Challenge
Method validation following USP 232/233 guidelines for the determination of elemental impurities in folic acid using ICP-MS

Solution
The PlasmaQuant MS Q offers outstanding sensitivity for the determination of elemental impurities in pharmaceuticals

Intended audience
For ICP-MS customers in pharma: Learn about elemental impurities and USP 233 compliance in this app note

Determination of Elemental Impurities in Pharmaceuticals by ICP-MS in accordance with ICH Q3D and USP 232 and 233 using the Example of Folic Acid

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 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].
As discussed below, ICP technology is now a compendial method for the quantification of trace elemental impurities and is becoming the routine method of choice for manufacturers and suppliers of pharmaceutical products, including raw materials, drug substances and excipients. Challenges within this field of application include a large variety of sample types with diverse 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 offers the measurement of a wide concentration range. In this regard, the plasma system needs to be able to handle any sample type without compromises in plasma stability and robustness. The accurate and reliable quantification of trace elemental impurities also requires a high sensitivity of the system, as well as the ability to resolve polyatomic interferences that are common in ICP-MS.
Within this study, the PlasmaQuant MS Q is used to determine elemental impurities in pharmaceutical products containing folic acid as the active pharmaceutical ingredient (API). Folic acid is used as the API in tablets as well as in liquid pharmaceutical products, which are administered orally or via injection (parenteral).
The PlasmaQuant MS Q allows for an interference-free analysis of trace elements in various matrices while being compliant to USP 232/233 guideline in all analytical steps. Furthermore, the high plasma robustness and high

sensitivity allow for the analysis of pharmaceutical products with high accuracy and high precision. The all-digital detector offers the measurement of a wide concentration range within a single measurement. This often avoids the need for measuring several sample dilutions in order to collect data for elements in both the low µg/L and the high mg/L range, providing savings in expenditure and time.

Overview of the USP Chapters <232>, <233>, and ICH Q3D

Chapter <232> Elemental Impurities – Limits and ICH Q3D

Chapter <232> and ICH Q3D specify maximum limits for the amount of elemental impurities permitted in drug products, which is defined to be the final form of the medicine which the patient takes. The elemental impurities may be present in either the drug substances, the active ingredients and/or excipients. Impurities may occur naturally in the raw material, derived from the production catalysts or introduced inadvertently throughout the manufacturing process. Compliance with the specified limits is required for all drug products, with the exceptions as listed in Chapter <232>. If elemental impurities are known to be present, have been added intentionally, or there is a known potential for introduction, it must be shown that compliance with defined limits is assured. Otherwise, a risk-based control strategy may also be considered.
Table 1 shows a total of 24 elemental impurities and the maximum permitted daily exposure (PDE) level in micrograms per day for oral, parenteral and inhalation drug delivery, as listed in chapter <232>.

Element Classification

The Elemental Impurities chapters classify the elements into three groups. The first group, or Class 1 elements, consist of the toxic elements Ar, Cd, Pb, and Hg. These elements must always be considered in the risk assessment and should always be measured. Class 2 elements are divided into two subgroups. Subclass 2A elements must also be included in all assessments, due to their ubiquity and relative toxicity. Subclass 2B elements need to be considered in the risk assessment only if they are known to be present or are intentionally added during the manufacturing process of the final pharmaceutical product. Class 3 elemental impurities have relatively low toxicity by oral administration but require assessment if delivered through the parenteral or inhalational routes.

Table 1: Permitted Daily Exposures (PDE) for elemental impurities as provided in USP chapter <232> [3]

Element	Class	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalation PDE (µg/day)
Cadmium	1	5	2	3
Lead	1	5	5	5
Arsenic	1	15	15	2
Mercury	1	30	3	1
Cobalt	2A	50	5	3
Vanadium	2A	100	10	1
Nickel	2A	200	20	5*
Thallium	2B	8	8	8
Gold	2B	100*	100*	1*
Palladium	2B	100	10	1
Iridium	2B	100	10	1
Osmium	2B	100	10	1

Continuation of table 1: Permitted Daily Exposures (PDE) for elemental impurities as provided in USP chapter <232> [3]

