



Analysis of pharmaceutical products for their elemental impurities with the Thermo Scientific iCAP RQ ICP-MS

Authors

Julian Wills and Daniel Kutscher
Thermo Fisher Scientific,
Bremen, Germany

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Goal

To demonstrate the use of the Thermo Scientific™ iCAP™ RQ ICP-MS to accurately determine concentrations of elemental impurities in pharmaceutical products brought into solution using microwave digestion. All sample preparation, measurement and data evaluation to be compatible with the guidelines defined in USP chapters <232> Elemental Impurities – Limits and <233> Elemental Impurities – Procedures.

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. United States Pharmacopeia (USP) General Chapter <231>, introduced in 1905, is a colorimetric test involving the co-precipitation of ten sulfide-forming elements and a visual color comparison to a 10 ppm lead standard. The limitations of this test are well understood (non-specificity, the test is based on limited understanding of trace metal toxicity, etc.) so that consequently the USP published two new general chapters to replace <231> starting January 1st, 2018.

- Chapter <232> Elemental Impurities¹ – Limits; defines the maximum limits of fifteen elements in pharmaceutical products
- Chapter <233> Elemental Impurities² – Procedures; defines how the testing for these elements should be performed.

From that date onward, all elemental impurity testing and all elemental impurity testing must instead conform to the limits set out in Chapter <232>, using the procedures set out in Chapter <233>.

In addition to the requirements described in the USP documents, any analytical system used for the creation of analysis data for pharmaceuticals must also comply with the US Food and Drug Administration's (FDA) 21 CFR Part 11 regulations regarding electronic records and validation of electronic signatures. These regulations are concerned with ensuring the integrity and authenticity of any electronic records and electronic signatures that 'persons create, modify, maintain, archive, retrieve or transmit'³. Control software used by analytical instruments in pharmaceutical production must therefore incorporate tools to maintain the integrity of the analytical method and subsequent results. In order to provide a transparent pathway to data generation, the control software should include support for audit trails and electronic signatures as well as security features to ensure that alterations cannot be made without clear indication of what has been changed, who changed it and why.

This note describes the effective application of the Thermo Scientific™ iCAP™ RQ single quadrupole (SQ) ICP-MS, to the detection and quantification of the 15 target elements specified in USP <232>, in accordance with the ICP-MS procedures described in USP <233>. In order to generate data compliant with the procedures described in 21 CFR Part 11, the Thermo Scientific Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software includes comprehensive features for the pharmaceutical industry, such as user access levels, audit trails, support for electronic signatures as well as integrated, secure data management.

Sample preparation

It has been demonstrated that direct aqueous dissolution is suited for the preparation of water soluble pharmaceutical samples before subsequent USP <233> compliant ICP-MS analysis. Indirect dissolution via closed vessel microwave digestion, however, is recognized as the most universal sample preparation method for materials for subsequent elemental analysis by ICP-MS. An important advantage of the closed vessel microwave approach is the retention of volatile elements, in particular mercury that might otherwise be lost.

Three pharmaceutical products were selected for analysis as part of this study:

Drug A: a phytotherapeutic (herbal) medicine

Drug B: a vascular medicine

Drug C: an antianxiety medicine

All three drugs were brought into solution via a microwave digestion procedure using an UltraWAVE closed vessel microwave digestion system (Milestone Inc., Shelton, CT, USA). Different microwave recipes are available to address specific sample matrices making this the most universal method of sample preparation for subsequent elemental analysis.

Samples of each drug (0.5 g) were weighed into 15 ml disposable glass vials. For Drugs A and B, 3 ml of HNO₃ was added to each tube. For Drug C, 2 mL of HNO₃ and 1 mL of H₂SO₄ was added to each vial. In compliance with the repeatability requirements defined in USP <233>, six separate preparations of each material were prepared.

Sample vials were transferred into the microwave digestion system which was then closed, pressurized with nitrogen at 40 bar and the temperature program shown in Table 1 was launched. High pressure digestions are recommended due to the use of lower temperatures minimizing the loss of volatile elements.

Table 1. Closed vessel microwave temperature program used for the dissolution of pharmaceutical products.

