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MULTIELEMENTAL DETERMINATION OF TRACE ELEMENTS IN RADIOPHARMACEUTICALS PRODUCED AT THE RADIOPHARMACY CENTER USING ICP-OES TECHNIQUE

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ABSTRACT

The control of chemical impurities in radiopharmaceuticals is critical to their safety and efficacy. According to the U.S. Pharmacopeia (USP), the elemental impurities with potential toxicity must be quantified. A proposed revision of USP 35 introduces the technique of atomic emission spectrometry for the analysis of elements. The aim of this work was to study the concentration of chemicals elements in FDG-Fluor-18, IPEN-TEC GENERATOR and MIBI-TEC using an ICP-OES technique. One analytical curve composed by 27 elements (Ag, Al, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Pd, Se, Sr, Te, Tl, Zn) was constructed in two analytical ranges. The analyses were carried out simultaneously in an ICP-OES Vista MPX (Agilent). Some parameters for analysis and method validation were evaluated. The cleaning and maintenance of equipment influenced the emission intensity of the elements. 1.2 kW power and 10s sample uptake resulted in a consumption of 1.6 mL of sample and Mg II / Mg I ratio relation equal to 9.40. Linearity, LOD and LOQ were determined. The analyses were performed using 1:40 dilution with purified water. The main elements studied in this work were Al, Cu and Zn. The %recovery was determined with final concentrations of 0.3, 0.5 and 0.8 μg mL^{-1}. The analyses were performed in triplicate with three different batches. The %recovery was between 96.65 and 117.61% and the values for precision (CV) were less than 5% indicating good accuracy of the method.

1. INTRODUCTION

Radiopharmaceuticals are administered orally, inhaled or intravenously and therefore they must meet the requirements of Good Manufacturing Practices (GMP) for conventional pharmaceuticals [1,2]. One of the objectives to be achieved during the production of pharmaceuticals is to ensure the quality and safety of the products [1].

In radiopharmaceuticals, elemental impurities, particularly metal ions, may come from raw material, reagents or solutions added in the production steps, equipment or containers used to prepare the batches or from the decomposition of the substances. This kind of impurity can alter the organoleptic characteristics, affect the radiolabeling and change the diagnosis, influence the safety and efficacy of the radiopharmaceuticals, and cause some adverse effect. This fact justifies the importance of knowing the concentration of these elements in the radiopharmaceuticals [3].

According to the Pharmacopeial Forum (USP), the elemental impurities can be divided in two classes according to the degree of toxicity, Class 1 (As, Cd, Pb, Hg) and Class 2 (Cr, Cu, Mn, Mo, Ni, W, Pd, Pt) elements [4].

The impurities classified as Class 1 must be essentially absent in drug products and excipients because they are highly toxic to health and environment. These substances, if present, should be in very strict levels [4]. The Class 2 elements are less toxic than Class 1 and the concentrations are less limited in drug; elemental impurities need be quantified only when they are added during the manufacture of the drug products [4].

Analytical methods for the determination of metal ions in raw materials, intermediates and drug have been developed and published [5]. Currently, the most widely used instrumental methods to analyse metals are neutron activation analysis (NAA), atomic absorption spectroscopy (AAS), grafite furnace atomic absorption spectroscopy (GFAAS), inductively coupled plasma-optical emission spectrometry (ICP-OES) or inductively coupled plasma-mass spectrometry (ICP-MS). ICP-OES technique has advantages such as the determination of low concentrations of elements (at ppm levels) with high sensitivity and speed of analysis [5,6].

A proposed revision of USP <233> has suggested the introduction of the ICP-OES technique for the analysis of elemental impurities [7].

The development of an analytical methodology involves the evaluation and optimization of several parameters, such as linearity, limit of detection (LOD) and quantification (LOQ) and % recovery [1-2,6].

In Brazil, three main government regulation must be followed in order to perform the validation of analytical methods in the pharmaceutical industry, that is, RE No. 899 of May 29, 2003: "Guia para validação de métodos analíticos e bioanalíticos", RE No. 27 of May 27, 2012: “Requisitos mínimos para a validação de métodos bioanalíticos empregados em estudos com fins de registro e pós registro de medicamentos” and INMETRO CGCRE DOQ-008-2003:" Orientação sobre a validação de métodos de ensaios químicos"[8-10].

The aim of this study was to develop a multielemental ICP-OES method to analyse the concentration of elemental impurities (mainly metals) in some radiopharmaceuticals produced at IPEN/CNEN-SP.

2. EXPERIMENTAL

2.1. Instrumentation

Before starting the test the ICP-OES removable parts must be cleaned. The torch was immersed in aqua regia ($HCl:HNO_3$ in the ratio 3:1) for 24 hours. After that the torch and nebulizer were kept in extran for 2 hours, transferred to a flask containing 20% HNO_3 for 2 hours and rinsed with purified water. All glassware dried at room temperature.

