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Development and validation of an ICP-MS method for the determination of elemental impurities in TP-6076 active pharmaceutical ingredient (API) according to USP ⟨232⟩/⟨233⟩

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ABSTRACT

The new guidelines of the United States pharmacopeia (USP), European pharmacopeia (EP) and international conference on harmonization (ICH) regulating elemental impurities limits in pharmaceuticals signify the end of unspecific analysis of metals as outlined in USP ⟨231⟩. The new guidelines specify both daily doses and concentration/limits of elemental impurities in pharmaceutical final products, active pharmaceutical ingredients (API) and excipients. In chapter USP ⟨233⟩ method implementation, validation and quality control during the analytical process are described. We herein report the use of a stabilising matrix that overcomes low spike recovery problem encountered with Os and allows the determination of all USP required elemental impurities (As, Cd, Hg, Pb, V, Cr, Ni, Mo, Cu, Pt, Pd, Ru, Rh, Os and Ir) in a single analysis. The matrix was used in the validation of a method to determine elemental impurities in TP-6076 active pharmaceutical ingredient (API) by ICP-MS according to the procedures defined in USP⟨233⟩ and to GMP requirements. This validation will support the regulatory submission of TP-6076 which is a novel tetracycline analogue effective against the most urgent multidrug-resistant gram-negative bacteria. Evaluation of TP-6076 in IND-enabling toxicology studies has led to the initiation of a phase 1 clinical trial.

1. Introduction

The current United States pharmacopeia (USP) method for monitoring inorganic contaminants in pharmaceutical samples as defined in general chapter ⟨231⟩ is over a 100 year-old colorimetric test. This method, known as the "heavy metals limit test", is based on precipitation of 10 sulfide-forming elements (Ag, As, Bi, Cd, Cu, Hg, Mo, Pb, Sb and Sn), in a reaction with a reagent such as thioacetamide [1]. The resulting coloured precipitate is compared visually to a 10 μg/g Pb standard to determine compliance with the heavy metal limit. Besides the obvious potential variability associated with a subjective visual comparison, USP⟨231⟩ is a limit test based on the sum of the 10 elements, and so does not give individual concentrations for each element. The current USP method, ⟨231⟩ "heavy metals limit test", is acknowledged to be inadequate and is due to be replaced with new general chapters USP⟨232⟩ (limits) and ⟨233⟩ (procedures) which are currently official and due to be enforced in 2018. Similar regulations were also issued by the European pharmacopeia (EP) and international conference on harmonization (ICH) regarding the limitation of elemental impurities, such as heavy metals and catalyst residues, produce an urgent need for robust and efficient ICP-MS analytical methods [2–6].

USP⟨232⟩ defines permitted daily exposure (PDE) limits for a wide range of inorganic (elemental) impurities: As, Cd, Hg, Pb, V, Cr, Ni, Mo, Cu, Pt, Pd, Ru, Rh, Os and Ir. The daily exposure limits must be scaled for the recommended maximum daily dose of the drug under investigation, so for a product with a daily dose of 10 g, the elemental impurity level in the dosage form (measured in μg/g) must be 10 times lower than the limits shown. The required limits can easily be measured directly with modern instrumental techniques such as ICP-MS referenced in USP⟨233⟩ [7,8].

However, many drug products/substances will require acid digestion with its associated dilution of the original sample to obtain solutions suitable for ICP-MS analysis. This represents a challenge as the digestion process is material consuming and many novel drugs are based on increasingly sophisticated and costly

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Table 1
Actual elemental impurities target limits[a] in TP-6076 active pharmaceutical ingredient amended for the maximum daily dose and the expected elemental contaminants in the excipients.

Element	Concentration limits (μg/g) for parenteral drug products with a maximum daily dose of ≤10 g/day daily dose of injection <100 mL	Concentration limits (μg/g) for parenteral drug products with a maximum daily dose of ≤0.5 g/day daily dose of injection <100 mL	Actual target limit (μg/g)
Cd	0.25	5	2.5
Pb	0.5	10	5
As	0.15	3	1.5
Hg	0.15	3	1.5
Ir	1	20	10
Os	1	20	10
Pd	1	20	10
Pt	1	20	10
Rh	1	20	10
Ru	1	20	10
Cr	Not a safety concern	Not a safety concern	100
Mo	1	20	10
Ni	5	100	50
V	1	20	10
Cu	10	200	100

[a] Limits are as per USP232 and official from February 1, 2013 till December 1, 2015.

