) for CE separation was composed of histidine (20 mmol L^{-1}) and lactic acid (22 mmol L^{-1}), pH 4.7. Standard stock solutions of metal ions at concentrations of 10 mmol L^{-1} were prepared by dissolving the respective chloride salts in methanol, except for the LiCl solution that was prepared in deionized water and used as an internal standard. To obtain the standard curves, working standard solutions, containing the four analyzed metal ions and the internal standard, were prepared by dilution of the stock solutions with a 10-fold diluted BGE. This diluted BGE solution was also used in the liquid-liquid extraction procedure. The analyzed VOO samples were acquired at local markets of Campinas (Brazil).

CE Instrumentation and Procedure

The CE separations were performed with a homemade CE system equipped with C^4D. A bore fused-silica capillary column with a 50 µm i.d. and 50.0 cm in length (42.0 cm effective) was used for CE separations. The samples were hydrodynamically injected into the capillary column by pressure (11 kPa) for a period of 10 s. The separation potential was 25 kV and the C^4D operated at 600 kHz (sinusoidal) and 1.5 V (peak to peak amplitude). All operations were carried out at ambient temperature (20 to 25°C).

Before the first analysis of the day, the fused-silica capillary was sequentially washed with 0.1 mol L^{-1} NaOH, water, and BGE (5 min each). After each running, the capillary was flushed with BGE for 1 min. Standard curves were obtained by injecting (in triplicate) six working standard solutions containing a mix of the metal ions at concentration levels from 0 to 8.02 mg L^{-1}. Lithium solution was added (1.04 mg L^{-1}) to all solutions as internal standard. The areas under the peaks in the electropherograms were integrated and the standard curves were then plotted as the ratio of peak area of metal ion to that of internal standard *versus* analyte concentration. A linear regression was performed on the standard curves using the least-square method and the obtained regression equations were used to estimate the concentrations of the analytes in the VOO samples. The peak integration and the statistical analysis were carried out with the software Origin 8.1 (OriginLab, Northhampton, MA, USA).

Liquid-Liquid Extraction Procedure

All the extractions were performed using 5 (±0.0001) g of VOO samples weighted directly in 15-mL capped polyethylene centrifuge tubes (Falcon®). For the recovery tests, a stock standard solution containing the metal ions (1 mmol L^{-1} each) was used to spike the samples, which were then equilibrated for 10 min before the extraction. After this period, 1 mL of the aqueous extraction solution (10-fold diluted BGE) was added to the samples. The solutions were manually homogenized for 2 min and then sonicated (Unique, UltraCleaner 1450) for 15 min. During the sonication, the tubes were manually stirred (5 s) 3 times per min in order to avoid separation between the oil and aqueous phases. Finally, the samples were centrifuged (Centribio, model 80-28) for 10 min at 4000 rpm for separation of the phases. By using a micropipette, 425 µL of the aqueous phase was collected and the internal standard solution (Li^+) was added (1.04 mg L^{-1}). Before analysis, all samples were filtered through polyethylene membrane filters with 0.22 µm pore diameter.

Results and Discussion

CE separation

Figure 1a displays an electropherogram of a standard solution containing the analyzed metal ions, while Fig. 1b and 1c exhibit the electropherograms of the extracts obtained from a VOO sample with and without spiking with the metal ions, respectively. The separation was attained within a short time (less than 3 min). The noticeable asymmetry in the shape of the peaks can be attributed to electrodispersion, an intrinsic phenomenon in the electrophoretic process caused by differences in the mobilities between the analyte zones and the BGE. Despite the electrodispersion, good peak resolutions were achieved. This high separation efficiency can be ascribed to the presence of lactate in the BGE composition that forms complexes with Ca^{2+} and Mg^{2+}, decreasing their electrophoretic mobilities.[26] The slight shifts in the migration times observed among the electropherograms (Fig. 1) can more likely be attributed to the variation in the electroosmotic flow intensity. Nevertheless, the identities of the analyte peaks were confirmed by spiking experiments. The chosen internal standard (Li^+) was considered suitable because its peak resolution was good, and this metal was not found in detectable amount in the VOO samples.

