2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-05-2**

DETERMINATION OF RADIOCHEMICAL YIELD OF ^{99m}Tc RADIOPHARMACEUTICAL PREPARATIONS USING GAMMA COUNTER AND LINEAR RADIOCHROMATOGRAPHY SCANNER

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ABSTRACT

The radiochemical purity (RCP) evaluation is a prerequisite for radiopharmaceuticals before the administration in patients. RCP is defined as the proportion of the total radioactivity in the product that is present in the specified chemical form. The most widely used techniques for RCP determination in radiopharmaceutical preparations are thin layer chromatograpy (TLC-Al), instant thin layer chromatography (ITLC-SG) and paper chromatography (PC). These techniques combined with radioactivity detection are one of the most important tools in the RCP of the radiopharmaceutical compounds. Several methods are used for the determination of the spatial distribution of radioactivity on the strips. The aim of this study was to compare two methods for radioactivity measurement in the determination of RCP in ^{99m}Tc radiopharmaceuticals using gamma counter and linear radiochromatography scanner. Lyophilized radiopharmaceuticals were labeled with ^{99m}Tc. The analysis was carried out using TLC-Al and high performance thin layer chromatography (HPTLC-Cellulose) sheets, ITLC-SG and 3MM Whatman PC. The radioactivity distribution was determined by counting each strip during 1 minute in a radiochromatography TLC scanner. For comparison, the strips were cut into small pieces and each one was separately measured in a gamma-counter during 0.20 minutes in 70-210 KeV ^{99m}Tc window. USP 36 and FDA specify that not less than 90% of the total radioactivity must be in the spot corresponding to ^{99m}Tc labeled compound. In conclusion, the procedure for RCP determination of ALBUMINA-TEC, DEX500-TEC, ECD-TEC, MACRO-TEC and MIBI-TEC can be faster using radiochromatography.

1. INTRODUCTION

Technetium-99m is the most widely used radionuclide in nuclear medicine services because it has both chemical and physical characteristics: monoenergetic gamma ray of 140 KeV, that is easily collimated and yields a sufficient number of photons, physical half-life of 6.0 h long enough to obtain diagnostic information in many procedures and short enough to cause minimal radiation dose to the patient and operator. The eluate of $Na^{99m}TcO_4$ obtained from an enclosed and sterile ^{99}Mo-^{99m}Tc generator system does not need further processing or purification and can be directly administered or used for the preparation of ^{99m}Tc-radiopharmaceuticals [1].

The use of radiopharmaceuticals *in vivo* requires radiochemical purity (RCP) testing to be carried out just before administration to the patient. The methods used for quality control should be characterized by the highest accuracy and reliability, as well as being easy to perform, safe and quick in order to ensure the use in a busy laboratory or in emergency situations. This is especially true for ^{99m}Tc radiopharmaceuticals, as they are usually labeled in the hospital using commercially available cold kits and generators [2].

RCP, the fraction of radioactivity present in the specified chemical form, is frequently carried out by chromatographic methods as thin layer chromatography (TLC), instant thin layer chromatography-silica gel (ITLC-SG), paper chromatography (PC), mini columns or high performance liquid chromatography (HPLC). These techniques combined with radioactivity detection are one of the most important tools in the RCP determination of the radiopharmaceutical compounds [3].

Impurities may arise during preparation and storage of the radiopharmaceuticals and can modify organ distribution and specificity, possibly leading to an incorrect diagnosis. Radiochemical impurities rarely produce a serious adverse toxic reaction but may degrade image quality giving unsatisfactory diagnostic [4].

US Pharmacopeia (USP) specifies that not less than 90% of the total radioactivity is correspondent to ^{99m}Tc-radiopharmaceuticals but the monographies don't specify anything about the method of radioactivity counting [5].

Several methods are used for the determination of the spatial distribution of radioactivity on the strips as dose calibrator, gamma counter, TLC scanner and film autoradiography [6].

The aim of this study was to compare the efficiency, accuracy and rapidity of gamma counter with NaI detector and linear radiochromatography scanner in the radioactivity measurement for the determination of RCP in ^{99m}Tc radiopharmaceuticals.

2. EXPERIMENTAL

Lyophilized cold kits were labeled with ^{99m}Tc as described by the manufacturer (IPEN/CNEN-SP, Brazil). The cold kits were labeled with 55.5 MBq/mL (1.5 mCi/mL) or 370 MBq/mL (10 mCi / mL). The RCP was determined 30 minutes after labeling.

The analyses were carried out using TLC-Al, TLC Reverse Phase (TLC-RP) and High Performance TLC-Cellulose (HPTLC-Cellulose) sheets from Merck (Germany), ITLC-SG from Varian Inc. (Australia) and PC with 3MM Whatman from GE Healthcare (UK).

