ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
https://www.icf.ro/rrch/

Rev. Roum. Chim.,
2024, *69*(3–4), 223–232
DOI: 10.33224/rrch.2024.69.3-4.12

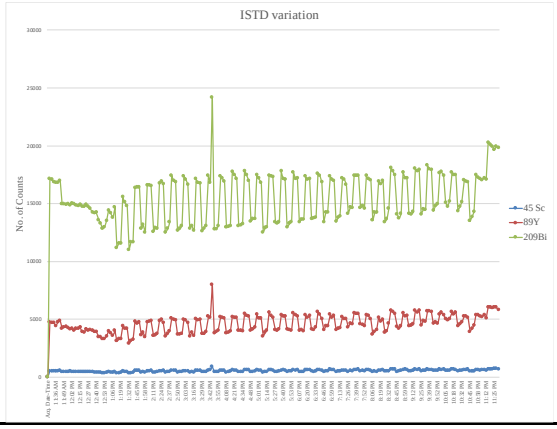
A NEW METHOD FOR SCREENING ELEMENTAL IMPURITIES IN MEDICO-PHARMACEUTICAL DISINFECTANTS

Florin ALBOTA,[a] Andreea SERBAN,[a*] Erhan IONUZ,[a,b] Marian VIRGOLICI,[a] Catalin DONEA,[c] Ioana DOBRESCU[c] and Ioan-Valentin MOISE[a]

[a]Horia Hulubei National Institute for Nuclear Physics and Engineering, P.O. Box MG-6, RO-077125 Bucharest-Magurele, Roumania
[b] Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Chemical Engineering and Biotechnologies, University Politehnica of Bucharest, Gh. Polizu St. 1–7, 011061 Bucharest, Roumania
[c]Klintensiv Ldt.544 Alexandriei Road, Bragadiru, Ilfov County, Roumania

Received January 15, 2024

Healthcare associated infections consist a major concern in South-East European countries. As hospital activities try to eliminate the possible risks of infections, the quality of the disinfectants used in surgical sites, microbiological laboratories and other aseptic places must be ensured by using a proper product. In this context, the PEROKLIN product produced by Klintensiv Ldt., Romania, was tested using ICP-MS technique at the IFIN-HH Chemical and Physical Test Laboratory in terms of determining the elemental concentrations of several elements of interest. A dedicated chemical protocol was developed for that purpose and the method was validated according to international guidelines.



INTRODUCTION

One of the major concerns of the healthcare system is represented by associated infections (HCAI) that patients are affected by. Studies have reported that between 7 and 10 patients develop at least once a HCAI, especially those that follow treatment in intensive care units.[1] Although surgical site infections are life-threatening, hospital hygiene activities face challenges when trying to eliminate the risk. Such challenges consist in limited resources for acquiring cleaning products suitable for hospital disinfection, lack of trained personnel and lack of social awareness, such as public reporting relevant surveillance data.

Recently, the European Network to promote Infection Prevention for Patient Safety (EUNETIPS), published the results of a survey that aimed to identify the most critical issues regarding the Healthcare infections that occur in south eastern European countries.[2] It was reported that all countries that participated in the survey were facing staff shortage, and therefore personnel training difficulties and poor supply services. Also, the study highlighted the importance of the quality

* Corresponding author: andreea.serban@nipne.ro

of the disinfection products to be correlated with institutional guidelines for preventing HCAIs.

As patient safety and the prevention of HCAI is progressing,[3,4,5] Klintensiv Ltd. from Romania developed a hydrogen peroxide disinfectant such that to be used for cleaning and disinfecting hard surfaces in areas with a high risk of contamination such as the laboratory for microbiological analyses, aseptic spaces for patients with severe burns or dialysis/transfusion equipment. In this case, the chemical purity of the PEROKLIN BIOC069 product had to be demonstrated. The elemental analysis was performed at the National Institute for Research and Development in Physics and Nuclear Engineering (IFIN-HH), using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique.

The Physical and Chemical Test Laboratory, part of Department of Technological Irradiations, developed an analytical method to determine the elements of interest (Cd, Pb, As, Hg, Co, V, Ni, Tl, Au, Pd, Ir, Rh, Ru, Se, Ag, Pt, Li, Sb, Ba, Mo and Fe). This paper presents the step-by-step process in developing the analytical procedure and to validate the chemical protocol in terms of accuracy (chemical recovery), precision, ruggedness and limits of detection and quantitation.[6–10]

INSTRUMENTATION

The instrument used in the present validation study is represented by the single quadrupole Agilent 7700s Inductively Coupled Plasma Mass Spectrometer (ICP-MS), which is used by the laboratory as a routine tool for determining the elemental concentrations in pharmaceutical samples. The instrument operates with a collision/reaction cell with He (purity 99.9995%), that allows the selection of isotopes that are nor isobars with other elements, such that achieving results at ppb (µg/kg) level. Therefore, the instrument was operated thought this analysis exclusively in "He mode".

