



*Dedicated to Professor Ion Grosu
on the occasion of his 65th anniversary*

A FREE NON-SPECTRAL INTERFERENCES METHOD BASED ON INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY FOR MULTIELEMENTAL DETERMINATION IN MULTIMINERAL/MULTIVITAMIN PREPARATIONS

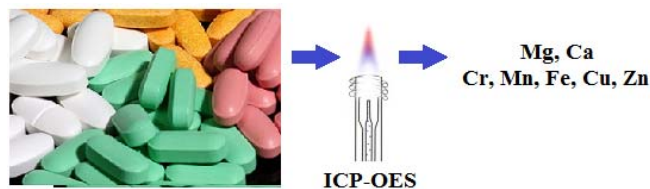
Dorina CASONI,^{a,b} Michaela PONTA,^{a,b} Mihaela-Nicoleta BIBICA,^a Ioana-Violeta LAZAR^a
and Tiberiu FRENTIU^{a,b,*}

^a Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany Janos 11, 400028 Cluj-Napoca, Roumania

^b Research Center for Advanced Chemical Analysis, Instrumentation and Chemometrics, Arany Janos 11,
400028 Cluj-Napoca, Roumania

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A free non-spectral interferences method based on inductively coupled plasma optical emission spectrometry was characterized in terms of figures of merit for multielemental determination in multiminerals/multivitamin preparations. The method was validated for the determination of Mg, Ca, Cr, Mn, Fe, Cu and Zn based on the analysis of a standard reference material (SRM 3280) and different brands of commercially available multiminerals/multivitamin supplements. The study showed that a simple 100-fold dilution of the sample ensures overcoming of non-spectral effects in complex matrices such as multiminerals/multivitamin preparations. Detection limits were in the range 0.0009–0.0037 mg g⁻¹. The recovery was within 93%–110% for certified reference materials and 94%–105% for real samples of marketed supplements. Precision of measurements was in the range 1.3–12.1%.



INTRODUCTION

Dietary supplements, defined as products intended to supplement the diet, are considered by consumers as drug alternatives for various reasons, including their relatively low cost, safety and effectiveness.¹ They contain vitamins, minerals, amino acids, herbs and other botanicals, and are used to increase essential nutrients in the body with beneficial effects for human health.^{2,3} Multivitamin/multimineral preparations are the largest supplement category of marketed supplements the use of which has considerably increased.⁴ Among the

well-known vitamins (A, B-complex, C, D, E, K), nutrients commonly contained in such preparations are minerals like magnesium, zinc, calcium, iodine, selenium, copper, manganese, chromium, molybdenum, etc. Commercial preparations differ quite a lot in terms of composition. On the other hand, dietary supplements could be a potential source of toxic elements (lead, cadmium, mercury and arsenic) thus representing a risk for consumers. In this context, multielemental analysis is important to assess the quality of these products. Usually their quality is estimated based on the labeled contents declared by the manufacturer. The

* Corresponding author: ftibi@chem.ubbcluj.ro

accuracy of the declared minerals concentration is of great concern, since dietary supplements are regulated as food, and producers are not required to register them before sale.⁵ Due to the various elements in a wide range of concentrations contained in complex matrix, the analysis of dietary supplements is often a challenge and there is no official method for elemental determination. Numerous analytical techniques and different strategies for sample preparation have been used for this purpose. Analytical methods including flame atomic absorption spectrometry,^{6–8} graphite-furnace atomic absorption spectrometry,^{9,10} laser induced breakdown spectrometry,^{11,12} visible molecular absorption spectrophotometry¹³ and stripping voltammetry¹⁴ have been proposed for the analysis of pharmaceuticals and dietary supplements in order to check accuracy of the declared element contents. Among spectrometric techniques, inductively coupled plasma optical emission spectrometry (ICP-OES) is widely used in the analysis of pharmaceuticals and dietary supplements.^{15–20} The advantages of this technique relate to extended dynamic range, simultaneous multi-element capability, high sensitivity and low detection limits.²¹ As most of spectrometric methods, ICP-OES generally requires conversion of the solid samples into a representative solution that is introduced by pneumatic nebulization into plasma. Conventional hot plate acid digestion and microwave-assisted acid digestion are the most used sample preparation methods for this purpose.^{22–25} Slurry nebulization, involving the direct aspiration of suspended sample directly into ICP-OES is a less commonly reported alternative.²⁶ Besides sample preparation, overcoming of