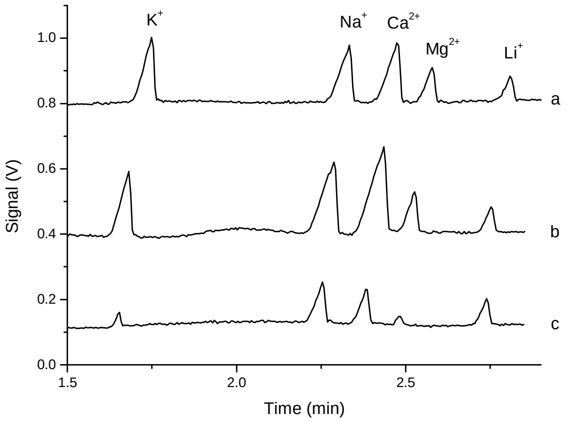

Figure 1. Electropherograms of a (a) standard solution of the analyzed metal ions (3.9, 2.3, 4.0, and 2.4 mg L^{-1} for K^+, Na^+, Ca^{2+} and Mg^{2+}, respectively) and of the extraction solutions for a VOO sample with (b) and without (c) spiking with the metal ions (0.92, 0.54, 0.94, and 0.57 mg kg^{-1} for K^+, Na^+, Ca^{2+}, and Mg^{2+}, respectively). Li^+ solution was added (1.04 mg L^{-1}) in the solutions as the internal standard. Separation conditions: BGE: 20 mmol L^{-1} histidine and 22 mmol L^{-1} lactic acid , pH 4.7. Fused silica capillary column with 50 µm i.d. and 50.0 cm length (42.0 cm effective). Separation voltage of 25 kV; pressure injection at 11 kPa for 10 s. C^4D working at 600 kHz and 1.5 V (peak to peak amplitude).

Optimization of the Liquid-Liquid Extraction

A quick separation between the oil and aqueous phases was observed during the sonication step. This phase separation reduced the surface contact area between the liquids and decreased the extraction efficiency, resulting in low recoveries of the analytes. This drawback was overcome by manually stirring (5 s) the centrifuge tubes at 3 times/min during the sonication.

The influence of the sonication time on the kinetics and efficiency of the extraction was evaluated. Figure 2 displays the recoveries obtained for a spiked VOO sample that was submitted to extractions under the sonication times of 5, 10, 15, and 20 min. One can note that 5 min of sonication provided the worst extraction efficiency, most likely because this time was not enough to perform an exhaustive extraction of the analytes. No significant differences among the recovery results for 10, 15, and 20 min were observed. However, the relative standard deviations were slightly smaller using 15 min of sonication. Thus, this time was chosen as the best one for further experiments.

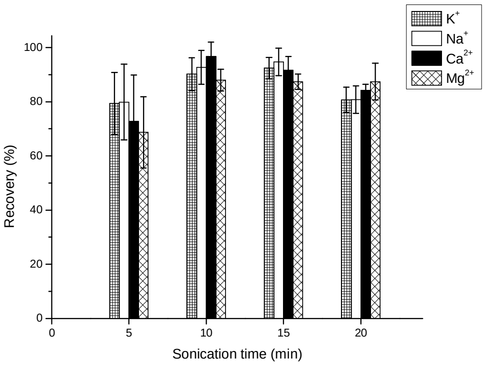

Figure 2. Average recoveries (%) and relative standard deviations (n = 3) of the metal ions extracted from a spiked VOO sample under different sonication times (5, 10, 15, and 20 min).