The sample introduction system consisted in a peristaltic pump that delivered a constant flow rate into the nebulizer. The autosampler consist in 4 trays which allow 84 samples of 50 mL each (maximum 6 injection of 7 mL per sample).

In order to ensure that the detector's switch from pulse mode (signal < 1 Mcps) to analog (> 1 Mcps) does not affect the linearity of calibration curves, the instrument was tuned using a solution obtained by mixing in a 100 mL PFA volumetric flask: 1 g 7500 Series PA Tuning 1, 1 g 7500 Series PA Tuning 2 and 98 g MX1. For this tuning (called PA factor tuning) to be acceptable, the number of isotopes which are successfully tuned must be higher than the number of isotopes which are unsuccessfully tuned (displaying the messages "Sensitivity too low" or "Sensitivity too high"). The results of the PA factor tuning are found in Table 1 below.

Table 1

PA factor tuning results

No.	Mass	Element	PA Factor	No.	Mass	Element	PA Factor
1	9	Be	0.134	16	88	Sr	Sensitivity too high
2	23	Na	0.148	17	89	Y	0.185
3	24	Mg	0.156	18	95	Mo	0.183
4	27	Al	0.161	19	101	Ru	0.188
5	45	Sc	0.165	20	105	Pd	0.192
6	47	Ti	Sensitivity too low	21	111	Cd	0.194
7	51	V	0.166	22	115	In	Sensitivity too high
8	52	Cr	0.173	23	118	Sn	0.195
9	55	Mn	0.177	24	121	Sb	0.194
10	59	Co	0.181	25	137	Ba	0.195
11	60	Ni	0.182	26	172	Yb	0.201
12	63	Cu	0.186	27	175	Lu	Sensitivity too high
13	66	Zn	0.185	28	193	Ir	0.205
14	72	Ge	0.184	29	205	Tl	0.207
15	75	As	0.182	30	208	Pb	Sensitivity too high
				31	209	Bi	0.208

After PA factor tuning, the instrument's parameters are tuned in "no gas" mode (there is no gas flowing into the collision cell) and afterwards in collision mode (the collision cell is filled with a constant flow rate of He), the results being displayed in Table 2 below.

For the entire measurement, an internal standard (a mix of Sc, Y and Bi in MX1, each at 10 ng/g) are used in order to improve signal stability and improve repeatability. The relevant instrument parameters are presented below in Table 3.

Table 2

Results for He mode tuning

m/z	Range	Counts	Average	RSD(%)
59	10,000	7,602	7,532.5	2.9
89	5,000	3,809	4,318.1	3.5
205	50,000	17,687	20,164.8	2.1

Table 3

Relevant ICP-MS instrument parameters

Parameter	Value
Plasma gas (Ar)	15 mL/min
Carries gas (Ar)	0.7 mL/min
Makeup gas (Ar)	0.5 mL/min
Nebulizer pump	0.1 RPS
Collision gas (He)	4 mL/min

REAGENTS AND MATERIALS

All the labware used in this research is made from polytetrafluoroethylene (PTFE) materials. Prior to any preparation, the labware was clean with vapours of nitric acid and ultrapure water. The reagents used for sample preparation, respectively nitric and hydrochloric acid, are of ultrapure grade, free of elemental impurities that could affect the final results, having concentrations of 60% and 30%,[6–10]

Table 4

Concentration of the elements of interest in the spike solutions and calibration points C1-C5

