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COMPARISON OF CHROMATOGRAPHY SYSTEMS FOR RADIOCHEMICAL PURITY DETERMINATION OF LYOPHILIZED REAGENTS LABELED WITH TECHNETIUM-99m

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ABSTRACT

A variety of lyophilized reagents (LR) labeled with ^{99m}Tc has been developed for determining organ function or assessing disease status by imaging methods. Usually, the quality of the radiopharmaceutical preparations is evaluated by paper chromatography (PC), thin layer chromatography (TLC), instant thin layer chromatography-silica gel (ITLC-SG), high performance liquid chromatography (HPLC) on reverse-phase columns and capillary electrophoresis (CE). PC and TLC have been applied due to the low cost and short time in the determination of pertechnetate ($^{99m}TcO_4^-$) and technetium dioxide ($^{99m}TcO_2$). The present study reports the comparison between PC and TLC chromatographic methods for determination of the radiochemical purity of LR labeled with ^{99m}Tc from IPEN-CNEN/SP (Brazil). PC was performed with Whatman 3MM/1MM paper chromatography strips and TLC with ITLC-SG sheets or reversed phase (RP). RP was used only for ECD. Although the radioactivity profile of the separation of the species on both stationary phases was satisfactory, the difference in results for % $^{99m}TcO_4^-$ and $^{99m}TcO_2$ was up to 4.2 % using PC for ECD and PYP. ITLC supports gave better resolution than conventional PC supports for these products. In ECD analysis, the comparison was performed between RP and ITLC-SG stationary phases for determination of $^{99m}TcO_4^-$, $^{99m}TcO_2$ and other impurities. It was observed that the sheet length as described in the United States Pharmacopeia (USP) was not sufficient for a good separation of the product and the impurities. The results showed that there were not significant differences between PC and TLC chromatographic stationary phases are going to be accomplished.

1. INTRODUCTION

Among the conveniently available radionuclides, technetium has by far the best nuclear properties for diagnostic imaging, as 6-hr physical half-life, monochromatic 140 keV photons and administration of milicurie of ^{99m}Tc radioactivity without significant radiation dose to the patient [1]. The advent of commercial generator systems, instant technetium, innovations in chelation, and new chelating agents, has caused a marked expansion in the use of ^{99m}Tc labeled compounds. Chemical forms of ^{99m}Tc are present in the most widely used radiopharmaceuticals for radionuclide imaging of the brain, liver, lung and skeleton, and in thyroid scintigraphy [2].

In evaluating the quality of a radiopharmaceutical preparation, several properties must be tested to assure its safety and efficacy and fulfill requisites of the Good Manufacturing Practices (GMP) and others because of radioactivity [3]. Properties like radionuclidic, radiochemical and chemical purity; sterility and apyrogenicity are essential considerations when the radiopharmaceutical is intended for parenteral administration [4]. One important quality parameter is the radiochemical purity of the radiolabeled product. This is defined as

the proportion of the total radioactivity in a sample associated with the desired radiolabeled species [1, 5]. Radiochemical impurities in a radiopharmaceutical preparation would rarely produce a serious toxic reaction but may lead to serious error in diagnosis [4]. A variety of lyophilized reagents (LR) labeled with ^{99m}Tc has been developed for determining organ and tissue functions or assessing the disease status. MIBI-^{99m}Tc (hexakis methoxy isobutyl isonitrile) and PYP-^{99m}Tc (sodium pyrophosphate) are used in studies of myocardial perfusion [1, 6], HSA-^{99m}Tc (human serum albumin) for blood pool imaging [7] ECD-^{99m}Tc (ethylysteinate dimer) for brain scintigraphy, DISIDA-^{99m}Tc (diisopropyliminodiacetic acid) for hepatobiliary imaging and EC-^{99m}Tc (ethylenedicysteine) for renal function imaging [1].

Usually, the quality of the radiopharmaceutical preparations is evaluated by paper chromatography (PC), thin layer chromatography (TLC), instant thin-layer chromatography (ITLC), high performance liquid chromatography (HPLC) on reverse-phase columns and capillary electrophoresis (CE). PC and TLC are techniques for adsorption chromatography. The adsorbent in paper chromatography is a strip or sheet of paper across which a liquid phase migrates. In TLC, the stationary phase is a thin coating of dry adsorbent applied to aluminum, polymer or glass sheet. Silica gel and alumina are the most commonly used adsorbents. The instant thin-layer chromatography (ITLC) had been improved with the development of a glass microfiber sheet [4].

