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Validation of the ICP-MS Method for Determination of Trace Elements Provided by ICH Q3D Guideline in Fosamprenavir Calcium

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Abstract

Fosamprenavir calcium is an active pharmaceutical ingredient (API) in which trace elements must be monitored by ICH Q3D Guideline. It delivers appropriate method to ensure the quality of the product and its safe use. Elemental impurities in API are separated in three classes based on their toxicity and probability of their occurrence in the product. ICP-MS has proven to be a suitable technique with the appropriate sample preparation method. Due to the presence of oxidising agents in the sample preparation, determination of osmium is problematic. In the presence of oxidising nitric acid, a highly volatile and toxic osmium tetraoxide is formed. Due to its high volatility, the recovery of osmium has reached the value of 287 %, which exceeds recovery limit (70–150 %). However, osmium can be stabilised by complexation with thiourea in the hydrochloric acid solution (recovery = 87 %). In that way, the loss of osmium is reduced and good results are achieved in terms of usability, accuracy, and precision.

A method with addition of thiourea has been successfully validated through main validation parameters: linearity, quantitation limit, selectivity, accuracy, precision (which included repeatability and intralaboratory reproducibility), and robustness. Each of these validation parameters met the acceptance criteria, and therefore it was concluded that the method is suitable for the determination of trace elements in fosamprenavir calcium by ICP-MS.

Keywords

Microwave digestion, ICP-MS, API, fosamprenavir calcium, osmium

1 Introduction

During the synthesis of pharmaceuticals, significant contamination with some impurities may occur. They can be either intentionally added to the process (*e.g.*, residual catalyst or reactants) or introduced from the equipment or the drug packages.[1] Either way, they need to be controlled. Many chemical reactions are accelerated by using metal-based catalysts. In addition, most of the equipment is made of metal materials (reaction vessels, mixing tanks, filters, *etc.*). Therefore, it is highly expected that traces of metals will appear in pharmaceutical products.[2,3] Metals present in the active pharmaceutical ingredient (API) are of great concern due to the possible toxicity of the metals present in the product, which could put human health at high risk.[4]

Elemental impurities control is a segment of pharmaceutical quality control, which ensures that elemental impurities stay within the permitted daily exposure (PDE) limits for specific pharmaceutical product. The European Medicines Agency (EMA) has issued a Guideline (Q3D) by the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), in which the steps to control the elemental impurities in the pharmaceuticals are given.[5]

Before guideline ICH Q3D, total metal content in API was analysed following British and American Pharmacopeias.[6,7] According to them, total metal content in API is measured through the reactions between the metal in the sample and thioacetamide reagent. It precipitates metals in the form of coloured sulfides. The colour is compared with a parallel treated standard solution of lead. The total heavy metal content is expressed relative to the standard lead solution. Although still in use, this method is non-specific and insensitive, time-consuming, and very often has low usability. Non-specificity means that this method provides no information about which heavy metal gives a positive result, which is a great disadvantage of the method.[8]

On the other hand, ICH Q3D Guideline separates 24 elemental impurities in three classes, based on their toxicity (expressed as PDE) and probability of their occurrence in the product. Table 1 shows the PDE limits of elemental impurities for oral consumption for the purpose of evaluation of elemental impurities contamination, with the maximum dosage of 10 g day^{-1}.

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Table 1 – Oral consumption limits for daily (10 g) pharmaceutical intake issued by ICH Q3D Guidelines
Tablica 1 – Dopuštene koncentracije unosa metala za dnevni unos lijekova (10 g) prema smjernicama ICH Q3D

Element	Class	Oral consumption limits / $\mu g\,g^{-1}$
Cd, Pb	1	0.5
As	1	1.5
Hg	1	3
Co	2A	5
V	2A	10
Ni	2A	20
Tl	2B	0.8
Au, Pd, Ir, Os, Rh, Ru, Pt	2B	10
Se, Ag	2B	15
Li	3	55
Sb	3	120
Ba	3	140
Mo	3	300
Cu	3	300
Sn	3	600
Cr	3	1100