Analysis of elemental impurities was performed using Vista-MPX simultaneous ICP-OES (Agilent Inc., Australia) equipped with axially viewed plasma, concentric glass nebulizer and

megapixel CCD detector. Argon was used as the plasma and auxiliary gas. The ICP-OES instrumental conditions and the analytical wavelengths used for the metal determinations are given in Table 1 and 2.

Table 1: ICP instrumental conditions

Parameter	Setting
Power (kW)	1.2
Plasma gas flow (L min $^{-1}$)	15.0
Auxiliary gas flow (L min $^{-1}$)	1.5
Nebulizer gas flow (L min $^{-1}$)	0.75
Replicate read time (s)	5
Long-term stability (s)	15
Sample uptake (s)	10

Table 2: Analytical wavelength

Element	Analytical wavelength (nm)	Element	Analytical wavelength (nm)
Ag	328.068	Li	610.365
Al	396.152	Mg	280.270, 285.213
B	249.678	Mn	293.931
Ba	233.527	Mo	281.615
Be	234.861	Na	568.821, 588.995
Bi	223.061	Ni	230.299
Ca	373.690, 422.673	Pb	220.353
Cd	226.502	Pd	340.458
Co	238.892	Se	196.026
Cr	267.716	Sr	460.733
Cu	327.395	Te	214.282
Fe	259.940	Tl	377.572
Ga	287.423	Zn	213.857
K	766.491		

2.2. Reagents and Standard Solution Preparation

100 μg mL^{-1} multielementar solution, single component solutions of Ca, Na, Mo, Pt, Pd (each one with 1000 μg mL^{-1} concentration), 65% HNO_3 purchased from Merck Millipore were used.

An analytical curve composed by 27 elements was constructed in two analytical ranges: 0.025, 0.05, 0.1, 0.5, 1.0 μg mL^{-1} range for Ag, Al, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, Li, Mg, Mn, Mo, Ni, Pb, Pd, Se, Sr, Te, Tl, Zn and 1.0, 2.0, 5.0, 10.0, 20.0 μg mL^{-1} for K, Na and Ca in 3% HNO_3.

2.3. Sample Preparation

125 µL of FDG-Fluor-18 and IPEN-TEC GENERATOR were diluted to 5 mL final volume (1:40 dilution) with purified water, MIBI-TEC cold kit was dissolved with 1 mL and 125 µL of MIBI-TEC was diluted to 5 mL final volume (1:40 dilution).

The %recovery was determined using 125 µL of the radiopharmaceutical sample by adding separately of 15 µL, 25 µL and 40 µL of the multielementar solution in order to obtain a 5.0 mL final volume, and element concentration of 0.3, 0.5 and 0.8 µl mL^{-1}. The %recovery was calculed according to the equations described in RE 899 [8].

2.4. Experimental Procedure

The influence of the glassware cleaning was evaluated comparing the analytical curves before and after the cleaning procedure.

The influence of instrumental conditions in the analytical curve was evaluated, varying the conditions of power supply and sample uptake, as described in Table 3.

Table 3: ICP-OES instrumental conditions

Parameter	Condition
Power (kW)	1.2 ; 1.3; 1.4; 1.5
Sample uptake (s)	10; 20; 30

The ratio Mg (II) at 285nm / Mg (I) at 280nm was calculated in each condition described in Table 3.

The linearity of the method was determinated using the multielementar analytical curve. Analyses of the three analytical curves were performed in triplicate.

The quantification of elemental impurities in FDG-Fluor-18, IPEN-TEC GENERATOR and MIB-TEC samples were evaluated with multielementar curve. Precision was calculated using the data from the %recovery of elemental impurities that have the highest concentrations. The limit of detection (LOD) and limit of quantification (LOQ) were calculated for each metal, according to the equations of RE 899. [8].

3. RESULTS AND DISCUSSION

The influence of glassware cleaning was evaluated by comparing the equations of the emission wavelenghts of each one of the 27 elements before and after the cleaning procedure. The results of parameters **a** (intersection with the y axis), **b** (angular coefficient) and **r** (correlation coefficient) are shown in Table 4.