active pharmaceutical ingredients (APIs), which may only be available in very small amounts [9–12]. The dilution associated with the preparation of these milligram-scale sample weights means that careful consideration needs to be given to the experimental design to minimise errors. Also, the digestion sample preparation approach is based on the complete break-down of the sample matrix often by oxidising mineral acids which will cause problems in the determination of osmium traces, since this element forms different species of varying volatility under such conditions, leading to uncontrolled losses of Os [13,14]. Although Os is frequently stabilised by the addition of chloride or HCl, it can still be present as various species in different oxidation states and chemical composition in hydrochloric matrices leading to significant losses of the analyte during storage or sample preparation [15]. A recent study on the topic demonstrates that the accurate quantification of total Os concentrations together with all elements required by the USP chapter ⟨232⟩ following oxidative digestion is difficult and prone to errors [16].

We herein report the use of a mixture to stabilise Os in an oxidizing environment by using an aqueous solution of 5% HCl (v/v), 0.1% acetic acid (v/v), 0.076% thiourea (w/v) and 0.01% ascorbic acid (w/v). This digestion free approach has been applied in the present study and showed the capability to analyse the full range of elements as required by the USP using one simple dissolution process/method. The approach was used in the determination of Cd, Pb, As, Hg, Ir, Os, Pd, Pt, Rh, Ru, Cr, Mo, Ni, V, and Cu content in TP-6076 active pharmaceutical ingredient (API) by ICP-MS according to the validation procedures defined in USP⟨233⟩. The elemental impurities limits are based on the maximum daily dose and the expected elemental contaminants in the excipients used; these are listed in Table 1. This validation will support the regulatory submission of TP-6076 (compound developed by Tetraphase Pharmaceuticals, Inc. and selected for clinical development from more than 3000 analogues) which is a novel tetracycline analogue effective against the most urgent multidrug-resistant gram-negative bacterial health threats identified by the CDC in a September 2013 report. Pathogens targeted include carbapenem-resistant strains of *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Escherichia coli*. Evaluation of TP-6076 in IND-enabling toxicology studies has led to the initiation of a phase 1 clinical trial. The randomized, placebo-controlled, double-blind, single-ascending dose phase 1 study is evaluating the safety and pharmacokinetics of TP-6076. The study is being conducted under a clinical trial authorisation in a single centre in up to 32 healthy volunteers.

2. Experimental

2.1. Chemical and laboratory reagents

Trace metal grade concentrated acetic acid (Fisher Chemical), trace metal grade concentrated hydrochloric acid (Fisher Chemical), TraceSELECT grade thiourea (Fluka), multi-element standard Sc, Li6, Y, In, Tb and Bi (Inorganic Ventures), multi-element standard Ir, Os, Pd, Pt, Rh and Ru (IV-Stock-38 from Inorganic Ventures), multi-element standard Cd, Pb, As, Hg, Mo, Ni, V and Cu (IV-Stock-41 from Inorganic Ventures), Ni standard (Inorganic Ventures), V standard (Inorganic Ventures), Cd standard (Inorganic Ventures), Pt standard (Inorganic Ventures), Pd standard (Inorganic Ventures), Mo standard (Inorganic Ventures), Hg standard (Inorganic Ventures), Cr standard (Inorganic Ventures), Pb standard (Inorganic Ventures), Cu standard (Inorganic Ventures), Cr standard (Inorganic Ventures), As standard (Inorganic Ventures), Os standard (Inorganic Ventures), Ir standard (Inorganic Ventures), Rh standard (Inorganic Ventures), Ru standard (Inorganic Ventures), TP-6067 active pharmaceutical ingredient (Tetraphase Pharmaceuticals).

Test materials were stored at the recommended storage conditions provided on the supplier's certificate of analysis. All solutions prepared from the test materials were stored at room temperature. All analytical chemicals sourced by Almac Sciences were of trace analysis grade or equivalent. All volumetric flasks used in preparations were polymethylpentene (PMP), polypropylene (PP) or equivalent quality polymer. All sample preparation steps and measurements at Almac Sciences- Mass Spectrometry laboratory (FDA and MHRA certified) were carried out under GMP.

2.2. Sample preparation

Two replicates were prepared by dissolving approximately 10 mg of TP-6076 in 10 mL of diluent [5% HCl (v/v), 0.1% acetic acid (v/v), 0.076% thiourea (w/v) and 0.01% ascorbic acid (w/v)] to give a 1 mg/mL solution.

2.3. Internal standard

A 200 ng/mL mixed internal standard (containing scandium, lithium6, yttrium, indium, terbium and bismuth) was introduced online into the spray chamber using a peristaltic pump.