non-spectral effects coming from complex matrices such as dietary supplements seems to be a challenge even in ICP-OES analysis.

The aim of this study was to develop and characterize a simultaneous, free from non-spectral effects analytical method, for multielemental determination in complex matrices such as food supplements using ICP-OES. The method was characterized in terms of limit of detection (LOD), limit of quantification (LOQ), accuracy and precision. It was checked whether the simple dilution of sample is effective to remove the non-spectral effects in Mg, Ca, Cr, Mn, Fe, Cu and Zn determination for their control in multimineral/multivitamin preparation.

RESULTS AND DISCUSSION

Limit of detection, accuracy and non-spectral interference

The method performance in terms of LOD, LOQ and parameters of the calibration curves achieved in ICP-OES are summarized in Table 1. The LOD and LOQ values in dry mass were calculated considering the sample preparation protocol.

A good linearity over the calibration range with determination coefficients in the range 0.9978–0.9999 was obtained for all the determined elements. The average LODs were between 0.0009 mg g⁻¹ for Mn and 0.0037 mg g⁻¹ for Cu. Excepting Cr, LODs were better than those reported by Krejčova *et al.* in the analysis of multimineral/multivitamin preparations.²⁷

Table 1

Figures of merit of the ICP-OES method used in the multielemental analysis of multimineral/multivitamin preparations

Element	λ (nm)	Calibration range (mg L ⁻¹)	R ²	LOD (mg L ⁻¹)	LOD ^a (mg g ⁻¹)	LOQ ^b (mg g ⁻¹)
Mg	285.213	0–1.0	0.9993	0.032	0.0021	0.0063
Ca	422.673	0–1.0	0.9988	0.045	0.0030	0.0090
Cr	267.716	0–1.0	0.9998	0.017	0.0012	0.0036
Mn	260.569	0–1.0	0.9999	0.013	0.0009	0.0027
Fe	259.940	0–1.0	0.9997	0.021	0.0014	0.0042
Cu	324.754	0–1.0	0.9978	0.056	0.0037	0.0111
Zn	213.856	0–1.0	0.9980	0.054	0.0036	0.0108

^a LOD in dry sample corresponds to 1500 mg digested sample diluted to 100 mL

^b LOQ was calculated as 3xLOD

Table 3

Results (mg/tablet) for the element contents in multiminerals/multivitamin preparations by ICP-OES

Element	Sample Identification code	Labeled (mg/tablet)	Sample dilution			
			Undiluted		100-fold dilution	
			Found \pm CI (mg/tablet)	Recovery \pm RSD (%)	Found \pm CI (mg/tablet)	Recovery \pm RSD (%)
Mg	A	80	17 \pm 0.7	21 \pm 4	78 \pm 0.5	98 \pm 3
	B	-	-	-	-	-
	C	75	10.5 \pm 0.5	14 \pm 5	71 \pm 3.2	95 \pm 6
Ca	A	120	18 \pm 0.5	15 \pm 3	116 \pm 2.5	97 \pm 2
	B	-	2.7 \pm 0.04	-	-	-
	C	-	1.2 \pm 0.02	-	2 \pm 0.01	-
Mn	A	2	0.4 \pm 0.04	20 \pm 12	2.1 \pm 0.1	105 \pm 5
	B	-	0.02 \pm 0.01	-	0.1 \pm 0.01	-
	C	-	-	-	-	-
Fe	A	14	8 \pm 0.6	57 \pm 11	14 \pm 0.4	100 \pm 2
	B	9.9	8 \pm 0.9	81 \pm 2	9.7 \pm 0.3	98 \pm 3
	C	7	6.4 \pm 0.3	91 \pm 8	7.3 \pm 0.23	104 \pm 2
Cu	A	1	0.8 \pm 0.02	80 \pm 6	1.0 \pm 0.2	100 \pm 5
	B	-	-	-	-	-
	C	0.1	0.1 \pm 0.05	100 \pm 7	0.1 \pm 0.04	100 \pm 3
Zn	A	10	5.0 \pm 0.01	50 \pm 8	10 \pm 0.2	100 \pm 6
	B	10.6	5.1 \pm 0.04	48 \pm 11	10 \pm 0.3	94 \pm 4
	C	7.5	2.8 \pm 0.05	37 \pm 2	7.5 \pm 0.45	100 \pm 6