Reagents from Merck were used to prepare the mobile phases: acetone, 70% and 85% methanol, 0.9%, 20% and 30% (w/w) sodium chloride, 0.5 mol L^{-1} acetic acid, ethyl acetate:ethanol (3:7) and acetonitrile:methanol: 0.5 mol L^{-1} ammonium acetate: tetrahidrofuran (THF) (4:3:2:1).

Table 1 shows the characteristics of stationary phase for radioactivity measurement in NaI detector and linear radiochromatography scanner.

Table 1. Characteristics of Stationary Phases for Radioactivity Measurement in NaI Detector and Linear Radiochromatography Scanner

Stationary Phases	Strip Size (mm)	Number of Segments in NaI Gamma-Counter	Length for Area Integration in Radiochromatography (mm)
TLC-Al	100	10	100
TLC-RP	100	10	100
HPTLC-Cellulose	70	7	85
ITLC-SG	150	15	200
3MM Whatman	70 or 100	2 or 10	85 or 150

The chromatographic systems used in this work are recommended by the manufacturer or described in the USP and are summarized in Table 2.

Table 2. Cromatographic Systems for RCP Determination of ^{99m}Tc Radiopharmaceuticals

PRODUCT	MOBILE/STATIONARY PHASE SYSTEM A ($^{99m}TcO_4^-$)	MOBILE/STATIONARY PHASE SYSTEM B ($^{99m}TcO_2$)
ALBUMINA-TEC	Acetone – TLC-Al	-
DEX-500-TEC	Acetone – 3MM Whatman	-
DISIDA-TEC	30 % (w/w) NaCl – 3MM Whatman	85 % Methanol – HPTLC-Cellulose
EC-TEC	Acetone – ITLC-SG	0.5 mol L^{-1} acetic acid – ITLC-SG
ECD-TEC	20 % (w/w) NaCl– TLC-Al	ethyl acetate:ethanol (3:7) – HPTLC-Cellulose
GLUCOHEPTONATO-TEC	Acetone – ITLC-SG	0.9 % (w/w) NaCl – ITLC-SG
MACRO-TEC	70 % Methanol – 3MM Whatman	-
MDP-TEC	Acetone – 3MM Whatman [(1)]	0.9 % (w/w) NaCl – 3MM Whatman [(2)]
MIBI-TEC	Acetonitrile: methanol: 0.5 mol L^{-1} ammonium acetate: THF (4:3:2:1) - TLC-RP	
PIRO-TEC	Acetone – 3MM Whatman	0.9 % (w/w) NaCl – HPTLC-Cellulose

[(1)] 70 mm; [(2)] 100 mm

After the development of the mobile phase, the strips were dried and rolled up into adhesive tape.

The radioactivity distribution was determined by counting each strip during 1 minute on a radiochromatography TLC scanner (AR-2000, BioScan, USA) equipped with a high efficiency or high resolution collimator and P10 gas.

For comparison, the strips were cut into 2, 7, 10 or 15 small pieces depending on the radiopharmaceutical and each one was placed into a tube, sequentially measured in a gamma-counter with NaI detector (Cobra II AutoGamma, Perkin Elmer Inc., USA) during 0.20 minutes in 70-210 KeV window. The data obtained with NaI detector were expressed in *counts per minute* (cpm).

The Rf value was 0.0 for $^{99m}TcO_2$ and in 0.8-1.0 range for $^{99m}TcO_4^-$, respectively. For MIBI-TEC, a single chromatographic system was used to determine both impurities.

The $^{99m}TcO_2$ and $^{99m}TcO_4^-$ impurities were calculated as the percentage of the radioactivity corresponding to the Rf of the impurity divided by the total radioactivity on the strip. The RCP was found by subtracting the impurities percentage from 100.

Using the TLC scanner based on P10 gas, the integration of the areas of the product and the impurity peaks was performed using WIN SCAN software. The RCP was calculated using the Equations 1, 2 and 3.

$$\% \ ^{99m}TcO_2 = {}^{99m}TcO_2 \text{ Area / Total Area x 100} \quad (1)$$

$$\% \ ^{99m}TcO_4^- = {}^{99m}TcO_4^- \text{ Area / Total Area x 100} \quad (2)$$

$$\% \text{ Product} = 100 - \% \ ^{99m}TcO_2 - \% \ ^{99m}TcO_4^- \quad (3)$$

Using the gamma counter with NaI detector, $^{99m}TcO_2$ impurity was found in the 1st segment, i.e., 1 cm after the sample application point (Table 2, System B) and the last two segments represented $^{99m}TcO_4^-$ (Table 2, System A). The RCP was calculated using the Equations 4, 5 and 3:

$$\% \ ^{99m}TcO_2 = \text{1st segment / sum of the countings x 100} \quad (4)$$

$$\% \ ^{99m}TcO_4^- = \text{two segments / sum of the countings x 100} \quad (5)$$

3. RESULTS AND DISCUSSION

USP 36 and FDA specify that not less than 90% of the total radioactivity must be in the spot corresponding to the ^{99m}Tc labeled compound.