2.4. Spiked samples preparations

Spiked samples were prepared at 50% (*0.5 J*), 100% (*1 J*) and 150% (*1.5 J*) of the target limit by spiking 10 mg of TP-6076 with 250, 500 and 750 μL of the spiking solution and make the volume to 10 mL using the diluent. Six preparations at each spiking level were prepared. The spiking solution (Ir, Os, Pd, Pt, Rh, Ru, Mo, V at 200 ng/mL, Cu and Cr at 2000 ng/mL, As and Hg at 30 ng/mL, Cd at 50 ng/mL, Pb at 100 ng/mL and Ni at 1000 ng/mL) was prepared by mixing certified parent standards for each element. *J* is the limit for each elemental impurities in the final analysis solution.

2.5. Calibration standards preparation

The calibration standards were prepared at 10% *(0.1J)*, 25% *(0.25J)*, 50% *(0.5J)*, 100% *(1J)*, 200% *(2J)* and 500% *(5J)* of the target limit for each elemental impurities in the final analysis solution.

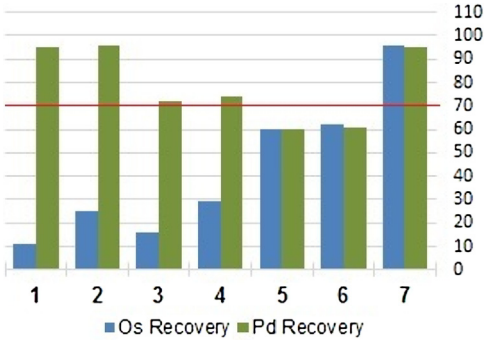


Fig. 1. The impact of different sample preparation techniques on Os and Pd recovery (%).

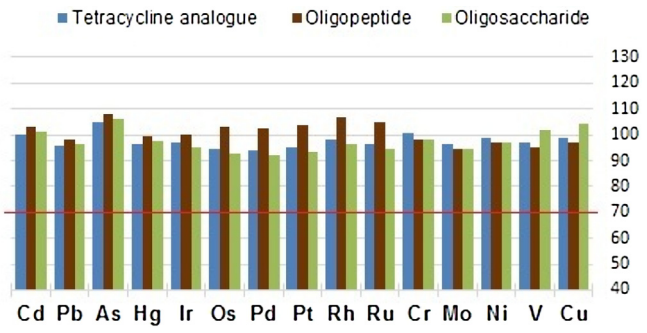


Fig. 2. Recovery (%) of the 15 USP elements from three different sample types spiked at equal concentration (J) using the stabilising mixture.

This encompassed the specification limit as described in USP Chapters <233/232> in the final working solution *(J)*. To verify that the calibration standards were prepared correctly, an independent quality control (IQC at *1J*) was run against the calibration line.

2.6. Instrumentation

An Agilent 7900 ICP-MS was used for all measurements. The instrument was equipped with standard nickel sampling and skimmer cones, a glass concentric nebulizer, quartz spray chamber and quartz torch with 2.5 mm id injector. The instrument also features a fourth generation collision/reaction cell, the ORS4. The ORS4 includes a standard helium (He) mode cell gas line which provides effective removal of most common polyatomic interferences (He gas flow is approximately 5 mL/min). He mode provides sensitivity for the detection of elements at the levels defined in the USP. The advanced high energy helium mode (HEHe) was also used during the validation. This mode utilises He gas flow >10 mL/min and is useful for elements under significant polyatomic interferences. An Agilent ASX-520 auto-sampler was used to deliver the samples. The measured analyte masses/internal standard masses were $^{51}V/^{45}Sc$, $^{52}Cr/^{45}Sc$, $^{60}Ni/^{45}Sc$, $^{63}Cu/^{45}Sc$, $^{75}As/^{89}Y$, $^{95}Mo/^{89}Y$, $^{101}Ru/^{89}Y$, $^{103}Rh/^{89}Y$, $^{105}Pd/^{159}Tb$, $^{111}Cd/^{159}Tb$, $^{189}Os/^{209}Bi$, $^{193}Ir/^{209}Bi$, $^{195}Pt/^{209}Bi$, $^{202}Hg/^{209}Bi$ and $^{208}Pb/^{209}Bi$.

