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Electrothermal atomic absorption spectrometric determination of aluminium, cadmium, chromium, copper, iron, manganese, nickel and lead in olive oil

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A simple and fast procedure for the direct ETAAS determination of Al, Cd, Cr, Cu, Fe, Mn, Ni and Pb in olive oil is proposed. *N*,*N*-Hexamethylenedithiocarbamic acid, hexamethyleneammonium salt (HMDC-HMA), was used as a universal modifier with two functions: isoformation of the chemical species of the investigated elements, present in olive oil and their thermal stabilisation during the pre-treatment step. Various organic solvents were studied and 1,4-dioxane was found to be the most suitable solvent for oil dilution prior to ETAAS analysis. 1,4-Dioxane improves the decomposition of triglycerides during the pre-treatment step and permits aqueous standard solutions to be used for calibration. Uncoated graphite tubes with platforms are proposed as atomisers for the ETAAS determination of Cd, Cu, Fe, Mn and Fe, and pyrolytic graphite coated graphite tubes with grooves are the most suitable atomisers for Al, Cr and Ni. Instrumental parameters optimised according to pre-treatment and atomisation curves, obtained in the presence of the olive oil matrix and with HMDC-HMA as modifier, are presented. The proposed method permits the determination of 0.1 μg g^{-1} Fe, 0.05 μg g^{-1} Ni, 0.02 μg g^{-1} Al, Cu, Cr and Pb, and 0.01 μg g^{-1} Cd and Mn with relative standard deviations of about 8–10% for all analytes in this concentration range.

Keywords: *Olive oil; electrothermal atomic absorption spectrometry; dithiocarbamate; trace elements*

Trace levels of metal ions (Cu, Fe, Ni, Mn) are known to have adverse effects on the oxidative stability of edible oils.[1–5] Transition metals (Cu, Fe) catalyse the decomposition of hydroperoxides and lead to more rapid formation of undesirable substances. Taking into account also the metabolic role of some metals, the development of fast and accurate analytical methods for trace element determination in olive oil is important from the view point of both production quality control and food analysis.

The content of metals and their chemical forms in edible oils depend on several factors. The metals might originate from the soil and fertilisers and be intimately incorporated in the oil. They might be introduced during the production process (by processing actions such as bleaching, hardening, refining and deodorization) or by contamination from the metal processing equipment and thus be suspended in the oil. Procedures based on preliminary wet or dry matrix digestion have been proposed for trace analyte determination.[6–9] Trace element extraction by using different extractants has also been used as a separation procedure.[10–14] These methods are often laborious and suffer from disadvantages such as the possible loss of volatile metal species during ashing, contamination in the course of the digestion or chelation process, or non-quantitative recoveries especially when numerous extraction steps are involved.

The application of a direct spectrometric technique, particularly ETAAS, is the best way to effect such a difficult analysis.[15–19] The main problems in olive oil analysis by ETAAS are connected with the high values of non-specific absorption and with the observed depression of the atomisation of the analytes due to the olive oil matrix. The different chemical forms of trace elements in olive oil, and hence species with different thermal behaviour, make the optimisation of temperature programmes for the graphite furnace difficult.

The aim of this paper was to investigate the use of diethyl ether, isobutyl methyl ketone (IBMK), xylene, heptane and 1,4-dioxane as solvents for olive oil dilution and *N*,*N*-hexamethylenedithiocarbamic acid, hexamethyleneammonium salt, as a modifier for the isoformation of different chemical species of metals present in olive oil and also their thermal stabilisation during the pre-treatment step. The atomisation behaviour of Al, Cd, Cr, Cu, Fe, Mn, Ni and Pb was studied using various atomisers in order to establish the optimum conditions for their accurate determination in olive oil by direct ETAAS.

EXPERIMENTAL

Apparatus

The measurements were carried out on a Perkin-Elmer (Norwalk, CT, USA) Zeeman 3030 spectrometer with an HGA-600 graphite furnace. The light sources used were hollow cathode lamps for Al, Cr, Cu, Fe, Mn, Ni and Pb (Perkin-Elmer) and an electrodeless discharge lamp for Cd (Perkin-Elmer). The spectral bandpass for all analytes was 0.7 nm. Standard uncoated graphite tubes, uncoated tubes with pyrolytic platforms, pyrolytic graphite coated graphite tubes with grooves (Perkin-Elmer) and tungsten-impregnated graphite tubes[20] were used as atomisers. Micropipettes (Eppendorf, Westbury, NY, USA) were used for manual injections of 20 μl sample solutions into the graphite atomisers. Atomic absorption signals were recorded on an Anadex (Camarillo, CA, USA) printer. Only peak areas were used for quantification. The graphite furnace operating parameters are presented in Tables 1–2.

