Determination of Elemental Impurities in Antibiotics according to USP 232 and 233 by HR ICP-OES

Challenge

Determination of elemental impurities in oral antibiotics.

Solution

ICP-OES with exceptionally high sensitivity, an industry leading high-resolution optical system and a wide working range for a trouble-free determination of elemental impurities.

Introduction

As of January 2018, pharmaceutical products must comply with specified limits for the allowed exposure to certain trace elemental impurities. The maximum permitted exposure limits and the analytical methods in order to quantify the listed trace elemental impurities are described in the United States Pharmacopeia (USP) chapters <232> Elemental Impurities – Limits[1] and <233> Elemental Impurities – Procedures[2] and are aligned with the International Conference on Harmonization (ICH) Q3D Step 4 guidelines[3]. The described regulations define the list of analytes and the maximum permitted exposure limits taking into account the route of drug administration. The use of closed vessel sample digestion and modern instrumental techniques is also introduced to ensure the accurate recovery and determination of individual target element concentrations.

The quantification of trace elemental impurities by ICP instrumentation is becoming a routine task for manufacturers and suppliers of pharmaceuticals. Challenges within this field of application comprise a large variety of sample types with different analyte combinations and target limits. This, in turn, requires ICP instrumentation that can handle a large variety of sample types with varying matrix loading and solvent types (e.g., aqueous or solvent-based) and allows the measurement of a wide concentration range. In this regard, the plasma system needs to be able to handle any sample type without compromising plasma stability and robustness.

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The accurate and reliable quantification of trace elemental impurities also requires a high sensitivity of the system as well as the ability to resolve spectral interferences that are common in ICP-OES.

The here described method analyzes cadmium, lead, arsenic, mercury, cobalt, vanadium and nickel in an antibiotic product. The developed method is validated according to the requirements of USP <233>, quantitative procedures[2]. Here, the exceptionally high spectral resolution and sensitivity of the PlasmaQuant 9100 Elite by Analytik Jena allows for an interference-free analysis of trace elements in varying matrices and elemental constellations. Furthermore, the high plasma robustness of the device's High-Frequency Generator and the sample introduction system with its centerpiece, the V-Shuttle torch, guarantee a highly accurate and precise analysis of pharmaceutical products.

USP Chapter <233> describes two analytical procedures, including sample preparation procedures, instrumental methods, and validation studies and requirements for measuring elemental impurities. The two compendial procedures are the inductively coupled plasma-based spectrochemical techniques, ICP-OES and ICP-MS.

USP chapter <232> defines that all elements of Classes 1 and 2A must be analyzed in oral pharmaceuticals, such as antibiotics (Table 1). Elements of Classes 2B and 3 are only to be analyzed if they are intentionally added to the manufacturing process or to the raw materials. Table 1 shows the permitted daily exposure (PDE) level in micrograms per day for oral drug delivery.

Table 1: Permitted Daily Exposures (PDE) for elemental impurities for oral administration

Element	Class	Oral PDE [µg/day]	If intentionally added	If not intentionally added
Cadmium	1	5	yes	yes
Lead	1	5	yes	yes
Arsenic	1	15	yes	yes
Mercury	1	30	yes	yes
Cobalt	2A	50	yes	yes
Vanadium	2A	100	yes	yes
Nickel	2A	200	yes	yes
Thallium	2B	8	yes	no
Gold	2B	100	yes	no
Palladium	2B	100	yes	no
Iridium	2B	100	yes	no
Osmium	2B	100	yes	no
Rhodium	2B	100	yes	no
Ruthenium	2B	100	yes	no
Selenium	2B	150	yes	no
Silver	2B	150	yes	no
Platinum	2B	100	yes	no
Lithium	3	550	yes	no
Antimony	3	1,200	yes	no
Barium	3	1,400	yes	no

Element	Class	Oral PDE [µg/day]	If intentionally added	If not intentionally added
Molybdenum	3	3,000	yes	no
Copper	3	3,000	yes	no
Tin	3	6,000	yes	no
Chromium	3	11,000	yes	no

