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APPLICATION NOTE 44385

Analysis of elemental impurities in drug products using the Thermo Scientific iCAP 7400 ICP-OES Duo

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Goal

Trace elemental impurities in pharmaceutical products are potentially harmful and thus their determination is of great importance. The work described here demonstrates compliance with 21 Code of Federal Regulation (CFR) Part 11 and analysis according to latest implementation of United States Pharmacopeia (USP) General Chapters <232> and <233>.

Introduction

Impurities in pharmaceutical products are of great concern not only due to the inherent toxicity of certain contaminants, but also due to the adverse effects that contaminants may have on drug stability and shelf-life. This necessitates the monitoring of organic and inorganic impurities throughout the pharmaceutical manufacturing process, from raw ingredients to final products.

Recently, USP announced measures to modernize (and replace) the USP General Chapter for Heavy Metals <231> by proposing two new General Chapters:

<232> *Elemental Impurities – Limits* (1)

<233> *Elemental Impurities – Procedures* (2)

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The rationale behind introducing the new chapters was to provide a modern equivalent to USP General Chapter <231>, which is based on a more than one hundred-year-old colorimetric test ('heavy metals test') involving the precipitation of ten sulfide-forming elements and visually comparing the color of the resulting precipitate to that of a 10 $mg \cdot kg^{-1}$ lead standard. There are several known deficiencies with the method including: the inability to differentiate between the levels of individual contaminants, use of potentially hazardous solvents such as thioacetamide and the use of a furnace during the preparation of certain samples, which results in significant loss of volatile contaminants such as tin and mercury.

USP General Chapter <232> sets out the permissible levels of twenty-four elements in final drug products according to limits established in ICH guideline Q3D (3). The guideline uses toxicological data to set the limits, which are then expressed in terms of a permissible daily exposure (PDE) limit. The route of administration (oral, parenteral, or inhalation) is taken into account when setting the PDE, with orally administered drugs having a higher permissible limit than drugs that are delivered parenterally or by inhalation. Elements included in the chapter have been placed into three classes, based on their toxicity and likelihood of occurrence in the drug product.
The classification scheme is intended to focus the risk assessment on those elements that are the most toxic but also have a reasonable probability of inclusion in the drug product. For element PDEs and classification, see Table 1.

Table 1. PDE limits for elemental impurities in drug products.

Element	Element symbol	Class	Oral PDE ($\mu g \cdot day^{-1}$)	Parenteral PDE ($\mu g \cdot day^{-1}$)	Inhalation PDE ($\mu g \cdot day^{-1}$)
Cadmium	Cd	1	5	2	2
Lead	Pb	1	5	5	5
Arsenic	As	1	15	15	2
Mercury	Hg	1	30	3	1
Cobalt	Co	2A	50	5	3
Vanadium	V	2A	100	10	1
Nickel	Ni	2A	200	20	5
Thallium	Tl	2B	8	8	8
Gold	Au	2B	100	100	1
Palladium	Pd	2B	100	10	1
Iridium	Ir	2B	100	10	1
Osmium	Os	2B	100	10	1
Rhodium	Rh	2B	100	10	1
Ruthenium	Ru	2B	100	10	1
Selenium	Se	2B	150	80	130
Silver	Ag	2B	150	10	7
Platinum	Pt	2B	100	10	1
Lithium	Li	3	550	250	25
Antimony	Sb	3	1200	90	20
Barium	Ba	3	1400	700	300
Molybdenum	Mo	3	3000	1500	10
Copper	Cu	3	3000	300	30
Tin	Sn	3	6000	600	60
Chromium	Cr	3	11000	1100	3

USP General Chapter <233> deals with sample preparation strategies and analytical detection techniques to measure the elements defined in chapter <232>. This includes the choice between two ICP-based technologies (ICP-OES and ICP-MS), as well as a protocol to establish alternative test procedures.

The official date for implementation of the new chapters was January 1, 2018 and marks the date on which <232> and <233> are applicable to drug product producers. A further consequence of the implementation process for General Chapters <232> and <233> is the complete removal of USP General Chapter <231> *Heavy Metals* from the compendia on January 1, 2018. Past January 1, 2018 chapter <231> will no longer be valid and testing must instead conform to the limits set out in chapter <232>, using the procedures set out in chapter <233> (analysis by ICP-OES, ICP-MS or an acceptable alternative procedure).

In future all drug products produced and sold in the U.S. must comply with the limits set by USP General Chapter <232>. Drug substances and excipients will be tested and reported for elemental impurities. Similarly, nutraceutical products must comply with the limits set by USP General Chapter <2232> (4), which extends only to arsenic, mercury, cadmium and lead. Speciation of organic and inorganic elemental forms is critical for the analysis of Dietary Supplements.

