A proposal for validation of the radiochemical purity of Technetium Tc99m Pentetate

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INTRODUCTION

Radiopharmaceutical manufacturers must assure that their products comply with the requirements of Good Manufacturing Practices for Radiopharmaceuticals. Validation of manufacturing processes and analytical methods in the pharmaceutical industry are slow and time-consuming procedures that ensure that safe and effective products are obtained for the intended use. The objective of validation of an analytical procedure is to demonstrate that it is suitable for the intended purpose. The assays to be performed in the validation of analytical method should be selected depending on the goal, i.e., identification tests, quantitative tests and limit tests for the control of impurities and quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product. Every test should be designed to accurately reflect the sample characteristics. Radiochemical purity (RCP) is defined as the proportion of the total radioactivity of the radionuclide present in the stated chemical form. Impurities as $^{99m}TcO_4^-$ or $^{99m}TcO_2$ may arise during the labeling/ storage of a lyophilized reagent with the eluate of $^{99}Mo/^{99m}Tc$ generator. Radiochemical impurities in a radiopharmaceutical preparation would rarely produce a serious toxic reaction but may lead to errors in the diagnosis. These impurities may degrade image quality, increase absorbed radiation dose, or be localized in areas other than those intended, ultimately giving incomplete or incorrect information. The objective of this study was to present a proposal for the complete validation of the RCP method used at IPEN for Technetium Tc99m Pentetate.

METHODS

Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained by elution of a $^{99}Mo/^{99m}Tc$ generator (IPEN-CNEN/SP, Brazil). DTPA-TEC lyophilized reagent was analyzed after labeling with $^{99m}TcO_4^-$, obtaining 55.5 MBq mL^{-1} of radioactive concentration. 3MM Whatman paper chromatography (PC) strips were used for $^{99m}TcO_2$ and $^{99m}TcO_4^-$ determination. The sample was applied to the strip at 1.0 cm from origin. The mobile phases for determination of $^{99m}TcO_2$ and $^{99m}TcO_4^-$ were saline and acetone, respectively. After the chromatographic separation, the strips were dried and cut in two pieces and the radioactivity was sequentially counted in an Auto-Gamma Cobra II gamma counter, 5002 Series, Perkin Elmer. Figure 1 shows the tests performed for method validation.

