Application Note
Pharmaceutical

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Elemental Impurities in Aspirin: USP <232>/<233> and ICH Q3D Methods Using ICP-OES

Validation of the USP <232>/<233> method on an Agilent ICP-OES

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Verified for Agilent
5800 and 5900 ICP-OES

Introduction

Pharmaceutical manufacturers are required to control elemental impurities in drug products. Sources of elemental contamination include raw materials, manufacturing processes, and packaging and container closure systems (CCS).

The previous US Pharmacopeia (USP) method for trace metals, USP<231> (heavy metals limit test) was not quantitative and was incapable of providing adequate information regarding the potential toxicity of contaminants. To address the limitations of USP<231>, USP released updated methods to provide quantitative measurements of inorganic impurities in pharmaceutical products using current analytical instrumentation.

The USP General Chapters <232> (Elemental Impurities – Limits) (*1*) and <233> (Elemental Impurities – Procedures) (*2*) specify the limits and procedures for measuring elemental impurities in drug products and their ingredients. The methods are harmonized with the Technical Requirements for Pharmaceuticals for Human Use Guidelines, issued by the International Council for Harmonization ICH Q3D (*3*)

USP<232> Elemental Impurities—Limits

Both the ICH Q3D and USP<232> chapters specify the target analytes and Permitted Daily Exposure (PDE) limits for elemental impurities in drug products that pharmaceutical companies must comply with.

Table 1 shows the PDE limits for elemental impurities in drug products, according to their route of administration. The potential toxicity class of each element is also indicated, as specified in the USP/ICH methods.

Table 1. The permitted daily exposure (PDE) limits for elemental impurities in oral, parenteral, and inhalation formulations.

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

USP<233> Elemental Impurity—Procedures

USP General Chapter <233> recommends the use of either ICP-OES or ICP-MS for the analysis of elemental impurities in drug products and their ingredients. Several methods for sample preparation are specified. The most appropriate solubilization procedure should be selected for the pharmaceutical product being analyzed. The options include:

- Direct analysis
- Dilution/solubilization in a suitable aqueous solvent, such as water or dilute acid
- Dilution/solubilization in a suitable organic solvent
- Closed-vessel microwave acid digestion for insoluble samples

Agilent ICP-OES for pharmaceutical applications

The Agilent 5900 Synchronous Vertical Dual View (SVDV) or or Agilent 5800 VDV ICP-OES are ideal instruments for the determination of elemental impurities in bulk raw materials and for oral formulation final products (*4*). Designed for the measurement of the most challenging samples, the instruments include:

- A vertically oriented torch that allows the analysis of a wide range of samples with little or no sample dilution, including samples with total dissolved solids upwards of 25%.
- The Vista Chip III detector: a high-speed detector with a wide wavelength coverage that can measure all wavelengths from 167 to 785 nm in a single measurement. The wide wavelength coverage allows analysts to choose wavelengths that are free from interferences.
- High speed of analysis. Without compromising performance, the Agilent ICP-OES instruments can measure up to 2500 samples every 24 hours. The instruments are suitable for both low and high production laboratories.
- A choice of different background correction techniques: Fitted Background Correction (FBC) and Fast Automated Curve fitting Technique (FACT). FBC simplifies method development and reduces complexity for the analyst. FACT corrects for spectral interferences and complex background structures.
- Ease-of-use. The intuitive Agilent ICP Expert software, is supplied with preset methods for elemental impurity analysis in pharmaceutical samples (version 7.4 and later). The methods support compliance with the requirements of ICH-Q3D and USP<232>/<233>.

This study describes the analytical procedures and validation studies required by USP <233>/ ICH-Q3D. It includes the analysis of the 24 elements of interest in aspirin samples using ICP-OES.

Experimental

Instrumentation

This work was performed using the 5110 SVDV ICP-OES, but the method is also compatible with the Agilent 5800 VDV or Agilent 5900 SVDV ICP-OES. Both instruments use a vertically orientated torch and a solid-state RF system, operating at 27 MHz, to deliver a plasma with the stability and robustness necessary for the analysis of complex samples.