Element	Class	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalation PDE (µg/day)
Rhodium	2B	100	10	1
Ruthenium	2B	100	10	1
Selenium	2B	150	80	130
Silver	2B	150	10*	7
Platinum	2B	100	10	1
Lithium	3	550	250	25
Antimony	3	1200	90	20
Barium	3	1400	700	300
Molybdenum	3	3000	1500	10
Copper	3	3000	300	30
Tin	3	6000	600	60
Chromium	3	11000	1100	3

1 human toxicants
2A route-dependent human toxicants (high probability of occurrence)
2B route-dependent human toxicants (reduced probability of occurrence)
3 low toxicities (by oral route, may need consideration for inhalation and parenteral routes)
* revisions to the PDEs for nickel (inhalation: 6), gold (oral: 300, parenteral: 300, inhalation: 3), and silver (parenteral: 15) have been made in 2021 to correct calculation errors [4]

Chapter <233> Elemental Impurities – Procedures

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.

The criteria for acceptable alternative procedures, i.e. trace-element techniques such as Flame-AAS or GF-AAS, are also included. Alternative procedures must meet the described validation requirements in order to be used.

It must be emphasized that in addition to the system suitability requirements for the compendial ICP-OES and ICP-MS methods, before any procedure (including compendial) is initially used, the overall analytical procedure including sample preparation (if not otherwise indicated in the monograph) should be confirmed to be appropriate, for both the instrument being used and the samples being analyzed. This is done by meeting the requirements for alternate procedure validation, as described in Chapter <233>.

Method Validation

Meeting the requirements for the alternate procedure validation, as described in Chapter <233>, is critical as all aspects of the analytical procedures including the instrumental technique and sample preparation process must be validated and shown to be acceptable. As defined in Chapter <233>, the validation parameters for acceptability of the alternative procedure depend on whether the procedure is a “Limit Procedure” or a “Quantitative Procedure”.

Since the ICP-MS procedure is a “Quantitative Procedure” the requirements for the following validation parameters must be met: accuracy, precision (repeatability and ruggedness), specificity and limit of quantitation, range and linearity (demonstrated by meeting the accuracy requirement).

Meeting the performance requirements defined in these tests must be demonstrated experimentally using an appropriate system suitability procedure and reference material. The suitability of the method is determined by conducting studies with the material under test, supplemented or spiked with known concentrations of each target element of interest at the appropriate acceptance limit concentration.

Materials and Methods

Samples and Reagents

The API tested in this study is folic acid. It can be administered orally in the form of tablets or via injection (parenteral) in liquid form. Due to the different possible routes of administration and different formulation processes, multiple classes (as described above) of elemental impurities should be analyzed in folic acid products. For this reason, this study includes all 24 elements specified within the USP and ICH guidelines as well as a full validation of the applied methodology. For single products, only a subsection of these elements may be of interest, e.g., class 1 and 2A elements for oral drugs.

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 0.5 g of the folic acid drug product was accurately weighed and transferred into a digestion vessel (CX 100). The sample was spiked with 5 mL of conc. nitric acid, 1 mL of 30% hydrogen peroxide and 1 mL of conc. hydrochloric acid. The mixture was then shaken carefully and left standing for 10 minutes before the vessel was closed. Subsequent digestion was performed in the Speedwave XPERT microwave with the following program:

Table 2: Digestion program for folic acid pharmaceutical product

Step	[°C]	p_{max} [bar]	Ramp time [min]	Hold time [min]
1	160	80	5	10
2	190	80	5	10
3	210	80	5	20
4	50	80	-	20

After complete digestion and cooling to room temperature the clear solution was filled up to 50 mL with deionized water. For analysis of folic acid sample spikes were prepared according to the J-values listed in table 3.
For matrix-matched calibration, blanks and sample dilution the following composition was chosen:

- 2% HNO_3 (for stabilizing Li, V, Cr, Co, Ni, Cu, As, Se, Ag, Cd, Ba, Hg, Tl and Pb)
- 0.5% HCl (for stabilizing Mo, Ru, Rh, Sn, Sb, Os, Ir, Pt and Au, which are unstable in diluted HNO_3)

In order to correct long-term signal drifts and matrix effects, scandium, yttrium, terbium, and bismuth were added online as internal standards into the sample solution via a Y-piece.

