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APPLICATION NOTE 43445

Analysis of trace elements in nutraceuticals in compliance with USP chapter <2232> *Elemental Contaminants in Dietary Supplements*

Authors

Sanja Asendorf,
Application Specialist,
Thermo Fisher Scientific,
Bremen, Germany

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Goal

To demonstrate the ability of the Thermo Scientific™ iCAP™ 7000 Plus Series ICP-OES to determine trace elements in nutraceutical products in compliance with the new United States Pharmacopeia (USP) chapter <2232> *Elemental Contaminants in Dietary Supplements*

Introduction

Nutraceuticals can be defined as a range of isolated nutrients, dietary supplements and herbal products, as well as functional food, promoting health benefits beyond the basic nutritional function. As this definition is very diverse, effective and harmonized global regulations for the monitoring of nutraceuticals are not yet in place.

In the USA nutraceuticals are monitored and regulated as dietary supplements under the Dietary Supplement Health and Education Act (DSHEA, 1994). Functional foods, however, do not have a specific definition or regulation and can be regulated as conventional foods, food additives, dietary supplements, drugs, medical foods or food for special dietary use, depending on the claimed health benefits on the product labels.

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European Union (EU) law relating to food neither offers a regulatory framework for functional foods nor for nutraceuticals. However, several EU directives are in place that cover one or the other product that is within the definition range of nutraceuticals. The EU Food Supplement Directive (FSD) 2002/46/EC, for example, was set up to approximate the laws relating to food supplements and lays down specific rules for vitamins and minerals used in food supplements. Another European Regulation (1925/2006) establishes common rules concerning the addition of vitamins, minerals and other substances to food.

Japan is the pioneer nation when it comes to the regulation of nutraceuticals and functional foods. Under the concept of Foods for Special Health Use (FOSHU), foods must be approved by the Ministry of Health and Welfare and can have the three functions: nutrition, sensory satisfaction and physiological improvements.

Some dietary supplements may not only be recognized as a nutraceutical but also be labeled as conforming to USP (United States Pharmacopoeia) or NF (National Formulary) standards. The USP general chapters <232> *Elemental Impurities – Limits* and <233> *Elemental Impurities – Procedures* will replace the current USP Chapter <231> *Heavy Metals* and specify the technique of Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) as one of the methods for analysis. Along with these two chapters, USP general chapter <2232> *Elemental Contaminants in Dietary Supplements* has been created to limit the amounts of elemental contaminants in finished dietary supplement dosage forms and concentrates on the four major elements of toxicological concern: arsenic, cadmium, mercury and lead. The permitted daily exposure limits (PDE; Table 1) are derived from the Provisional Tolerable Weekly Intake that is recommended by the Food and Agriculture Organization on the United Nations and World Health Organization (FAO/WHO). The latest versions of the chapters become official on December 1, 2015 and are supposed to be implemented by January 1, 2018. In this application note we demonstrate how to comply with USP chapter <2232> when analyzing a nutraceutical with the Thermo Scientific™ iCAP™ 7000 Plus Series ICP-OES.

Table 1. Permitted daily exposures (PDE) in dietary supplements according to USP chapter <2232>.

Element	PDE ($\mu g \cdot day^{-1}$)
Arsenic (inorganic)	15
Cadmium	5
Lead	5
Mercury (total)	15

Instrumentation

For the sample analysis, the Thermo Scientific iCAP 7400 ICP-OES Duo was used together with an aqueous sample introduction kit, and an internal standard kit for online addition of the internal standard. The duo configuration was chosen for its ability to detect trace elements in axial view and major components in radial view. A Teledyne CETAC ASX-560 Autosampler was used to transfer the sample to the introduction system of the ICP-OES. The Thermo Scientific Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software simplifies method development and provides easy options for post-analysis data manipulation.