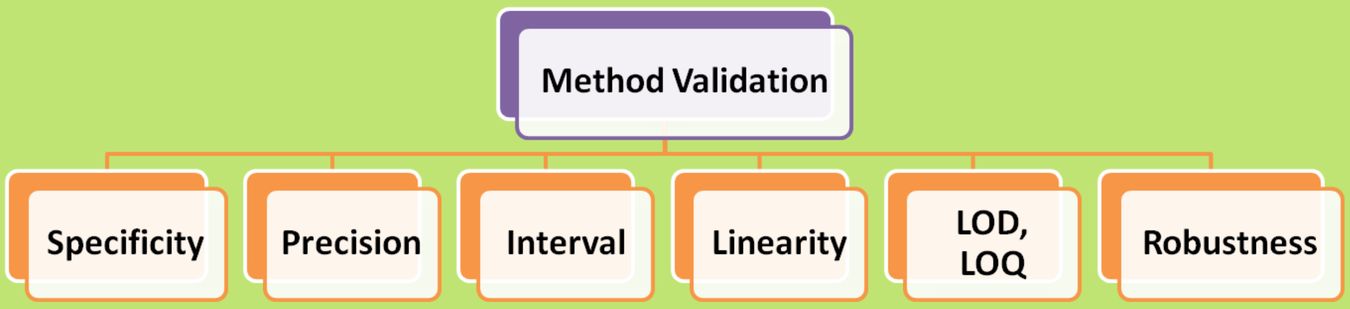

Figure 1 – Flowchart of tests

The specificity was evaluated by adding Al^{3+} or $^{99}MoO_4^-$ in the labeling step. DTPA-TEC RCP, $\%^{99m}TcO_4^-$ and $\%^{99m}TcO_2$ were determined in the absence of Al^{3+} or $^{99}MoO_4^-$ and in the presence of 1, 3 or 5 µg mL^{-1} Al^{3+} concentrations and in the presence of 185, 370 and 555 KBq $^{99}MoO_4^-$. The precision assays were performed in the same day. For repeatability, 6 RCP analyses were performed in a day with the same analyst and for the intermediate precision, other 6 RCP analyses from the same batch used in the repeatability test were performed by a different analyst. To determine the interval, $^{99m}TcO_4^-$ was applied to PC strips in 18.5, 37, 55.5, 111, 185 MBq mL^{-1} radioactive concentration. Calibration curve and linearity range was obtained relating the counts in cpm (y axis) *versus* the radioactivity concentration in mCi mL^{-1} (x axis). Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using linear and angular coefficients of calibration curve. The robustness of RCP method was evaluated varying length (8, 12, 15 cm) and width (1, 1.5 and 2.5 cm) of the paper strips ; mobile phase for $^{99m}TcO_4^-$ (50, 70 and 90% acetone); mobile phase for $^{99m}TcO_2$: (0.9, 15, 20 and 30% NaCl); sample application mode (capillary and micropipette) and sample volume (2, 5, 10 µL).

RESULTS

The RCP results for the sample contaminated with Al^{3+} or $^{99}MoO_4^-$ up to the limit allowed in international monographs indicated that the PC system is specific for $^{99m}TcO_4^-$ determination. Repeatability and intermediate precision were challenged and the results were lower than 5% with two different analysts performing six replicates of the RCP assay, in the same day. The calibration curve determined the range, LOD and LOQ of ^{99m}Tc concentration in which the gamma counter can linearly measure the radioactivity (cpm), with $r^2>0.99$. In the robustness, strip length and strip width, solvent composition and volume of sample spot were evaluated for $^{99m}TcO_2$ or $^{99m}TcO_4^-$, in three different conditions, in triplicate and all results presented coefficient variation lower than 5% for RCP. It was verified that small changes in the experiment conditions did not result in significant errors. It was not possible to perform experiments to determine the accuracy of the method because there is not an appropriate radioactive standard that can be used.

Table 1 – Validation results

Specifity		Robustness						
$[Al^{3+}]$ (µg mL^{-1})		$^{99m}TcO_4^-$	[ ] Mobile phase	Average ± SD	Volume	Average ± SD	Dimensions (cm)	Average ± SD
0	97.86 ± 0.54		Acetone PA	99.73 ± 0.16	Capilar	99.73 ± 0.16	8 x 100	99.73 ± 0.16
1	98.05 ± 0.57		Acetone 90%	99.89 ± 0.02	2 µL	99.93 ± 0.01	12 x 1.0	99.90 ± 0.02
3	98.76 ± 0.49		Acetone 70%	99.89 ± 0.02	5 µL	99.89 ± 0.03	15 x 1.0	99.89 ± 0.01
5	98.86 ± 0.19		Acetone 50%	99.53 ± 0.02	10 µL	99.90 ± 0.04	8 x 1.5	99.92 ± 0.03
							8 x 2.0	99.66 ± 0.14
$^{99}MoO_4^-$ (µCi)		$^{99m}TcO_2$	NaCl 0,9%	99.89 ± 0.01	Capilar	99.89 ± 0.01	8 x 1.0	99.90 ± 0.04
0	97.86 ± 0.54		NaCl 15%	99.80 ± 0.21	2 µL	99.81 ± 0.06	12 x 1.0	99.90 ± 0.04
5	99.33 ± 0.29		NaCl 20%	99.84 ± 0.13	5 µL	99.86 ± 0.11	15 x 1.0	99.90 ± 0.04
10	99.36 ± 0.21		NaCl 30%	99.90 ± 0.02	10 µL	99.83 ± 0.03	8 x 1.5	99.85 ± 0.03
15	99.11 ± 0.25						8 x 2.0	99.45 ± 0.28
Precision		Repeatability	Intermediate precision		LOD	0.21	LOQ	0.69
		99.90 ± 0.04	99.84 ± 0.11					
Linearity		$y = 176004.75 + 1.23762 . 10^6 x$; $r^2 = 0.9991$						

CONCLUSIONS

The results of the validation of the RCP method were within the acceptance criteria established by the requirements of RE 899 - Resolution for Validation of Analytical Methods in Brazil.