Class of Toxicity	Element	Spike 50%	Spike 100%	Spike 150%	Target Limit J*	C1	C2	C3	C4	C5
		Concentration, ppm (mg/kg)								
1	Cd	0.013	0.026	0.039	0.030	0.000253	0.000063	0.000025	0.000006	0.000003
1	Pb	0.033	0.067	0.100	0.075	0.002665	0.000666	0.000266	0.000065	0.000027
1	As	0.100	0.201	0.301	0.225	0.015292	0.003820	0.001529	0.000375	0.000153
1	Hg	0.020	0.040	0.061	0.045	0.000920	0.000230	0.000092	0.000023	0.000009
2A	Co	0.033	0.067	0.100	0.075	0.005076	0.001268	0.000508	0.000125	0.000051
2A	V	0.062	0.125	0.188	0.150	0.009502	0.002374	0.000950	0.000233	0.000095
2A	Ni	0.133	0.267	0.400	0.300	0.005325	0.001330	0.000533	0.000131	0.000053
2B	Tl	0.053	0.107	0.160	0.120	0.005726	0.001430	0.000573	0.000141	0.000057
2B	Au	0.682	1.364	2.047	1.500	0.103955	0.025967	0.010395	0.002551	0.001039
2B	Pd	0.066	0.133	0.199	0.150	0.002247	0.000561	0.000225	0.000055	0.000022
2B	Ir	0.067	0.134	0.201	0.150	0.006397	0.001598	0.000640	0.000157	0.000064
2B	Rh	0.067	0.134	0.201	0.150	0.010204	0.002549	0.001020	0.000250	0.000102
2B	Ru	0.066	0.133	0.199	0.150	0.001729	0.000432	0.000173	0.000042	0.000017
2B	Se	0.543	1.086	1.629	1.200	0.019459	0.004861	0.001946	0.000477	0.000195
2B	Ag	0.066	0.133	0.199	0.150	0.005247	0.001311	0.000525	0.000129	0.000052
2B	Pt	0.067	0.133	0.200	0.150	0.003429	0.000857	0.000343	0.000084	0.000034
3	Li	1.716	3.433	5.149	3.750	0.242150	0.060488	0.024214	0.005942	0.002421
3	Sb	0.613	1.226	1.839	1.350	0.053464	0.013355	0.005346	0.001312	0.000535
3	Ba	4.797	9.597	14.396	10.500	0.048187	0.012037	0.004819	0.001182	0.000482
3	Mo	10.279	20.564	30.846	22.500	0.249439	0.062308	0.024943	0.006121	0.002494
3	Cu	2.037	4.075	6.112	4.500	0.214497	0.053580	0.021449	0.005264	0.002145
3	Sn	4.104	8.210	12.316	9.000	0.150325	0.037550	0.015032	0.003689	0.001503
3	Cr	7.514	15.033	22.550	16.500	0.959403	0.239653	0.095937	0.023543	0.009593
N/A	Fe	2.556	5.114	7.671	4.500	0.357102	0.089202	0.035709	0.008763	0.003571

For the validation study presented in this paper, single element solutions containing elements such as Cd, Pb, As, Hg, Co, V, Ni, Tl, Au, Pd, Ir, Rh, Ru, Se, Ag, Pt, Li, Sb, Ba, Mo, Cu, Sn, Cr and Fe ICP CRM (single element 1000 mg/kg Certified Reference Materials

solutions, provided by Merck, Germany) were used to prepare spike solutions of 50%, 100% and 150%, with resulted concentration presented in Table 4 along with the Targer Limit Concentration (J*, according with ICH guide [17] J is the target limit for a specific elemental impurity derived from PDE – maximum permitted daily exposure dose and maximum drug quantity permitted per day, < *e.g.* for 100 g of drug per day, J concentration in ug/g would be PDE in ug/day divided by g of drug/day >; Chapter 5.6 of ICH Guide relates to – Risk Assessment Analysis – and defines an extra control threshold J* = 30% J, as a criteria for Customer Decision of batch to batch testing or periodic screening); other ICP Certified Reference Materials were used to prepare multi-element ICP-MS tuning (Ce, Co, Li, Tl, Y), PA tuning 1 (*e.g.*: As, Be, Cd, Zn, Mg, Ni, Pb, Al, Ba, Bi, Co, Cr, Cu, In, Lu, Mn, Na, Sc, Sr, Tl, V, Y, Yb), PA tuning 2 (*e.g.*: Ge, Mo, Pd, Ru, Sb, Sn, Ir, Ti) and internal standards (Sc, Y and Bi) solutions in the same matrix as the analysed samples,respectively 5% HNO_3 + 0.5% HCl.

Calibration solutions C1-C5 were prepared based on the spike 100% solution by consecutive dilution, according to Table 5 below:

Table 5

Practical masses for preparing calibration solutions C1 – C5

CALIB STD	**Spike 100% solution Mass, g**	***C1***	***C3***	***MX1* Mass, g**	**Total Mass, g**
C1	27.05		–	327.97	355.02
C2	–	60.70	–	182.30	243.00
C3	–	29.54	–	265.87	295.41
C4	–	–	59.46	182.84	242.30
C5	–	–	25.59	230.33	255.92

The raw data has been used to obtain the calibration curves for each isotope of interest, such that to corelate the number of counts to a concentration value. Figure 1 below illustrates the example for ^{111}Cd calibration curve:

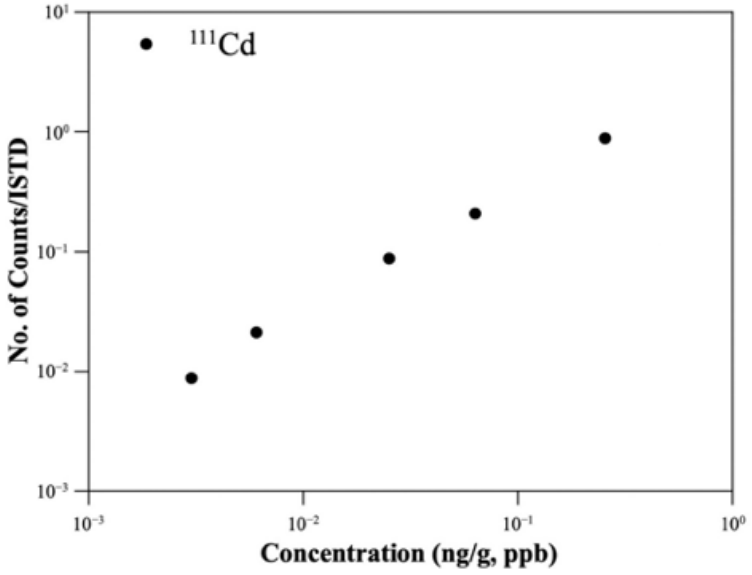


Fig. 1. – ^{111}Cd calibration curve.