The ICH Q3D Guideline follows the suggestions of USP h233i very closely.[6] It applies traditional sample preparation (such as microwave digestion) with inductively coupled plasma (ICP). Heavy metals in pharmaceuticals are nowadays most commonly analysed by ICP-MS technique. The advantages of this technique are very simple sample preparation, small quantities of the samples needed for the analysis, good sensitivity, and possibility to screen a wide spectrum of elements in a relatively short time.[9,10,11,12] For many elements, these approaches deliver excellent results in terms of precision and accuracy.[13] On the other hand, oxidising agents in sample preparation may cause a problem in determination of osmium traces. Osmium can occur in pharmaceuticals as a residual of catalyst or can be found in catalysts based on some other metals.[14] In certified reference standards (CRS), osmium is found dissolved in diluted HCl solution as salt ($(NH_4)_2OsCl_6$).When exposed to the nitric acid at elevated temperatures, osmium forms highly volatile and extremely toxic osmium tetraoxide (OsO_4). Additionally, due to its volatility, a sample loss during the sample preparation is possible. If the preparation is fast and precise, the results of osmium analysis can give falsely elevated responses. It evaporates even more in the nebulizer, after which it reaches the plasma, and eventually comes to the detector.[15]

Thus, for the collective determination of all elements required by ICH Q3D Guideline, including osmium, a simple and effective method of stabilisation for osmium is required.[13] A literature search revealed that osmium tetraoxide could be complexed with thiourea prepared in diluted HCl solution, and thus stabilized.[16]

In this study, the samples of API fosamprenavir calcium were prepared by several microwave digestion methods for the determination of the trace elements. The most suitable microwave digestion method was applied, and the ICP-MS method validated.

2 Experimental

2.1 Chemicals

Fosamprenavir calcium as tested API was obtained by Pliva Croatia d. o. o., and used in the form of powder. ICP-MS method validation was performed with the Certificated Reference Standards (CRS, Inorganic Ventures, USA). The CRS with their nominal concentrations are presented in Table 2.

Table 2 – Certificated Reference Standards (CRS)
Tablica 2 – Certificirani standardi

Name	γ / ppm	Name	γ / ppm	Name	γ / ppm	Name	γ / ppm
Ag	1000	Cu	10000	Pd	1000	Tl	10
As	10	Hg	1000	Pt	1000	V	1000
Au	1000	Ir	1000	Rh	1000	Sc	1000
Ba	10000	Li	1000	Ru	1000	Ge	1000
Cd	10	Mo	10000	Sb	1000	Dy	1000
Co	1000	Ni	1000	Se	1000	In	1000
Cr	10000	Pb	10	Sn	10000	Os	1000

As dissolving agents, following chemicals were used: nitric acid (69.0–70.0 %, trace element analysis grade, J. T. Baker, USA), perchloric acid (69–72 %, trace element analysis grade, J. T. Baker, USA), hydrochloric acid (36.5–38.0 %, trace element analysis grade, J. T. Baker, USA), and hydrogen peroxide (30 %, Merck, USA).

Thiourea (≥99.999 %, Sigma-Aldrich, USA) was used in sample preparation procedure for osmium determination. During all of the experiments, ultra-pure water was used (18.2 MΩ cm, Mili-Q Advantage, Merck, USA).

2.2 ICP-MS system

For trace metal analysis, Agilent Technologies ICP-MS 7700x and 7900 were used (Agilent Technologies, Santa Clara, CA, USA). Since both devices are equivalent, their characteristics are described jointly. The sample flow of quartz concentric pneumatic sprayer was ~0.2 ml min^{-1}. Quartz low-volume Scott-type chamber with double passage was used for liquid separation. For sample intake, internal standard intake, and disposal drainage from liquid separation chamber was performed by peristaltic pump. Radio-frequent generator was characterised with the 27 MHz variable frequency impedance, and the working power range of 500–1600 W. Plasma torch was made of

2.5 mm quartz glass. A pair of cones were made of copper with nickel tip: first cone for sampling with 1 mm gap, and second cone as the skimmer cone with 0.45 mm gap. Argon was used as carrier gas with flow rate of 1.0 l min^{-1}, while helium was used as collision gas with flow rate of 5.0 ml min^{-1}. Octopole 4th generation reaction system provided fast gas exchange and effective interference elimination with kinetic energy-based discrimination in collision helium-mode for ^{51}V, ^{52}Cr, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{75}As, and ^{78}Se. Mass spectrometry system had hyperbolic quadrupole analyser with high frequency working mode (3 MHz), and the orthogonal detector system with high linear dynamic range of 11 orders of magnitude.