Calibration

Calibration solutions were prepared from multi element solutions (TraceCERT®, according to ICH Q3D oral, standard 1, 2 and 3, SIGMA-ALDRICH®). Calibration was done matrix-matched by using the abovementioned solution.

Target limit (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, based on the daily dosage of the drug, and appropriately diluted to the working range of the instrument after completion of the sample preparation procedure.

As an example, the PDE limit for Cd in an oral medication as defined in Chapter <232> is 5 µg/day (see table 1). If the maximum dosage of the final drug product is 1 g per day this is equivalent to 5 µg of Cd / 1 g of drug product. If 0.5 g of the drug product is digested or dissolved (sample preparation above) and made up to 50 mL and 25-fold diluted prior to analysis (dilution factor 2500), the J-value for Cd in this example is equal to 2 µg/L (see table 3 below).
The method then recommends using a calibration made up of two standards: standard 1 = 0.5 J, standard 2 = 1.5 J. For Cd, this is equivalent to 1 µg/L for standard 1 and to 3 µg/L for standard 2. The calibration ranges for all elements are displayed in table 3 in accordance with the J-value calculated for each element.

In the Results and Discussion section the calculated J-values are compared with the limits of quantification (LOQ), see table 12. The LOQ values should be well below the target limits of each target element. Should this not be the case, it may be necessary to use an alternative sample preparation procedure (e.g., different starting mass of sample for dilution, different dilution factor, etc.) or a different analysis technique.

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

Element	Concentration limits for oral drug with a maximum daily dose of ≤ 1 g/day [µg/g]	0.5 J [µg/L]	0.8 J [µg/L]	1 J [µg/L]	1.5 J [µg/L]
Cd	5	1	1.6	2	3
Pb	5	1	1.6	2	3
As	15	3	4.8	6	9
Hg	30	6	9.6	12	18
Co	50	10	16	20	30
V	100	20	32	40	60
Ni	200	40	64	80	120
Tl	8	1.6	2.56	3.2	4.8
Au	100 (300*)	20 (60*)	32 (96*)	40 (120*)	60 (180*)
Pd	100	20	32	40	60
Ir	100	20	32	40	60
Os	100	20	32	40	60
Rh	100	20	32	40	60
Ru	100	20	32	40	60
Se	150	30	48	60	90
Ag	150	30	48	60	90
Pt	100	20	32	40	60
Li	550	110	176	220	330
Sb	1200	240	384	480	720
Ba	1400	280	448	560	840
Mo	3000	600	960	1200	1800
Cu	3000	600	960	1200	1800
Sn	6000	1200	1920	2400	3600
Cr	11000	2200	3520	4400	6600

Calculated for 1 dose per day, 1 g per dose and day, 0.5 g sample weight, 50 mL final volume, 25-fold sample dilution and a final dilution factor of 2500.

* revision to the PDE for gold (oral) [4]

Instrument settings

Table 4: PlasmaQuant MS Q instrument settings

Parameter	Specification
Nebulizer	MicroMist, 0.4 mL/min
Spray chamber	Scott double-pass spray chamber
Torch	Standard ICP-MS torch with 2.4 mm injector
Cones	Ni sampler and skimmer
iCRC gases	He, 120 mL/min
Autosampler	CETAC ASX 560

Method parameters

Table 5: Method parameters

Parameter	Specification
Plasma gas flow	9 L/min
Auxiliary gas flow	1.25 L/min
Sheath gas flow	0 L/min
Nebulizer gas flow	1.0 L/min
RF power	1.27 kW
Pump rate	15 rpm
Stabilization delay	30 s
Spray chamber temperature	3 °C
Sampling depth	6.0 mm

Evaluation Parameters

Polyatomic interferences were removed by utilizing the iCRC technology of Analytik Jena (integrated Collision Reaction Cell) applying helium as collision gas. All analytes were measured using one condition set (iCRC He mode).
For data recording, five average values were calculated, each average composed of twenty single scans. The resulting mean value and standard deviation were obtained from the average of the five replicates. Pb was determined using the sum of the intensities for its three major isotopes (^{206}Pb, ^{207}Pb, ^{208}Pb) to account for isotopic variation in the samples and standards. The analyzed isotopes were: ^{7}Li, ^{51}V, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{75}As, ^{78}Se, ^{98}Mo, ^{101}Ru, ^{103}Rh, ^{105}Pd, ^{107}Ag, ^{111}Cd, ^{121}Sb, ^{137}Ba, ^{189}Os, ^{193}Ir, ^{195}Pt, ^{197}Au, ^{202}Hg, ^{205}Tl, $^{206\text{-}208}$Pb.