The results obtained for determination of elements in standard reference material SRM 3280 multivitamin/multielement tablets presented in Table 2 show good recovery in the range of 93–110% for individual tablets and mixed tablets analyzed in 100-fold and 1000-fold diluted digests, respectively. In both cases the *t*-test indicates no significant difference between the found and certified values ($t_{\text{calc},95\%}$ in the range 0–3.29). According to Association of Official Agricultural Chemists (AOAC), a method should provide recovery within the range 80–120% to be considered appropriate for the elemental determination.²⁸ In our case it was demonstrated that a simple dilution of sample was able to compensate for non-spectral matrix effects and allowed the accurate determination of Mg, Ca, Cr, Mn, Fe, Cu and Zn in multivitamin/multielement formulations by ICP-OES. Under these circumstances, it is not necessary to use the standard addition method or internal standard method as recommended by some authors in the case of complex matrices.²⁷ Precision expressed as relative standard deviation calculated from measurements on the standard reference material was in the range 1.3–9.4% in compliance with the AOAC guidelines.²⁸

Overall, the results obtained for the SRM analysis demonstrated that ICP-OES method is robust in terms of accuracy and precision for the multielemental analysis of dietary supplements

after a dilution of at least 100-fold of the digested sample.

Analysis of commercial multiminerals/multivitamin formulations

Results obtained for the analysis of individual tablets of different commercially multiminerals/multivitamin formulations and the declared composition are given in Table 3.

The concentration of the determined elements corresponded generally to the labeled content with average recovery in the range 94–105% and measurements precision expressed as relative standard deviation in the range 1.5–12.1%. It is worth mentioning that in some preparations elements not labeled by the manufacturer (Ca, Mn) were however found in small concentrations. Thus the ICP-OES method was found to be suitable for the determination of Mg, Ca, Cr, Mn, Fe, Cu and Zn in multiminerals/multivitamin dietary supplements.

EXPERIMENTAL

Reagents and standards

Chemicals used throughout the work were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany). Concentrated nitric acid (69%) and hydrogen peroxide (30%) were used for samples digestion. Deionized water was used throughout the work. ICP multielement

standard solution IV (CertiPUR IV, traceable to NIST, Merck, Darmstadt, Germany) (23 elements in diluted nitric acid) 1000 mg L⁻¹ was used for preparation of calibration standards in the range 0–1.0 mg L⁻¹ element by serial dilution with 2% (v/v) nitric acid. The standard reference material SRM 3280 Multivitamin/Multielement Tablets (National Institute of Standards and Technology - NIST, Canada) was used to check the accuracy of the method.