3. Results and discussion

3.1. Method development

Through the course of this study a number of problems were overcome, primarily the poor recoveries of some elements due to incompatibility with the analysis matrix. The room temperature digestion and microwave accelerated digestion (Mars5 system form CEM) were investigated as potential sample preparation techniques using either nitric acid or a mixture of 80:20 nitric acid: hydrochloric acid. Although the microwave digestion gave excellent Pd recoveries (90–95%) and the ambient digestion gave acceptable Pd recoveries (71–75%), the digestion based approach was not suitable as Os recoveries were < 30% (Fig. 1). Low Os recoveries are due to the formation of volatile Os oxide species in the nitric acid medium during digestion. Simple dissolution of the sample in 5% hydrochloric acid resulted in better Os spike recovery of approximately 60% possibly due to the formation of the soluble hexachloro anionic complex $[OsCl_6]^{2-}$ in a dilute HCl media which will minimise osmium loss. Nevertheless, the achieved 60% recovery was less than the USP minimum required recovery of 70%. Also, the good spike recovery for Pd that was achieved when digestion was used dropped to <70% in the dissolution approach, potentially due to the formation of a complex between the tetracyclic structure of TP-6076 and Pd.

Ascorbic acid and thiourea were found, through their reducing properties, to stabilise osmium and prevent oxidation in the presence of acids to osmium tetroxide which is very difficult to analyse as well as being dangerous at high levels. The addition of 0.076% thiourea (w/v) and 0.01% ascorbic acid (w/v) to the 5% HCl (v/v), 0.1% acetic acid (v/v) yielded acceptable Os recoveries around 95% meeting USP requirements. The addition of thiourea has also helped in stabilising palladium and the platinum group elements (PGE's) as it forms a complex with PGE's through sulfur within the molecule. The complex assists with ionisation of the PGE's leading to improved recoveries [17–19].

Since the recovery can be sample specific and to demonstrate the suitability of the stabilising mixture across different chemical classes, the spike recoveries of Cd, Pb, As, Hg, Ir, Os, Pd, Pt, Rh, Ru, Cr, Mo, Ni, V, and Cu from TP-6076 (Tetracycline analogue) were compared to the corresponding recoveries obtained from an oligosaccharide and oligopeptide compounds as illustrated in Fig. 2. All elements displayed recoveries in the range of 94–109% which is well within the USP requirements of 70–150%. The results clearly indicate that the use of the stabilising mixture is ideal for elemental impurities determination in pharmaceutical products after dissolution and will meet accuracy regulatory requirements.

The stabilising matrix was used in the validation of a method to determine elemental impurities in TP-6076 active pharmaceutical ingredient by ICP-MS according to the validation procedures defined in USP⟨233⟩ and as described in the following sections.

Microwave digestion (HNO_3), **2**: Microwave digestion (HNO_3/HCl), **3**: Room temperature digestion (HNO_3), **4**: Room temperature digestion (HNO_3/HCl), **5**: Simple dissolution in 5% HCl, **6**: Simple dissolution in 5% HCl, 0.1% acetic acid, **7**: Simple dissolution in 5% HCl, 0.1% acetic acid, 0.076% thiourea, 0.01% ascorbic acid. USP requirements of at least 70% recovery is marked as red line.

USP requirements of at least 70% recovery is marked as red line.

3.2. Accuracy and precision (including repeatability)

The endogenous content in the un-spiked sample (Table 3) was subtracted from the results of the spiked samples before accuracy and precision were determined. The results are presented in Table 2. All acceptance criteria described in USP for accuracy were met as the spike recovery for the mean of all six preparations, at each spiking concentration for each element was within 70–150%. Also, USP requirements for precision (repeatability) were met as the%RSD for each of the six preparations at each of the spiking concentrations for each element was ≤20%. The method gave results well within USP requirements as recoveries for all elements (except Pd at *0.5J level)* were within 90–110%, with relative standard deviations (RSDs) of <10%. Pd spike recovery of 86.6% (He mode) and 89.3% (HEHe mode) may be explained by the interaction with

Table 2
Accuracy (mean spike recovery%) and precision (repeatability as%RSD) for all USP restricted elements in [He] and [HEHe] modes.

