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Methodology of trace metals analysis in reference, generic and similar medicines: a comparison

Metodologia da análise de metais trace em medicamentos de referência, genéricos e semelhantes: uma comparação

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ABSTRACT

Trace metals are, among the toxic substances, some that cause intoxication of the human organism, they are highly reactive and bioaccumulative; however, living things require small amounts of some metals, including Co, Cu, Mn, Mo, V, Sr and Zn to perform vital functions. Other metals such as mercury, lead and cadmium, have no function to organic systems, and their accumulation may cause several pathologies. Trace metals are also part of pharmaceutical preparations found as contaminants from machinery, handling or medicinal plants' extracts. The presence of metals in medicines has become a relevant subject to evaluate the degree of exposure and possible consequences for human health. The aim of the work being carried out at the Northeastern Regional Center for Nuclear

Sciences in conjunction with the Department of Nuclear Energy is to obtain a qualitative and quantitative analysis of trace metals in medicines and comparing with specific parameters of the Brazilian Legislation. To help regulatory agencies to have a greater control of medicines sold based on the elaborated methodology.

Keywords: drugs, trace elements, contamination in production.

RESUMO

Entre às substâncias tóxicas que causam intoxicação ao organismo humano está à presença de metais-traço altamente reativos e bioacumuláveis. De acordo com Bezerra Neto e Barreto (2000) os seres vivos necessitam de pequenas quantidades de alguns metais, incluindo Co, Cu, Mn, Mo, V, Sr, e Zn, para a realização de funções vitais no organismo. Porém níveis excessivos desses elementos podem ser extremamente tóxicos. Outros metais como o Hg, Pb e Cd não possuem função relacionada aos sistema orgânicos, e sua acumulação pode provocar patologias. Metais-traço fazem parte das preparações farmacêuticas encontradas como contaminantes provenientes de máquinas, manuseio ou extratos de plantas medicinais. O controle da presença de metais em medicamentos tem se tornado um assunto relevante para se avaliar o grau de exposição e possíveis consequências para a saúde humana, devido à toxicidade inerente de cada um desses metais, sendo que alguns podem atuar na potencialização de patologias. O objetivo do trabalho que vem sendo desenvolvida no Centro Regional de Ciências Nucleares do Nordeste em conjunto com o Departamento de Energia Nuclear é obter uma análise qualitativa e quantitativa de metais-traço em medicamentos e comparar com parâmetros específicos da Legislação Brasileira e ajudar os órgãos fiscalizadores, baseados na metodologia elaborada, a ter um maior controle dos medicamentos vendidos.

Palavras-chave: drogas, elementos traço, contaminação na produção.

1. INTRODUCTION

Among the toxic substances that cause intoxication to the human organism is the presence of highly reactive and bioaccumulable trace metals (EC COMMISSION, 2006). Living beings require small amounts of some metals, including Co, Cu, Mn, Mo, V, Sr, and Zn, to perform vital functions in the organism (BEZERRA NETO; BARRETO, 2000). However, excessive levels of these elements can be extremely toxic. Other metals such as Hg, Pb and Cd have no function related to organic systems, and their accumulation can cause pathologies (WILLIAMS et al., 2000; SHAW, 1997). Trace metals are part of pharmaceutical preparations found as contaminants from machinery, handling or extracts of medicinal plants (ERNST, 2002). Trace-metal contamination in long-term medications, such as antihypertensives, accumulate throughout the individual's life (LEHNER-GALLA, 2011), antihypertensive drugs include captopril, which is an inhibitor of the enzyme angiostensin convertor, indicated for patients with hypertension, congestive heart failure, myocardial infarction and diabetic nephropathy (SILVA, 2006).

Some vaccines contain aluminum and thimerosal, a preservative based on mercury. The presence of the metals may not be accidental, for example, Na and Mg, in part, come from the excipients used in the capsules (SILVEIRA et al. 2007).