Step	Time (min)	Temperature (°C)	Power (W)
1	15	200	1500
2	10	200	1500

When sufficiently cooled, the clear, colorless digested material was transferred to polypropylene vials and made up to 50 ml with ultrapure water. Each sample was then diluted by a factor of five into 15 ml polypropylene autosampler vials in a matrix of 1.2% HNO₃ and 0.5% HCl + 200 µg·L⁻¹ of gold to give a total dilution factor of 500 from the original solid sample. This diluent was used to ensure stability of the target elements in solution and efficient washout of these elements between samples from the sample introduction system.

The samples were measured using an external calibration approach against calibration solutions prepared in the same diluent as the samples. The calibration solutions contained all of the elements listed under the Oral daily dose PDE (in µg·g⁻¹) in USP <232>. Internal standardization was applied, using Ga, In and Tl internal standards at 5, 10 and 10 µg·L⁻¹ respectively, added online via a T-piece.

Calibration solution preparation

Sample analyses were carried out in accordance with the requirements described in USP <233> Elemental Impurities – Procedures. This document specifies that the elements to be measured should be calibrated against standard solutions at concentrations of blank, 0.5J and 2J where J = the concentration (w/w) of the element(s) of interest at the target limit, appropriately diluted to the working range of the instrument².

Target limits for each of the USP <232> controlled elements were calculated by dividing the permitted daily exposure based on a 50 kg person (PDE) by the maximum daily dose. For the three drugs used in this work, the maximum daily dose is 10 g.

Table 2. Target limits (J) for the fourteen elements specified in USP <232>.

Element	Oral daily dose PDE* ($\mu\text{g}\cdot\text{day}^{-1}$)	Target limit J ($\mu\text{g}\cdot\text{g}^{-1}$)
Cadmium	5	0.5
Lead	5	0.5
Inorganic arsenic	15	1.5
Inorganic mercury	30	3
Iridium	100	10
Osmium	100	10
Palladium	100	10
Platinum	100	10
Rhodium	100	10
Ruthenium	100	10
Chromium	11000	1100
Molybdenum	3000	300
Nickel	200	50
Vanadium	100	20
Copper	3000	300

* PDE = permitted daily exposure based on a 50 kg person

With this target limit taken into account, and as the samples were diluted by a factor of 500 from the original sample, two multielemental calibration solutions were prepared at the concentration levels 0.5J and 2J in 2% HNO_3 .

Results

Calibration Curves

Linear calibrations with low (sub $\text{ng}\cdot\text{g}^{-1}$) blanks were obtained for all elements. Example calibration lines for the ‘big four’ elements are shown in Figure 1.

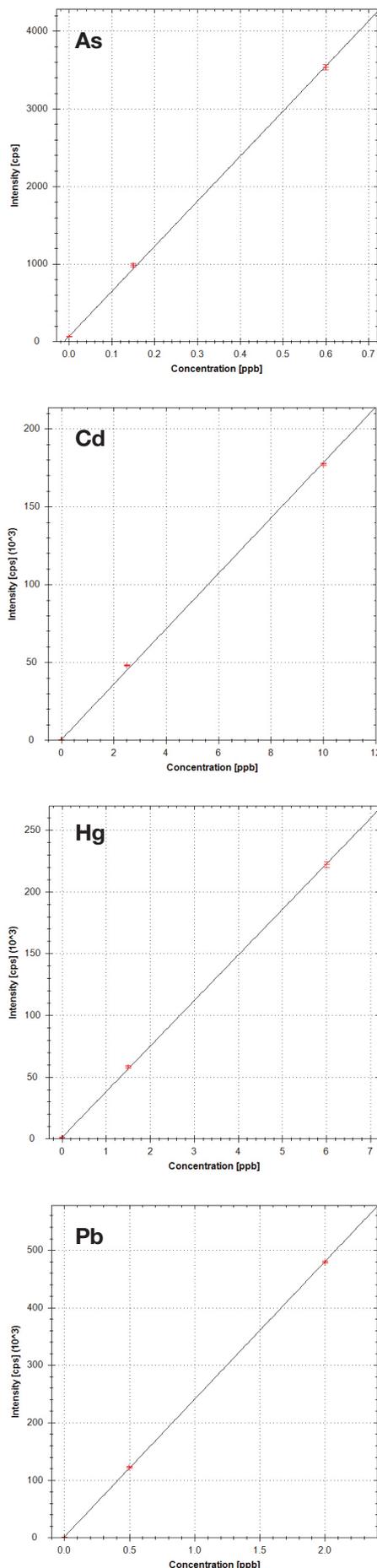


Figure 1. Example calibrations for the ‘big four’ elements: As, Cd, Hg and Pb.