Materials and Methods

Samples and reagents

According to the USP <233> recommendation on the use of "strong acids" for digestion of insoluble samples, the preferred approach is closed vessel microwave digestion. For the microwave digestion 1.0 g of the antibiotic product was accurately weighed and transferred into a digestion vessel (CX 100). The sample was spiked with 4 mL of conc. nitric acid, 1 mL of hydrogen peroxide and 1 mL of hydrochloric acid. The mixture was then shaken carefully and left standing for 10 minutes before the vessel was closed. Subsequent digestion was performed in Analytik Jena's TOPwave microwave with the following program:

Table 2: Digestion program for folic acid pharmaceutical product

Step	T [°C]	p_{max} [bar]	Ramp time [min]	Hold time [min]
1	170	80	5	10
2	210	80	5	20
3	50	80	-	20

After complete digestion and cooling to room temperature, the clear solution was filled up to 50 mL with deionized water.
Spiked samples with concentration levels of 0.5 J, 0.8 J, 1.0 J and 1.5 J were prepared by adding element concentrations according to USP <232> and <233> (Table 3) using single-element standards (Sigma Aldrich).

J-Value

In order to assess the suitability of the technique for the analytical task, it is important to know the PDE limit for each target element, and in particular what the USP calls the J-value. The J-value is defined as the PDE concentration of the element of interest, appropriately diluted to the working range of the instrument after completion of the sample preparation procedure.
As an example, the PDE limit for arsenic in an oral medication as defined in Chapter <232> is 15 µg/day based on a suggested dosage of <3 g of the drug product per day, that is equivalent to 5 µg/g of arsenic. If 1.0 g of sample is digested or dissolved and made up to 50 mL (50-fold dilution), the J-value for arsenic in this example is equal to 100 µg/L.

Table 3: J-values in accordance to oral PDE with a maximum daily dose of ≤1 g/day and the method calibration standards

Element	Concentration limits for oral drug with a max. daily dose of ≤3 g/day [µg/g]	0.5 J [µg/L]	0.8 J [µg/L]	1.0 J [µg/L]	1.5 J [µg/L]
As	5	50	80	100	150
Cd	2	20	32	40	60
Hg	10	100	160	200	300
Pb	2	20	32	40	60
Co	20	200	160	400	600
Ni	70	700	1,120	1,400	2,100
V	35	350	560	700	1,050

Instrumentation

Instrument settings

For the analysis a PlasmaQuant 9100 Elite ICP-OES equipped with a standard sample introduction kit was used in combination with a Teledyne Cetac ASX 560 autosampler incl. ENC-560DC enclosure. The detailed system configuration is shown in Table 4.

Table 4: Configuration of the PlasmaQuant 9100 Elite equipped with standard kit

Parameter	Specification
Plasma power	1,200 W
Plasma gas flow	12.0 L/min
Auxiliary gas flow	0.5 L/min
Nebulizer gas flow	0.5 L/min
Nebulizer	Seaspray, concentric, 1.0 mL/min, borosilicate
Spray chamber	Cyclonic spray chamber, 50 mL, borosilicate
Outer tube/Inner tube	Quartz/Quartz
Injector	Quartz, ID: 2mm
Pump tubing	PVC (black, black)
Sample pump rate	1.0 mL/min
Sample uptake ime	55 s

Method parameters

Table 5: Overview of method-specific evaluation parameters

Element	Line [nm]	Plasma view	Integration mode	Read time [s]	Evaluation			
					No. of pixel	Baseline fit, pixel no.	Polyn. degree	Correction
As	188.979	axial	Peak	10	3	ABC[1]	auto	-
Cd	228.802	axial	Peak	3	3	ABC	auto	-
Hg	184.886	axial	Peak	3	3	ABC	auto	-
Pb	220.353	axial	Peak	10	3	ABC	auto	-
Co	228.615	axial	Peak	3	3	ABC	auto	-
Ni	231.604	axial	Peak	3	3	ABC	auto	-
V	309.311	axial	Peak	3	3	ABC	auto	-

[1] Automatic Baseline Correction

Results and Discussion

In order to demonstrate that the ICP-OES procedure, including sample preparation as described above is appropriate for the samples being analyzed, the following measurements, tests and validations were performed as per USP <233>:

- Calibration and System Suitability
- Method Validation
  - Accuracy
  - Precision (repeatability and ruggedness)
  - Specificity
  - Limits of quantitation and sensitivity
  - Range and linearity (demonstrated by meeting the accuracy requirement)

Calibration and system suitability

USP <233> recommends using a calibration made up of two standards: standard 1 = 0.5 J, standard 2 = 1.5 J. The calibration ranges for all elements are shown in Table 3 in accordance to the J-value calculated for each element. All calibration functions of Class 1 and 2A target elements are displayed in Figure 1.