The sample introduction system consisted of a SeaSpray nebulizer, double-pass cyclonic spray chamber, and a 1.8 mm i.d injector torch. An Agilent SPS 4 autosampler was used for sample introduction. Instrument operating parameters are shown in Table 2.

Table 2. Agilent 5110 SVDV ICP-OES instrument and method parameters.

Parameter	Setting
Read time (s)	30
Replicates	3
Sample uptake delay (s)	30
Stabilization time (s)	15
Rinse time (s)	36
Pump speed (rpm)	12
Fast pump during uptake and rinse (rpm)	On
RF power (kW)	1.50
Aux flow (L/min)	1.00
Plasma flow (L/min)	12.0
Nebulizer flow (L/min)	0.7
Viewing mode	SVDV
Sample pump tubing	White/white
Waste pump tubing	Blue/blue
Internal standard pump tubing	Orange/green

Standards and sample preparation

The J-value

The maximum level of elemental impurities in finished drug products is expressed as a maximum PDE. The PDE limit considers the concentration of the element present in the drug products and the maximum recommended daily dose for the medicine.

For materials that require digestion or dilution in a solvent before analysis, the PDE limit (in µg/day) must be converted to a concentration limit (in µg/L) as measured in the prepared test sample. This concentration is calculated after multiplying the PDE by the dilution factor. The dilution factor used can be optimized to bring the analyte or analytes within the analytical range of the instrument.

The target concentration value in the prepared sample, referred to as the "J-value", defines the maximum permitted concentration limit for the analyte in that sample, where:

$$J = \frac{PDE}{Total\ Dilution \times Max\ Daily\ Dose}$$

The J-value is also used to define the calibration levels and concentrations of quality control (QC) solutions. For example, calibrations must be prepared at concentration levels of between 0.5 and 1.5 J. Detectability (for the Limit Procedures described in USP<233>) must be demonstrated using a sample spiked at 80% of the J value (0.8 J). Spike recovery tests must be performed at concentrations ranging from 50 to 150% of the J value (i.e. between 0.5 and 1.5 J).

Standard preparation

Calibration standards were prepared at 0.5, 1.0, and 1.5 J for each target analyte using multi-element standards from the Agilent ICH Q3D/USP <233> Elemental Impurities kit (*5*). The kit consists of five Certified Reference Materials (CRMs). Each CRM contains a group of elements (target elements) selected by ICH/USP class, chemical compatibility, and the relative mandated concentrations. The calculated J-values for each analyte in aspirin (based on the maximum daily dose = 3000 mg/day) are shown in Table 4.

The multi-element CRM standards were diluted in Millipore 18.2 M Ω polished water. All blanks and standards were matrix matched to the samples in 10% HNO_3 and 10% HCL.

Sample preparation

Approximately 1.0 g of the aspirin sample was accurately weighed into borosilicate tubes. The samples were digested in 5 mL HNO_3 and 1 mL HCL using an UltraWAVE Single Reaction Chamber Microwave Digestion system (Milstone Inc., Shelton CT). The heating conditions are given in Table 3. After microwave digestion, 5 mL of HCL was added to the sample and it was then diluted with Nanopure water to a final volume of 50 mL.

Table 3. Parameters for microwave digestion (T1 and T2 are the programmed initial and final vessel temperatures).

Step	Time (Min)	T1 (°C)	T2 (°C)	Nitrogen Gas Pressure (bar)	Power (W)
1	15	250	60	140	1500
2	15	250	60	140	1500

Spiked sample solutions

Three spiked solutions of the aspirin sample were prepared before digestion:

- Spiked sample solution 1: an aspirin sample was spiked with the 1.0 J standard solution.
- Spiked sample solution 2: an aspirin sample was spiked at a concentration of 0.8 J.
- Spiked sample solution 3: an aspirin sample was spiked at a concentration of 0.5 J.