In ICP-MS techniques, internal standards (ISTD) can be used for tracking system stability and possible analyte loss. Good candidates for internal standards are usually elements not normally expected in the matrix and not analytes of interest, but which have good response and stability. In this research, ISTD was prepared in 25 mg l^{-1} mass concentration. It was assumed that the same effect would equally change the response of analyte of interest, as well as the internal standard response. Therefore, used ICP-MS software automatically corrected concentration of each analyte with regard to added internal standard, the result being the relative ratio of the analyte signal and the internal standard signal. As internal standards, Sc, Ge, Dy, and In were used: ^{7}Li/^{45}Sc, ^{51}V/^{45}Sc, ^{52}Cr/^{45}Sc, ^{59}Co/^{45}Sc, ^{60}Ni/^{45}Sc, ^{63}Cu/^{45}Sc, ^{75}As/^{72}Ge, ^{78}Se/^{72}Ge, ^{95}Mo/^{115}In, ^{101}Ru/^{115}In, ^{103}Rh/^{115}In, ^{105}Pd/^{115}In, ^{107}Ag/^{115}In, ^{111}Cd/^{115}In, ^{118}Sn/^{115}In, ^{121}Sb/^{115}In, ^{138}Ba/^{115}In, ^{189}Os/^{163}Dy, ^{193}Ir/^{163}Dy, ^{195}Pt/^{163}Dy, ^{197}Au/^{163}Dy, ^{202}Hg/^{163}Dy, ^{205}Tl/^{163}Dy, ^{208}Pb/^{163}Dy.[17,18]

For data analysis, MassHunter computer software was used (Agilent Technologies, Santa Clara, CA, USA).

2.3 Preparation of standard solutions

Standard solutions were used for ICP-MS calibration and linearity testing, as well for method performance testing as standard addition (spiking of samples with standard solutions). Working standard solutions were made to have exactly 0, 10, 30, 50, 100, 150, and 200 % concentration of each element, according to ICH Q3D requirement for oral consumption (Table 1). Diluent solution 1 contained 2.5 % v/v HCl, and it was used for sample preparation of fosamprenavir calcium in which Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V would be determined. Diluent solution 2 contained 0.01 M thiourea in 2.5 % v/v HCl. Thiourea was added to complex volatile and toxic osmium tetraoxide, which is the product of osmium and nitric acid reaction. Therefore, diluent solution 2 was used to determine the trace osmium in the samples.

2.4 Sample preparation

Fosamprenavir calcium has very low solubility in water, which improves with the acidification and has its peak at pH 3–4. Regardless, the sample must be completely dissolved in order to be analysed on ICP-MS systems. Therefore, different combinations of various acids and microwave digestion under increased pressure and temperature conditions were tested using microwave chamber (UltraWave, Milestone, Italy).

Fosamprenavir calcium powder was homogenised, weighed 50 mg (± 10 %) (analytical balance Mettler Toledo AX205 DeltaRange, OH, USA), and put into quartz cuvettes. Mixture of acids (Table 3) and 100 µl of ISTD were added to the sample in the cuvettes. Cuvettes were closed, shaken, and placed for microwave digestion according to the program described in Table 3. After microwave digestion, samples were quantitatively transferred to 50-ml polypropylene volumetric flasks, and filled with dilution solution 1 to the mark. Results showed that, in most combinations of acids, the sample remained undissolved under microwave digestion. Combination of nitric acid and perchloric acid dissolved the sample. For further analysis and method validation, 5 ml of HNO_3 and 250 µl of $HClO_4$ was used.

Table 3 – Microwave digestion program and combination of acids

Tablica 3 – Program mikrovalne digestije i kombinacije kiselina

#	t/min	P/W	T_1/°C	T_2/°C	p/bar
1	25	1500	210	70	130
2	20	1500	210	70	130

Combination of acids for microwave digestion	Sample state after digestion
5.00 ml HNO_3	Undissolved
5.00 ml HCl	Undissolved
4.00 ml HNO_3 + 1.00 ml HCl	Undissolved
4.00 ml HNO_3 + 0.25 ml $HClO_4$ + 1.00 ml HCl	Undissolved
4.00 ml HNO_3 + 0.25 ml $HClO_4$	Undissolved
4.00 ml HNO_3 + 0.50 ml $HClO_4$	Dissolved
5.00 ml HNO_3 + 0.25 ml $HClO_4$	Dissolved

The blank sample was made in the same manner, containing the mixture of acids (5 ml of HNO_3, 250 µl of $HClO_4$) and 100 µl of ISTD, but without the addition of fosamprenavir calcium. After microwave digestion, it was transferred to 50-ml polypropylene volumetric flasks, and filled with dilution solution 1 to the mark.