Results and Discussion

In order to demonstrate that the sample preparation and ICP-MS procedure is appropriate for the samples being analyzed, the following measurements, tests, and validations must be performed, as per USP <233>:

- Calibration and system suitability
- Method validation
    - Detectability
    - Precision (Repeatability)
    - Accuracy
    - Specificity
    - Intermediate precision (Ruggedness)

Remark:
USP stated in 2021 that revisions to the PDEs were done for nickel (inhalation: 6), gold (oral: 300, parenteral: 300, inhalation: 3), and silver (parenteral: 15) to correct calculation errors [4]. Since we focus on oral PDE in this work, only the change for Au is relevant here. Due to the increase of the value from 100 to 300, the required method limit of quantification has also increased. We kept the previous low value since the measurement was done with the concentrations according to the respective J-value. This also allowed us to showcase that even the lower limit is reached due to the high instrument sensitivity.

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 with the J-value calculated for each element. 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. The obtained deviations are well within the required 20% (Table 6):

Table 6: Results of system suitability tests

Element	Standard 1 at start of sequence [µg/L]	Standard 1 at end of sequence [µg/L]	Deviation [%]	Pass/Fail
Cd	2.98	2.85	4.6	Pass
Pb	3.05	3.04	0.3	Pass
As	8.78	8.72	0.7	Pass
Hg	18.32	17.89	2.4	Pass
Co	29.32	29.4	0.3	Pass
V	58.13	58.88	1.3	Pass
Ni	117.39	117.39	0.0	Pass
Tl	4.88	4.77	2.3	Pass
Au	61.76	59.81	3.3	Pass
Pd	59.64	58.72	1.6	Pass
Ir	61.86	59.98	3.1	Pass
Os	61.33	57.35	6.9	Pass
Rh	59.87	57.41	4.3	Pass
Ru	59.78	58.91	1.5	Pass
Se	91.22	86.82	5.1	Pass
Ag	89.21	88.46	0.8	Pass
Pt	61.28	58.94	4.0	Pass
Li	322.99	300.6	7.4	Pass
Sb	712.62	687.32	3.7	Pass
Ba	826.18	811.4	1.8	Pass
Mo	1776.03	1711.83	3.8	Pass
Cu	1750.61	1688.54	3.7	Pass
Sn	3593.86	3478.48	3.3	Pass
Cr	6464.68	6307.78	2.5	Pass

Method Validation

Detectability

The instrumental detectability test (acceptance criterion 1) involves a comparison of the average value of three replicate measurements of a 1.0 J spiked sample solution with the 1 J standard solution. The recovery needs to be within 85% to 115%. The non instrumental detectability test (acceptance criterion 2) requires a comparison of the concentration of 1.0 J spiked sample solution against 0.8 J spike. The test passes if the spiked concentration value of 0.8 J is less than that of the 1.0 J spike. The spike concentrations need to be corrected by subtracting the concentration of the unspiked sample solution. All elements were within the acceptable criteria (Table 7).

Table 7: Detectability

Element	1 J standard	0.8 J spike (corrected) [µg/L]	1 J spike (corrected) n=3 [µg/L]	1 J recovery [%]	Pass/Fail criterion 1	Pass/Fail criterion 2
Cd	1.98	1.6	1.93	97.3	Pass	Pass
Pb	2.01	1.66	2.02	100.3	Pass	Pass
As	5.87	4.77	5.72	97.4	Pass	Pass
Hg	11.98	9.3	11.36	94.8	Pass	Pass
Co	19.84	15.9	19.62	98.9	Pass	Pass
V	39.65	31.81	38.83	97.9	Pass	Pass
Ni	78.92	64.21	78.13	99.0	Pass	Pass
Tl	3.2	2.58	3.20	99.9	Pass	Pass
Au	41.15	33.12	40.70	98.9	Pass	Pass
Pd	40.11	32.33	39.60	98.7	Pass	Pass
Ir	40.53	32.92	40.52	100.0	Pass	Pass
Os	41.35	28.83	36.02	87.1	Pass	Pass
Rh	40.04	32.4	39.11	97.7	Pass	Pass
Ru	40.29	32.21	39.78	98.7	Pass	Pass
Se	60.05	49.23	59.57	99.2	Pass	Pass
Ag	60.2	47.3	58.31	96.9	Pass	Pass
Pt	40.02	32.89	39.98	99.9	Pass	Pass
Li	211.43	169.07	199.02	94.1	Pass	Pass
Sb	457.52	374.65	451.10	98.6	Pass	Pass
Ba	545.79	441.35	524.91	96.2	Pass	Pass
Mo	1170.05	914.82	1105.69	94.5	Pass	Pass
Cu	1152.56	932.28	1122.23	97.4	Pass	Pass
Sn	2305.75	1895.81	2323.25	100.8	Pass	Pass
Cr	4226.18	3448.8	4179.68	98.9	Pass	Pass