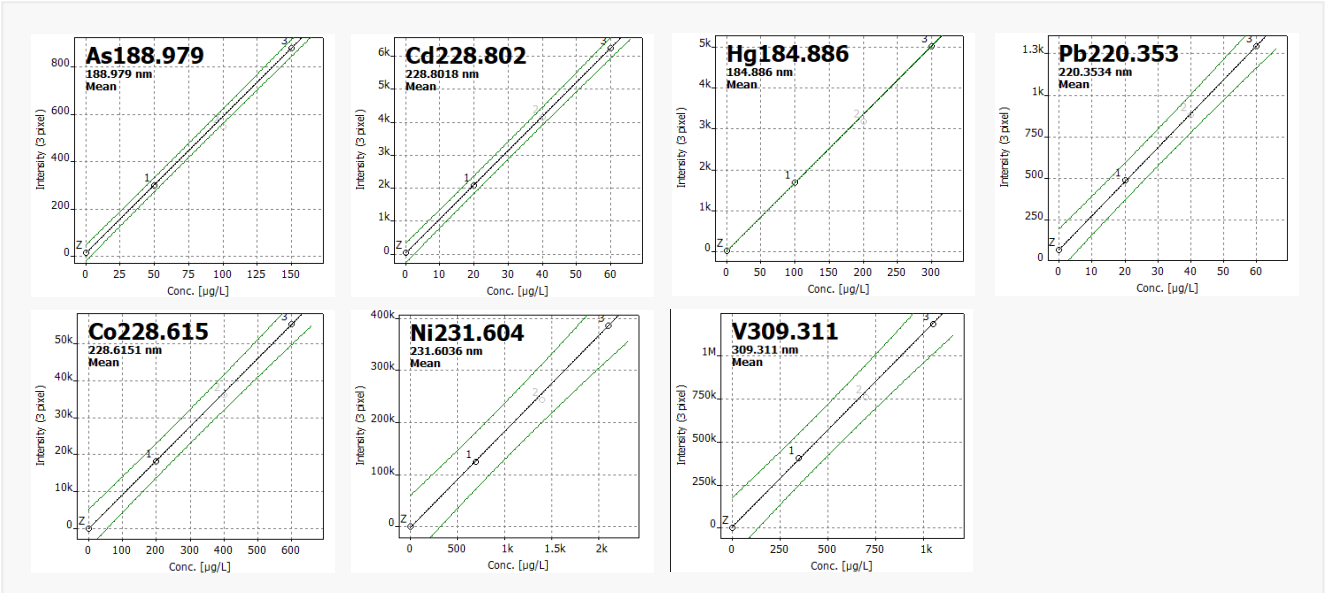

Figure 1: Calibration curves for Class 1 and 2A elemental impurities; black – calibration curve, green – confidence level

The system suitability test described in USP <233> requires a QC check standard with the concentration of 1.0 J to be measured before and after a batch of samples. The acceptance criteria defined in USP <232> for this test is a deviation of less than 20% for each target element.

Table 6: Results of system suitability test

Element	Standard solution 1 start of sequence [µg/L]	Standard solution 1 end of sequence [µg/L]	RSD [%]
As	142	146	2.7
Cd	60.1	61.1	1.7
Hg	314	300	4.5
Pb	61.7	60.8	1.4
Co	586	594	1.3
Ni	2,076	2,170	4.5
V	1,090	1,049	0.8

The obtained RSD values are well within the required 20% (Table 6). Within this study a batch of samples covering a full working day of 8 hours was measured in-between the QC check standards.

Method validation

Spike recovery – Accuracy

In accordance to USP <233> guidelines, the accuracy of the method can be assessed by spike recoveries. Figure 2 shows averaged spike recoveries for all samples prepared in triplicate at the three levels, 0.5 J, 1.0 J and 1.5 J.