Results and discussion

Calibration linearity

Linear calibrations were obtained for all 24 elements analyzed. Figure 1 shows calibration curves for Class 1 analytes (As, Cd, Hg, and Pb), which must be controlled in all drug products. Wavelengths and working calibration range for all elements are shown in Table 4.

Table 4. Wavelengths, background correction method used, the calculated 1J value, and the working calibration range used for each of the 24 target elements. Two wavelengths per element is not mandatory but have been included to contribute to the specificity test.

Element and Wavelength (nm)	Background Correction	1 J Value (µg/L)	Calibration Range (ppm)
As 188.980	FACT	100	0.0 - 0.15
As 193.696	Fitted	100	0.0 - 0.15
Cd 214.439	Fitted	33	0.0 - 0.05
Cd 226.502	Fitted	33	0.0 - 0.05
Hg 184.887	Fitted	200	0.0 - 0.30
Hg 194.164	Fitted	200	0.0 - 0.30
Pb 220.353	Fitted	33	0.0 - 0.05
Ag 328.068	FACT	667	0.0 - 0.10
Ag 338.289	FACT	667	0.0 - 1.00
Au 242.794	Fitted	667	0.0 - 1.00
Au 267.594	Fitted	667	0.0 - 1.00
Co 228.615	Fitted	333	0.0 - 0.05
Co 238.892	Fitted	333	0.0 - 0.05
Ir 212.681	Fitted	667	0.0 - 1.0
Ir 224.268	Fitted	667	0.0 - 1.0
Ni 216.555	Fitted	1333	0.0 - 2.0
Ni 231.604	Fitted	1333	0.0 - 2.0
Os 225.585	Fitted	667	0.0 - 1.0
Os 228.228	Fitted	667	0.0 - 1.0
Pd 229.651	Fitted	667	0.0 - 1.0
Pd 340.458	Fitted	667	0.0 - 1.0
Pt 203.646	Fitted	667	0.0 - 1.0
Pt 214.424	Fitted	667	0.0 - 1.0
Rh 343.488	FACT	667	0.0 - 1.0
Rh 369.236	FACT	667	0.0 - 1.0
Ru 245.657	Fitted	1000	0.0 - 1.5
Ru 267.876	Fitted	1000	0.0 - 1.5
Se 196.026	Fitted	1000	0.0 - 1.5
Se 203.985	FACT	1000	0.0 - 1.5
Tl 190.794	Fitted	53	0.0 - 0.8
V 292.401	Fitted	667	0.0 - 1.0
V 309.310	Fitted	667	0.0 - 1.0
Ba 455.403	Fitted	9333	0.0 - 1.4
Ba 493.408	Fitted	9333	0.0 - 1.4
Cr 205.560	Fitted	73333	0.0 - 110
Cr 267.716	Fitted	73333	0.0 - 110
Cu 324.754	Fitted	20000	0.0 - 30
Cu 327.395	Fitted	20000	0.0 - 30
Li 610.365	FACT	3667	0.0 - 5.5
Li 670.783	Fitted	3667	0.0 - 5.5
Mo 202.032	Fitted	20000	0.0 - 30
Mo 204.598	Fitted	20000	0.0 - 30
Sb 206.834	FACT	8000	0.0 - 12
Sb 217.582	Fitted	8000	0.0 - 12
Sn 189.925	Fitted	40000	0.0 - 60
Sn 283.998	Fitted	40000	0.0 - 60

table continues...