Materials and methods

All solutions were prepared from single element solutions (1000 $mg \cdot kg^{-1}$, SPEX CertiPrep Group, Metuchen, US). The individual solutions were made up with ultra-pure water (18 MΩ) and nitric and hydrochloric acids (TraceMetal Grade, Fisher Chemical, Loughborough, UK) to a final concentration of 8.3% HNO_3 and 1.2% HCl. All spike solutions and an internal standard solution of yttrium (10 $mg \cdot kg^{-1}$) were prepared in the same way. To validate the developed method for use in compliance with USP chapter <2232> the standard reference material (SRM) 3280 – Multivitamin Tablets (NIST, Gaithersburg, MD, USA) was used as a Standard Reference Material. The concentrations for the elements of interest at the *Target Limit*, diluted accordingly to the dilution of the material in sample solution (also referred to as *J* value) were calculated with the maximum daily dose of 1 tablet (1.5 g) and are displayed in Table 2.

Table 2. *J* value for the *Target Elements* (concentrations at the *Target Lim*it in $\mu g \cdot kg^{-1}$).

Element(s)	*J*
Cd, Pb	33
As, Hg	100

Sample preparation

To ensure a homogenous sample, 15 multivitamin tablets were ground and this powder was dried (80 °C for 12 hours). For the determination of accuracy, three replicates of sample were spiked at 0.5 *J* and 1.5 *J*, respectively, and to demonstrate repeatability six samples were spiked at 1 *J* before digestion. For the digestion, the ground multivitamin tablets powder was weighed (~0.5 g) into a PTFE high pressure vessel and 6.4 mL HNO_3 as well as 1.6 mL HCl were added. If material adhered to the walls of the vessel, it was washed down carefully with the acid. The digestion was conducted in a Milestone Ethos EZ microwave equipped with an SK-10 segmented rotor and a temperature sensor according to the protocol in Figure 1. After digestion the digest was transferred to a 50 mL volumetric flask. The digestion vessels were rinsed with ultra-pure (18 MΩ) water and after transferring this to the flask as well, it was filled up with ultra-pure water to the measured mark. Additionally, for each digestion cycle a duplicate of a digestion blank was run. This contained only acids and after digestion was treated the same way as the samples.

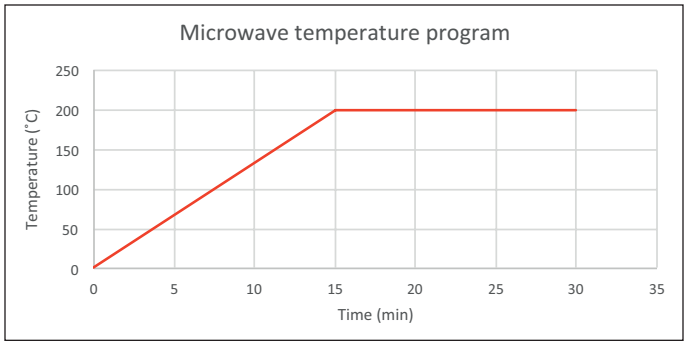

Figure 1. Temperature program of the digestion.

Method development

A method was created in Qtegra ISDS Software. The wavelengths used for the analysis are shown in Table 7, these were selected as they were free from interferences and provided the sensitivity to quantify the elements of interest in the expected concentration range. The wavelengths of the internal standard yttrium were applied according to the plasma view and wavelength range (UV or Vis). The parameters used for the method can be found in Table 3. The plasma was ignited and the instrument allowed to warm up for a period of 15 minutes. A spectrometer optimization was performed directly before each analysis.

Table 3. Instrument parameters.

Parameter	Setting	
Pump Tubing (Standard Pump)	Sample Tygon® orange/white Drain Tygon® white/white Internal standard Tygon® orange/blue	
Pump Speed	50 rpm	
Spray Chamber	Glass cyclonic	
Nebulizer	Glass concentric	
Nebulizer Gas Flow	0.55 L·min^{-1}	
Coolant Gas Flow	12 L·min^{-1}	
Auxiliary Gas Flow	0.5 L·min^{-1}	
Center Tube	2 mm	
RF Power	1150 W	
Plasma View	Axial	Radial
Exposure Time	UV 15 s, Vis 5 s	UV 15 s, Vis 5 s

Following method development, the instrument was calibrated and the samples analyzed. A method detection limit study was carried out by analyzing a digestion blank with ten replicates and multiplying the standard deviation of this analysis by three. This was repeated three times and the average values for detection limits were calculated.