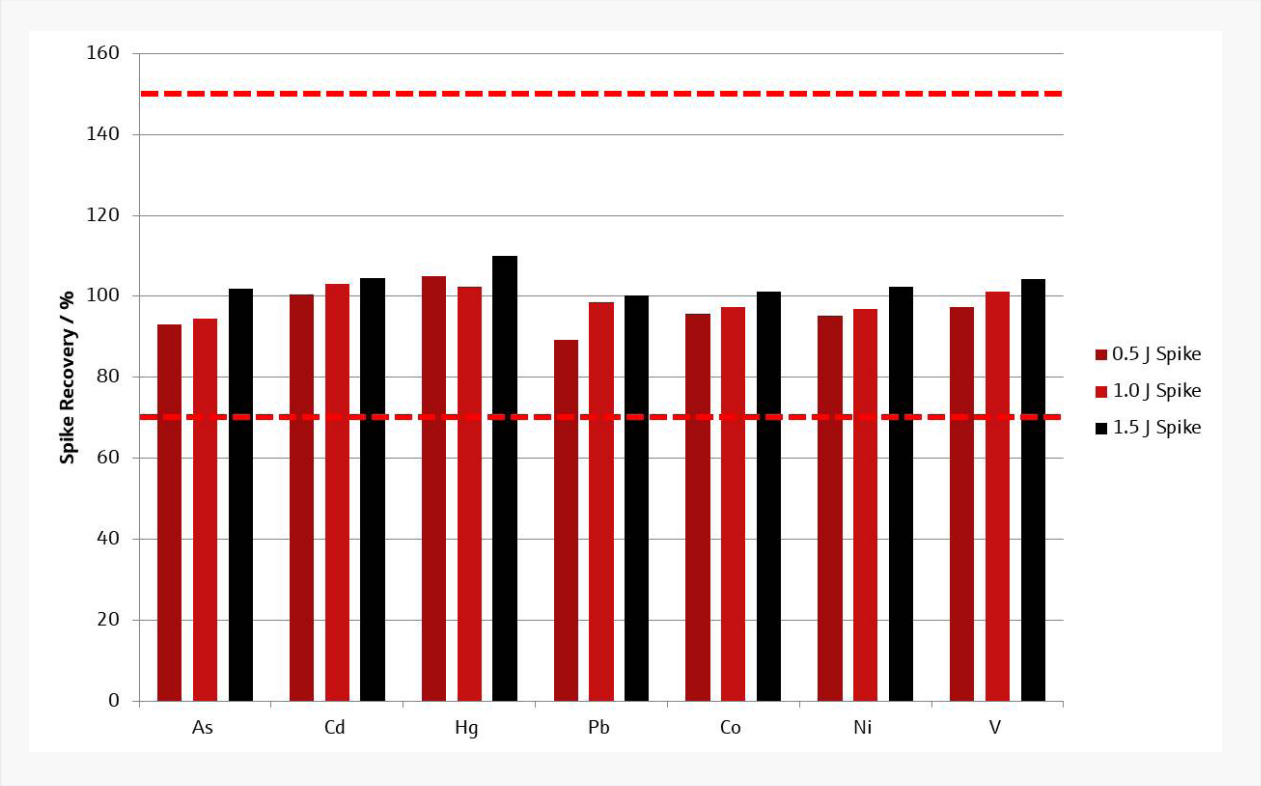

Figure 2: Spike recovery results for 0.5 J, 1.0 J and 1.5 J

The acceptance criteria defined in USP <232> for this kind of test are recoveries of between 70% and 150%. Figure 2 clearly shows that these criteria are easily met using the PlasmaQuant 9100 Elite, with average recoveries ranging from 90% to 110%.

Precision (repeatability and ruggedness)

In terms of repeatability, six independent aliquots of each sample were spiked with concentration 1.0 J. The excellent repeatability achieved with RSD values well below 7.5% from six independent preparations illustrates the robustness and reliability of the method being well below the acceptance criteria of 20%.

In terms of ruggedness, the results of twelve repeat analyses for each sample from twelve independent aliquots spiked with target value 1.0 J were analyzed over two non-consecutive days with a different operator, new calibration and re-optimization of the instrument. The criterion of 25% RSD in terms of ruggedness is easily achieved with the PlasmaQuant 9100 Elite, as precision values of less than 10% were achieved for the spiked antibiotic samples. These results over two non-consecutive days illustrate the robustness and reliability of the method (Table 7).