Analytical Parameters of the Method

The main analytical parameters of the CE-C^{4}D method were obtained (Table 1) according to the recommendations of the literature.[27] The relative standard deviations (RSD) of the migration times were about 3.4%. The instrumental limits of detection (LOD) and quantification (LOQ) are comparable or better than those obtained by reported atomic-spectrometric methods.[14, 15, 28] The LOQ of the method was calculated by dividing the instrumental LOQ by 5, the approximate preconcentration factor obtained in the liquid-liquid extraction (5 g of sample and 1 mL of extraction solution). The linearity of the standard curves over the evaluated concentration ranges were validated by the lack-of-fit test[29] (data not shown) and the acceptable coefficients of determination (R^2), which ranged from 0.9978 to 0.9995. The accuracy and intra-day precision of the CE-C^{4}D method were evaluated by recovery tests performed at three concentration levels of the added metals in the analyzed samples of VOO. The recovery results (Table 2) ranged from 80.5 to 119.6% with RSD of 0.6-18.1%. According to the recommendations of the literature[27] for the validation of analytical separation methods, recoveries between 70 and 120% with RSD lower than 20% are acceptable. Thus, the results of the recovery tests indicated the CE-C^{4}D method is sufficiently accurate for the determination of the analyzed metal ions in VOO.

Application of the CE-C^{4}D Method for VOO Analysis

The analyses of 5 different commercial VOO samples were carried out in triplicate and the electropherograms of two extracts of the analyzed samples are shown in Fig. 3. The slight shifts observed in the migration times have already been discussed elsewhere in this paper. The analysis results are summarized in Table 3. The RSD (n=3) for the analyses ranged from 1.0 to 19.2%. Sodium and calcium ions were detected in all samples and their concentrations ranged from 0.14 to 0.65 mg kg^{-1} for the Na^+ and 0.11 to 1.18 mg kg^{-1} for the Ca^{2+}. On the other hand, K^+ and Mg^{2+} were below the LOQ in certain samples but detected in other samples in concentrations that varied from 0.17 to 0.43 mg kg^{-1} and 0.06 to 0.220 mg kg^{-1}, respectively.

By comparing the concentrations reported in the literature[12, 14, 15, 17, 28] for determination of K^+ (0.05 to 2.14 mg kg^{-1}), Na^+ (0.76 to 30.03 mg kg^{-1}), Ca^{2+} (0.63 to 76.0 mg kg^{-1}), and Mg^{2+} (0.056 to 4.61 mg kg^{-1}) in VOO by atomic-spectrometric methods, the obtained results were in agreement for K^+, Ca^{2+}, and Mg^{2+} concentrations, but Na^+ levels were lower than those previously reported. It is important to highlight that the concentrations of these analyzed metals in VOO can vary significantly according to the geographical origin (olive variety and soil composition) of the oil.[10, 28, 30]

Currently, there are no limits for concentrations of these elements in VOO established by the IOOC[1].

Table 1. Analytical parameters of the CE-C^{4}D method

Parameter	Metal ions			
	K^+	**Na^+**	**Ca^{2+}**	**Mg^{2+}**
Migration time(min)[a]	1.73 ± 0.06	2.41 ± 0.08	2.57 ± 0.09	2.70 ± 0.09
Regression equation[b]	y = 0.0273x + 0.0349	y = 0.0187x + 0.3929	y = 0.0288x + 0.1998	y = 0.0135x + 0.1231
R^2	0.9982	0.9986	0.9995	0.9978
Range (mg L^{-1})	0-6.02	0-4.60	0-8.02	0-4.86
LOD[c] (mg L^{-1})	0.043	0.043	0.051	0.067
LOQ[d] (mg L^{-1})	0.14	0.14	0.17	0.22
LOQ[e] (mg kg^{-1})	0.029	0.029	0.033	0.044

[a] mean ± standard deviation for 5 consecutive replicate runs.

[b] x = concentration of corresponding metal ion (mg L^{-1}); y = ratio of peak area of metal ion to that of internal standard.

[c] Instrumental limit of detection (S/N = 3).