Between each to consecutive points, a linear equation was used to determine the concentration value, as following:

$$I_i = a_i \cdot conc_i + b_i \quad (1)$$

where I_i stands for the signal intensity for isotope of interest *i*, corrected with the internal standard signal, $conc_i$ represents the concentration of isotope *i* (measured in ng/g, or ppb, as indicated in Fig. 1 above) in the calibration solution, and a_i and b_i represent the slope, and respectively intercept, for isotope *i*.

SAMPLE PREPARATION

The testing sample consist in PEROKLIN (product code: BIOC069) which is a mix of hydrogen peroxide, quaternary ammonium salt, non-ionic surfactant and water. Hydrogen peroxide-based disinfectants are reported as being effective against bacteria, C. auris and C. difficile spores, in approximately 1 minute of exposure, and also do not cause secondary effects of hospitals surfaces that contact the patient's skin (ex: bedside tabletop or mattress).[11–12] Also, hydrogen peroxide is effectively used in cleaning hospital water tanks in which bacteria can colonize in time.[13]

This preparation procedure was designed for samples that must be digested in concentrated acid using a closed-vessel digestion system. Closed-vessel digestion minimizes the loss of volatile impurities. For this validation study it was used the UltraWave closed vessel microwave digestion system, produced by Milestone.

In terms of the sample preparation chemical protocol, replicates of approximately 0.20 ± ± 0.04 g were collected from the testing BIOC069 sample and weighted accurately in PTFE digestion vials on an analytical balance. In the following order there were added to each vial 0.20 g of MX1, 7.15 g HNO_3 60% and 1.12 g HCL 30%. For the validation study presented in this paper, 6 replicates of samples were collected and chemical prepared as described above.

Positive Quality Control solution were prepared using 18 replicates of sample with the addition of 0.20 g of spike solution (12 replicates with spike 100%, 3 replicates with spike 100% and 3 replicates with spike 150%), 7.15 g HNO_3 60% and 1.12g HCL 30%. Additionally, there were prepared 6 Negative Quality Control with 0.20 g of MX1, 7.15 g HNO_3 60% and 1.12 g HCL 30%, such that to determine the background contamination level.

Following the chemical sample preparation protocol, the digestion vials were capped and placed in the UltraWave system in 1L PTFE tank containing 150g ultrapure water and 6.5 g HNO_3 60% (boiling medium). A specific program of pressure and temperature, with parameters listed in Table 6, was designed such that to ensure the complete digestion of the testing materials.

Table 6

Digestion parameters used with the UltraWave system

Parameter	**Value**
Pressure	40 bar (N_2)
Microwave power	1500 W
From room temperature to 50°C	5 min
Ramp to 250°C	40 min
Isothermal at 250°C	40 min

After digestion, samples were diluted with 20g of 0.5% HCl, resulting in a matrix of approximately 5% HNO_3 + 0.5% HCl, weighed on the analytical balance. The resulting solution is used as-is for ICP-MS measurement.

VALIDATION PROCEDURE

The developed chemical protocol was validated din terms of accuracy (chemical recovery), precision, ruggedness and limits of detection and quantitation, as suggested by the EURACHEM/CITAC guide[14,15] and the European Pharmacopoeia.[16]

1. Internal standard (ISTD) stability

For the entire measurement, an internal standard (a mix of ^{45}Sc, ^{89}Y and ^{209}Bi in MX1, each in concentration of 10 ng/g) was used in order to monitor signal stability.