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. A Teledyne CETAC Technologies ASX-560 Autosampler was used to transfer the sample to the introduction system of the ICP-OES.

The iCAP 7400 ICP-OES Duo is well suited to this type of application due to its low detection capabilities for the elements of interest, as well as for its ability to resolve complex spectra. Both of these points are critical in relation to the low limits stipulated for elements such as arsenic and mercury. In addition, elements such as palladium, platinum, osmium and iridium produce many emission lines when excited in the plasma, which need to be resolved effectively to avoid spectral interferences. The Thermo Scientific™ iCAP™ 7000 Series Qualification Kit and Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software were used to ensure that the analysis can meet the requirements of the FDA 21 CFR Part 11 regulations relating to the use and control of electronic records (5).

Standard and sample preparation

Standards and spikes

Two standard stock solutions with different element compositions were prepared from single element solutions (1000 $mg \cdot kg^{-1}$ and 10000 $mg \cdot kg^{-1}$, SPEX CertiPrep Group, Metuchen, USA). Stock solution A contained Ag, As, Ba, Cd, Co, Cu, Cr, Hg, Li, Mo, Ni, Pb, Sb, Se, Sn, Tl and V whereas stock solution B contained Au, Ir, Os, Pd, Pt, Rh and Ru. The individual solutions were made up with ultrapure water (18 MΩ) and hydrochloric acid (TraceMetal™ Grade, Fisher Chemical, Loughborough, UK) to a final concentration of 5% HCl. All spike solutions and an internal standard solution of yttrium (5 $mg \cdot kg^{-1}$) were prepared in the same way. To stabilize mercury in stock solution A, 10 $mg \cdot kg^{-1}$ gold was added to it. As a calibration blank 5% HCl was used. Two sets of standardization solutions and sample spike solutions were prepared, one for elements contained in stock solution A and one for those in stock solution B.

Samples

To validate the developed method for use in compliance with USP General Chapter <233> a cough medicine from a local pharmacy (acetylcysteine, ACC 600) in the form of an effervescent tablet was analyzed. One gram of the tablet was diluted in a few mL of ultrapure water (18 MΩ) and the developing CO_2 was allowed to degas. After the reaction had subsided, the aliquot was acidified to a final concentration of 5% HCl, spiked accordingly for the various tests of the validation procedure and filled up with ultrapure water to a final volume of 50 mL.

Target Elements and calculation of the J value

Elements with the potential of being present in the material under test are called Target Elements within USP General Chapters <232> and <233>. In any case, arsenic, cadmium, lead, and mercury have to be included in the Target Element evaluation when testing is done to demonstrate compliance. Target Elements should also include any elements that may be added through material processing or storage.

The accepted concentration value for the elemental impurity being evaluated is called the Target Limit. Exceeding the Target Limit indicates that a material under test exceeds the acceptable value. To calculate the Target Limit, the permissible daily exposure (PDE) limit is divided by the maximum daily serving size or maximum daily dose (MDD). Due to sample preparation techniques and different working ranges of the specified instrumentation, the concentration of the elements of interest at the Target Limit has to be calculated including the dilution factor. This concentration is known as the J value:

$$J\ Value = \frac{PDE}{MDD \cdot Dilution\ factor}$$

With an MDD of 2 gram per day and a dilution factor of 50, the J value concentrations were calculated for the cough medicine according to Table 2.

Table 2. J value for ACC effervescent tablets with MDD of 2 gram per day and a dilution of 50x.

Target Element	Concentration ($mg \cdot kg^{-1}$)	Target Element	Concentration ($mg \cdot kg^{-1}$)
Cd	0.05	Rh	1
Pb	0.05	Ru	1
As	0.15	Se	1.5
Hg	0.3	Ag	1.5
Co	0.5	Pt	1
V	1	Li	5.5
Ni	2	Sb	12
Tl	0.08	Ba	14
Au	1	Mo	30
Pd	1	Cu	30
Ir	1	Sn	60
Os	1	Cr	110

Method development

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	
Analysis Pump Speed	50 rpm	
Spray Chamber	Glass Cyclonic	
Nebulizer	Burgener Mira Mist	
Nebulizer Gas Flow	0.5 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	Vis 5 s

A method was created in Qtegra ISDS Software. The wavelengths used for the analysis are shown in Table 4. These were selected as they were mostly free from interferences and provided the sensitivity to quantify the elements of interest in the expected concentration range. The observed interferences were corrected for by inter-element corrections (IEC) which were set up in the software. The wavelengths of the internal standard yttrium were applied according to the plasma view and wavelength range (UV or Vis).