Instrumental and Method Detection Limits

Single digit $\mu\text{g}\cdot\text{g}^{-1}$ instrumental detection limits (LoD) are typically obtained for all of the USP <232> defined elements (Table 3). Background equivalent concentrations (BEC) for the 1.2% HNO_3 and 0.5% HCl calibration solution were also calculated. Low or sub $\mu\text{g}\cdot\text{g}^{-1}$ detection limits (LOD) highlight the excellent detection power of the iCAP RQ ICP-MS for single mode He KED analysis for the USP <232> required elements.

However, while the instrumental detection limits in Table 3 illustrate the detection capabilities of the iCAP RQ ICP-MS for the analysis of the USP <232> required elements, they are not representative of what can practically be achieved on a routine basis. In order to assess this, method detection limits (MDL) were determined from the analysis of three (microwave digestion) procedural blanks from three separate analytical runs performed on different days. Three times the standard deviation of the mean of the blanks from each day was calculated, corrected for dilution and are compared to the Target Limit (J) in the solid (from Table 2). The comparison shows that the attainable MDLs for all elements are at least 50 times lower than the target limit in the solid.

Table 3. Instrumental detection limit (LOD, based on 3 x the standard deviation of the calibration blank), background equivalent concentration (BEC) (reported as ng/g) and resulting MDLs (reported as $\mu\text{g/g}$) for the USP <232> defined elements.

Isotope	LOD ($\text{ng}\cdot\text{g}^{-1}$)	BEC ($\text{ng}\cdot\text{g}^{-1}$)	MDL ($\mu\text{g}\cdot\text{g}^{-1}$)	Target limit J ($\mu\text{g}\cdot\text{g}^{-1}$)
⁵¹ V	0.0035	0.0629	0.014	10
⁵² Cr	0.007	0.042	0.008	1100
⁶⁰ Ni	0.0012	0.0163	0.100	20
⁶³ Cu	0.0049	0.0910	0.186	300
⁷⁵ As	0.0009	0.0087	0.0005	1.5
⁹⁵ Mo	0.0026	0.0013	0.027	300
¹⁰¹ Ru	0.0003	0.00005	0.025	10
¹⁰³ Rh	0.0001	0.00005	0.026	10
¹⁰⁵ Pd	0.0036	0.0351	0.044	10
¹¹¹ Cd	0.00001	0.00009	0.006	0.5
¹⁸⁹ Os	0.0007	0.0003	0.043	10
¹⁹³ Ir	0.0005	0.0045	0.023	10
¹⁹⁵ Pt	0.0001	0.0002	0.024	10
²⁰² Hg	0.0099	0.0290	0.018	3
²⁰⁸ Pb	0.0009	0.0035	0.009	0.5

Sample analysis results

The final concentrations determined for each target element in the pharmaceutical products tested (six repeat analyses per sample) are shown in Table 4. MDL and target limit (J) values are provided for comparison. Determined concentrations found to be less than the MDL are marked as '<MDL'.

Table 4. Final concentrations obtained for each target element from the six replicate analyses of the three drugs tested.

Element	Drug A ($\mu\text{g}\cdot\text{g}^{-1}$)	Drug B ($\mu\text{g}\cdot\text{g}^{-1}$)	Drug C ($\mu\text{g}\cdot\text{g}^{-1}$)	MDL ($\mu\text{g}\cdot\text{g}^{-1}$)	Target Limit J ($\mu\text{g}\cdot\text{g}^{-1}$)
Cadmium	<MDL	<MDL	<MDL	0.006	0.5
Lead	0.134	0.171	0.017	0.009	0.5
Inorganic arsenic	0.056	0.091	0.065	0.001	1.5
Inorganic mercury	0.032	<MDL	<MDL	0.018	3
Iridium	<MDL	<MDL	<MDL	0.023	10
Osmium	<MDL	0.107	0.161	0.043	10
Palladium	0.073	<MDL	<MDL	0.044	10
Platinum	<MDL	<MDL	<MDL	0.024	10
Rhodium	<MDL	<MDL	<MDL	0.026	10
Ruthenium	<MDL	<MDL	<MDL	0.025	10
Chromium	<MDL	<MDL	<MDL	0.008	1100
Molybdenum	0.121	0.647	0.073	0.027	300
Nickel	0.780	1.92	12.8	0.100	50
Vanadium	0.224	0.402	0.509	0.014	20
Copper	29.2	5.53	0.965	0.186	300