Validation of the procedure

In order to validate the used method, the tests defined in USP chapter <233> under “Alternate Procedure Validation” – “Quantitative Procedures” were conducted.

Accuracy

For the accuracy test, the instrument was calibrated with standard solutions containing 0.5 *J* and 1.5 *J* of the *Target Elements*. Six samples were spiked before the microwave digestion with three times each, 0.5 *J* and 1.5 *J* of each *Target Element*. According to the acceptance criteria, spike recoveries of the mean of the three replicates recoveries have to be within 70-150% at each concentration. As the recoveries were within 72-96% (Table 4) the acceptance criterion for accuracy of the method is fulfilled. Moreover, limit of quantification, range and linearity are demonstrated to be suitable by meeting the accuracy requirements.

Table 4. Average measured concentrations of the *Target Elements* of three spiked sample solutions at each, 0.5 *J* and 1.5 *J* and percentage recoveries. Concentrations in µg·kg^{-1}.

Element and wavelength (nm)	Plasma view	0.5 *J* Spike			1.5 *J* Spike		
		Average measured concentration	Spiked concentration	Recovery (%)	Average measured concentration	Spiked concentration	Recovery (%)
As 189.042	Axial	46.39	50.00	92.8	142.32	150.00	94.9
Cd 228.802	Axial	16.03	16.67	96.2	46.56	50.00	93.1
Hg 184.950	Axial	43.35	50.00	86.7	133.51	150.00	89.0
Pb 220.353	Axial	13.45	16.67	80.7	36.05	50.00	72.1

Precision

Precision was tested by means of repeatability and ruggedness of the method. For the repeatability test, six independent samples of material under test were spiked at a concentration of *1* J for each *Target Element* before the microwave digestion. The acceptance criterion in USP chapter <233> states a relative standard deviation (RSD) of not more than 20% between the repeats for each *Target Element*. The calculated RSDs are clearly in the required range, varying between 0.4-2.3% (Table 5).

Ruggedness of the method was determined by performing the repeatability experiment on three different days. The total RSD of the repeated analysis (n=18) was 0.8-3.2% (Table 6) and is therefore clearly below the acceptance criterion of not more than 25% RSD for each *Target Element*.

Table 5. RSD of the *Target Elements* in percent for six sample solutions, spiked at 1 *J*. Concentrations in µg·kg^{-1}.

	As 189.042	Cd 228.802	Hg 184.950	Pb 220.353
1	106.6	35.16	103.4	28.78
2	103.9	35.17	102.9	30.09
3	108.9	35.76	102.2	28.62
4	107.9	35.02	103.0	28.28
5	109.7	35.54	103.0	28.76
6	107.0	35.22	103.1	28.28
RSD (%)	1.9	0.8	0.4	2.3

Table 6. RSD of the *Target Elements* in percent for six sample solutions, spiked at 1 *J* and measured on three different days (n=18). Concentrations in µg·kg^{-1}.

	As 189.042	Cd 228.802	Hg 184.950	Pb 220.353
RSD (%)	1.2	0.9	0.8	3.2

Specificity

According to USP chapter <233> specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, including other *Target Elements* and matrix components. To ensure the identity of the analyte, several wavelengths for each element were analyzed and the subarrays examined carefully for any interferences. As the accuracy and repeatability tests show appropriate results the specificity of the method is verified.

Results

As all the requirements for method validation were met, the SRM 3280 sample was analyzed for its major and minor components as referenced in the NIST Certificate of Analysis, including the *Target Elements* of USP chapter <2232>. Minor components (< 1% in undigested material; As, B, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Se) were analyzed directly after digestion, major components (> 1% in undigested material; Ca, Fe, K, Mg, P, Zn) were diluted with a factor of 100 to get the sample concentration into the linear range of the instrument. The instrument was calibrated with standard solutions at a concentration of 0.5 *J* and 2 *J* and a drift sample at the concentration of 2 *J* was analyzed before and after sample analysis. The system suitability criterion in USP chapter <233> requires a drift of not more than 20% which is met by the exhibited drift sample recoveries of 98.2-102.1% for all sample analyses.