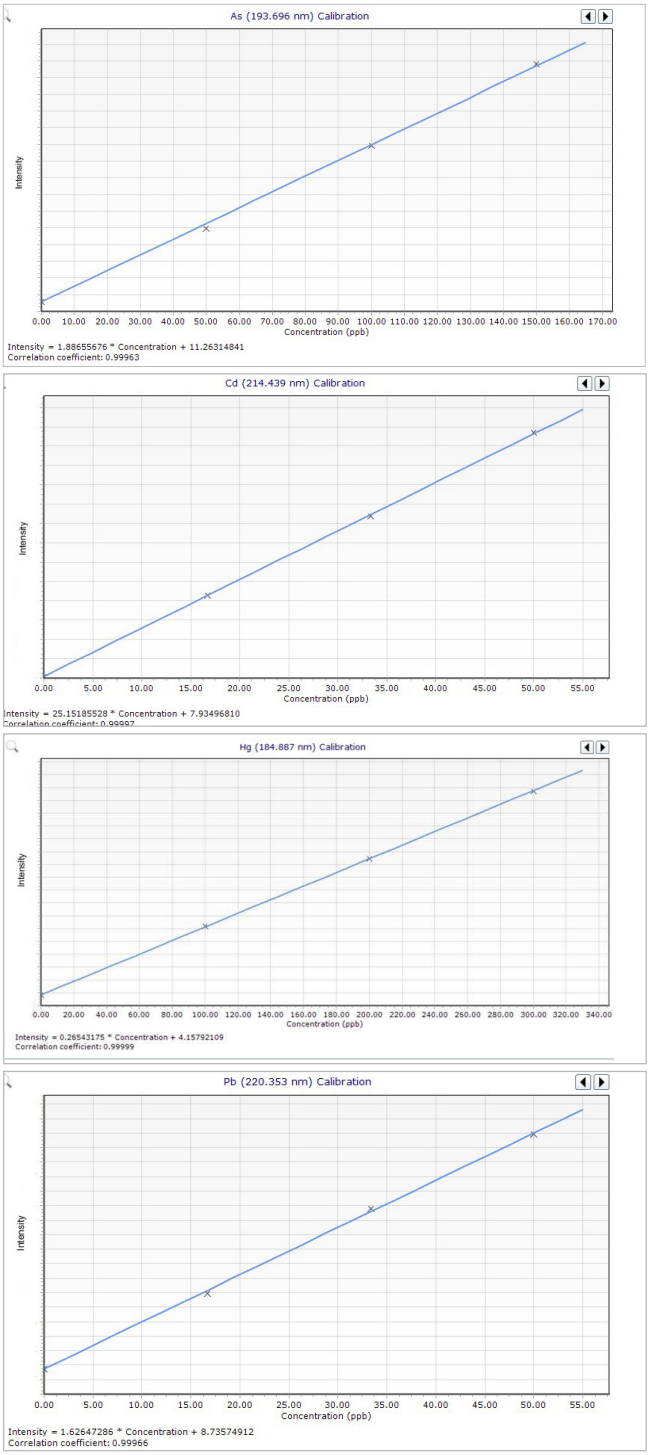

Figure 1. Calibration curves for the four Class 1 elements: As 193.696 nm, Cd 214.439 nm, Hg 184.887 nm, and Pb 220.353 nm.

Validation procedure

USP <233> lists two options for validating the analytical procedure used to determine the levels of elemental impurities: limit procedures and quantitative procedures. The limit procedures are often used by final product pharmaceutical manufacturers while the quantitative procedures are used by active pharmaceutical ingredient (API) and excipient producers. This study illustrates both options, but only one is required.

Quantitative procedures

The quantitative procedures include: accuracy, precision (repeatability, ruggedness) and specificity.

Accuracy

The accuracy test requires measurements of three spike levels in a sample: 0.5 J, 1.0 J, and 1.5 J for each target element. For the aspirin samples, each spike level was prepared in triplicate for a total of nine measurements. The results, shown in Table 5, show that all target elements were within the acceptance criteria for the mean of three replicate preparations at each concentration.

Table 5. Accuracy test results for measurement of three individual readings of aspirin spiked at three different levels 0.5J, 1.0J, and 1.5J. Average of three spikes limits should be within 70-150%.