In each sample some elements were found to be below the calculated MDL but no element was found to be above the Target Limit, J.

Drift

Following the requirement detailed in USP <233>, the read back concentrations for one of the calibration standards analyzed before and after the sample solutions were compared. This comparison is made to ensure that the initial calibration remains valid over the entire analysis. The test is deemed to pass if the relative difference between two analyses of the calibration solution is less than 20%. All elements were found to be reproducible over the complete analysis period (three hours in total) with relative standard deviation (RSD) between 0.1% to maximum 4%, and hence well within the USP <233> defined limit for the calibration solution containing a 2J spike.

Validation procedure

The USP requires that the analytical procedure used to determine elemental impurities in each individual pharmaceutical product passes a series of validation tests before being accepted as suitable. In order to demonstrate the applicability of the iCAP RQ ICP-MS based method described above, its performance was assessed by testing the USP <233> defined criteria (accuracy, precision (repeatability), and ruggedness)² for the analysis of the three drugs used in this test.

USP <233> states that the acceptance criteria for this test are recoveries of between 70 and 150% for the mean of the three repeat analyses of each sample at both spike levels.

Figures 2a and 2b show that these criteria are easily met using the iCAP RQ ICP-MS, with average recoveries at both spike levels ranging from 92 to 128%.

Accuracy test

In order to assess the accuracy of the method, a series of spike recovery tests were made following the guidelines set out in USP <233>. The spike recoveries for each repeat of all three samples at the 0.5J and 1.5J spike levels are given in Figures 2a and 2b.

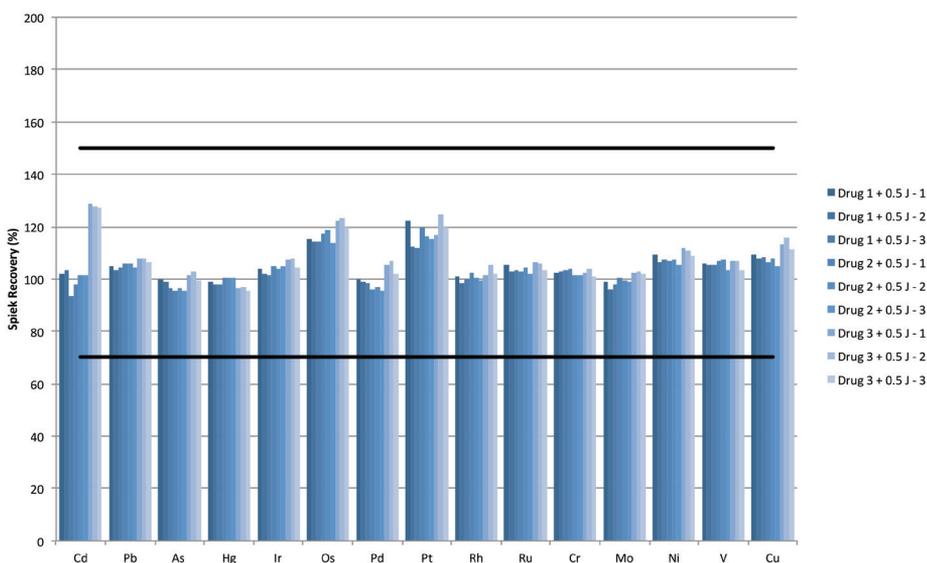


Figure 2a. Recoveries (in %) for the 0.5J spike level.