Table 1 Recommended conditions for HGA programme for the Zeeman 3030 instrument; 20 μl sample aliquot

	Step					
Parameter	1	2	3	4	5	6
Temperature/°C	50	120	450	Variable*	Variable*	Variable*
Ramp time/s	10	10	20	20	0	2
Hold time/s	10	10	30	20	5	3
Read	—	—	—	—	On	—
Internal gas flow/ml min^{-1}	0	300	300	300	0	300

* See Table 2.

Table 2 Optimum pre-treatment and atomisation temperatures for oil analysis by wall and platform atomisation without and with HMDC-HMA as modifier; 20 μl sample aliquot

	Temperature/°C							
	Wall atomisation				Platform atomisation			
Analyte	Step 4 without HMDC-HMA	Step 4 with HMDC-HMA	Step 5	Step 6	Step 4 without HMDC-HMA	Step 4 with HMDC-HMA	Step 5	Step 6
Al	1200	1200	2400	2600	1200	1300	2500	2600
Cd	300	450	1200	2000	400	600	1600	2000
Cd*	350	550	1500	2200	—	—	—	—
Cr	1200	1200	2400	2600	1200	1300	2500	2650
Cu	900	1100	2200	2500	1000	1200	2400	2500
Fe	1000	1100	2300	2500	1100	1200	2500	2600
Fe*	1100	1200	2400	2500	—	—	—	—
Mn	900	1000	2100	2300	1000	1200	2400	2500
Ni	1100	1200	2400	2500	1100	1200	2500	2600
Pb	400	700	1800	2300	600	800	2300	2500
Pb*	450	800	2100	2300	—	—	—	—

* Wall atomisation using tungsten-impregnated graphite tube.

Table 3 Influence of olive oil matrix on the ratio b_m/b_o of the slopes of calibration graphs for wall and platform atomisation: b_o = slope of calibration graph obtained with aqueous standard solutions, b_m = slope of calibration graph obtained in the presence of 0.03, 0.05, 0.1 and 0.2 g ml^{-1} olive oil in the sample solution

	$b_m/b_o \pm s$							
	0.03 g ml^{-1} olive oil		0.05 g ml^{-1} olive oil		0.1 g ml^{-1} olive oil		0.2 g ml^{-1} olive oil	
Element	Wall	Platform	Wall	Platform	Wall	Platform	Wall	Platform
Al	0.99±0.02	—	0.99±0.02	—	0.95±0.03	—	0.83±0.04	—
Cd	0.94±0.04	0.97±0.04	0.83±0.05	0.91±0.04	0.6±0.08	0.75±0.05	*	0.52±0.08
Cr	0.99±0.02	—	0.99±0.02	—	0.96±0.03	—	0.92±0.03	—
Cu	0.99±0.04	0.99±0.03	0.93±0.05	0.99±0.04	0.82±0.07	0.95±0.06	0.77±0.07	0.91±0.06
Fe	0.99±0.05	0.99±0.04	0.88±0.07	0.95±0.06	0.74±0.08	0.82±0.06	0.68±0.09	0.76±0.06
Mn	0.99±0.02	0.99±0.02	0.99±0.03	0.99±0.03	0.92±0.04	0.95±0.03	0.82±0.05	0.91±0.04
Ni	0.99±0.02	—	0.92±0.02	—	0.87±0.04	—	0.81±0.05	—
Pb	0.99±0.03	0.99±0.03	0.92±0.04	0.95±0.04	0.83±0.06	0.91±0.05	0.72±0.07	0.88±0.05

* Very high values of non-specific absorption.

Reagents

Stock standard solutions, 1000 μg ml^{-1} (Merck, Darmstadt, Germany), were used for the preparation of working aqueous standard solutions by appropriate dilution. Multi-element standard, dissolved in oil, containing 0.1 g kg^{-1} Al, Cr, Cu, Fe, Mg, Ni and Pb (Merck), Cd standard, dissolved in oil, 0.1 g kg^{-1} (Merck), and Mn standard, dissolved in oil, 0.1 g kg^{-1} (Merck), were used for the preparation of working organic standard solutions by appropriate dilution. Organic solvents used in sample preparation were additionally purified by sub-boiling distillation. *N*,*N*-Hexamethylenedithiocarbamic acid, hexamethyleneammonium salt (HMDC-HMA), (*pro analysi*, Merck), was purified by two recrystallizations from 1,4-dioxane. All other reagents were of analytical-reagent grade. Doubly distilled water was used throughout.