Two different kinds of detectors for measuring the radioactivity on TLC and PC sheets were used in this study. Although the Equations for %impurity calculation were different, the counting of the radioactivity was performed in the same place in the strips for both techniques.

Figure 1 shows the profile of the radioactivity on the strips of ALBUMINA-TEC, DEX500-TEC, ECD-TEC, GLUCOHEPTONATO-TEC, MACRO-TEC and MIBI-TEC respectively, obtained with TLC scanner.

The comparison of the results of RCP, obtained with the measurement of radioactivity using the NaI and P10 gas detectors, is described in Tables 3 and 4.

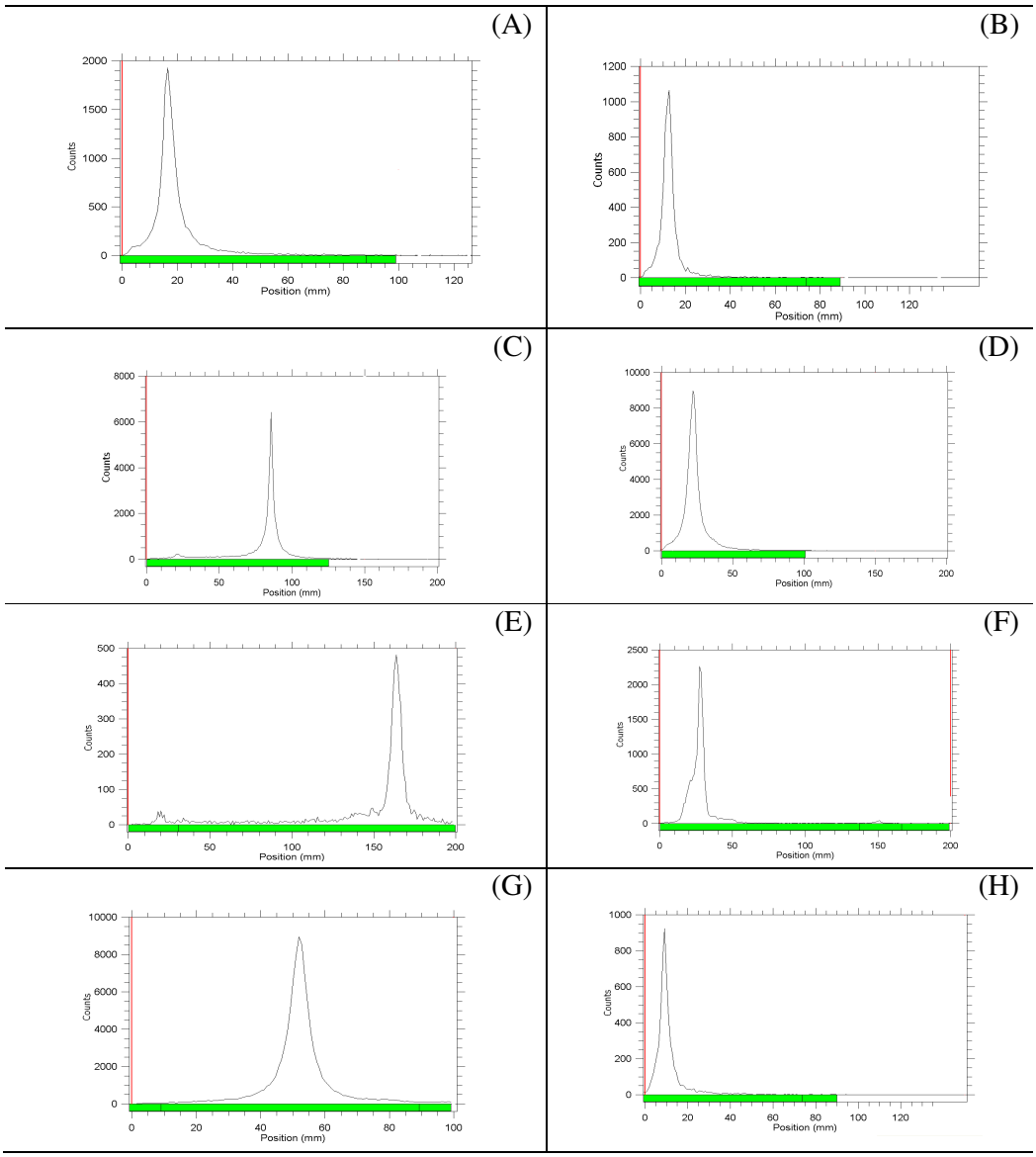

Figure 1: Profile of the radioactivity for: (A) ALBUMINA-TEC; (B) DEXTRAN-TEC;(C) ECD-TEC - Ethyl Acetate:Ethanol (3:7)/HPTLC-Cellulose; (D) ECD-TEC – 20% NaCl/TLC-Al; (E) GLUCOHEPTONATO-TEC - 0.9 % NaCl/ITLC-SG, (F) GLUCOHEPTONATO-TEC – Acetone/ITLC-SG; (G) MIBI-TEC; (H) MACRO-TEC, obtained with scanner.

The product peak was well defined and presented good resolution of the impurity peaks in the radiochromatograms represented in Figure 1. A good separation between the peaks indicated that the chromatographic system was adequated.

The noise was higher when the radiopharmaceutical was labeled with 1.5 mCi/mL, as for GLUCOHEPTONATO-TEC (Figure 1).

Table 3. Results of RCP for ^{99m}Tc Radiopharmaceuticals labeled with 55.5 MBq/mL (1.5 mCi/mL) and using a high efficiency collimator on the TLC scanner.

PRODUCT	$^{99m}TcO_4^-$		$^{99m}TcO_2$		RCP		Deviation
	NaI	P-10	NaI	P-10	NaI	P-10	
DISIDA-TEC	0.64	1.06	1.99	6.54	97.37	92.40	4.97
	1.13	1.72	4.85	10.7	94.02	87.58	6.44
ECD-TEC	0.73	1.56	0.16	0.08	99.11	98.36	0.75
	2.46	4.47	0.07	0.02	97.47	95.51	1.96
GLUCOHEPTONATO-TEC	7.37	2.95	0.2	0.53	92.43	96.52	4.09
PIRO-TEC	0.82	3.05	0.61	2.44	98.57	94.51	4.06
	0.64	2.83	0.76	2.08	98.60	95.09	3.51

Table 4. Results of RCP for ^{99m}Tc Radiopharmaceuticals labeled with 370 MBq/mL (10 mCi / mL) and using a high resolution collimator on the TLC scanner.

