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Quantification of Active Pharmaceutical Ingredient in SAH-TEC Radiopharmaceutical by UV-Visible Spectrophotometry

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1. Introduction

Radiopharmaceuticals are pharmaceutical preparations with diagnostic or therapeutic purposes that, when ready for use, contain one or more radionuclides. Lyophilized reagents (LR) are reconstituted with Tc-99m generator eluate to be used by Nuclear Medicine as a diagnostic radiopharmaceutical for a series of illnesses. RDC no. 301/2019 and IN no. 37/2019 of ANVISA (National Health Surveillance Agency) establish the minimum requirements for industrial production of radiopharmaceuticals, in which final products must have a qualitative and quantitative composition in accordance with the described in drug registration. Because of this, active pharmaceutical ingredient (API) of each LR needs to be identified and quantified, in order to comply with GMP (Good Manufacturing Practices) [1-3].
There are many methods to determinate total protein concentration, and the most commonly used are based on UV-visible light absorption properties of proteins complexed with biuret, Lowry, Bradford, or Smith reagents. The biuret method was firstly proposed in 1915 and is based on the reagent composed by a mixture of copper, sodium hydroxide and sodium tartrate, as complexant of copper. Copper, in an alkaline medium, reacts with proteins forming a planar square complex. The product of the reaction has two absorption bands, one at 270 nm and another at 540 nm. Although the absorption band with maximum in 270 nm increases the sensitivity of the biuret method in six times, the band in 540 nm region is the most used for analytical purposes because several substances can cause interference in 270 nm region [4, 5].
Technetium Tc-99m Human Albumin Injection (SAH-TEC®) is a sterile, pyrogen-free, aqueous suspension of Human Albumin to be labeled with Tc-99m. It is used for lymphoscintigraphy of limbs and for the diagnosis of sentinel lymph node and as a blood compartment agent for radionuclide ventriculography, for suspicion or existence of coronary artery disease; assessment of congestive heart failure; assessment of cardiac function in patients undergoing chemotherapy; assessment of ventricular function in patients with valvular disease [6].
The objective of this work was to evaluate the method described in US Pharmacopeia based on biuret reagent to quantify SAH-TEC® LR API by UV-visible spectrophotometry.

2. Methodology

Traceable standard of human serum albumin (HSA) (Sigma) was used to obtain the analytical curve in 0.5 to 15 mg range of HSA. Calibration solutions and LR samples, using blank solution in reference cell, were analyzed at 540 nm wavelength using 1900i UV-vis Shimadzu spectrophotometer. Three vials of five batches of SAH-TEC® LR produced by IPEN/CNEN-SP was solubilized with 9 g . L^{-1} NaCl and an aliquot was placed into a vial to react with biuret reagent prepared according to US Pharmacopoeia, and the absorbance of the solution was measured. The quantification of HSA was calculated using calibration curve equation.

3. Results and Discussion

Figure 1 shows the scheme of the biuret test reaction and Figure 2 represents the UV-visible spectrum of a HSA-biuret reaction.

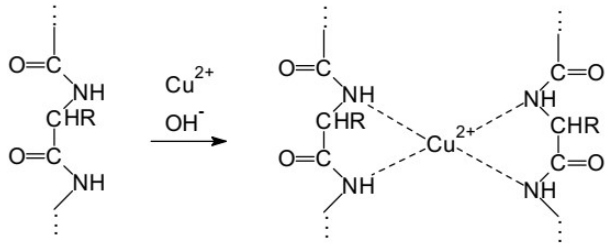

Figure 1. Biuret test reaction scheme [5]

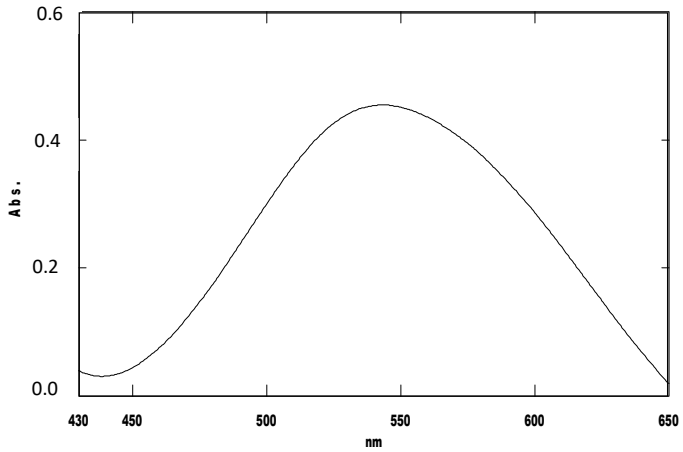

Figure 2. UV-Vis absorption spectrum of HSA-biuret complex

Figure 3 shows the analytical curve for HSA API quantification in SAH-TEC®. The linear equation that represents 0.5-15.0 mg range is A= 0.0432 [HSA] + 0.0103, with coefficient of determination of $r^2 = 0.998$.

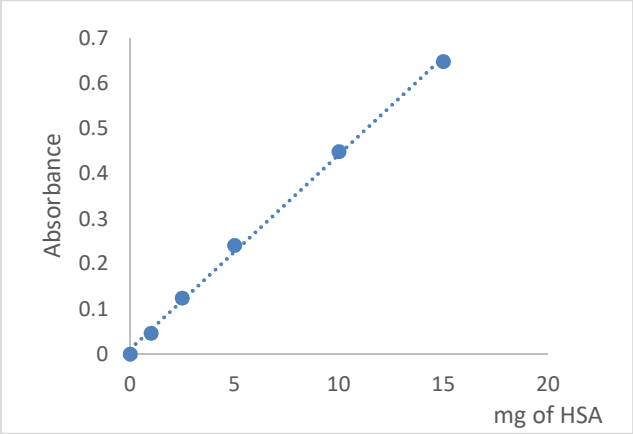

Figure 3. Calibration curve for HSA by using biuret test.

SAH-TEC® LR produced by IPEN-CNEN/SP must contain not less 9 mg and not more than 11 mg of HSA. Table I shows the results of HSA quantification. Only one of the analyzed five batches was above 90-110% range.

Table 1: HSA concentration results and %recovery in LR.

Batch	Concentration (mg)	Recovery (%)
1	10.97 ± 0.20	110 ± 2
2	10.83 ± 0.43	108 ± 4
3	11.13 ± 0.04	111 ± 0.4
4	10.57 ± 0.07	106 ± 0.7
5	10.92 ± 1.39	109 ± 1

(n=3)

4. Conclusions

The biuret method described in US Pharmacopoeia [7] was reproducible, easy to perform and allowed HSA API quantification in SAH-TEC®.

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