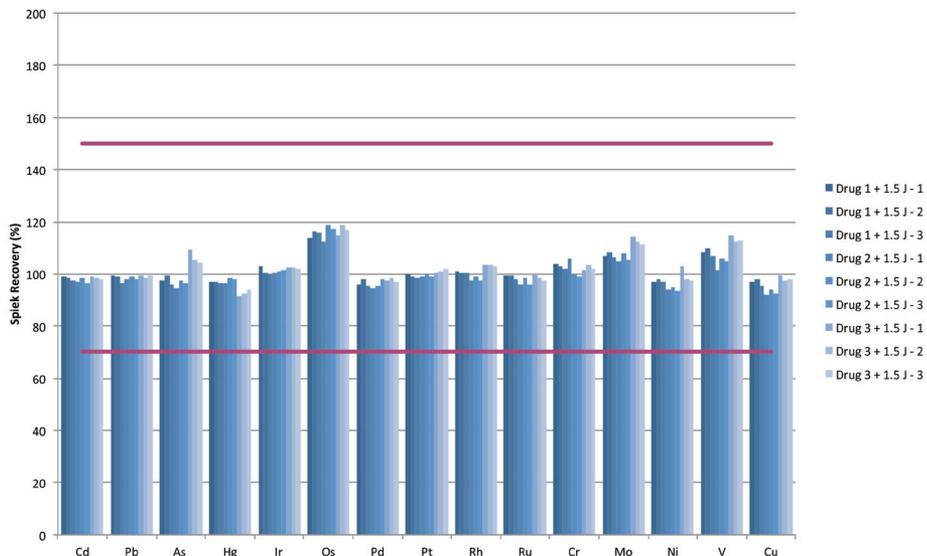


Figure 2b. Recoveries (in %) for the 1.5J spike level.

Precision test

The precision (repeatability) of the method was assessed by measuring six independent aliquots of each of the three materials tested spiked with the fourteen USP defined elements at the target limit (J). The results from these tests are shown in Tables 5a, 5b and 5c.

USP <233> defines that the precision (% RSD) from the six repeat analyses should not be greater than 20%.

Table 5a. Precision for six separate measurements of Drug A spiked at the target limit (J), expressed as percent recovery.

Element	Drug A - 1	Drug A - 2	Drug A - 3	Drug A - 4	Drug A - 5	Drug A - 6	Mean	RSD (%)
Cadmium	99.0	100.0	98.6	97.0	97.8	99.2	98.6	1.1
Lead	112.6	110.6	109.6	113.6	112.6	109.6	111.4	1.5
Inorganic arsenic	118.0	114.6	114.6	114.6	111.3	114.6	114.6	1.8
Inorganic mercury	99.3	98.7	98.0	98.0	96.3	97.7	98.0	1.0
Iridium	101.5	100.0	100.5	100.5	99.0	100.0	100.3	0.8
Osmium	115.0	112.5	112.5	114.0	112.5	114.0	113.4	0.9
Palladium	98.5	99.0	98.5	97.0	97.0	97.2	97.9	0.9
Platinum	96.5	94.5	96.5	94.0	93.5	93.0	94.7	1.6
Rhodium	101.5	102.5	102.0	99.0	101.0	100.5	101.1	1.2
Ruthenium	100.0	101.5	101.0	99.5	100.0	99.5	100.3	0.8
Chromium	102.6	101.9	103.1	102.8	101.7	103.4	102.6	0.7
Molybdenum	109.0	112.0	110.5	109.0	109.5	109.0	109.8	1.1
Nickel	99.4	101	97.7	99.4	98.0	98.4	99.0	1.2
Vanadium	108.0	107.0	106.5	106.5	105.5	107.0	106.8	0.8
Copper	112.4	112.4	110.2	110.2	106.8	110.7	110.5	1.9

Table 5b. Precision for six separate measurements of Drug B spiked at the Target Limit (J), expressed as percent recovery.

