

## EVALUATION OF DITHIOL SPECTROPHOTOMETRIC METHOD FOR TIN DETERMINATION IN LYOPHILIZED REAGENTS

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## INTRODUCTION

Production of radiopharmaceuticals classified as radionuclides (such as the Mo-99/Tc-99m generator, for example), ready-to-use radioisotopes and non-radioactive components for labeling with a radioactive component (known as lyophilized reagent, LR) must comply with Good Practices of Manufacturing (GMP) [1].

In Brazil, National Sanitary Surveillance Agency (ANVISA) regulates the production of medicines through RDC 301/2019. Radiopharmaceuticals require specialized techniques in handling and are also regulated by IN 37/2019, to ensure that these products have necessary characteristics for their safe and correct use in Nuclear Medicine services. Quality Control (QC) is part of GMP, responsible for sampling and testing of raw materials, active ingredients, excipients, and package materials to ensure that qualitative and quantitative characteristics comply with the acceptance limits, before releasing for use [1, 2]. Radiopharmaceuticals must be analyzed according to the tests and must meet the specifications described in monographs, i.e. radiochemical purity, radionuclidic purity, chemical purity, bacterial endotoxins and sterility test [3-6]. Tin is used in the range of some microgram to milligrams as stannous chloride in almost all LR formulations. There are many well-established methods for tin quantification, as ICP (inductively coupled plasma) analysis, redox titration, and polarographic methods, but a specific requirement for tin, based in the reaction with 4-methyl-1,2-dimercaptobenzene (dithiol) is described in European Pharmacopoeia (EP) LR monographs [4]. The reaction of tin with dithiol was described by Clark and modified by Farnsworth and Pekola [7, 8].

The objective of this work was to evaluate the EP dithiol spectrophotometric method of described for tin determination to be used in

LR radiopharmaceuticals.

## METHODOLOGY

The preparation of dithiol, thioglycolic acid and sodium lauryl sulfate (SDS) (Sigma-Aldrich) solutions were made according to LR monographs and reagent preparation procedure described in the European Pharmacopoeia [4].

Calibration curves of Sn(II) and Sn(IV) in 1.0 - 60.0  $\mu\text{g mL}^{-1}$  concentration range, in the presence and absence of thioglycolic acid and SDS were obtained by using 1900i Shimadzu UV-VIS spectrophotometer, measuring the absorbance at 540 nm wavelength.

## RESULTS AND DISCUSSION

Clark studied the use of 4-methyl-1,2-dimercaptobenzene (dithiol) as reagent for spot test and colorimetric determination of tin and observed that a pink or red color developed within seconds if stannous tin is present, and with tannic tin the color took longer to develop and the test was less sensitive. If a trace of thioglycolic acid was added to the liquid to be tested, the whole tin was rapidly reduced and the sensitivity of the test became independent of the initial state of oxidation of the tin [7-8]. Williams and Whitehead introduced Teepol X as dispersing agent to improve the analytical range and the stability of tin-dithiol color complex [9].

In this work, stannous chloride and stannic chloride were separately used as reagent for standard solution preparation of calibration curves in 1.0 - 60.0  $\mu\text{g mL}^{-1}$  concentration range, in the presence or absence of thioglycolic acid, in the presence or absence of SDS and the equations and  $r^2$  were determined (Table I)

Reagents	Equation	Range (r2)
Sn(IV)/dithiol/thioglycolic acid	A= 0.0777 [tin] + 0.0162	1.0-20.0 µg mL <sup>-1</sup> (0.996)
Sn(II)/dithiol/thioglycolic acid	A= 0.0635 [tin] + 0.0691	1.0-20.0 µg mL <sup>-1</sup> (0.995)
Sn(IV)/dithiol/SDS	A= 0.0860 [tin] + 0.0144	1.0-20.0 µg mL <sup>-1</sup> (0.999)
Sn(II)/dithiol/SDS	A= 0.0643 [tin] + 0.0496	1.0-20.0 µg mL <sup>-1</sup> (0.999)
Sn(II)/dithiol/thioglycolic acid/SDS	A= 0.0507 [tin] + 0.0389	1.0-20.0 µg mL <sup>-1</sup> (0.999)

Table I: Analytical curve of tin-dithiol reaction

The analytical curves for Sn(II) and Sn(IV) were linear in 1.0-20.0 µg mL<sup>-1</sup> range, with correlation coefficient greater than 0.99, and they were not linear up to 60.0 µg mL<sup>-1</sup>, standard solution concentration used in European pharmacopeia to interpolate and calculate tin concentration in LR.

The reaction of tin and dithiol were not influenced by the presence of thioglycolic acid or SDS and the complex did not presented changes in the spectrum when Sn(II) or Sn(IV) was used, confirming that the reaction did not distinguish the oxidation state of tin.

## CONCLUSIONS

Dithiol spectrophotometric method described in European Pharmacopoeia is useful for total tin determination but it is necessary to change the tin standard solution concentration for routine analysis, considering that the analytical range is not linear up to 60.0 µg mL<sup>-1</sup>. The reaction did not allow the speciation of Sn(II), important to guarantee the stability of the LR.

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