Samples preparation

Three brands of multimineral/multivitamin dietary supplements commercially available in drugstores in Cluj-Napoca town, Romania, were analyzed for total content of Mg, Ca, Cr, Mn, Fe, Cu and Zn. Each brand was assigned a code for identification (A, B and C). According to preparation labels, dietary supplements contain generally vitamins (A, B1, B2, B6, B12, C, D, E and K1), biotin, nicotinamide, pantothenic acid, folic acid, rutin, lutein, Ginseng extract, minerals (Ca, Mg, Fe, Cu, Zn, Mo, Se), coenzyme Q10, lecithin, pollen, saffron oil, wheat germ oil, anti-caking agents, dyes, emulsifiers and acidity correctors. The reproducibility in terms of element content was checked by analyzing individual tablets of SRM 3280. Two different sample preparation approaches were used. In the first case five tablets were randomly selected, accurately weighted, grounded and homogenized, and individually mineralized. In the second approach, five tablets from each bottle were randomly selected, grounded and homogenized. Amounts of 1.5 g homogenized powder accurately weighed (5 parallel samples) were subjected to microwave-assisted digestion in closed vessels using 15 mL of HNO₃ 69%, 3 mL of H₂O₂ and 25 mL demineralized water for such kind of samples. The digest was quantitatively transferred to a volumetric flask, made up to 100 mL with demineralized water, filtered and stored in polyethylene flask.

Instrumentation

The SPECTRO CIROS^{CCD} spectrometer (Spectro, Kleve, Germany) was used for the ICP-OES measurements. Instrumental details and operating conditions are summarized in Table 4. Determination of elements was based on external calibration using a 6-point linear calibration curve over the range 0–1.0 mg L⁻¹ element in 2% HNO₃. The emission

signals as peak heights were measured for 48 s integration time and background correction using the two points model approach. The most sensitive emission lines free of spectral interferences were selected.

Berghof MWS3+ (Berghof, Germany) high-pressure microwave digestion system was used for sample mineralization.

Method performance

The analytical performance of the ICP-OES method was evaluated in terms of the limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and matrix effect. LOD was calculated using the (3σ) criterion and parameters of the calibration curve, while LOQ was considered as 3xLOD. The standard deviation of background was assessed from 10 measurements of blank. The concentrations in solid sample were calculated taking into account the sample preparation protocol. The accuracy of the ICP-OES method was assessed in a recovery test by analyzing the multivitamin/multielement standard reference material SRM 3280 and found results were compared with certified values for 95% confidence level. The precision of the method expressed as relative standard deviation (RSD) was evaluated from three parallel measurements of three different brands of multimineral/multivitamin formulations. Accuracy and precision of the method were compared to values recommended by the Association of Official Agricultural Chemists (AOAC).²⁸

CONCLUSIONS

The study revealed that a simple 100-fold to 1000-fold sample dilution is effective for the determination of Mg, Ca, Cr, Mn, Fe, Cu and Zn in multimineral/multivitamin preparations by ICP-OES, and makes the analysis much easier. The ICP-OES method was characterized for limit of detection, accuracy and precision that fulfill the AOAC recommendations. The proposed ICP-OES method can be used for the control of multielemental content in multimineral/ multivitamin preparations.

Table 4

Working conditions for the SPECTRO CIROS^{CCD} ICP optical emission spectrometer

Component	Characteristics
Generator	Free-running 27.12 MHz operated at 1400 W
Plasma torch	Axial viewing, torch positioning (mm) X = - 3.9, Y = + 3.6, Z = + 2.6. Argon flow rates: Outer gas 12 L/min Intermediate gas 0.6 L/min Nebulizer gas 1 L/min
Sample introduction system	Four channels peristaltic pump, K2 cross-flow nebulizer, double pass Scott type spray chamber, 2 mL/min sample uptake rate
Polychromator	160 – 600 nm double grating Paschen-Runge mounting, chamber filled with Ar
Detector	22 CCDs
Data processing	Background correction: linear and square two points models, best SNR strategy, integration time 48 s and three successive measurements for each sample.
Analytical wavelengths	Mg, 285.213 nm; Ca, 422.673 nm; Cr, 267.716 nm; Mn, 260.569 nm; Fe 259.94 nm; Cu, 324.754 nm; Zn, 231.856 nm

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