In terms of repeatability, the acceptance criteria defined in USP <232> requires the relative standard deviation of six independent aliquots of 1 J spiked sample to be below 20%. The excellent repeatability achieved with RSDs between 2.0 and 3.7% from six independent preparations, illustrates the robustness and reliability of the method being well below the acceptance criteria (Table 8):

Table 8: Results of the repeatability test

Element	Spike 1 [µg/L]	Spike 2 [µg/L]	Spike 3 [µg/L]	Spike 4 [µg/L]	Spike 5 [µg/L]	Spike 6 [µg/L]	RSD [%]	Pass/Fail
Cd	1.92	2.03	1.85	1.99	1.84	1.98	3.7	Pass
Pb	2.02	2.05	1.99	2.02	1.91	2.04	2.3	Pass
As	5.99	6.05	5.86	5.84	5.59	5.89	2.5	Pass
Hg	12.24	12.55	12.12	12.36	11.69	12.27	2.2	Pass
Co	19.83	20.46	19.59	19.83	19.0	19.83	2.2	Pass
V	39.27	40.33	38.73	39.33	37.46	39.29	2.2	Pass
Ni	78.52	81.39	77.28	78.94	75.29	79.12	2.4	Pass
Tl	3.18	3.29	3.2	3.28	3.1	3.22	2.0	Pass
Au	41.29	42.39	41.62	42.02	39.82	41.36	2.0	Pass
Pd	39.92	40.64	39.42	39.85	37.88	40.1	2.2	Pass
Ir	40.11	41.52	40.14	40.87	38.67	40.42	2.2	Pass
Os	36.67	37.93	36.8	37.46	35.32	36.79	2.2	Pass
Rh	39.82	40.02	40.16	39.48	37.5	40.73	2.6	Pass
Ru	40.03	40.98	39.38	39.96	37.69	39.95	2.5	Pass
Se	59.81	61.59	59.41	60.9	57.81	58.84	2.1	Pass
Ag	59.53	60.41	58.29	59.0	56.26	59.06	2.2	Pass
Pt	39.67	40.96	39.9	40.5	38.18	39.96	2.2	Pass
Li	206.56	216.33	203.17	209.93	201.41	206.53	2.3	Pass
Sb	466.09	480.84	457.09	460.72	429.68	454.12	3.3	Pass
Ba	556.22	560.15	528.2	547.78	522.42	559.69	2.8	Pass
Mo	1180.69	1201.77	1137.45	1160.33	1094.45	1167.84	2.9	Pass
Cu	1154.32	1167.52	1130.22	1151.88	1088.51	1147.37	2.2	Pass
Sn	2366.27	2388.41	2273.13	2333.4	2240.86	2368.95	2.3	Pass
Cr	4249.17	4402.65	4194.26	4321.2	4079.18	4307.16	2.4	Pass

Accuracy

In accordance with USP <233> guidelines, the accuracy of the method can be assessed by spike recoveries. Table 9 shows averaged spike recoveries for all samples independently prepared in triplicate at the levels 0.5 J, 1.0 J and 1.5 J. The acceptance criteria defined in USP <232> for this kind of test are recoveries between 70 and 150%. This criterion was easily met using the PlasmaQuant MS Q, with average recoveries ranging from 89 to 106%.