PRODUCT	$^{99m}TcO_4^-$		$^{99m}TcO_2$		RCP		Deviation
	NaI	P-10	NaI	P-10	NaI	P-10	
ALBUMINA-TEC			1.25	0.89	98.75	99.12	0.36
			0.56	0.34	99.44	99.66	0.22
DEX-500-TEC			0.19	1.24	99.81	98.76	1.05
EC-TEC	1.57	1.43	1.96	3.22	96.47	95.35	1.12
MACRO-TEC			0.47	2.08	99.53	97.92	1.61
MDP-TEC	0.41	0.40	0.28	3.19	99.31	96.41	2.90
	0.34	1.73	0.40	2.23	99.26	96.04	3.22
MIBI-TEC	0.50	0.72	0.73	1.45	98.76	97.83	0.93
	0.32	0.70	0.71	2.21	98.96	97.10	1.87

According to the Tables 3 and 4, the RCP results obtained with NaI and P10 detectors presented a deviation less than 2% for ALBUMINA-TEC, DEX500-TEC, EC-TEC, ECD-TEC, MACRO-TEC and MIBI-TEC while the deviation was higher than 3% for DISIDA-TEC, GLUCOHEPTONATO-TEC, MDP-TEC and PIRO-TEC.

The difference between the RCP results for EC-TEC was lower than 2% but the radiochromatograms showed that the product was carried along the strips in the two chromatografic systems. DISIDA-TEC and MDP-TEC showed the same problem on Whatman 3MM strips and PIRO-TEC on the HPTLC-Cellulose, in the determination of $\%^{99m}TcO_2$.

Most of the results with differences higher than 3% were observed in the radiopharmaceuticals labeled with 55.5 MBq/mL (1.5 MBq/mL). For these products, the %impurity was lower when the NaI detector was used.

Further studies need to be carried out to evaluate the limitations and the potentialities of TLC scanner in quality control of ^{99m}Tc radiopharmaceuticals.

4. CONCLUSIONS

The procedure for RCP determination of ALBUMINA-TEC, DEX500-TEC, ECD-TEC, MACRO-TEC and MIBI-TEC can be faster using radiocromatography.

In the analyzed kits, the RCP was in accordance to the allowable limit described in USP 36 and FDA.

ACKNOWLEDGMENTS

The authors thank the Production of Lyophilized Reagents Group of the Radiopharmacy Center in IPEN-CNEN/SP.

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