3 Results and discussion

3.1 ICP-MS method validation

Analyses performed in this work included rigorous system suitability testing. Critical performance characteristics were measured and verified according to appropriate acceptance criteria for every analyte (Table 4).

Table 4 – Validation acceptance criteria
Tablica 4 – Prihvatljivi kriteriji validacije

Validation parameter	Acceptance criteria
Specificity	
Recovery	70 – 150 %
Linearity	
Correlation coefficient	≥ 0.9900
Quantitation limit	
Value	10 % PDE
RSD (10 % PDE)	≤ 20 %
Accuracy	
Recovery level 1 (30 % PDE)	70 – 150 %
Recovery level 2 (100 % PDE)	
Recovery level 3 (150 % PDE)	
Precision	
Repeatability	≤ 20 %
Reproducibility	≤ 20 %
Total	≤ 25 %
Robustness	
Error	≤ 10 %

Specificity is the ability to unambiguously determine the analyte in the presence of components that can be expected in a prepared sample. Usually, this includes impurities, degradation products, matrix ingredients, *etc.* Specificity is, therefore, the parameter that indicates how much a component in the complex sample matrix interferes with analyte determination. It is expressed as recovery of the lowest investigated concentration, in this case 10 % PDE.

Osmium is found in the dissolved form in the used CRS as ammonium salt of complex: $[OsCl_6]^{2-}$. When exposed to oxidative conditions of nitric acid, especially at elevated temperatures, osmium forms a highly volatile and extremely toxic osmium tetraoxide (OsO_4). The main issue with OsO_4 is the loss of analyte during sample preparation due to its volatility and consequently its falsely elevated response. Volatility causes an increased extent in the nebulizer, resulting in an elevated concentration when reaching the detector.[13]

In literature, it was found that OsO_4 can be complexed with thiourea prepared in diluted HCl solution. Therefore, preliminary tests were performed using a dilution solution containing 0.01 mol l^{-1} thiourea in 2.5 % v/v HCl (dilution solution 2). Results had significantly improved compared to those obtained by analysis with dilution solution containing only 2.5 % v/v HCl (dilution solution 1).

Regression analysis data for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in fosamprenavir calcium with the addition of thiourea analysed by the ICP-MS technique are given in Table 5. The developed method gives linear results for all the examined elements and satisfies the acceptance criterion for linearity, which demands a correlation factor greater than or equal to 0.9900. Obtained results showed that silver had the lowest correlation factor of 0.9989. Limit of detection was calculated from RSD of the lowest concentration investigated.

Table 5 – Regresion analysis data for trace elements in fosamprenavir calcium with the addition of thiourea analysed by the ICP-MS
Tablica 5 – Podatci regresijske analize ICP-MS određivanja elemenata u tragovima u fosamprenavir kalciju uz dodatak tiouree

Element	*R*	*LOD* / ng ml^{-1}	Slope	Intercept
Li	0.9995	0.0032	0.0047	$1.5739 \cdot 10^{-4}$
V	0.9999	0.0635	0.0741	0.0547
Cr	1.0000	0.0350	0.1063	0.0227
Co	0.9999	0.0006	0.2064	$1.6291 \cdot 10^{-4}$
Ni	0.9999	0.0125	0.0594	0.0059
Cu	0.9998	0.0406	0.1602	0.1676
As	0.9994	0.0141	0.0122	$4.3694 \cdot 10^{-4}$
Se	0.9998	0.2192	$4.7553 \cdot 10^{-4}$	$1.8680 \cdot 10^{-4}$
Mo	0.9993	0.0100	0.0028	$1.6241 \cdot 10^{-4}$
Ru	0.9997	0.0023	0.0033	$7.4044 \cdot 10^{-6}$
Rh	0.9992	0.0004	0.0173	$8.9351 \cdot 10^{-6}$
Pd	0.9997	0.0042	0.0036	$1.2636 \cdot 10^{-4}$
Ag	0.9989	0.0031	0.0069	$2.2655 \cdot 10^{-5}$
Cd	0.9997	0.0003	0.0025	$3.6556 \cdot 10^{-6}$
Sn	0.9996	0.0388	0.0043	0.0021
Sb	0.9996	0.0017	0.0058	$8.9905 \cdot 10^{-5}$
Ba	0.9998	0.0013	0.0143	$1.2134 \cdot 10^{-4}$
Os	0.9999	0.0173	0.0113	$6.0422 \cdot 10^{-4}$
Ir	0.9999	0.0012	0.0338	$2.5141 \cdot 10^{-4}$
Pt	0.9999	0.0031	0.0120	$2.0969 \cdot 10^{-5}$
Au	0.9999	0.0042	0.0174	$1.6016 \cdot 10^{-4}$
Hg	0.9999	0.0051	0.0055	$1.6007 \cdot 10^{-4}$
Tl	0.9997	0.0017	0.0422	$4.4806 \cdot 10^{-4}$
Pb	0.9998	0.0018	0.0609	$2.7836 \cdot 10^{-4}$