Element	Drug B - 1	Drug B - 2	Drug B - 3	Drug B - 4	Drug B - 5	Drug B - 6	Mean	RSD (%)
Cadmium	100.6	101.6	101.4	100.4	99.4	98.6	100.3	1.2
Lead	117.9	114.9	116.9	115.9	115.9	115.9	116.2	0.9
Inorganic arsenic	117.3	118.5	116.9	118.1	117.8	117.2	117.6	0.5
Inorganic mercury	98.0	97.7	98.0	98.7	98.0	98.2	98.1	0.3
Iridium	100.5	100.5	101.5	103.5	1001.0	101.2	101.2	1.2
Osmium	116.5	114.5	117.5	118.0	117.5	117.8	117.0	1.1
Palladium	97.5	98.5	99.5	98.0	97.5	97.5	98.1	0.8
Platinum	97.2	97.0	97.5	99.2	98.5	97.4	97.8	0.9
Rhodium	101.5	101.0	100.7	101.2	100.0	100.8	100.9	0.5
Ruthenium	100.8	101.1	101.4	100.6	99.8	100.9	100.8	0.5
Chromium	104.6	103.5	103.8	102.9	103.6	104.1	103.8	0.6
Molybdenum	117.5	117.2	116.8	116.5	115.9	116.1	116.7	0.5
Nickel	98.5	97.5	99.5	100.0	98.2	97.6	98.6	1.0
Vanadium	105.8	108.0	108.6	107.7	107.4	106.8	107.4	0.9
Copper	99.2	98.5	100.2	99.8	98.0	96.7	98.7	1.3

Table 5c. Precision for six separate measurements of Drug C spiked at the Target Limit (J), expressed as percent recovery.

Element	Drug C - 1	Drug C - 2	Drug C - 3	Drug C - 4	Drug C - 5	Drug C - 6	Mean	RSD (%)
Cadmium	100.1	98.6	99.4	99.6	99.8	99.6	99.5	0.5
Lead	100.4	99.8	100.7	100.5	100.5	101.3	100.5	0.5
Inorganic arsenic	116.9	117.5	117.9	115.5	118.1	117.1	117.2	0.8
Inorganic mercury	91.3	90.7	93.0	92.7	91.0	93.0	92.0	1.2
Iridium	100.1	100.9	104.5	102.8	102.1	102.5	102.2	1.5
Osmium	115.5	117.1	119.4	117.5	119.9	118.7	118.0	1.4
Palladium	96.5	97.8	100.4	99.8	100.6	99.9	99.2	1.7
Platinum	95.5	96.7	99.1	97.2	97.4	98.5	97.4	1.3
Rhodium	102.3	102.8	105.1	103.7	105.3	104.8	104.0	1.2
Ruthenium	98.0	99.1	100.0	99.4	100.8	99.7	99.5	0.9
Chromium	101.8	102.5	102.0	103.1	101.5	102.3	102.2	0.6
Molybdenum	112.4	113.8	114.2	113.6	114.8	114.6	113.9	0.8
Nickel	108.2	109.0	111.2	111.8	114.1	112.2	111.1	2.0
Vanadium	110.8	111.1	114.2	113.8	114.2	114.7	113.1	1.5
Copper	96.1	95.5	99.2	99.0	98.7	99.8	98.1	1.8

Tables 5a, 5b and 5c show that a precision of < 20% is easily achieved.

Ruggedness test

The ruggedness of the method was assessed by measuring six independent aliquots of each of the three materials tested spiked with the fourteen USP defined elements at the target limit (J), on three separate days. A final average and % RSD were calculated from the averages of the values obtained on each day. The results from these tests are shown in Tables 6a, 6b and 6c.

USP <233> defines that the ruggedness (% RSD) from three repeat analyses on different days should not be greater than 25%.

Table 6a. Ruggedness for three repeat measurements of Drug A spiked at the target limit (J), expressed as percent recovery.

Element	Drug A - 1	Drug A - 2	Drug A - 3	Mean	RSD (%)
Cadmium	98.4	96.8	98.0	97.7	0.9
Lead	97.2	95.2	93.2	95.2	2.1
Inorganic arsenic	95.0	96.0	97.0	96.0	1.0
Inorganic mercury	98.0	97.3	96.0	97.1	1.0
Iridium	100.0	100.0	98.0	99.3	1.2
Osmium	113.0	10.2	97.0	103.4	8.2
Palladium	97.6	95.8	96.5	96.6	0.9
Platinum	94.4	95.8	95.6	95.3	0.8
Rhodium	101.0	99.0	100.0	100.0	1.0
Ruthenium	99.9	98.7	99.0	99.2	0.6
Chromium	102.1	103.2	102.9	102.7	0.6
Molybdenum	109.0	107.0	106.0	107.3	1.4
Nickel	98.6	95.6	94.6	96.3	2.2
Vanadium	106.0	98.0	98.0	100.7	4.6
Copper	95.8	92.0	89.8	92.5	3.3



Table 6b. Ruggedness for three repeat measurements of Drug B spiked at the target limit (J), expressed as percent recovery.