	He mode						HEHe mode					
	0.5J		1J		1.5J		1.5J		1.5J		1.5J	
	Mean%	RSD%	Mean%	RSD%	Mean%	RSD%	Mean%	RSD%	Mean%	RSD%	Mean%	RSD%
Cd	101.4	2.7	100.0	1.3	99.1	1.5	101.9	2.8	100.3	1.3	99.1	1.5
Pb	98.0	2.5	95.5	1.4	93.8	1.7	98.5	2.5	95.8	1.3	93.9	1.5
As	107.6	3.1	105.1	1.8	103.0	1.7	109.0	2.6	105.2	1.3	103.5	1.8
Hg	98.1	2.9	96.5	1.9	94.8	1.2	100.7	2.7	97.6	1.8	95.2	2.1
Ir	98.6	2.8	97.0	1.6	96.1	1.6	98.3	2.6	96.3	1.3	95.0	1.5
Os	96.7	2.7	94.7	1.3	93.4	1.6	97.4	2.5	95.7	1.5	94.4	1.4
Pd	86.6	3.9	93.8	1.4	95.3	1.3	89.3	8.5	93.8	2.6	95.8	1.3
Pt	97.4	2.8	95.4	1.3	93.9	1.6	96.7	2.4	95.0	1.4	93.5	1.5
Rh	100.1	2.7	98.1	1.0	97.8	1.1	99.8	2.6	97.8	1.4	96.1	1.6
Ru	98.2	2.3	96.2	0.8	95.3	1.2	98.0	2.7	95.7	1.3	94.3	1.6
Cr	102.3	2.9	100.4	1.0	99.0	1.7	102.0	2.7	99.5	1.6	97.9	1.4
Mo	98.6	2.9	96.2	1.2	95.3	1.4	98.9	2.8	96.3	1.5	95.0	1.6
Ni	101.6	2.8	99.1	1.0	98.1	1.5	99.9	2.8	97.3	1.6	96.3	1.5
V	100.6	2.4	97.0	1.3	96.4	1.5	99.9	2.0	96.1	1.6	95.3	1.6
Cu	101.9	2.9	99.1	1.0	97.5	1.4	99.5	2.7	97.6	1.4	96.4	1.5

(n = 6 corresponds to six independently prepared spiked samples at each spiking concentration from the solid material). All values meet the USP⟨233⟩ acceptance criteria of 70–150% recovery and%RSD ≤ 20% [4].

TP-6076 structure leading to partial metal complexation and consequently less plasma induced ionisation in the spiked samples. During the method development stage and before the addition of thiourea to the analysis matrix, palladium recoveries were in the approximate range of 55–65%. When Pd was stabilised with the use of thiourea, which competes with TP-6076 API for Pd to form a counter complex assisting in the ionization, better recoveries that meet USP requirements were obtained. The thiourea stabilisation reagent used for Pd also helped in the stabilisation of Hg as it is a soft acid cation. Chelating agents with sulfur ligands provides excellent rinse-out while maintaining mercury (II) in the oxidised state [20].

Excellent Os spike recoveries ranging from 93.4% to 97.4% were obtained due to the stabilising effect of HCl acid and the antioxidant effect of ascorbic acid as it prevents the formation of volatile Os species. During the development phase, arsenic spike recoveries were slightly boosted by the positive matrix effect of the TP-6076 API organic residual in the sample analysis solution. Acetic acid was added to the matrix (as recommended by USP⟨730⟩) and this helped balance the accuracy of the analysis as acetic acid boosts arsenic response in the calibration standards (matrix matching) and will compensate for the matrix effect.

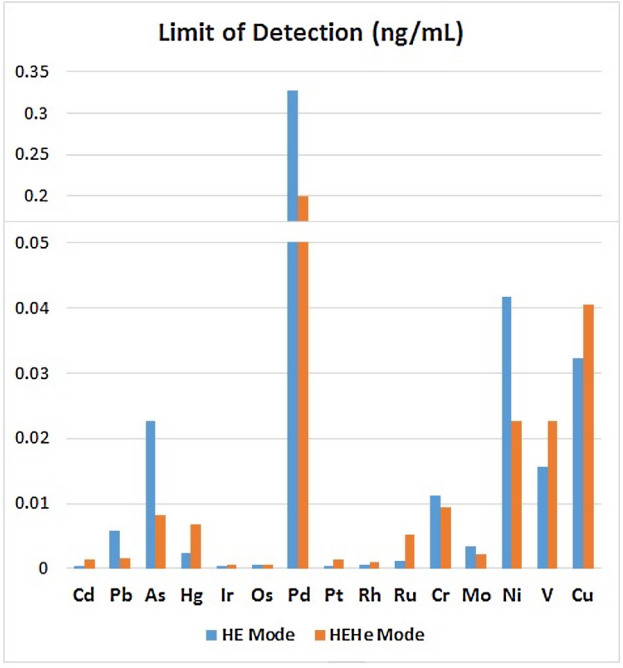
Table 3
Un-spiked sample (endogenous) results.

	He Mode	HEHe Mode
	endogenous content μg/g	endogenous content μg/g
Cd	0.00	0.00
Pb	0.04	0.04
As	0.04	0.03
Hg	0.00	0.00
Ir	0.07	0.08
Os	0.00	0.00
Pd	0.00	0.00
Pt	0.00	0.00
Rh	0.00	0.00
Ru	0.00	0.01
Cr	1.17	1.15
Mo	0.08	0.08
Ni	0.30	0.30
V	2.20	2.19
Cu	0.41	0.37

Mean of two independently prepared un-spiked samples.