Modifier solution. A modifier solution of HMDC-HMA was prepared by dissolution of the appropriate amount of the substance in the organic solvent used for sample preparation in order to obtain a 0.1 mol l^{-1} solution.

Sample preparation

An accurately weighed olive oil sample of 1 g was dissolved in 5 ml of modifier solution. Solutions with a matrix content of 0.02–0.2 g ml^{-1} were then prepared by appropriate dilution with modifier solution. Standard additions were obtained by addition of specific volumes of organic or aqueous standard solution (1 ng g^{-1} Cd; 10 ng g^{-1} Al, Cr and Mn; 20 ng g^{-1} Cu and Pb; 40 ng g^{-1} Ni; and 100 ng g^{-1} Fe) to the set of samples prepared.

RESULTS AND DISCUSSION

Effect of the solvent media

Sample injection using an autosampler is the best means of sample introduction in ETAAS. However, most autosamplers are not adapted for use with organic solvents. In this study, manual injection into the hot (50 °C) furnace was used and the RSD of sampling was around 4–5%. The injection of 20 μl of organic solution into the furnace is optimum. Spreading of the sample makes the injection of larger volumes almost impossible. In general, it is better to introduce smaller volumes of concentrated samples than larger volumes of diluted samples. Different solvents were investigated for oil dilution, *viz*., diethyl ether, IBMK, 1,4-dioxane, xylene and heptane. Diethyl ether is unsuitable for practical application owing to its extremely high volatility. When xylene and heptane were used as solvents for olive oil dilution, very high values of non-specific absorption were observed, probably due to the organic solvent itself.[21] IBMK and 1,4-dioxane are the most suitable solvents for olive oil analysis by ETAAS. The advantage of 1,4-dioxane is that peroxide compounds formed during the decomposition of the solvent assist in the destruction of triglycerides of olive oil during the ashing step and thus lower the value of non-specific absorption in the atomisation step. However, it should be noted that, even in the presence of 1,4-dioxane, a pre-treatment temperature of around 1200 °C is required for complete matrix decomposition.

Effect of chelating agent and ashing temperature

It might be assumed that addition of a strong chelating agent to the sample will convert all the different chemical species of