3.2 Quantitation limit

Quantitation limit was determined at 10 % PDE (Provided by ICH Q3D, Table 1). The obtained response values were converted into mass fractions of each individual contaminant in the sample, and in relation to the actually added quantities of elements, the individual recoveries (%) were determined. RSD (%) of six measurements with and without addition of thiourea were determined (Fig. 1). As results showed, the ICP-MS method in the presence of thiourea for analysis of trace elements in fosamprenavir calcium yielded good results with values of RSD (10 % PDE) lower than 10 %. Analyses of Os and Hg, which showed values of RSD greater than 10 %, had significantly improved with the addition of thiourea. In case of Pb, there was an opposite

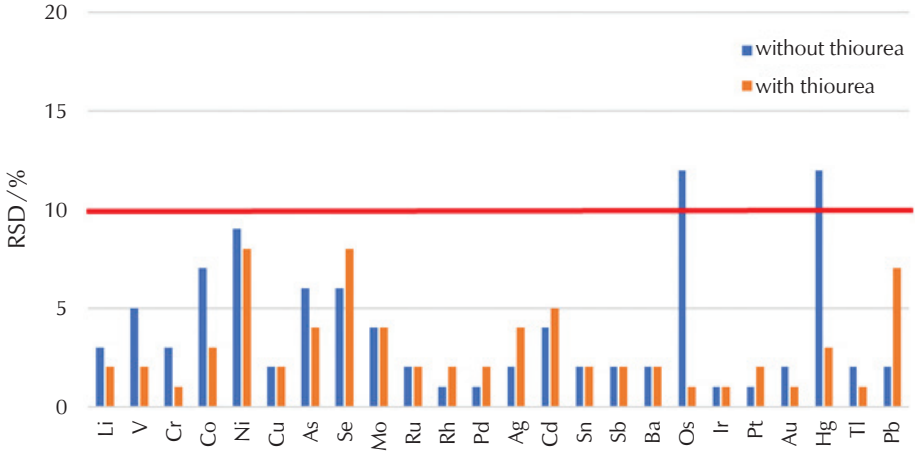

Fig. 1 – Recovery (%) of each trace element on 10 % PDE; with and without addition of thiourea

Slika 1 – Relativna standardna devijacija (%) za sve elemente u tragovima na 10 % PDE, s dodatkom i bez dodatka tiouree

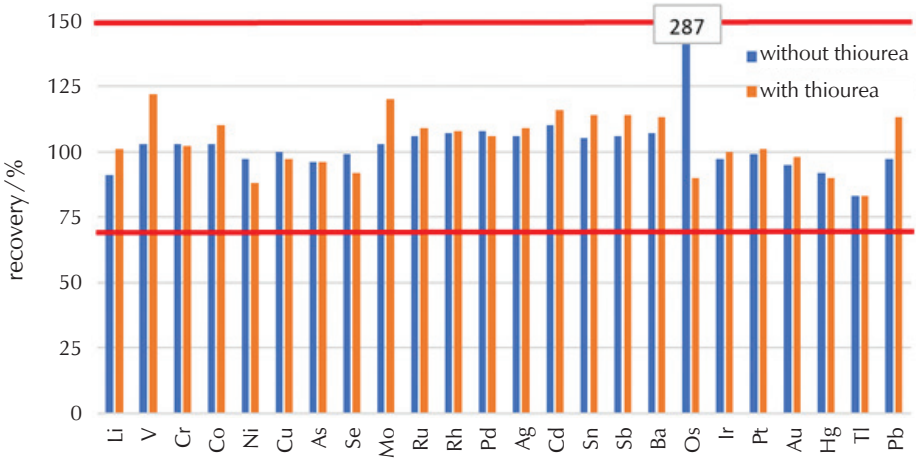

Fig. 2 – RSD (%) of each trace element on 10 % PDE; with and without addition of thiourea