3.3. Linearity and range

The correlation coefficients for each element in [He] and [HEHe] modes were R ≥0.99 and therefore met the requirement of USP as per Table 4. The limits of detection for all elements (except Pd) were < 50 ppt. Pd's limit of detenction (LOD) was approximately 328 ppt for He mode and 200 ppt for HEHe mode (Fig. 3, LOD are automatically calculated by Agilent's MassHunter software). The big four (toxic) elements Cd, Hg, As, Pb were detected with excellent LODs – less than 25 ppt in [He] and [HEHe] modes.



$$LOD = \frac{3\ x\ Blank\ Counts\ Standard\ Deviation\ x\ \ Concentration\ of\ Standard}{Counts\ of\ Standard - Counts\ of\ blank}$$

Fig. 3. Limit of detection for the 15 USP elements.

Table 4
Linearity data for the 15 USP elements.

	He Mode				HEHe Mode			
	R	LOD ng/mL	Slope	Intercept	R	LOD ng/mL	Slope	Intercept
Cd	1.0000	0.0004741	0.0027	+6.1625E-6	1.0000	0.001338	0.0034	+7.9339E-6
Pb	1.0000	0.005803	0.0447	+4.9712E-4	1.0000	0.001664	0.0362	+4.0979E-4
As	1.0000	0.02269	0.0062	+3.4703E-4	1.0000	0.008285	0.0039	+4.4407E-5
Hg	1.0000	0.002434	0.0110	+5.8788E-5	1.0000	0.006863	0.006863	+5.5991E-6
Ir	1.0000	0.0003754	0.0672	+5.6470E-5	1.0000	0.0006671	0.0189	+1.5182E-5
Os	1.0000	0.0006135	0.0180	+9.8110E-6	1.0000	0.0006831	0.0067	+3.9668E-6
Pd	0.9997	0.3277	0.0095	+0.0159	0.9997	0.1993	0.0067	+0.0116
Pt	1.0000	0.0003768	0.0276	+1.6105E-5	1.0000	0.001298	0.0060	+4.7216E-6
Rh	1.0000	0.0004931	0.3389	+3.0937E-4	1.0000	0.0009209	0.1193	+4.5831E-4
Ru	1.0000	0.001225	0.0571	+3.4884E-4	1.0000	0.005183	0.0216	+5.8109E-4
Cr	1.0000	0.01121	0.3298	+0.0277	1.0000	0.009462	0.2450	+0.0225
Mo	1.0000	0.003317	0.0387	+1.6773E-4	1.0000	0.002129	0.0181	+6.3521E-5
Ni	1.0000	0.04176	0.1830	+0.0604	1.0000	0.02263	0.1044	+0.0390
V	1.0000	0.01565	0.2244	+0.1026	1.0000	0.02266	0.1928	+0.0711
Cu	1.0000	0.03237	0.5355	+0.1313	1.0000	0.04053	0.2660	+0.0794

$R = correlation \quad coefficient, LOD = \frac{3 \times Blank\ Counts\ \ Standard\ \ Deviation \times Concentration\ \ of\ \ Standard}{Counts\ \ of\ \ Standard - Counts\ \ of\ \ blank}$

3.4. Ruggedness

A second analyst on a different day prepared and analysed six preparations of the approximately 100% target limit spiked samples *(J)* with freshly prepared spiking solutions. The samples were quantified against fresh IQCs and calibration standards.

Ruggedness was established as operator/day to operator/day precision change in the mean. The results determined for ruggedness are summarised in Table 5. For each element, the second analyst/day mean for all six preparations was within ±20% of the mean result for the first analysis. The% RSD for all preparations (n = 12) for each target element was ≤25%. All acceptance criteria described in USP Chapters <233/232> for ruggedness were met.

3.5. Stability

3.5.1. Spiked samples stability

The second analyst ran the six preparations of the approximately 100% spiked samples *(J)*, which were stored at ambient temperature for approximately 54 h. The concentrations were calculated and the mean for each element was compared with the previous mean value that was calculated when the samples were fresh in order to establish solution stability in the spiked samples. The spiked sample stability results are presented in Fig. 4. The change in mean was within ±20% from initial result for all the elements and the sample solutions are consequently considered stable for 54 h in both He and HEHe modes. For detailed results, refer to supplementary data – Section 1.

Table 5
Ruggedness as operator/day to operator/day change in mean results (n = 6 for each operator/day) and precision (% RSD for all preparations n = 12).