Table 9: Results of accuracy test

Element	Spike recovery [%]			Pass/Fail
	0.5 J spike	1.0 J spike	1.5 J spike	
Cd	94.3	96.7	97.1	Pass
Pb	103.0	100.8	99.7	Pass
As	100.6	95.7	94.0	Pass
Hg	100.6	98.8	96.7	Pass
Co	101.5	98.1	96.7	Pass
V	101.8	98.0	96.9	Pass
Ni	101.2	97.7	96.6	Pass
Tl	102.7	100.1	98.8	Pass
Au	106.2	103.3	101.0	Pass
Pd	100.6	99.1	97.9	Pass
Ir	104.0	101.4	99.2	Pass
Os	94.1	91.9	89.7	Pass
Rh	99.6	97.8	96.2	Pass
Ru	101.1	99.5	98.3	Pass
Se	99.1	99.4	96.6	Pass
Ag	98.4	97.3	95.2	Pass
Pt	103.1	100.0	97.7	Pass
Li	95.5	90.5	88.5	Pass
Sb	96.5	93.9	92.5	Pass
Ba	96.7	93.8	93.2	Pass
Mo	94.4	93.0	92.4	Pass
Cu	96.5	93.5	91.6	Pass
Sn	98.7	96.8	94.3	Pass
Cr	98.3	95.0	93.2	Pass

Specificity

The definition of specificity within USP <233> is that the established method must be able to unequivocally assess each target element in the presence of components that may be expected to be present, including other target elements and matrix components. Further definition and means to determine specificity is given in USP chapter <1225> [4]. Here, specificity is defined to serve the purpose of "ensuring that all of the analytical procedures performed allow an accurate statement of the content of impurities of an analyte" [5]. Hence it has to be validated that the obtained results are interference-free and no false-positive or false-negative results are obtained. The proposed determination of specificity for impurity procedures is: "by spiking the drug substance or product with appropriate levels of impurities and demonstrating that these impurities are determined with appropriate accuracy and precision." [4]

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 (Table 10). As acceptance criterion the concentration needs to increase with every level. 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 accuracy and repeatability tests described above and therefore prove that each target element is assessed unequivocally.

Table 10: Results of specificity test

Element	Unspiked sample [µg/L]	0.8 J spiked sample [µg/L]	1 J spiked sample [µg/L]	Pass/Fail
Cd	0.01	1.53	2	Pass
Pb	0.01	1.64	2.14	Pass
As	< DL	4.7	6.12	Pass
Hg	0.34	9.97	12.87	Pass
Co	0.11	16.23	20.88	Pass
V	< DL	32.35	41.73	Pass
Ni	0.11	64.63	83.01	Pass
Tl	0.01	2.58	3.43	Pass
Au	0.29	33.65	44.45	Pass
Pd	0.06	32.79	41.51	Pass
Ir	0.1	32.98	43.33	Pass
Os	0.53	30.28	39.4	Pass
Rh	0.17	32.61	41.64	Pass
Ru	0.02	33.03	41.66	Pass
Se	< DL	49.05	61.94	Pass
Ag	0.07	48.29	61.61	Pass
Pt	0.04	32.74	42.95	Pass
Li	0.28	170.47	221.15	Pass
Sb	0.97	377.26	466.83	Pass
Ba	2.26	460.93	554.03	Pass
Mo	31.87	933.88	1200.3	Pass
Cu	0.85	926.1	1199.53	Pass
Sn	2.65	1932.68	2417.25	Pass
Cr	2.41	3469.14	4474.67	Pass

Intermediate Precision (Ruggedness)

The results of 12 repeat analyses for each sample from six independent aliquots spiked with target value 1.0 J, were analyzed over two consecutive days with a different operator, new calibration and re-optimization of the instrument. The results for the folic acid samples over the two working days are shown in table 11. The RSDs refer to samples 1a – 6a (see repeatability, table 8) from the previous and samples 1b – 6b of the following measuring day. With a maximum RSD of 4.8% the acceptance criteria for ruggedness (RSD < 25%) was easily met.