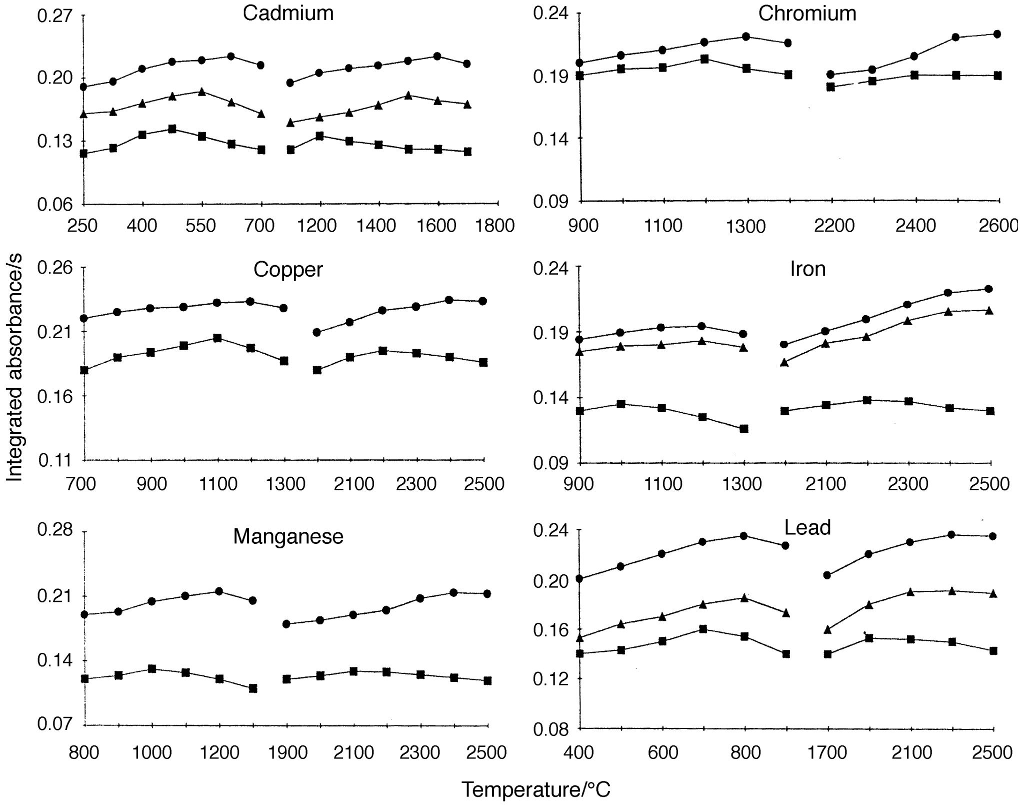

Fig. 1 Pre-treatment (left) and atomisation (right) curves for Cd, Cr, Cu, Fe, Mn and Pb in the presence of olive oil matrix and HMDC-HMA as modifier by using pyrolytic graphite coated graphite tubes with grooves (■), tungsten-impregnated tubes (▲) and uncoated tubes with platforms (●).

Table 4 Recoveries obtained for ETAAS trace element determination using aqueous and organic standard solutions (six parallel determinations)

	Recovery (%)				
	Aqueous standard solution		Organic standard solution		
Element	Mean	*s*	Mean	*s*	$t_{calc.}$
Al	96.8	0.8	96.1	0.9	2.03
Cd	96.3	0.7	96.2	0.8	0.43
Cu	100.2	0.9	99.6	0.8	0.83
Cr	99.8	0.7	99.5	0.7	1.49
Mn	98.8	0.8	99.3	0.8	1.91
Fe	95.7	0.9	95.0	0.9	2.11
Ni	96.5	0.8	95.8	0.8	1.90
Pb	95.7	0.9	96.1	0.8	1.35

$t_{crit(p=0.9,\ n=6)}=2.47$.

Table 5 ETAAS determination of trace elements in olive oil sample from Chios island

Element	Determined/ ng g^{-1}	Added/ ng g^{-1}	Found/ ng g^{-1}	Recovery (%)	RSD (%)
Al	65±6	70	138±8	102	9
Cd	<DL*	10.0	9.5±0.4	95	4
Cr	<DL	50	47±3	94	6
Cu	25±2	50	73±4	97	8
Mn	9.5±0.8	50	60.5±0.9	102	8
Ni	<DL	80	83±5	103	6
Pb	<DL	80	78±7	97	9

* Below detection limit.

the analytes into a single chelate complex. Among the various chelating agents used in analytical practice, HMDC-HMA was chosen because of the high stability constants of its complexes with the studied metals and previously published data for thermal stabilisation of volatile elements in the presence of dithiocarbamates.[22] Thermal pre-treatment–atomisation curves were prepared to define the appropriate temperature programme for each studied element in the presence of the oil matrix using HMDC-HMA as modifier. Different types of graphite tube, *viz.*, uncoated graphite tubes, tungsten-impregnated graphite tubes, pyrolytic graphite coated graphite tubes with grooves and uncoated graphite tubes with platforms, were used as atomisers. Typical examples of curves constructed for Cd, Cr, Cu, Fe, Mn and Pb are presented in Fig. 1 and results obtained for all elements are summarised in Table 2. In general, it is important to decompose the oil matrix in two ashing steps, one longer step at around 450 °C and a second shorter step at around 1200 °C. A comparison of loss-free pre-treatment temperatures obtained in the presence of HMDC-HMA as modifier with those obtained without HMDC-HMA (Table 2) confirms that HMDC-HMA thermally stabilises elements such as Cd, Cu, Fe, Mn and Pb and permits higher pre-treatment temperatures, thus minimising the values of non-specific absorption in the atomisation step. Uncoated graphite tubes are not suitable for oil analysis; very poor reproducibility was obtained for all the studied elements. A probable explanation is sample spreading and penetration into the graphite, leading to irreproducible vaporisation of the analytes during the atomisation step. Tungsten-impregnated tubes are the best atomisers for Fe, allowing good reproducibility and sensitivity close to that achieved with a platform. As was to be expected, higher sensitivity was achieved for volatile elements (Cd, Cu, Mn and Pb) by using platform atomisation. The enhancement of the values of the absorbance signals expressed as the ratio A_{pl}/A_{wall} ranges from 1.3 for Fe, Mn and Cu to 1.7 for Cd and Pb.