Element	Drug B - 1	Drug B - 2	Drug B - 3	Mean	RSD (%)
Cadmium	99.2	98.0	98.0	98.4	0.7
Lead	97.8	95.8	93.8	95.8	2.1
Inorganic arsenic	92.7	93.2	92.8	92.9	0.3
Inorganic mercury	97.3	96.7	95.3	96.4	1.1
Iridium	101.0	102.0	99.0	100.7	1.5
Osmium	116.0	99.0	104.0	106.3	8.2
Palladium	97.0	95.7	95.5	96.1	0.8
Platinum	96.7	97.8	96.6	97.0	0.7
Rhodium	100.0	99.0	98.0	99.0	1.0
Ruthenium	99.6	98.6	98.1	98.8	0.8
Chromium	103.5	102.9	103.2	103.2	0.3
Molybdenum	115.0	113.0	111.0	113.0	1.8
Nickel	97.4	95.2	94.4	95.7	1.6
Vanadium	106.0	98.1	99.0	101.0	4.3
Copper	97.9	95.6	94.1	95.9	2.0

Table 6c. Ruggedness for three repeat measurements of Drug C spiked at the target limit (J), expressed as percent recovery.

Element	Drug C - 1	Drug C - 2	Drug C - 3	Mean	RSD (%)
Cadmium	98.8	95.2	97.6	97.2	1.9
Lead	100.0	98.1	96.7	98.3	1.7
Inorganic arsenic	116.7	115.2	116.4	116.1	0.7
Inorganic mercury	91.3	89.3	90.7	90.4	1.1
Iridium	102.0	103.1	98.9	101.3	2.1
Osmium	117.1	107.8	99.2	108.0	8.3
Palladium	98.5	95.1	97.0	96.9	1.8
Platinum	96.7	99.5	95.6	97.3	2.1
Rhodium	102.8	102.1	99.7	101.5	1.6
Ruthenium	98.8	97.6	96.2	97.5	1.3
Chromium	101.5	102.8	103.5	102.6	1.0
Molybdenum	113.0	110.8	105.6	109.8	3.5
Nickel	123.4	117.4	119.6	120.1	2.5
Vanadium	113.7	105.0	102.2	107.0	5.6
Copper	97.5	94.3	92.2	94.7	2.8

Tables 6a, 6b and 6c show that a precision of < 25% across three days is easily achieved. The excellent measurement stability for $\mu\text{g}\cdot\text{L}^{-1}$ levels of Mercury in each drug (< 1% precision over 3 days) is a result of the sample preparation method described and the stability of the iCAP RQ ICP-MS.

Conclusion

This application note has shown that the iCAP RQ ICP-MS is an ideal tool for elemental determination in pharmaceutical products after dissolution by microwave digestion. For the three drugs tested, method detection limits fifty times lower than the target limits were produced showing that the iCAP RQ ICP-MS is easily capable of accurately and precisely measuring all fourteen of the specified elements at the target limits listed in USP <232>. Based on this, when considering the continual change in regulations defined by USP and other National and International bodies, ICP-MS represents a future-proof investment for pharmaceutical laboratories embarking on elemental impurity analyses. The described method exceeds the analytical performance criteria described in USP <233> by a wide margin.

Finally, the range of security features, data management and audit trailing tools included in the advanced and flexible Qtegra ISDS Software provides the necessary support to meet the demands of 21 CFR Part 11 in the highly regulated pharmaceutical industry environment.

References

1. General Chapter <232> Elemental Impurities - Limits, United States Pharmacopeia.
2. General Chapter <233> Elemental Impurities - Procedure, United States Pharmacopeia.
3. 21CFR11 2017, Food and Drug Administration.

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