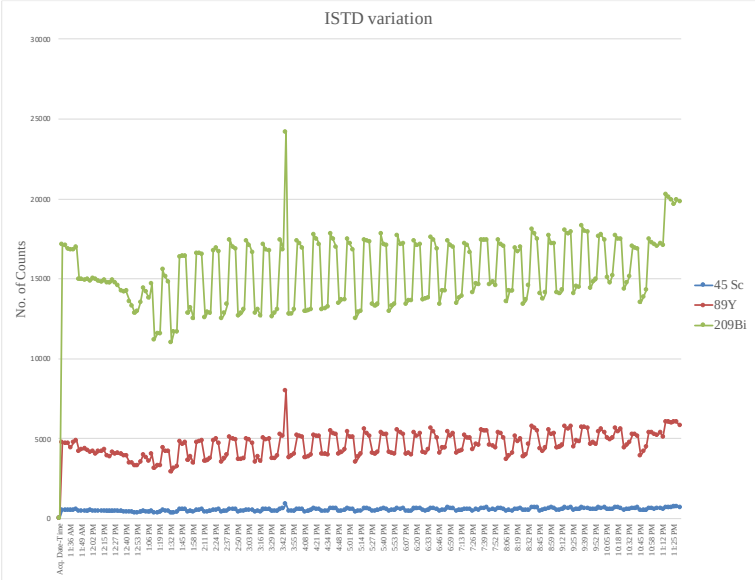


Fig. 2. – Internal standard variation.

The variability of the signal was measured 17.02% for ^{45}Sc, 16.19% for ^{89}Y and respectively 13.55% for ^{209}Bi.

2. Chemical Recovery

Spike chemical recovery, or accuracy, is defined by the quantity of an analyte measured in a spiked sample, compared to the practical concentration of the analyte at the moment of preparation. Each digestion batch contained 1 of each spiked sample and 1 unspiked sample. Unspiked sample subtraction was done only for samples digested in the same batch (*e.g.* replicate 1 unspiked is subtracted from each replicate 1 of spiked 50%, 100% and 150% J*, respectively).

Table 7

Accuracy for samples spiked at 50%, 100% and 150% of the specified limits (J*)

Class	Target Isotope	Spiked sample 50%		Spiked sample 100%		Spiked sample 150%	
		Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %
1	^{111}Cd	90.9	5.3	93.0	2.6	93.0	2.6
1	^{208}Pb	116.2	1.4	114.3	2.7	108.9	1.0
1	^{75}As	78.7	2.0	82.0	4.6	88.1	2.5
1	^{202}Hg	93.8	7.9	87.5	4.3	92.3	3.5
2A	^{59}Co	99.9	1.4	97.7	2.1	94.4	2.7
2A	^{51}V	82.8	2.8	89.0	4.2	89.1	4.0
2A	^{60}Ni	104.2	24.2	91.4	18.3	91.0	5.0
2B	^{205}Tl	114.6	1.8	115.0	1.7	110.0	0.8
2B	^{197}Au	82.0	1.4	81.8	2.9	88.5	4.0
2B	^{105}Pd	98.5	1.9	100.9	1.9	96.5	1.1
2B	^{193}Ir	94.7	0.9	97.7	2.4	101.3	2.2
2B	^{103}Rh	105.5	2.0	104.4	2.1	98.8	1.1
2B	^{101}Ru	105.9	2.0	106.7	2.2	101.1	1.0
2B	^{78}Se	84.1	3.0	83.5	6.1	83.4	2.7
2B	^{107}Ag	99.2	1.5	100.2	3.1	95.4	1.4
2B	^{195}Pt	74.1	4.2	80.0	2.9	90.2	4.4
3	^{7}Li	122.5	3.3	119.1	3.0	109.8	1.6
3	^{121}Sb	88.6	1.4	90.7	2.0	93.6	1.0
3	^{135}Ba	111.4	2.9	112.3	2.3	104.7	2.0
3	^{95}Mo	103.7	1.5	103.7	2.1	102.1	1.9
3	^{63}Cu	92.4	1.8	90.3	2.3	88.8	3.4
3	^{118}Sn	106.2	1.4	104.9	2.1	102.7	1.1
3	^{52}Cr	104.7	1.6	102.9	2.0	100.1	2.9
N/A	^{56}Fe	107.7	7.1	101.3	4.3	98.6	3.3

The concentration of the spike recovered was calculated as following:

$$conc_{sr} = \frac{(cps_{i,ss} - cps_{i,ns}) \cdot \alpha_i \cdot df_{ss}}{Ab_i} \quad (2)$$

where $conc_{sr}$ represents the concentration of spike recovered, $cps_{i,ss}$ stands for the signal intensity for target isotope *i*, in spiked samples, $cps_{i,ns}$ represents the signal intensity for target isotope *i* in unspiked samples, α_i is slope of target isotope *i*, df_{ss} is the dilution factor of spiked sample and Ab_i represents the natural isotopic abundance of target isotope *i*.

The measure of accuracy, Acc_E is obtained by comparing recovered spike with the practical spike concentration.

$$Acc_E = \frac{conc_{sr}}{conc_{E,p}} \quad (3)$$

where $conc_{E,p}$ represents the practical concentration of element (or isotope) E spike listed in listed in Table 4.

The accuracy was calculated based on samples spiked at 50%, 100% and 150% of the specified limits (J*), presented in Table 7 below. Acceptance criteria was considered to be 70–150 % for the mean of the 2 replicates at each concentration. Each replicate is the average of 2 injections from the same sample solution.