Table 7: Results of repeatability and ruggedness test

Element	RSD [%]	
	Repeatability (n=6) over 1 day	Ruggedness (n=12) over 2 days
As	4.9	6.2
Cd	6.4	6.5
Hg	4.8	5.4
Pb	5.0	9.7
Co	7.2	6.6
Ni	6.5	6.7
V	4.5	6.4

Specificity

Within this study the validation for specificity was undertaken by measuring the unspiked sample and two spiked samples with different levels of spiked target elements at 0.8 J and 1.0 J. Figure 3 depicts the obtained results normalized to the respective J-value of each impurity. For each analyte, the spikes show a distinctive increase in signal in comparison to the unspiked sample. Also, the 1.0 J spike shows a significantly greater signal in comparison to the 0.8 J spike. Both spike recoveries fulfill the requirements of the above described accuracy and repeatability tests and therefore prove that each target element is assessed unequivocally.

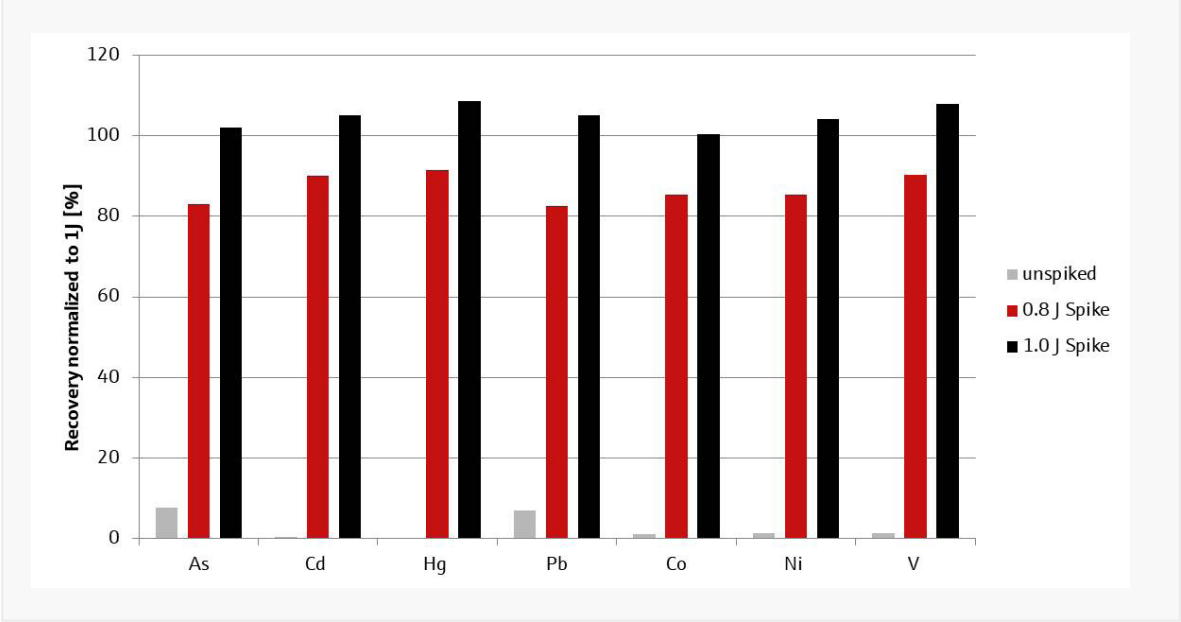

Figure 3: Results of specifity test (concentrations normalized to respective J-value

Further confidence in the accuracy of the results is obtained by the high-resolution spectra of the PlasmaQuant 9100 Elite. Figure 4 displays a spectral overlay of the unspiked sample and the two respective spikes at 0.8 J and 1.0 J for two target elements (cadmium and cobalt). It can be seen that these peaks clearly gain in signal intensity with increasing target element concentration and that the peaks are clearly separated from any adjacent peak that might interfere and cause a false-positive result.