Table 11: Results of ruggedness test

Element	Spike 1a [µg/L]	Spike 2a [µg/L]	Spike 3a [µg/L]	Spike 4a [µg/L]	Spike 5a [µg/L]	Spike 6a [µg/L]
Cd	1.92	2.03	1.85	1.99	1.84	1.98
Pb	2.02	2.05	1.99	2.02	1.91	2.04
As	5.99	6.05	5.86	5.84	5.59	5.89
Hg	12.24	12.55	12.12	12.36	11.69	12.27
Co	19.83	20.46	19.59	19.83	19.0	19.83
V	39.27	40.33	38.73	39.33	37.46	39.29
Ni	78.52	81.39	77.28	78.94	75.29	79.12
Tl	3.18	3.29	3.2	3.28	3.1	3.22
Au	41.29	42.39	41.62	42.02	39.82	41.36
Pd	39.92	40.64	39.42	39.85	37.88	40.1
Ir	40.11	41.52	40.14	40.87	38.67	40.42
Os	36.67	37.93	36.8	37.46	35.32	36.79
Rh	39.82	40.02	40.16	39.48	37.5	40.73
Ru	40.03	40.98	39.38	39.96	37.69	39.95
Se	59.81	61.59	59.41	60.9	57.81	58.84
Ag	59.53	60.41	58.29	59.0	56.26	59.06
Pt	39.67	40.96	39.9	40.5	38.18	39.96
Li	206.56	216.33	203.17	209.93	201.41	206.53
Sb	466.09	480.84	457.09	460.72	429.68	454.12
Ba	556.22	560.15	528.2	547.78	522.42	559.69
Mo	1180.69	1201.77	1137.45	1160.33	1094.45	1167.84
Cu	1154.32	1167.52	1130.22	1151.88	1088.51	1147.37
Sn	2366.27	2388.41	2273.13	2333.4	2240.86	2368.95
Cr	4249.17	4402.65	4194.26	4321.2	4079.18	4307.16

Continuation of table 11: Results of ruggedness test

Element	Spike 1b [µg/L]	Spike 2b [µg/L]	Spike 3b [µg/L]	Spike 4b [µg/L]	Spike 5b [µg/L]	Spike 6b [µg/L]	RSD (day a & b) [%]	Pass/Fail
Cd	2	1.97	2.05	1.92	2	2	3.3	Pass
Pb	2.08	2.03	2.08	2.04	2.07	2.04	2.2	Pass
As	6.16	6.21	6.17	6.2	6.2	6.08	3.1	Pass
Hg	12.25	12.2	12.52	12.01	12.42	12.21	1.8	Pass
Co	20.69	20.32	20.72	20.27	20.7	20.32	2.5	Pass
V	40.95	40.25	41.63	40.43	40.75	39.9	2.7	Pass
Ni	82.56	81.89	83.03	80.91	82.97	80.77	2.9	Pass
Tl	3.28	3.24	3.33	3.24	3.28	3.26	1.8	Pass
Au	42.29	41.21	43.1	41.44	42.1	41.2	1.9	Pass
Pd	40.61	39.96	41.25	40.28	40.56	40.01	2.0	Pass
Ir	40.64	39.88	41.58	40.12	40.35	40.03	1.8	Pass
Os	39.71	39.44	40.65	39.75	39.34	39.37	4.1	Pass
Rh	40.75	40	41.35	40.25	40.25	40.02	2.2	Pass
Ru	40.75	39.89	41.5	40.09	40.48	40.1	2.2	Pass
Se	62.98	62.64	62.21	62.09	62.39	61.93	2.6	Pass
Ag	59.2	56.34	61.98	50.95	59.88	55.51	4.8	Pass
Pt	40.51	39.68	41.28	39.81	40.31	39.74	1.9	Pass
Li	222.14	219.67	226.67	219.16	222.09	218.24	3.8	Pass
Sb	500.49	485.86	504.97	485.9	497.46	481.02	4.5	Pass
Ba	578.31	562.83	584.46	560.89	579.27	559.72	3.2	Pass
Mo	1242.08	1205.92	1262.22	1205.91	1245.47	1205.64	3.8	Pass
Cu	1232.11	1206.93	1239.56	1195.81	1228.49	1196.2	3.7	Pass
Sn	2512.8	2435.58	2562.95	2445.25	2493.49	2421.76	3.8	Pass
Cr	4531.32	4442.66	4541.05	4403.32	4532.69	4385.84	3.1	Pass

Limit 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 the calibration blank and is defined as ten times the standard deviation of the blank measurements divided by the slope of the calibration curve (sensitivity). The LOQs were measured under routine laboratory conditions. Table 12 shows that the levels of all target elements are well below the given limits. Additionally, the PlasmaQuant MS Q achieves LOQs both well below the required concentration limits and ensures a secure quantification of all trace element concentrations requested for oral drugs.