Table 7. Measured concentrations in the SRM 3280 – Multivitamin Tablets, compared to the certified concentration, and limits of detection and quantification for the elements of interest.

Element and wavelength (nm)	Plasma view	Certified concentration ($mg \cdot kg^{-1}$)	Measured concentration ($mg \cdot kg^{-1}$)	Limit of detection ($\mu g \cdot kg^{-1}$)	Limit of quantification ($\mu g \cdot kg^{-1}$)
As 189.042	Axial	0.132 ± 0.044	< LOD	2.0	6.5
B 249.678	Axial	141 ± 7	141 ± 8.2	2.5	8.2
Ca 317.933	Radial	110700 ± 5300	113348 ± 20	5.9	20
Cd 228.802	Axial	0.08015 ± 0.00086	< LOQ	0.3	0.8
Cr 283.563	Axial	93.7 ± 2.7	93.6 ± 1.8	0.5	1.8
Cu 324.754	Axial	1400 ± 170	1313 ± 2.9	0.9	2.9
Fe 238.204	Axial	12350 ± 910	12750 ± 2.6	0.8	2.6
Hg 184.950	Axial	Not determined	< LOD	1.1	3.7
K 766.490	Radial	53100 ± 7000	56566 ± 1.1	84	281
Mg 280.270	Radial	67800 ± 4000	70073 ± 1.1	0.3	1.1
Mn 260.569	Axial	1440 ± 110	1423 ± 6.5	0.3	1.1
Mo 281.615	Axial	70.7 ± 4.5	67.0 ± 2.7	1.9	6.5
Ni 216.556	Axial	8.34 ± 0.3	8.23 ± 12	0.8	2.7
P 178.284	Axial	75700 ± 3200	75410 ± 6.6	3.7	12
Pb 220.353	Axial	0.2727 ± 0.0024	< LOQ	2.0	6.6
Se 203.985	Axial	17.42 ± 0.45	16.24 ± 0.9	0.9	3.1
Zn 206.200	Axial	10150 ± 810	10288 ± 6.5	0.3	0.9

LOD = Limit of detection, LOQ = Limit of quantification

Except for selenium, which is 3% below the certified range, all elements are inside the range of the certified concentrations for the reference material which demonstrates perfect accuracy of the analytical and the microwave digestion method. As selenium has many volatile components, some of these may have been lost with the vapors that escaped during opening the microwave digestion vessels. These vapors would still escape even after the finished digest had cooled to room temperature overnight. The detection limits are in the single digit to sub-ppb range for all elements except K which displays a value of 84 ppb and results from a strong argon background emission in the high visible region of the spectrum. The limits of quantification of the *Target Elements* are between one and two orders of magnitude lower than the *J* value. This indicates the suitability of the method to determine the *Target Elements* quantitatively at the *Target Limit*.

The concentrations of the *Target Elements* in the sample solution are below the quantification limits and can therefore not be quantified correctly. For the quantitative analysis of the *Target Elements* in this reference material an alternative technique like Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) should be applied, such as delivered by the Thermo Scientific™ iCAP™ RQ ICP-MS. However, according to USP <2232> requirements, this nutraceutical shows concentrations below the permitted daily exposure limit and can be considered as safe for human intake.

Conclusion

The analysis shows that the Thermo Scientific iCAP 7000 Plus Series ICP-OES delivers excellent accuracy and sensitivity for analyses of trace elements and major components in nutraceuticals in conformity with the present USP chapter <2232> about *Elemental Contaminants in Dietary Supplements*. The detection limits obtained prove the excellent ability of the instrument of handling complex acidic matrices. Moreover, the good spike recoveries indicate that no *Target Elements* are lost during the sample preparation demonstrating this microwave digestion technique is an excellent choice for sample preparation.

Find out more at **thermofisher.com/ICP-OES**

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