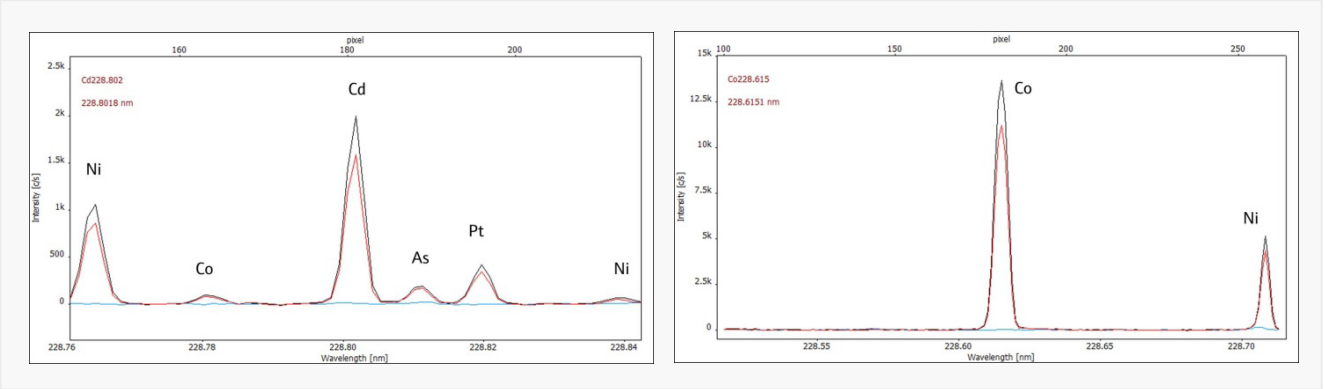

Figure 4: High resolution spectral overlay of Cd and Co; blue - sample, red – 0.8 J spike, black – 1.0 J spike

Limits of quantification and sensitivity

Low limits of quantification (LOQ) are particularly important for some of the potentially toxic trace elements defined in USP <232>, notably arsenic, cadmium, mercury and lead. The LOQ is based on the measurement of eleven blank solutions measured on two non-consecutive days and is defined as ten times the standard deviation of the eleven blank measurements and the dilution factor. Table 8 shows that the levels of all target elements are well below the requested limits. Additionally, the PlasmaQuant 9100 Elite achieves LOQs both well below the required concentration limits and ensures a secure quantification of all trace element concentrations requested for oral drugs.

Table 8: Comparison of limits of quantification (LOQ), concentration limits and sample concentrations

Element	Line [nm]	Method LOQ [µg/g]	Concentration limit [µg/g]	Sample concentration [µg/g]
As	188.979	0.0033	5	0.38
Cd	228.802	0.0001	2	0.01
Hg	184.886	0.0006	10	< LOQ
Pb	220.353	0.0013	2	0.14
Co	228.615	0.0003	20	0.20
Ni	231.604	0.0006	70	0.91
V	309.311	0.0003	35	0.50

Conclusion

This application note presents a simple and effective method for routine preparation and analysis of orally administered antibiotics by ICP-OES in combination with closed vessel microwave digestion. The methodology comprises a microwave digestion to mineralize the pharmaceutical product and a subsequent analysis of all Class 1 and Class 2A target elements by the PlasmaQuant 9100 Elite, a high-resolution ICP-OES.

With its high-frequency generator and its V-Shuttle torch, the PlasmaQuant 9100 Elite is able to reliably run samples with high matrix content, such as 20 g/L of antibiotics as described in the here presented methodology. The high matrix tolerance allows for lower dilution factors, which benefits the achievable method limits of quantification significantly. Together with the high sensitivity of the system, LOQs in the sub-ppm_w range are possible. At the same time, spectral interferences are resolved easily by the high-resolution optical system (2 pm @ 200 nm) ensuring high accuracy of the obtained results as well as high confidence in the developed methodology.

References

[1] General Chapter <232> Elemental Impurities—Limits, USP39. Publishing in Pharmacopeial Forum 42(2) [Mar.–Apr. 2016]

[2] General Chapter <233> Elemental Impurities—Procedures, Second Supplement to USP 38–NF 33, 2015

[3] International Conference on Harmonization, ICH Q3D Step 4 – Guideline for Elemental Impurities (ICH, Geneva, Switzerland, 2014)

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