Table 12: Comparison of limits of quantification (LOQ), defined concentration limits, and sample concentrations

Element	Isotope	Method LOQ [µg/g]	Concentration limits for oral drug with a maximum daily dose of ≤ 1 g/day [µg/g]	Sample concentration [µg/g]
Cd	111	0.055	5	< LOD
Pb	206-208	0.018	5	< LOQ
As	75	0.184	15	< LOD
Hg	202	0.231	30	0.551
Co	59	0.013	50	0.262
V	51	2.209	100	< LOD
Ni	60	0.112	200	0.1717
Tl	205	0.011	8	0.010
Au	197	0.044	100*	0.377
Pd	105	0.667	100	< LOD
Ir	193	0.024	100	0.118
Os	189	0.049	100	0.895
Rh	103	0.141	100	0.348
Ru	101	0.033	100	< LOD
Se	78	6.654	150	< LOD
Ag	107	0.043	150	0.067
Pt	195	0.007	100	0.074
Li	7	1.067	550	< LOQ
Sb	121	2.160	1200	< LOQ
Ba	137	6.808	1400	< LOQ
Mo	98	1.910	3000	42.069
Cu	65	3.878	3000	< LOD
Sn	118	2.840	6000	3.594
Cr	52	6.869	11000	< LOQ

* Revision to the PDE for gold (oral: 300) [4]

Summary

This application note shows a simple and effective method for routine preparation and analysis of pharmaceutical materials by ICP-MS in combination with closed vessel microwave digestion. The analysis of elemental impurities in pharmaceutical products by ICP techniques represents a routine task in QC labs of drug manufacturers and suppliers of materials involved in the manufacturing and handling process of these products. Each developed method to investigate such elemental impurities needs to be validated according to the guidelines and regulations of the International Conference on Harmonization (ICH) and the United States Pharmacopeia (USP).
The major challenges for this application include varying sample types in terms of matrix composition, varying matrix loading, drug specific target limits and analyte combinations, the possibility of polyatomic interferences, as well as the requirement of analyzing elements over a large concentration range (low µg/L to high mg/L) in a single run. The PlasmaQuant MS Q successfully meets all of these challenges and is well suited for the determination of elemental impurities in pharmaceutical materials by its ability to easily meet the target values and performance criteria as defined in the ICH Guideline and USP Chapter <232>.

Figure 4: PlasmaQuant MS Q

Recommended device configuration

Table 13: Overview of devices, accessories, and consumables

Article	Article number	Description
Microwave-assisted extraction		
speedwave XPERT - Microwave Pressure Digestion System		Set of 10 disposable tubes for AOF 18 x 6 mm, filled Set of 100 disposable tubes for AOF 16 x 8 mm, filled
Inductively Coupled Plasma Mass Spectrometry		
PlasmaQuant MS Q	818-08011-2	The PlasmaQuant MS Q is a high-performance ICP-MS, capable of measuring over 75 elements in a single measurement, from ultra-trace to major levels
Teledyne-Cetac ASX 560 Autosampler	810-88015-0	The Teledyne CETAC Technologies ASX-560, next generation autosampler with integrated rinse function is sleek and durable by design
21 CFR Part 11 Compliance Module for Aspect MS	810-88500-0	Software package for ASpect MS 4.X compliance with 21 CFR Part 11 requirements; consists of electronic signature, audit trail function, user-management and FDA-Certificate

References

[1] General Chapter <232> Elemental Impurities—Limits, USP39. Published 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)

[4] Chapter revision: ⟨232⟩ Elemental Impurities—Limits. Published in Pharmacopeial Forum 47(1) [Jan.-Feb. 2021]

[5] General Chapter <1225> Validation of compendial procedures, First Supplement to USP 40–NF 35

Headquarters
Analytik Jena GmbH+Co. KG
Konrad-Zuse-Strasse 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279

info@analytik-jena.com
www.analytik-jena.com

Version 1.0 · Author: FrLe
en · 07/2023