Slika 2 – Analitički povrat (%) za sve elemente u tragovima na 10 % PDE, s dodatkom i bez dodatka tiouree

effect but within the criteria limits. The quantitation limits for each tested element, determined by used method, meet the acceptability criteria, which stipulate efficiency in the range of 70 to 150 % and RSD of measurements lower than 10 %. The lowest average efficiency of the method was obtained for thallium and its value was 83 %, while the highest value of 110 % was obtained for cadmium.

3.3 Accuracy

The accuracy test was performed on 12 samples; the first three were prepared without standard addition, and the next three series with three samples, contained standard addition at 30, 100, and 150 % of limit values prescribed by the ICH Q3D guidelines for oral products. The measured concentrations were converted to mass fractions for each prepared sample, and compared to the value of the corresponding standard additive. The results are presented as recovery (%) for each individual element, as shown in Fig. 3. The accuracy for each element, determined by this method, at each tested level, satisfied the acceptance criteria, which require recovery in the range of 70 to 150 % in relation to the value of the standard addition. The lowest efficiency for the tested accuracy of 86 % was obtained for vanadium at the standard addition of 30 % of the maximum allowable amount, while the highest value of 130 % was obtained for molybdenum also at the standard addition of 30 % of the maximum allowable amount.

Recovery of osmium without addition of thiourea exceeded recovery range (287 %). This could be attributed to the high volatility of osmium in the presence of oxidising nitric acid. Addition of thiourea in hydrochloric acid to osmium achieved good results with 87 % recovery.

By satisfying the acceptance criteria results of the accuracy test, the specificity of the ICP-MS method for determining all investigated elements in fosamprenavir calcium were confirmed (first confirmation is provided in Fig. 2).

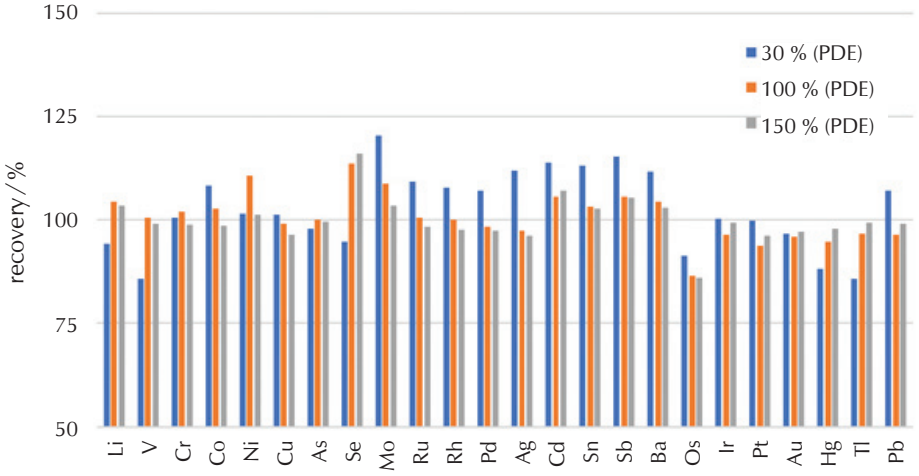

Fig. 3 – Accuracy of ICP-MS method expressed by recovery
Slika 3 – Preciznost ICP-MS metode izražene preko analitičkog povrata

3.4 Precision

The precision of the ICP-MS method was evaluated on two levels, as repeatability and intralaboratory reproducibility. The results are presented as relative standard deviations of repeated measurements on the same instrument (repeatability), and on another instrument the day after with another analyst. Repeatability was tested with six equally prepared samples and the standard addition of each individual test element with 100 % of the limit amount prescribed by the ICH Q3D guidelines. The results are presented as a relatively standard deviation of the obtained values, as shown in Table 6. Developed ICP-MS method for determination of trace elements in fosamprenavir calcium gave good results and satisfied the acceptance criteria, which require the RSD between the measurements less than or equal to 20 %. The highest RSD between the measurements for the performed repeatability test was obtained for Se with the value of 9 %.