Element and Wavelength (nm)	Aspirin + Spike 0.5J (%)	Sspirin + Spike 1.0J (%)	Aspirin + Spike 1.5J (%)	Total Mean %Rec (n=9)
As 193.696	92	96	99	96
Cd 214.439	98	99	98	98
Hg 184.887	93	91	94	93
Pb 220.353	108	104	108	106
Co 238.892	95	94	94	94
Ni 231.604	94	95	94	94
V 292.401	88	92	90	90
Ag 328.068	93	94	94	94
Au 242.794	94	96	96	95
Ir 212.681	92	93	93	93
Os 225.585	94	99	98	97
Pd 340.458	88	92	93	91
Pt 214.424	93	97	96	95
Rh 343.488	90	94	95	93
Ru 245.657	92	94	94	93
Se 196.026	102	98	99	100
Tl 190.794	80	86	88	85
Ba 455.403	95	95	93	94
Cr 205.560	99	96	91	95
Cu 327.395	92	93	93	93
Li 670.783	93	99	101	98
Mo 202.032	92	93	91	92
Sb 217.582	92	94	94	93
Sn 189.925	97	96	93	95

Precision

The precision test involves assessing the repeatability and ruggedness of the procedure. The repeatability of the procedure was assessed by analyzing six independent samples of aspirin, spiked at 1 J. Ruggedness was assessed by analyzing six independent samples of aspirin spiked at 1 J, across two different days. The acceptance limits for repeatability are <20% RSD for each target element and for ruggedness it is <25% RSD. All target elements met the acceptance criteria, as shown in Table 6.

Table 6. Repeatability and ruggedness results for measurement of six independent samples spiked at 1.0 J and captured across two days.

Element and Wavelength (nm)	Repeatability Day 1 Captured over 1 day (n=6) (%)	Repeatability Day 2 Captured over 2 days (n=12) (%)
As 193.696	1.00	1.90
Cd 214.439	1.90	1.60
Hg 184.887	2.60	2.30
Pb 220.353	2.30	2.60
Co 238.892	1.40	1.20
Ni 231.604	1.10	1.10
V 292.401	1.50	1.20
Ag 328.068	1.80	1.60
Au 242.794	1.20	1.10
Ir 212.681	0.90	0.80
Os 225.585	4.80	4.60
Pd 340.458	1.10	1.00
Pt 214.424	1.00	1.30
Rh 343.488	1.10	0.90
Ru 245.657	1.00	0.90
Se 196.026	1.30	1.20
Tl 190.794	2.50	2.10
Ba 455.403	1.00	1.00
Cr 205.560	0.90	0.80
Cu 327.395	1.40	1.20
Li 670.783	1.40	1.50
Mo 202.032	0.70	0.60
Sb 217.582	0.70	0.60
Sn 189.925	1.20	0.90

Specificity

The procedure must determine each target element in the presence of other components that could be present in the sample. These include other target elements and matrix components. (See Validation of Compendial Procedures <1225>). For the procedure to pass specificity criteria, it must be proven that the presence of other components does not impact on recovery for each target element. See Table 5 for the accuracy results.

An optional second way to assess specificity is by measuring the concentration of each element at multiple wavelengths. If the calculated concentration is the same for both wavelengths per element, it indicates that there is absolutely no interference. Table 7 contains the concentrations of the target elements, measured at two wavelengths in the aspirin samples.

Table 7. Specificity test results shows that two wavelengths for each element gave the same concentration result (within the allowed tolerance).