The generic medicinal product is the one containing the same active ingredient, in the same dose and in the pharmaceutical form, administered in the same way and with the same therapeutic indication of the reference medicine, presenting the same safety as the reference medicinal product. interchangeable. This interchangeability can only be performed by the responsible pharmacist, the pharmacy or drugstore and must be registered in the medical prescription (ANVISA, 2009a). Generics are copies of innovative medicines whose patents have expired (PRÓ-GENÉRICOS, 2011). Likewise, the same drug is the one containing the same or the same active principles, it has the same concentration, the same pharmaceutical form, the same route of administration, the same dosage and therapeutic indication, which is equivalent to the medicine registered with the federal agency responsible for sanitary surveillance, and may differ only in characteristics related to the size and shape of the product, shelf-life, packaging, labeling, excipients, vehicle and must always be identified by trade name or brand (ANVISA, 2009b).

Although the evaluation of the generic drug is a responsibility of ANVISA, and it is suggested the analysis of trace metals in these drugs by an atomic absorption spectrophotometer (AAS) and a plasma-based mass spectrometer (ICP-MS). We suggested using EDXRF to verify the concentrations of metals in these drugs (reference drugs, generic and similar) because the analysis of trace metals, by pharmacopoeia 5th edition, volume 1, 2010, does not describe the specific method for sample digestion, and standards to be used in in drugs. The adequacy of the methodology performed with precision will contribute to help the oversight agencies to have a greater control of the drugs sold. Either, the institution conducting the study may request validation of the methodology used for publication in the Pharmacopoeia.

2. METHODS

The samples were crushed in a mortar and packed in plastic bottles until analysis. Samples were transferred to specific polyethylene capsules for EDXRF analysis and sealed with polypropylene films at the base and at the top. As the analyzes were carried out in the vacuum, small respirators were made on the top polypropylene of the capsules with the aid of a needle to avoid tearing of the film during the chemical analyzes in the

atmosphere with air pressure less than 30 Pa. Measurements of the induced radioactivity occurred in triplicate with a voltage of 15 kV for the determination of the chemical elements with an atomic number less than 22 and a voltage of 50 kV for the heavier chemical elements.

The determination of trace metals was performed by EDXRF that uses an X-ray beam to promote excitation of the electrons of the trace metals in the samples. This excitation provides the emission of characteristic X-rays that are detected and through the generated spectra; the chemical elements were identified and quantified. The EDX-720 equipment from Shimadzu was calibrated from the energy and resolution calibration standards, A-750 and SUS. Samples were transferred to polyethylene capsules specific for EDXRF analysis and sealed with polypropylene films. The determination of trace elements in the samples was performed from analytical curves obtained by reference materials (SOUSA et al., 2013). For the quality control of the analytical procedure (validation of the analytical procedure), certified reference material was analyzed together with the samples (Table 1).

Table 1: Results from standard measured comparing with certificated values (SRM-1570)

Metals	Standard meansured (mg/kg)	SRM-1570 (mg/kg)
Al	309.0	310.0
Mn	65.50	74.80
Zn	88.15	86.20
Sr	60.00	56.04
Ni	2.20	2.08
Ca	13.09	14.60
P	4.93	5.12
K	23.05	28.7
Na	12.15	17.98

3. RESULTS AND DISCUSSION

The Brazilian Pharmacopoeia reports maximum values for some elements such as copper, manganese, nickel, and iron. (Table 2). Although the responsibility for evaluating

the generic drug is from ANVISA, it is suggested that trace metals be analyzed in these drugs to ascertain the concentrations of these metals in reference, generic and similar medicines, because the pharmacopoeia 5th edition, volume 1, 2010 does not describe a specific method for sample digestion, neither standards used for trace metals in drug analysis. And a methodology more appropriate, performed with more precision, will contribute to help oversight agencies to have a better control for drugs sold.

Table 2: Limited for oral metal consumed by Pharmacopeia.

Metals	**Limited for oral use (mg/kg)**
Cu	250
Mn	250
Ni	25
Fe	100

Tables 3, 4 and 5 report the results obtained in analysis of the chemical elements present in medicines, such as Furosemide, Simvastatin and Captopril.