Intralaboratory reproducibility was tested based on the samples prepared for the repeatability testing. These samples were re-analysed in the same manner the next day by the equivalent instrument. The results are presented as a RSD (%) of the obtained values, as shown in Table 6. The intralaboratory precision for each tested element determined by the developed method satisfied the acceptance criteria according to Table 4 (RSD ≤ 20 %). The highest RSD between measurements for the performed intralaboratory precision test was achieved for lithium with RSD of 11 %. The total precision results (total RSD (%)) are expressed as the sum of RSD (%) of method repeatability and method reproducibility (Table 6). The results meet the acceptability criteria where the total RSD must be less than or equal to 25 %. The lowest precisions were obtained for lithium, nickel, and selenium, with values of 14, 13, and 14 %, respectively.

3.5 Robustness

Developed ICP-MS method was subjected to a robustness test. Slight changes in microwave digestion conditions in

Table 6 – Preciznost izražena kao ponovljivost i unutarlaboratorijska obnovljivost
Tablica 6 – Precision expressed as repeatability and intralaboratory reproducibility

Precision			
Element	Method Repeatability RSD / %	Method Reproducibility RSD / %	Total RSD / %
Li	3	11	14
V	1	1	2
Cr	1	1	2
Co	5	1	6
Ni	5	8	13
Cu	1	1	2
As	1	2	3
Se	9	5	14
Mo	1	2	3
Ru	1	1	2
Rh	1	1	2
Pd	1	1	2
Ag	1	2	3
Cd	1	3	4
Sn	1	1	2
Sb	1	1	2
Ba	1	1	2
Os	3	3	6
Ir	0	1	1
Pt	2	1	3
Au	1	2	3
Hg	2	1	3
Tl	0	1	1
Pb	1	2	3

terms of temperature and concentration of acid added to samples were applied. After digestion, all samples were dissolved and analysed by ICP-MS according to the instrumental conditions prescribed for the developed method. The results presented in the form of the relative difference between the reference measurement (using the results obtained by repeatability measurement) and the measurements performed in robustness test are shown in Table 7. Column 1 describes the relative difference between the reference measurement results and the results obtained from the analysis of samples prepared with decreased microwave digestion temperature by 10 °C (200 °C), column 2 shows results after increasing microwave digestion temperature by 10 °C (220 °C). Columns 3 and 4 represent results obtained when concentrations of the nitric acid and the perchloric acid were reduced. Results obtained by robustness test met the acceptability criteria, which require relative differences in the results between the measurement of samples with modified preparation and the measurement of samples prepared under prescribed conditions to be less than or equal to 10 %. Each condition change that was tested for every examined element fulfilled the criteria and confirmed the resistance and robustness of the method used for the trace elements in fosamprenavir calcium analysis. In the case of osmium, microwave digestion at elevated temperature proved to be a critical parameter in the sample preparation process. This is understandable and expected, given the volatility of the osmium tetraoxide formed in contact with the oxidising nitric acid. Therefore, it is certainly important to pay attention to the stability of the microwave reactor. On the other hand, there was no significant effect of diluted nitric and perchloric acids on the obtained results. Generally, better yields were obtained by conducting the microwave digestion at lower temperatures and using the quartz cuvettes of smaller volume.

Table 7 – Robustness of the ICP-MS method for analysis of trace elements

Tablica 7 – Robustnost ICP-MS metode za analizu metala u tragovima

	1	2	3	4
	T_1 – 200 °C	T_2 – 220 °C	Lower concentration of HNO_3	Lower concentration of $HClO_4$
Li	8.32 %	1.32 %	1.54 %	0.78 %
V	1.22 %	0.87 %	1.53 %	0.78 %
Cr	0.97 %	0.50 %	1.92 %	0.86 %
Co	2.00 %	5.15 %	5.07 %	6.71 %
Ni	5.36 %	8.38 %	3.46 %	2.45 %
Cu	2.99 %	1.17 %	1.60 %	0.31 %
As	2.19 %	1.22 %	0.83 %	0.76 %
Se	2.02 %	4.20 %	0.06 %	1.46 %
Mo	0.51 %	2.05 %	0.07 %	0.90 %
Ru	1.49 %	1.06 %	1.73 %	1.79 %
Rh	2.09 %	1.13 %	0.80 %	0.69 %
Pd	2.25 %	0.93 %	1.17 %	1.81 %
Ag	4.98 %	4.94 %	3.32 %	3.02 %
Cd	4.02 %	6.86 %	7.94 %	8.50 %
Sn	0.76 %	1.10 %	1.25 %	1.82%
Sb	2.78 %	0.35 %	5.28 %	1.62 %
Ba	0.31 %	0.97 %	2.15 %	2.76 %
Os	5.26 %	9.48 %	3.60 %	0.43 %
Ir	3.02 %	2.93 %	2.99 %	2.61 %
Pt	0.54 %	1.22 %	0.74 %	1.20 %
Au	1.15 %	4.48 %	0.38 %	1.57 %
Hg	1.02 %	1.92 %	0.04 %	1.47 %
Tl	1.49 %	4.47 %	1.27 %	0.86 %
Pb	0.10 %	0.24 %	1.25 %	1.07 %