Table 4. Comparison between equations before and after cleaning

Element	Befor cleaning			After cleaning		
	a	b	r	a	b	r
Ag	54.9	10805.3	0.99997	122.6	25794.4	0.99992
Al	91.3	8036.8	0.99995	213.1	18771.4	0.99989
B	2043.3	499.4	0.99578	4825.4	6371.8	0.95369
Ba	41.1	17182.4	0.99999	1071.4	32429.4	0.98718
Be	16.6	32526.9	0.99999	883.8	62977.9	0.99963
Bi	8.6	486.4	0.99974	28.2	1203.8	0.99899
Ca	124.1	1637.1	0.99854	283.8	3987.2	0.99820
Cd	9.7	10127.7	0.99999	73.3	24799.4	0.99998
Co	9.3	3193.8	0.99999	31.7	8152.5	0.99998
Cr	42.3	12558.6	0.99999	135.0	30329.9	0.99998
Cu	54.1	8547.6	0.99998	86.0	20697.4	0.99996
Fe	39.3	6028.9	0.99997	152.4	15160.6	0.99996
Ga	29.1	850.4	0.99999	38.7	2074.5	0.99998
K	221.5	11857.1	0.99989	379.3	24038.1	0.99980
Li	-296.7	17781.3	0.99949	-314.7	38804.5	0.99993
Mg	45.9	8494.7	0.99998	133.0	20716.5	0.99995
Mn	65.1	13885.8	0.99999	143.9	32860.3	0.99993
Mo	1.2	4304.0	0.99986	-4.1	10499,6	0.99986
Na	3544.7	90477.5	0.99757	12531.1	218796.0	0.99771
Ni	12.6	1789.2	0.99999	32.2	4468.9	0.99994
Pb	9.7	462.2	0.99989	16.0	1076.6	0.99996
Pd	39.4	2849.7	0.99997	64.9	6959.0	0.99995
Se	6.2	74.1	0.99864	8.7	203,3	0.99776
Sr	90.0	24445.0	0.99999	1075.7	42213.9	0.99209
Te	7.4	151.7	0.99975	10.8	396.4	0.99989
Tl	82.0	6886.	0.99950	78.6	1680.2	0.99970
Zn	19.3	4776.8	0.99999	70.6	12245.5	0.99998

Most of the elements has an increase in the emission intensity after cleaning the equipment. This fact indicated that the maintenance is a prerequisite for better identification and quantification of the elements.

The analysis of the spectral lines of Mg has been used as an indicator of robustness of the ICP-OES method. A ratio of emission intensity Mg II (285nm) / I (280nm) Mg above 10 indicates low sensitivity of the method to matrix effects, whereas a value below 4 represents a high sensitivity to matrix effects [6]. The results for Mg II / Mg I obtained with the change in the ICP-OES operation conditions are described in Table 5.

Table 5. Ratio Mg II / Mg I

Parameter	Condition					
	A	B	C	D	E	F
Power (kW)	1.2	1.2	1.2	1.3	1.4	1.5
Sample uptake (s)	30	20	10	10	10	10
Sample consumption (mL)	2.9	2.2	1.6	1.6	1.6	1.6
Mg II / Mg I	9.42	9.47	9.40	10.01	10.37	10.80

The use of a power supply higher than 1.3 kW needs further maintenance of equipment and it is indicated mainly when there is a change in the chemical composition of the sample, such as in the case of an organic sample. The decrease of sample uptake time from 30 to 10 seconds reduced in 44.82% the sample consumption, resulting in 1.6 mL sample volume. This smaller sample volume is important for the analysis of radiopharmaceuticals due to a few sample volume for the quality control. The smaller the sample volume required for analysis, the lower dilution factor is needed. Because of this, the selected conditions for analysis of radiopharmaceuticals were described in Table 3, column C.
The parameters of analytical curves for 27 elements, LOD and LOQ are shown in Table 6.

Table 6. Parameters of the analytical curves for 27 elements

Element	**Emission line (nm)**	**a**	**b**	**r**	**LOD** $\mu g\ mL^{-1}$	**LOQ** $\mu g\ mL^{-1}$
Ag	328.068	136.1	24686.7	0.99992	0.002	0.006
Al	396.152	129.9	18141.0	0.99996	0.001	0.004
B	249.678	892.0	9371.3	0.99983	0.054	0.181
Ba	233.527	1064.1	31204.0	0.98534	0.002	0.005
Be	234.861	874.7	61718.6	0.99959	0.002	0.007
Bi	223.061	14.4	1167.6	0.99998	0.008	0.026
Ca	422.673	22169.8	45816.6	0.99864	0.043	0.143
Cd	226.502	58.8	24139.8	0.99995	0.001	0.004
Co	238.892	32.0	7806.1	0.99996	0.001	0.004
Cr	267.716	130.9	29493.3	0.99995	0.001	0.004
Cu	327.395	51.8	20247.2	0.99996	0.003	0.008
Fe	259.940	145.1	14484.9	0.99993	0.002	0.007
Ga	287.423	30.3	1952.0	0.99990	0.003	0.010
K	766.491	-5558.1	25479.3	0.99974	0.018	0.060
Li	610.365	-268.7	35338.6	0.99990	0.001	0.002
Mg	280.270	1465.6	75259.4	0.99942	0.002	0.006
Mg	285.213	101.0	19474.6	0.99994	0.001	0.001
Mn	293.931	129.3	31220.4	0.99994	0.001	0.005
Mo	281.615	49.8	10122.2	0.99995	0.002	0.006
Na	588.995	-315.7	208565	0.99998	0.005	0.018
Ni	230.299	34.7	4351.1	0.99990	0.001	0.003
Pb	220.353	14.6	1026.8	0.99987	0.002	0.008
Pd	340.458	78.9	6662,4	0.99991	0.004	0.015