Table 6 Determination of trace elements in olive oil by wet digestion ETAAS and by direct ETAAS after dissolution in 1,4-dioxane

Element	Wet digestion ETAAS (mean $\pm s$)/ng g^{-1}			ETAAS after dissolution in 1,4-dioxane (mean $\pm s$)/ng g^{-1}		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Al	65 ± 7	120 ± 11	185 ± 12	67 ± 6	112 ± 10	196 ± 13
Cu	45 ± 6	96 ± 10	210 ± 14	50 ± 7	110 ± 9	220 ± 15
Cr	< DL*	< DL	25 ± 5	< DL	< DL	30 ± 4
Fe	570 ± 60	1340 ± 130	1520 ± 150	600 ± 30	1290 ± 50	1540 ± 51
Mn	15 ± 4	20 ± 5	45 ± 5	11 ± 3	23 ± 4	47 ± 4
Pb	< DL†	< DL	25 ± 5	< DL	< DL	22 ± 3

* 0.03 ng g^{-1}. † 0.02 ng g^{-1}.

Matrix interferences

The sample dilution ratio was optimised for each studied element in order to obtain the lowest value of non-specific absorption during the atomisation step and also to reduce matrix interferences. Matrix interferences were evaluated from the ratio b_m/b_o of the slopes of calibration graphs; b_m is the slope of the calibration graph obtained in the presence of oil samples with different oil content and b_o is the slope of the calibration graph for organic standard solutions. Results obtained for pyrolytic graphite coated graphite tubes with grooves and uncoated graphite tubes with platforms are presented in Table 3. Platform atomisation ensured better reproducibility and lower interferences for relatively volatile elements such as Cd, Cu, Mn and Pb. Evidently, the most important factor for the accurate determination of these elements is the use of isothermal conditions in the atomisation step. Also, use of a platform avoids dispersion of the sample inside the tube. The accurate ETAAS determination of Cd is possible with 0.05 g ml^{-1} olive oil in the sample solution and for Cu, Mn and Pb in the presence of 0.2 g ml^{-1} olive oil. Results obtained for Fe revealed that the use of uncoated tubes with platforms or tungsten-impregnated tubes as atomisers minimised the matrix effect and correct results were obtained in the presence of 0.05 g ml^{-1} olive oil in the sample. For Al, Cr and Ni no significant difference was found between pyrolytic graphite tubes with grooves and platform atomisation. Tungsten-impregnated tubes are unsuitable for these elements in the presence of the oil matrix. Lower sensitivity was observed probably due to the formation of mixed carbides. Wall atomisation from pyrolytic graphite tubes with grooves permits the ETAAS determination of Al, Cr and Ni in the presence of 0.2 g ml^{-1} olive oil in the sample.

Calibration

Matrix interferences (Table 3) observed in oil analysis meant that the standard additions method was necessary for calibration. Organic standard solutions are not always available; therefore, experiments were performed with aqueous standard solutions. The organic solvent 1,4-dioxane is miscible with water; hence it is easy to dilute appropriate amounts of aqueous standard solutions in 1,4-dioxane and to use the obtained solution for standard additions to the oil sample. After addition of the ligand HMDC-HMA, all metal ions present in the sample and in the added standard solution will be converted into dithiocarbamate complexes, *i.e.*, their thermal behaviour will be similar. Thermal pre-treatment and atomisation curves constructed for those samples did not differ significantly from those presented in Fig. 1 for the samples prepared with organic standard solutions, thus demonstrating the possibility of using aqueous standard solutions for standard additions instead of organic solutions. Results from recovery studies performed for trace analyte determination in olive oil, using organic or aqueous standard solutions, are presented in Table 4. According to the Student's t-criterion, the obtained recovery values with organic standard solutions do not differ statistically from those obtained with aqueous standard solutions ($t_{calc.} < t_{crit.}$); consequently, aqueous standard solutions can be used for calibration in the ETAAS analysis of olive oil by the proposed method.

The proposed method permits the determination of 0.1 μg g^{-1} Al, 0.02 μg g^{-1} Cr and 0.05 μg g^{-1} Ni in olive oil using wall atomisation, and 0.01 μg g^{-1} Cd and Mn and 0.02 μg g^{-1} Cu and Pb in olive oil using platform atomisation.

The accuracy and precision of the proposed ETAAS method are presented in Table 5 for an olive oil sample from the island of Chios. The accuracy of the proposed method was evaluated by comparing the results with those obtained by wet digestion ETAAS (a 0.5 g olive oil sample was digested with a mixture of hydrochloric and nitric acid in a Kjehldal flask and then diluted to 25 ml). No significant differences between the results obtained were observed (Table 6).

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