[d] Instrumental limit of quantification (S/N = 10)

[e] Limit of quantification of the method considering the preconcentration factor of 5x

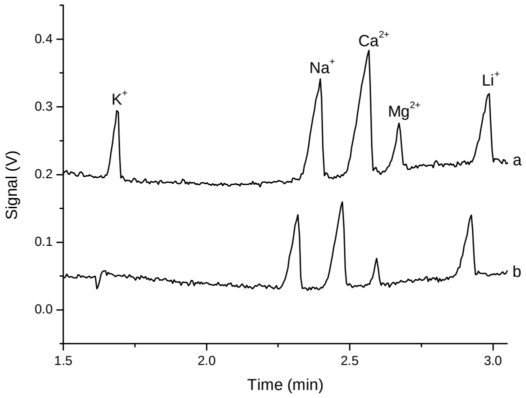

Figure 3. Electropherograms of the aqueous extraction solutions from the VOO samples 4 (a) and 3 (b). Separation conditions as in Figure 1.

Table 2. Recovery percentage (mean±RSD)[a] of the analyzed metal ions at three concentration levels

Metal ion	Concentration added (mg kg^{-1})	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
K$^+$	0.92	91.0 ± 2.5	82.3 ± 1.9	113.1 ±4.1	88.8 ± 7.7	93.5 ± 1.6
	0.46	95.8 ± 6.1	84.4 ± 5.7	119.6 ± 1.2	103.8 ± 15.6	100.3 ±6.1
	0.23	86.1 ± 5.6	83.7 ± 10.5	111.0 ± 3.0	91.6 ± 6.5	100.8 ± 9.3
Na$^+$	0.54	91.4 ± 1.7	95.2 ± 9.5	88.1 ± 12.9	84.9 ± 12.1	81.1 ± 1.1
	0.27	88.8 ± 6.3	95.8 ± 13.2	88.4 ± 15.2	105.0 ± 25.6	80.7 ±4.0
	0.14	95.1 ± 7.0	91.2 ± 9.9	84.1 ± 3.9	85.8 ± 8.8	87.1 ± 7.0
Ca^{2+}	0.94	92.2 ± 3.7	89.0 ± 8.5	116.0 ± 6.7	95.9 ± 15.4	86.8 ± 6.9
	0.47	88.3 ± 7.9	80.5 ± 14.0	117.4 ± 6.0	104.2 ± 4.9	93.6 ± 9.3
	0.24	92.1 ± 10.4	85.7 ± 18.1	106.8 ± 13.5	98.8 ± 16.2	89.1 ± 12.4
Mg^{2+}	0.57	90.7 ±1.4	95.9 ± 1.2	119.4 ± 1.2	85.9 ± 6.8	88.7 ± 3.4
	0.29	94.6 ± 5.1	98.8 ± 5.8	119.4 ± 0.6	91.3 ± 5.4	89.6 ± 2.9
	0.14	89.5 ± 7.5	101.8 ± 0.2	118.7 ± 1.8	86.7 ± 4.6	89.4 ± 4.5

[a] RSD = relative standard deviation (n = 3)

Table 3. Results for the determination of the analyzed metals ions in VOO samples.

Sample	Concentration (mg kg^{-1})[a] K$^+$	Na$^+$	Ca^{2+}	Mg^{2+}
1	<LOQ	0.16±0.02	0.11±0.01	<LOQ
2	<LOQ	0.61±0.03	0.47±0.09	<LOQ
3	<LOQ	0.400±0.004	0.59±0.01	0.06±0.01
4	0.43±0.01	0.65±0.01	1.18±0.03	0.220±0.004
5	0.17±0.02	0.14±0.01	0.98±0.02	0.13±0.01

[a] mean ± standard deviation (n = 3)

Conclusions

The proposed CE-C^{4}D method was demonstrated to be simple, rapid, and inexpensive for the determination of sodium, potassium, calcium, and magnesium ions in VOO. The liquid-liquid extraction of the analytes with aqueous solution was considered simple and efficient. Additionally, cleanup steps or organic solvents were not required. Although the VOO has a complex matrix, the proposed method provided acceptable precision and accuracy for the analysis of commercial samples of VOO.

Acknowledgements

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for scholarship to M.A.T.L. This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAFESP, grants nº 2008/57805-2 and 2013/22485-6) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grants nº 573672/2008-3 and 305318/2012-8).

Notes

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