Table 3: Quali-quantitative analysis of chemical elements in Furosemide drug.

Mean values of elements from Furosemide (mg/kg)			
Elements	Reference drug	Similar drug	Generic drug
Cl	23825.76	26578.73	23742.06
Mg	13734.26	10141.53	3556.23
Al	1007.83	920.96	672.93
K	143.96	181.47	160.86
Fe	36.06	42.67	ND
Zn	18.50	18.23	17.76
Ba	14.56	9.36	14.03
Sr	10.50	13.16	10.56
Cu*	13.10	8.83	8.93
Ni*	2.63	1.93	2.23

*Estimated values due to the low concentration of the elements in the samples, it would be necessary to use atomic absorption equipment to quantify them with better precision

Thorio, vanadium, cobalt, cerium, antimony, molybdenum, cesium, rubidium, selenium and titanium were also found in Furosemide drug, qualitatively. Analyzing the table, we find that the values of Cl, Mg, Al, K, Fe, Zn, Ba, Sr were similar for the Furosemide samples in reference and similar medicines.

Table 4: Quali-quantitative analysis of chemical elements in Simvastatin drug.

Mean values of elements from Simvastatin (mg/kg)			
Elements	Reference drug	Similar drug	Generic drug
Ba	1003.75	9122.63	9529.30
Fe	1143.00	188.27	570.50
La	606.35	6101.43	8082.70
Al	551.75	353.06	313.06
Mg	226.4	132803	1306.23
K	177.1	200.90	228.4
Cl	73.35	119.8	163.00
Ce	34.15	299.93	267.23
V	27,10	257.23	262.26
Zn	18.10	17.70	18.50
Sr	12.25	12.83	11.76
Cu	10.30	9.70	10.50
Rb	10.60	10.60	15.93
Ni*	2.20	2.30	2.50

*Estimated values due to the low concentration of the elements in the samples, it would be necessary to use atomic absorption equipment to quantify them with better precision

Molybdenum, antimony, cesium, uranium, thorium, selenium, nickel, molybdenum and cobalt were also found in simvastatin drug, qualitatively. The generic simvastatin and similar samples show similar results for trace metals.

Table 5: Quali-quantitative analysis of chemical elements in Captopril drug.

Mean values of elements from Captopril (mg/kg)		
Elements	Generic drug	Similar drug
Al	928.43	45.73
P	533.40	0.0
Mg	478.73	1193
Ti	364.56	313.16
K	163.96	209.00
Zn	19.23	16.50
Fe	23.70	1989.00
Ba	12.86	5.70
Cu	12.43	10.60
Sr	11.56	11.16
Ni*	2.70	3.20

* Estimated values due to the low concentration of the elements in the samples, it would be necessary to use atomic absorption equipment to quantify them with better precision

Cerium, Rubidium, Cobalt, Molybdenum, Uranium, Thorio, Cesium were found qualitatively in the captopril samples analyzed. Only Zn, Ti and Sr values were similar for generic and similar captopril samples, with high aluminum for generic captopril, whereas iron was elevated (above permitted) in similar captopril. High levels of zinc, aluminum and iron suggest contamination from machines. High concentrations of Aluminum and Iron, in the body, could cause neurodegenerative diseases like Alzheimer's disease and others degenerative diseases. Thorio and Uranium as radionuclides have been found in few amounts, so that, these metals do not cause health damages, however, other metals such as nickel, copper and chromium could cause gastrointestinal and respiratory problems and anemia in humans.

4 CONCLUSION

We concluded that trace metal determination in drug samples can be performed by the EDXRF technique and that even higher concentrations of iron than allowed were

found in samples of similar captopril. And high concentrations of aluminum were found in samples of generic captopril. However, these high values may be due to contamination of the drugs at the time of manufacture. Other drugs are being analyzed by Atomic Absorption Spectrometry as suggested by the pharmacopoeia.

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