Table 4. Analyte, internal standard wavelengths and view as well as interfering elements and correlation coefficient of the calibration curve (R^2).

Element and wavelength (nm)	View	Internal standard wavelength (nm)	Interfering elements	R^2
Cd 226.502	Axial	Y 224.306		1.0000
Pb 182.205	Axial	Y 224.306		0.9992
As 189.042	Axial	Y 224.306		1.0000
Hg 184.950	Axial	Y 224.306		0.9999
Co 228.616	Axial	Y 224.306		1.0000
V 309.311	Axial	Y 324.228		1.0000
Ni 221.647	Axial	Y 224.306		1.0000
Tl 190.856	Axial	Y 224.306		1.0000
Au 267.595	Axial	Y 324.228		0.9994
Pd 324.270	Axial	Y 324.228		0.9995
Ir 215.268	Axial	Y 224.306		0.9996
Os 228.226	Axial	Y 224.306	Mo	0.9995
Rh 343.489	Axial	Y 360.073		0.9989
Ru 266.161	Axial	Y 324.228	Cr	0.9994
Se 206.279	Axial	Y 224.306		1.0000
Ag 328.068	Axial	Y 324.228		1.0000
Pt 203.646	Axial	Y 224.306		0.9995
Li 610.362	Radial	Y 371.030	Na	0.9994
Sb 217.581	Axial	Y 224.306		1.0000
Ba 455.403	Radial	Y 371.030		0.9997
Mo 284.823	Radial	Y 324.228		0.9989
Cu 224.700	Radial	Y 360.073		1.0000
Sn 226.891	Axial	Y 224.306		1.0000
Cr 357.869	Radial	Y 360.073		0.9991

Table 5. Average recoveries (in percentages) of 3 replicate sample spikes at each 0.5 J and 1.5 J demonstrating accuracy of the method.

Element	Average spike recovery 0.5 J (n=3)	Average spike recovery 1.5 J (n=3)
Cd	96	97
Pb	94	94
As	97	103
Hg	93	97
Co	96	98
V	100	99
Ni	95	97
Tl	86	94
Au	100	100
Pd	98	99
Ir	98	99
Os	100	101
Rh	101	102
Ru	94	96
Se	106	106
Ag	94	100
Pt	98	98
Li	102	98
Sb	97	100
Ba	101	98
Mo	100	100
Cu	109	94
Sn	94	97
Cr	101	98

Validation procedure

In order to validate the used method, the tests defined in USP General 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 with three times each, 0.5 J and 1.5 J of all Target Elements. According to the acceptance criteria, the mean recovery of the three replicates has to be within 70-150% at each concentration. As the recoveries were within 86-109% (Table 5) 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.

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. The acceptance criterion in USP General Chapter <233> states a relative standard deviation (RSD) of not more than (NMT) 20% between the repeats for each Target Element. The calculated RSDs are clearly in the required range, varying between 0.7-2.5% (Table 6).

Ruggedness of the method was determined by performing the repeatability experiment on two different days. The total RSD of the repeated analysis (n=12) was 1.5-6.0% (Table 6) and is therefore clearly below the acceptance criterion of NMT 25% RSD for each Target Element.

Table 6. RSDs of 6 as well as 12 replicate sample spikes at 1 J demonstrating repeatability and ruggedness of the method.

Element	RSD (n=6) NMT 20%	RSD (n=12) NMT 25%
Cd	1.7	1.6
Pb	2.2	6.0
As	2.5	2.1
Hg	2.2	2.2
Co	1.7	1.7
V	1.2	1.8
Ni	1.7	1.7
Tl	2.1	2.5
Au	1.0	1.7
Pd	1.2	1.9
Ir	0.9	1.7
Os	0.7	1.6
Rh	0.9	1.7
Ru	1.3	1.7
Se	1.6	1.9
Ag	1.4	2.1
Pt	0.8	1.8
Li	2.5	5.2
Sb	2.0	1.7
Ba	2.4	2.5
Mo	1.8	1.5
Cu	2.1	2.6
Sn	1.8	1.7
Cr	2.1	1.7

Specificity

According to USP General Chapter <233>, specificity is the ability to assess the analyte unequivocally 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, two wavelengths for each element were analyzed and the subarrays examined carefully for any interferences. As the accuracy and precision tests show appropriate results within the defined ranges, specificity of the method is verified (see Table 7).

Table 7. Accuracy (shown as average spike recovery) and precision (RSD) results for the analysis of six replicate sample spikes at 1 J. All results are in percentages.