Se	196.026	8.2	208.7	0.99986	0.025	0.084
Sr	460.733	1025.5	44678.4	0.99237	0.001	0.005
Te	214.282	9.0	390.8	0.99990	0.10	0.033
Tl	377.572	74.2	1651.4	0.99990	0.015	0.048
Zn	213.857	43.7	12236.1	0.99998	0.001	0.004

Analyses of FDG-Fluor-18, IPEN-TEC GENERATOR and MIBI-TEC showed that only some elemental impurities were present in higher concentration, such as Al, Zn and particularly Cu in MIBI-TEC due to the raw material. Other elements, if present, were below the detection limit. Table 7 shows the results of analyses and related standard deviations with n= 3. The %recovery and CV for Al, Cu, and Zn are described in Tables 8 - 13.

Table 7: Element concentrations in radiopharmaceutical samples

Sample	Element	Concentration ($\mu g\ mL^{-1}$)
FDG-Fluor-18	Al	0.270 ± 0.014
	Zn	0.072 ± 0.011
IPEN-TEC GENERATOR	Al	0.027 ± 0.007
	Zn	0.068 ± 0.006
MIBI-TEC	Zn	0.086 ± 0.007
	Cu	1.972 ± 0.063

Table 8: % Recovery of Zn in FDG-Fluor-18

Zn addition ($\mu g\ mL^{-1}$)	Recovery ($\mu g\ mL^{-1}$)	%Recovery	CV (%)
0.3	0.361	117.61	2.8
0.5	0.573	107.5	1.2
0.8	0.883	102.4	2.5

Table 9: % Recovery of Al in FDG-Fluor-18

Al addition ($\mu g\ mL^{-1}$)	Recovery ($\mu g\ mL^{-1}$)	%Recovery	CV (%)
0.3	0.528	103.08	4.3
0.5	0.751	105.51	2.8
0.8	1.121	113.19	1.2

Table 10: % Recovery of Zn in GERADOR IPEN-TEC

Zn addition ($\mu g\ mL^{-1}$)	Recovery ($\mu g\ mL^{-1}$)	%Recovery	CV **(%)**
0.3	0.362	115.96	4.8

0.5	0.574	116.53	1.2
0.8	0.869	106.71	4.5

Table 11: % Recovery of Al in GERADOR IPEN-TEC

Al addition ($\mu g\ mL^{-1}$)	Recovery ($\mu g\ mL^{-1}$)	%Recovery	CV (%)
0.3	0.321	102.92	3.6
0.5	0.526	99.92	4.0
0.8	0.835	96.48	1.4

Table 12: % Recovery of Cu in MIBI-TEC

Cu addition ($\mu g\ mL^{-1}$)	Recovery ($\mu g\ mL^{-1}$)	%Recovery	CV (%)
0.3	2.222	102.14	1.1
0.5	2.543	100.52	4.0
0.8	2.751	96.65	2.5

Table 13: % Recovery of Zn in MIBI-TEC.

Zn addition ($\mu g\ mL^{-1}$)	Recovery ($\mu g\ mL^{-1}$)	%Recovery	CV (%)
0.3	0.393	111.80	4.4
0.5	0.597	112.41	3.3
0.8	0.883	105.54	2.9

In FDG-Fluor-18 and IPEN-TEC GENERATOR, CV was in the range 1.17 to 4.32% and 1.44 - 4.00%, respectively for Al and Zn. In MIBI-TEC, CV was in the range 1.14 and 4.40%, for Cu and Zn. Precision complies with that established by ANVISA (CV $\leq$ 5%), indicating repeatability of the analyses.

4. CONCLUSION

The optimization of the operation parameters of the ICP-OES allowed to determine the best conditions for analysis of elemental impurities in radiopharmaceuticals. The cleaning of glassware influenced in the emission intensity of the elements. The method for Al, Cu and Zn quantification in FDG-Fluor-18, IPEN-TEC GENERATOR and MIBI-TEC showed to be linear, precise and accurate in the studied range.

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