All target elements respect the acceptance criteria 70–150%, proving therefore that the chosen chemical protocol is suitable to determine these specific elemental impurities in the tested matrix.

3. Precision (Repeatability)

The precision was calculated for each target element based on six replicates of samples spiked with 100% J*. The acceptance criteria is considered no more than 20% for the relative standard deviation. The results are presented in Table 8 below.

Table 8

Precision results for six replicates of samples spiked with 100% J*

No	Class	Element	Mean over 6 replicates (ppm, mg/kg)	SD	RSD
1	1	*Cadmium*	0.026	0.001	2.5%
2	1	*Lead*	0.067	0.002	3.6%
3	1	*Arsenic*	0.202	0.008	4.0%
4	1	*Mercury*	0.039	0.001	3.0%
5	2A	*Cobalt*	0.067	0.002	2.4%
6	2A	*Vanadium*	0.125	0.004	3.1%
7	2A	*Nickel*	0.241	0.036	15.0%
8	2B	*Thallium*	0.106	0.001	1.3%
9	2B	*Gold*	1.349	0.037	2.7%
10	2B	*Palladium*	0.133	0.003	2.6%
11	2B	*Iridium*	0.132	0.003	2.4%
13	2B	*Rhodium*	0.135	0.004	2.7%
14	2B	*Ruthenium*	0.133	0.004	2.9%
15	2B	*Selenium*	1.066	0.072	6.8%
16	2B	*Silver*	0.134	0.005	4.0%
17	2B	*Platinum*	0.132	0.004	2.9%
18	*3*	*Lithium*	3.465	0.117	3.4%
19	3	*Antimony*	1.226	0.021	1.7%
20	3	*Barium*	9.656	0.268	2.8%
21	3	*Molybdenum*	20.522	0.603	2.9%
22	3	*Copper*	4.094	0.113	2.8%
23	3	*Tin*	8.229	0.231	2.8%
24	3	*Chromium*	15.062	0.375	2.5%
25	N/A	*Iron*	5.128	0.180	3.5%

Based on the results obtained, all the elements of interest respect the acceptance criteria, and therefore it is considered that the analytical method can provide reproductible results.

4. Ruggedness

Ruggedness was determined based on a combination between samples ran by Analyst 1

(6 samples spiked with 100% J*) and Analyst 2 (6 samples spiked with 100% J*) in different days (minimum 2 different days) during which samples were prepared. The results are presented in Table 9 below. The considered acceptance criteria consist in the relative standard deviation no more than 25% for each Target Element at 100% of the specified limits (J*), better suited for quatitative analysis.

Table 9

Ruggedness determined with respect to 12 replicates of samples spiked with 100% J*

Class	Element	Mean over 12 replicates (ppm, mg/kg)	SD	RSD
1	Cd	0.026	0.001	2.6%
1	Pb	0.066	0.002	2.7%
1	As	0.208	0.009	4.6%
1	Hg	0.043	0.005	12.4%
2A	Co	0.066	0.001	2.1%
2A	V	0.127	0.005	4.2%
2A	Ni	0.271	0.049	18.3%
2B	Tl	0.105	0.002	1.7%
2B	Au	1.374	0.039	2.9%
2B	Pd	0.131	0.003	1.9%
2B	Ir	0.133	0.003	2.4%
2B	Rh	0.132	0.003	2.1%
2B	Ru	0.131	0.003	2.2%
2B	Se	1.137	0.066	6.0%
2B	Ag	0.131	0.004	3.1%
2B	Pt	0.133	0.004	2.9%
3	Li	3.421	0.103	3.0%
3	Sb	1.241	0.024	2.0%
3	Ba	9.469	0.222	2.3%
3	Mo	20.251	0.422	2.1%
3	Cu	4.042	0.095	2.3%
3	Sn	8.114	0.172	2.1%
3	Cr	14.899	0.298	2.0%
N/A	Fe	5.168	0.221	4.3%

All the elements of interest fulfil the acceptance criteria for ruggedness. The criteria for ruggedness, along with accuracy and repeatability, ensure that the performed study is suitable not only to determine the elements of interest in the tested matrix, but also prove that the chemical protocol is reproduceable and is not influenced by the daily variations of the instrumentation.

5. Limits of Detection and Quantification

The Limit of Detection (LoD) and the Limit of Quantification (LoQ) were calculated based on calibration solution C5 and the target isotope and correlated with the element recovery coefficient (Table 7). The change from isotope concentration to element concentration was performed using the natural isotopic abundance of the isotope of interest.

$$conc_E = \frac{conc_i}{Ab_i} \quad (4)$$

where $conc_E$ represents the concentration of element E (ppm) and Ab_i represents the natural isotopic abundance of isotope i (%).

$$LoD_E = conc_E \cdot (1 + 3 \cdot RSD_i)/Acc_E \quad (5)$$

$$LoQ_E = conc_E \cdot \frac{1 + 10 \cdot RSD_i}{Acc_E} \quad (6)$$

where RSD_i stands for the relative standard deviation for isotope i, calculated based on three injections of calibration point C5 (which are presented in Table 10, reported as no. of counts/no. of counts ISTD), and Acc_E represents the element recovery coefficient (accuracy).