4 Conclusions

Impurities in pharmaceutical products in the form of trace elements must be monitored by appropriate methods to ensure the quality of the product and its safe use. ICP-MS has proven to be a suitable technique due to its characteristics of fast and multi-element analysis, and low detection limits necessary for the determination of trace elements. With good instrumental methods, sample preparation is the other factor for trace elements in API method development. Therefore, two sample preparation methods were developed for the determination of elemental impurities in fosamprenavir calcium. Based on the conducted experiments, the following can be concluded:

1. Sample preparation is a critical parameter in the analysis of elemental contaminants by ICP-MS technique. Careful preparation and usage of equipment, chemicals, reagents, and certified reference standards of high quality and purity is necessary to prevent the possible introduction of contaminants into the sample. It is also necessary to take into account all the procedures (weighing, reagent addition, microwave digestion, replenishment) that could lead to a loss of analyte in any way. It is necessary to strictly adhere to the prescribed procedures for each step in order to ensure quality and reliable results.

2. The interference present must be kept at a minimum using collision gas and the selection of suitable analyte isotopes and, where appropriate, by the introduction of additional procedures to eliminate interference present in the sample matrix.

3. Osmium, in the presence of oxidising nitric acid gives a highly volatile and toxic osmium tetraoxide, which can be stabilized by complexation with thiourea in hydrochloric acid solution. In that way, the loss of osmium is reduced, and significantly better results are achieved in terms of usability, accuracy and precision.

4. The validation procedure confirmed that the developed ICP-MS method obtained specific, accurate and precise results within the method of the defined working range for each determined element. In case of element osmium,

sample preparation was found to be critical factor. Addition of thiourea resolved the issue of volatile and poisonous osmium tetraoxide. With thiourea added to the sample, the whole method was successfully validated through main validation parameters: linearity, quantitation limit, selectivity, accuracy, precision (which included repeatability and intralaboratory reproducibility), and robustness. Each of these validation parameters met the acceptance criteria. Thus, it was concluded that the method is suitable for the determination of trace elements in fosamprenavir calcium by ICP-MS.

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SAŽETAK

Validacija ICP-MS metode za određivanje metala u tragovima prema ICH Q3D smjernicama u fosamprenavir kalciju

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Fosamprenavir kalcij je aktivna farmaceutska tvar (API) u kojoj se elementi u tragovima moraju nadzirati prema ICH Q3D smjernicama. ICH Q3D smjernice donose odgovarajuću metodu za osiguravanje kvalitete proizvoda i njegove sigurne uporabe. Elementarne nečistoće u API-ju razdvajaju se u tri razreda na temelju njihove toksičnosti i vjerojatnosti da se pojave u proizvodu. ICP-MS se pokazala kao prikladna tehnika ako se primijeni odgovarajuća metoda pripreme uzoraka. Zbog prisutnosti oksidirajućih sredstava u pripremi uzoraka problem se javlja kod određivanja osmija. Osmij u prisutnosti oksidirajuće dušične kiseline daje visoko hlapljiv i toksičan osmij tetraoksid. Zbog visoke hlapljivosti, iskorištenje osmija iznosi 287 %, što premašuje zadanu granicu iskorištenja (70 – 150 %). Međutim, osmij se može stabilizirati kompleksiranjem s tioureom u otopini klorovodične kiseline (povrat od 87 %). Na taj se način smanjuje njegov gubitak, a postižu se dobri rezultati u pogledu točnosti, preciznosti i robusnosti metode.

Ključne riječi

Mikrovalna digestija, ICP-MS, aktivna farmaceutska tvar, fosamprenavir kalcij, osmij

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