Element and wavelength (nm)	Accuracy (Recovery, n=6)	Precision (RSD, n=6)	Element and wavelength (nm)	Accuracy (Recovery, n=6)	Precision (RSD, n=6)
Cd 226.502	95.7	1.7	Rh 343.489	102.0	0.9
Cd 214.438	96.1	1.6	Rh 339.682	102.7	0.9
Pb 220.353	92.5	4.7	Ru 267.876	95.6	1.5
Pb 182.205	99.0	2.2	Ru 266.161	95.1	1.3
As 189.042	100.3	2.5	Se 196.090	105.9	2.0
As 228.812	100.9	2.8	Se 206.279	105.0	1.6
Hg 184.950	93.6	2.2	Ag 328.068	97.6	1.4
Hg 194.227	93.2	1.9	Ag 338.289	92.8	1.8
Co 228.616	95.8	1.7	Pt 203.646	97.8	0.8
Co 238.892	94.0	0.8	Pt 214.423	97.1	0.8
V 310.230	98.1	1.1	Li 610.362	93.7	2.5
V 309.311	98.6	1.2	Li 670.784	108.7	2.4
Ni 231.604	95.6	1.7	Sb 206.833	97.0	2.1
Ni 221.647	95.2	1.7	Sb 217.581	96.8	2.0
Tl 190.856	92.1	2.1	Ba 455.403	98.1	2.4
Au 242.795	98.9	1.0	Ba 493.409	94.7	3.1
Au 267.595	99.7	1.0	Mo 281.615	97.3	1.9
Pd 324.270	99.4	1.2	Mo 284.823	98.1	1.8
Pd 340.458	101.3	1.0	Cu 219.958	98.4	2.2
Ir 212.681	99.5	0.9	Cu 224.700	98.7	2.1
Ir 215.268	99.3	0.9	Sn 226.891	94.2	1.8
Os 228.226	100.1	0.7	Sn 283.999	94.7	1.9
Os 225.585	99.5	0.6	Cr 284.325	96.1	1.9
			Cr 357.869	97.2	2.1

Results

All requirements for method validation were met by analyzing a series of unspiked and spiked samples at different multiples of the J value. To show system suitability of the indicated *Procedure 1: ICP-OES*, the instrument was calibrated with a blank and two standardization solutions:

- Blank – Matched matrix: 5% HCl
- Standardization solution 1 - 1.5 J in 5% HCl
- Standardization solution 2 - 0.5 J in 5% HCl

The results obtained from standardization solution 1 before and after the analysis of the sample solutions were compared. The suitability criterion of NMT 20% drift between analyses was met according to Table 8.

Table 8. Analysis results for the determination of system suitability (drift).

Element	Before ($mg \cdot kg^{-1}$)	After ($mg \cdot kg^{-1}$)	RSD (%)
Cd	0.075	0.077	1.5
Pb	0.075	0.078	2.7
As	0.225	0.238	3.9
Hg	0.453	0.464	1.7
Co	0.753	0.773	1.9
V	1.50	1.52	0.7
Ni	3.01	3.08	1.5
Tl	0.120	0.124	2.3
Au	1.48	1.48	0.2
Pd	1.48	1.47	0.7
Ir	1.48	1.49	0.2
Os	1.48	1.48	0.1
Rh	1.47	1.46	0.3
Ru	1.48	1.48	0.0
Se	2.25	2.31	2.0
Ag	2.25	2.24	0.3
Pt	1.48	1.48	0.1
Li	8.37	8.09	2.5
Sb	18.1	18.4	1.4
Ba	21.1	21.0	0.5
Mo	45.9	45.6	0.5
Cu	45.0	44.3	1.1
Sn	90.2	92.3	1.6
Cr	168	163	1.9

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 drug products in conformity with the present USP General Chapters <232> *Elemental Impurities – Limits* and <233> *Elemental Impurities - Procedures*. The results obtained prove the excellent ability of the instrument to resolve complex sample spectra, and the achieved detection limits demonstrate the suitability of the instrument to analyze toxic trace elements like arsenic and mercury for which the stipulated limits are very low.

References

1. <232> Elemental Impurities—Limits, USP 40-NF 35, First Supplement
2. <233> Elemental Impurities—Procedures, USP 38-NF 33, Second Supplement
3. <2232> Elemental Contaminants in Dietary Supplements <2232>, USP 38-NF 33, Second Supplement
4. ICH Q3D Impurities: Guideline for Elemental Impurities, Current Step 4 Version (2014)
5. 21 C.F.R. 11 Revised as of April 1, 2017

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