Table 10

No of counts / No of counts ISTD for the 3 injections of calibration point C5

Class	Target isotope	Calibration solution C5		
		Inject 1	Inject 2	Inject 3
		[cps target / cps ISTD]		
1	^{111}Cd	0.00807	0.00827	0.01024
1	^{208}Pb	0.06713	0.06675	0.06821
1	^{75}As	0.01430	0.01344	0.01555
1	^{202}Hg	0.00627	0.00621	0.00546
2A	^{59}Co	1.27044	1.22524	1.18314
2A	^{51}V	1.42240	1.39232	1.23293
2A	^{60}Ni	1.41832	1.37839	1.49239
2B	^{205}Tl	0.19538	0.19074	0.19056
2B	^{197}Au	0.82831	0.83283	0.85730
2B	^{105}Pd	0.23602	0.22486	0.22752
2B	^{193}Ir	0.41338	0.42082	0.41953
2B	^{103}Rh	1.13656	1.08108	1.06599
2B	^{101}Ru	0.18659	0.18063	0.17603
2B	^{78}Se	0.00394	0.00214	0.00212
2B	^{107}Ag	0.40024	0.38584	0.39666
2B	^{195}Pt	0.18861	0.19079	0.19565
3	^{7}Li	0.17892	0.17854	0.19197
3	^{121}Sb	0.45600	0.44279	0.44798
3	^{135}Ba	1.22214	1.17419	1.18218
3	^{95}Mo	15.28415	14.57207	14.95352
3	^{63}Cu	59.16464	59.50755	59.19305
3	^{118}Sn	4.74042	4.55064	4.56683
3	^{52}Cr	110.79408	109.55445	109.60288
N/A	^{56}Fe	22.18886	22.09990	21.58705

Table 11 below presents the results obtained for the calculation of LoD and LoQ parameters .

Table 11

Limits of detection and quantification for Single Quadrupole instrument based on calibration solution C5

Element	Target isotope	RSD (%)	LoD (ppm)	LoQ (ppm)
Cd	^{111}Cd	13.6	0.000028	0.000047
Pb	^{208}Pb	1.1	0.000053	0.000057
As	^{75}As	7.3	0.000187	0.000265
Hg	^{202}Hg	7.5	0.000038	0.000054
Co	^{59}Co	3.6	0.000056	0.000069
V	^{51}V	7.6	0.000117	0.000167
Ni	^{60}Ni	4.1	0.000228	0.000285
Tl	^{205}Tl	1.4	0.000084	0.000093
Au	^{197}Au	1.9	0.001097	0.001232
Pd	^{105}Pd	2.5	0.000109	0.000127
Ir	^{193}Ir	1.0	0.000105	0.000112
Rh	^{103}Rh	3.4	0.000112	0.000137
Ru	^{101}Ru	2.9	0.000110	0.000131
Se	^{78}Se	38.4	0.001779	0.003999
Ag	^{107}Ag	1.9	0.000107	0.000120
Pt	^{195}Pt	1.9	0.000107	0.000120
Li	^{7}Li	4.2	0.002943	0.003708
Sb	^{121}Sb	1.5	0.000975	0.001072
Ba	^{135}Ba	2.2	0.007783	0.008883
Mo	^{95}Mo	2.4	0.016790	0.019411
Cu	^{63}Cu	0.3	0.003134	0.003203
Sn	^{118}Sn	2.3	0.006683	0.007681
Cr	^{52}Cr	0.6	0.011673	0.012186
Fe	^{56}Fe	1.5	0.004069	0.004473

CONCLUSIONS

The method proposed has been shown to be linear, accurate, robust and possesses a good level of precision, Limit of Detection and Limit of Quantitation. All acceptance criteria have been met for the parameters validated with this method, the results being significantly lower than the proposed limits. The accuracy calculated based on spike recovery for samples spiked with 150% J* has been 83–110%, for 100% J* has been 80–119 %; and for 50% J* has been 74–123 % (Table 4). These values meet the acceptance criteria for accuracy, which is spike recovery 70–150%, for all target elements.

The Precision (Repeatability) was calculated using 6 replicate 100% J* spike. Relative standard deviation has been 1–15 % (see Table 8). These values meet the acceptance criteria, which is NMT 20% for each Target Element.

The Intermediate Precision (Ruggedness) was calculated using 12 replicate 100% J* spike with a combination between two analysts and different days. Relative standard deviation has been maximum 18.3 % (see Table 9), meeting the ruggedness acceptance criteria (which is NMT 25% for each Target Element).