	He mode		HEHe mode	
	% Change in mean	%RSD	% Change in mean	%RSD
Cd	−0.9	1.4	−0.9	1.5
Pb	−8.0	10.7	−7.4	10.4
As	1.9	2.4	3.2	2.3
Hg	−2.2	2.0	−1.5	2.3
Ir	−4.7	2.8	−7.2	3.8
Os	−5.0	2.8	−6.1	3.3
Pd	−15.8	7.8	−15.3	7.7
Pt	−4.2	2.5	−7.2	3.8
Rh	−7.9	4.1	−6.3	3.5
Ru	−6.0	3.2	−5.5	3.0
Cr	−1.3	1.3	−1.2	1.5
Mo	−2.7	1.8	−2.0	1.7
Ni	−1.9	1.5	−3.0	2.1
V	−2.3	1.7	−1.9	1.7
Cu	−2.8	1.8	−3.5	2.2

3.5.2. Spiking solution stability

The second analyst prepared and analysed six new preparations of the approximately 100% target value spiked samples *(J)* that were spiked using the old spiking solution, which were stored at ambient temperature for approximately 54 h. The concentrations were calculated and the means were compared with the previous mean values that were calculated when the samples were spiked with the fresh spiking solution. Since the spiking solution and independent spiking solution contain the same matrix, this test will be used to infer stability for both the spiking solution and the independent spiking solution. The results are presented in Fig. 5. This test indicates the stability of the spiking solution while also reconfirming the ruggedness of the analytical method.

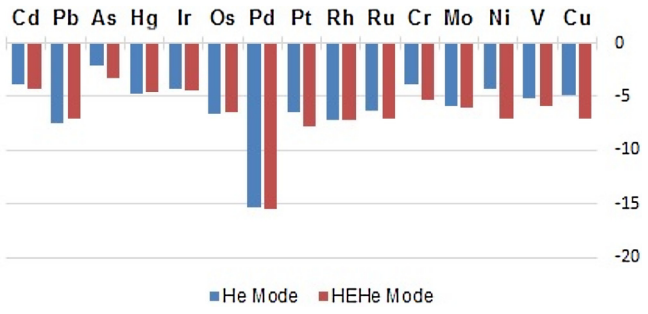


Fig. 4. Spiked samples stability results. Concentration change (ng/mL) over 54 h storage at ambient temperature (mean of n = 6).

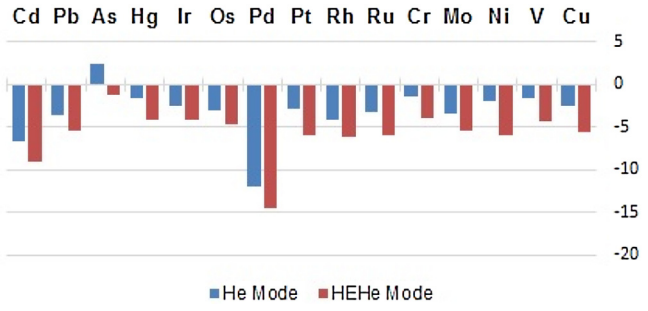


Fig. 5. Spiking solution stability results. Concentration change (ng/mL) over 54 h storage at ambient temperature (mean of n = 6).

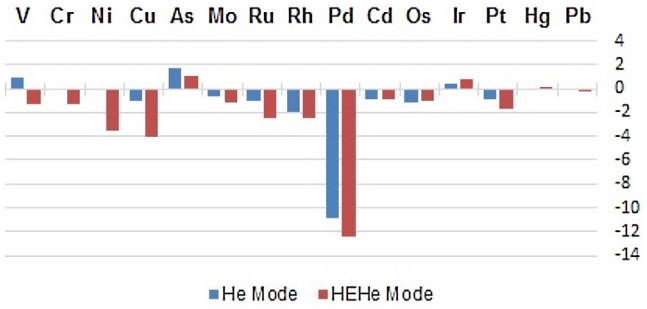


Fig. 6. Calibration standards and IQC stability results. Concentration change (ng/mL) over 54 h storage at ambient temperature.

A comparison between both means generated during the two analyses show that all elements meet the acceptance criteria of within 20% variation in both modes, He and HEHe. The spiking solution and the independent spiking solution are thus considered stable for up to 54 h. For detailed results, refer to supplementary data – section 2.