Element and Wavelength (nm)	1.0 J Value on Aspirin (ppb) (n-3)	Element and Wavelength (nm)	1.0 J Value on Aspirin (ppb) (n-3)
As 188.980	98	Rh 343.488	628
As 193.696	96	Rh 369.236	626
Cd 214.439	32	Ru 245.657	627
Cd 226.502	32	Ru 267.876	620
Hg 184.887	180	Se 196.026	977
Hg 194.164	186	Se 203.985	900
Pb 220.353	34	Tl 190.794	45
Ag 328.068	941	V 292.401	611
Ag 338.289	916	V 309.310	522
Au 242.794	639	Ba 455.403	8890
Au 267.594	624	Ba 493.408	8941
Co 228.615	313	Cr 205.560	70043
Co 238.892	317	Cr 267.716	69306
Ir 212.681	622	Cu 324.754	18765
Ir 224.268	631	Cu 327.395	18629
Ni 216.555	1256	Li 610.365	3384
Ni 231.604	1267	Li 670.783	3638
Os 225.585	663	Mo 202.032	18600
Os 228.228	671	Mo 204.598	18536
Pd 229.651	628	Sb 206.834	7389
Pd 340.458	614	Sb 217.582	7494
Pt 203.646	628	Sn 189.925	38327
Pt 214.424	641	Sn 283.998	37558

Limit procedures

The validation parameters for the limit procedures include: detectability (non-instrumental procedure and Instrumental procedure), precision (repeatability) and specificity. The precision and specificity parameters have been described above, in the quantitative procedures section.

Detectability

Detectability involves two parts: instrumental procedure and non instrumental procedure. The instrumental detectability procedure involves a comparison of the emission intensity of the average of three individual samples, spiked with 1.0 J. The recovery for the spiked samples needs to be within ±15% of the average value obtained for the replicate measurements of the 1.0 J standard solution. The results for the aspirin samples are shown in Table 8. All elements were within the acceptable criteria.

The non instrumental detectability involves a comparison of the signal intensity or concentration value of a sample spiked with 1.0 J against that measured for the sample spiked with 0.8 J. If the average signal intensity or concentration value of a 0.8 J spike is less than the 1.0 J spike, the test passes. The results for the non-instrumental test are also shown in Table 8.

Table 8. Detectability test results of a sample spiked at 1.0 J, compared to a 1.0 J standard (results should be within 15%). The fifth column shows the average concentration value of a 0.8 J spiked sample, which must be less than that of the 1.0 J spiked sample (third column) to pass.

Element and Wavelength (nm)	1.0 J Standard (ppb) (n=3)	1.0 J Spiked Sample (ppb)(n=3)	Difference (%)	0.8 J Value of Aspirin ppm (n-3)	Pass/ Fail
Ag 328.068	1000	938	6.25	756	Pass
As 193.696	100	96	1.40	74	Pass
Au 242.794	667	642	3.80	515	Pass
Ba 455.403	9333	8892	4.90	7117	Pass
Cd 214.439	33	32	0.30	26	Pass
Co 238.892	333	319	4.00	257	Pass
Cr 205.560	73333	70162	4.30	57642	Pass
Cu 327.395	20000	18714	6.40	14888	Pass
Hg 184.887	200	183	7.80	142	Pass
Ir 212.681	667	624	6.20	502	Pass
Li 670.783	3667	3638	0.80	2867	Pass
Mo 202.032	20000	18570	7.10	14811	Pass
Ni 231.604	1333	1271	5.00	1026	Pass
Os 225.585	667	651	2.90	501	Pass
Pb 220.353	33	34	4.20	26	Pass
Pd 340.458	667	617	7.30	489	Pass
Pt 214.424	667	643	3.00	512	Pass
Rh 343.488	667	631	5.40	498	Pass
Ru 245.657	667	632	5.10	507	Pass
Sb 217.582	8000	7512	6.10	6039	Pass
Se 196.026	1000	984	1.20	802	Pass
Sn 189.925	40000	38473	3.80	31036	Pass
Tl 190.794	53	45	14.10	36	Pass
V 292.401	667	613	8.10	485	Pass

Conclusion

The Agilent ICP-OES instrument easily met the requirements for determining elemental impurities in pharmaceutical products, according to the ICH Q3D and USP <232> and <233> procedures.

Validation studies for elemental impurity analysis in pharmaceutical samples were successfully completed for aspirin samples.

References

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