The analytical procedure proved its ability to obtain results which are directly proportional to the concentration, therefore ensuring the linearity of the method. This has been Demonstrated on 5-point calibration curves for all elemental impurities.

The Limit of Quantitation, calculated based on calibration solution C5, has been shown to be significantly lower than the concentration limits J* (Table 11 in correlation with 4).

The proposed method for sample preparation and analysis of "PEROKLIN" BIOC06 samples has been validated under the above parameters, in the conditions presented and is proposed for day-to-day testing as follows: for each sample the digestion batch will contain at least one blank, one sample replicate and one sample replicates with 100% J* spike (*e.g.* in a 15-position digestion batch can be accommodated maximum 7 samples and one common digestion blank).

The validation of the chemical protocol for determining elemental impurities in PERIKLIN BIOC069 demonstrated the product is suitable for use in the health medical system, for cleaning and disinfecting hard surfaces and laboratory equipment.

Acknowledgements. This work was supported by the project POC-G, ID: P_40_276 (SMIS 107514) "GAMMA-PLUS" – Enhancing competitiveness through innovation and improving manufacturing processes with gamma irradiation technology – subsidiary project no 45/2022 funded by European Regional Development Fund (FEDR), Minister of European Funds in Romania (MFE) and co-funded by Klintensiv Ltd. and IOSIN Programs are supporting IRASM gamma irradiators and laboratories of IFIN-HH.

REFERENCES

1. World Health Organization, Health care-associated infections. Fact Sheet. Available:http://www.who.int/gpsc/country_work/gpsc_ccisc_fact_sheet_en.pdf
2. M. Licker, L. Baditoiu, D. Lungeanu, R. Dobrevska, E. Szilagy, L. Raka, L. Markovic-Denic and S. Brusaferro, *J. Hosp. Infect*., **2017**, *66*, 85-88.
3. S. Brusaferro, Improving infection control and hospital hygiene in Europe: professional networks and European programs. 2015/C151/01. Available: http://eunetips.eu/pdf/eunetips.2015.06.26.brusaferro.pdf
4. European Union Council, Recommendation on patient safety, including the prevention and control of healthcare associated infections (2009). Available:http://ec.europa.eu/health/patient_safety/docs/council_2009_en.pdf
5. B. Allegranzi, S. P. Nejad, C. Combescure, W. Graafmans, H. Attar, L. Donaldson and D. Pittet, *Lancet*, **2011**, *377*, 228-241.
6. E. Rusen, G. Toader, A. Podaru, A. Diacon, A. Mocanu, O. Brincoveanu, M. Alexandru, F. L. Zorila, M. Bacalum, A.-M. Gavrila, F. Albota, B. Trica, T. Rotariu, M. Ionita and M. Istrate, *Polymers*, **2023**, *15*, 3176.
7. V. Moise, S. Vasilca, A. Baltac, C. Pintilie, M. Virgolici, M. Cutrubinis, C. Kamerzan, D. Dragan, M. Ene, F. Albota and S. Maier, *Rad. Phys. Chem.*, **2020**, *170*, 108658.
8. A. Serban, F. Albota, M. Virgolici, E. Ionuz, C. S. Tuta, I. Stanciu, V. Fugaru and O. Sima, *J. Radioanal. Nucl. Chem*., **2021**, *330*, 91-101**.**
9. A. Serban, F. Albota, I. Erhan, C. S. Tuta and M. Virgolici, *J. Radioanal. Nucl. Chem.*, **2023**, https://doi.org/10.1007/s10967-023-09189-5
10. M. Burducea, V. D. Zheljazkov, A. Lobiuc, C. A. Pintilie, M. Virgolici, M. Silion, M. Asandulesa, I. Burducea and M.-M. Zamfirache, *Scientia Horticulturae*, **2019**, *249*, 407–418.
11. J. L. Cadnum, B. S. Pearlmutter, M. F. Haq, A. L. Jencson and C. J. Donskey, *Am. J. Infect. Control.*, **2021**, *49*, 1572−1574.
12. Z. Han, E. Pappas, A. Simmons, J. Fox, C. J. Donskey and A. Deshpande, *Am. J. Infect. Control.*, **2021**, *49*, 34−39.
13 M. Biswal, P. Gupta, C. Singh, S. Vig and P. S. Saini MTech, *Am. J. Infect. Control*., **2023**, *51*, 18-22.
14. "Guide to Quality in Analytical Chemistry – An Aid to Accreditation": 2002, CITAC/Eurachem Guide.
15. "Quality Assurance for Research and Development and Non-routine Analysis" – Internet Edition 1.0 - 1998, CITAC/Eurachem Guide CG2.
16. The European Pharmacopoeia, 11th Edition, Published July 2022.
17. ICH Quality Guideline – Q3D(R2), Guideline for Elemental Impurities, Final version, 26 April 2022.