3.5.3. *Calibration standards and IQCs stability*

The second analyst tested the 100%*(1J)* calibration standard which was prepared and analysed by the first analyst and then stored at room temperature for approximately 54 h. The results generated from the stored standard solution were compared to the original results obtained from the fresh standard solution to establish solution stability for each element. Since all calibration standards and the independent quality control (IQC) contain the same matrix, stability can be inferred for the IQC based upon the results determined for the calibration standard. Results determined during the investigation of solution stability for the calibration standard are presented in Fig. 6. The comparison revealed that the change in response varied by no more than 20% from the initial result. Calibration standards and IQC solutions are considered stable for up to 54 h for all elements. For detailed results, refer to supplementary data – section 3.

3.6. *Specificity*

Specificity was assessed to show the absence of isobaric and polyatomic interference via quantifying the spiked samples against the calibration standard solutions and meeting the accuracy requirements. To further determine this parameter; specificity solutions, each missing a single target element, were used. Any interference observed at the measured mass of any target element from the elements present in the specificity test solutions were <10× background equivalent concentration (BEC as calculated by MassHunter software) or <10× blank concentration in both He and HEHe modes. From the data presented in Figs. 7 and 8, it is demonstrated that this method is specific with respect to all 15 elements and any interference caused is less than 10 x background noise. For detailed results, refer to supplementary data – section 4.

4. Conclusion

A method for the analysis of elemental impurities according to USP ⟨232⟩/⟨233⟩ has been developed and successfully validated for specificity, accuracy (over the range 50–150% of target value) precision (repeatability (n = 6)), ruggedness (on different days and a second analyst), linearity and solution stability. Complexation and stabilisation of all samples and standards was mandatory to obtain accurate Os and Pd results. An aqueous solution of 5% HCl (v/v), 0.1% acetic acid (v/v), 0.076% thiourea (w/v) and 0.01% ascorbic acid (w/v) was used for complexation and stabilisation. The mixture

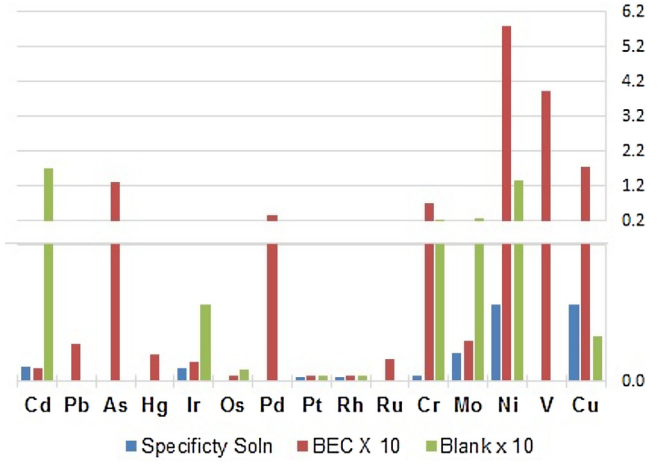


Fig. 7. Specificity solutions readings in He mode are less than 10 x BEC or 10 x blank (ng/mL).

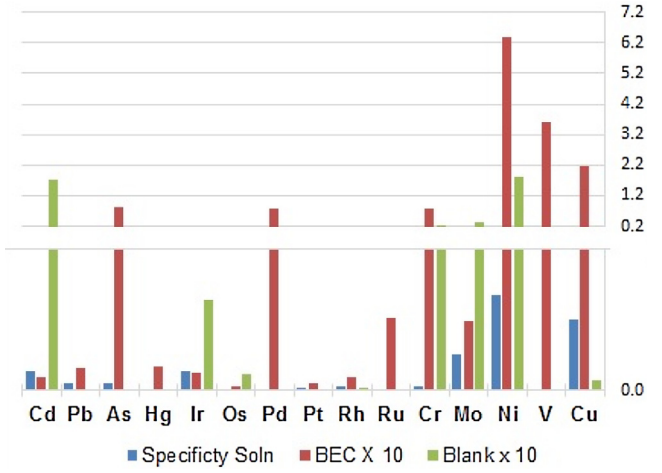


Fig. 8. Specificity solutions readings in HEHe mode are less than 10 x BEC or 10 x blank (ng/mL).

allowed the analysis of the full range of elements as required by the USP using one simple dissolution process. Ascorbic acid was found through its reducing properties to stabilise Os and prevent oxidation in the presence of acids. The thiourea stabilisation reagent used for Pd also helped in the stabilisation of Hg. All criteria described by USP ⟨232⟩/⟨233⟩ were met and Almac Sciences will use the method to test TP-6076 active pharmaceutical ingredient (API) for elemental impurities in support of Phase I and II studies.

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Conflict of interest

The authors declare that there is no conflict of interest.

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