The migration of individual compounds in sample is conveniently characterized by Retention factor (R_f) values. It is necessary to establish R_f values for both the desired compound and the probable impurities in the chromatography system to be used for quality control [4].
The radioactivity profile of paper or thin layer chromatograms is measured either by cutting the strip or counting each segment separately or by using a recording strip scanner with a radiation detector [4].

Specific methods for determining radiochemical impurities in commonly available radiopharmaceuticals are described in the literature and United States Pharmacopeia (USP) [4,8].

The objective of this work was to compare TLC and PC chromatographic methods for radiochemical purity determination of LR labeled with ^{99m}Tc.

2. EXPERIMENTAL

2.1. Materials

Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained by elution of a ^{99}Mo/^{99m}Tc generator (IPEN-CNEN/SP-Brazil) in saline solution. DISIDA, EC, ECD, HSA, MIBI and PYP LR were from IPEN-CNEN/SP (Brazil). Acetone, methanol, acetic acid, sodium chloride, ethyl acetate and ammonium acetate were P.A. reagents (Merck). PC was performed with 3MM or 1MM Whatman paper chromatography and TLC with ITLC-SG sheets from Pall Science or Reversed phase (RP) Partisil KC 18 plates from Whatman.

2.2. Methods

Two vials of each LR were analyzed in duplicate, at 30, 60 and 240 minutes after labeling with ^{99m}Tc.

17.5 cm length ITLC-SG and PC strips were used for determination of $^{99m}TcO_2$ or $^{99m}TcO_4^-$ in DISIDA, EC, ECD, HSA, MIBI and PYP labeled with ^{99m}Tc. The radioactive concentration was 55.5 MBq mL^{-1}. The sample was applied to the strip at 1.5 cm height from origin.

Other two systems were used for ^{99m}Tc-ECD analysis: 2.5 cm x 7.5 cm TLC-SG for separation of ^{99m}Tc-ECD and the impurities and 2.5 cm x 7.5 cm reversed phase for determination of ^{99m}Tc-ECD, ^{99m}Tc-(IV)-ECD, $^{99m}TcO_2$, $^{99m}TcO_4^-$, ^{99m}Tc-ethylene cisteinate monomer and ^{99m}Tc-EDTA. The sample was applied to the strip at 2.0 cm height from origin.

The mobile phases for determination of $^{99m}TcO_2$ were acetic acid for EC, acetone for ECD, 0.9% (w/v) NaCl for PYP and 85% (v/v) methanol for DISIDA; % $^{99m}TcO_4^-$ was determined using methanol:ethyl acetate (2:8, v/v) for ^{99m}Tc-MIBI, acetone for ^{99m}Tc-EC and 85% (v/v) methanol for ^{99m}Tc-HSA. ^{99m}Tc-ECD was determined using ethyl acetate and in the reversed phase method acetone: 0.5 mol L^{-1} ammonium acetate (60:40, v/v) was used.

After the chromatographic separation, the strips were dried and cut in 1.0 cm pieces and the radioactivity was sequentially counted in a gamma counter (Auto-Gamma Cobra II, 5002 Series, Perkin Elmer).

3. RESULTS AND DISCUSSION

Lately, ITLC-SG a stationary phase commonly used for radiopharmaceutical purity analysis, had its manufacturing been discontinued by the supplier, thus requiring the development of alternative radiochemical purity procedures in LR Quality Control.

Tables 1 to 6 show the comparison of results obtained by using ITLC-SG and PC strips in the radiochemical purity determination of ^{99m}Tc-DISIDA, ^{99m}Tc-EC, ^{99m}Tc-ECD, ^{99m}Tc-HSA, ^{99m}Tc-MIBI and ^{99m}Tc-PYP. Some impurities were not described in the reported results as the main objective of this work was to compare the PC and ITLC-SG chromatographic systems.

Table 1. Comparison of % radiochemical purity of ^{99m}Tc-DISIDA by ITLC-SG and PC.

Stationary phase	Time after labeling (min)	% Radiochemical purity	% $^{99m}TcO_2$ (R_f=0) 85% methanol
ITLC-SG	30	98.35 ± 0.72	0.22 ± 0.07
	60	98.19 ± 0.63	0.33 ± 0.09
	240	96.09 ± 1,65	0.18 ± 0.01
Whatman 1MM	30	97.63 ± 0.20	1.44 ± 0.15
	60	96.86 ± 0.58	1.89 ± 0.20
	240	95.03 ± 0.36	1.63 ± 0.09

Table 2. Comparison of % radiochemical purity of ^{99m}Tc-EC by ITLC-SG and PC

Stationary phase	Time after labeling (min)	% Radiochemical purity	% $^{99m}TcO_4^-$ (Rf=1) acetone	% $^{99m}TcO_2$ (R_f=0) Acetic acid
ITLC-SG	30	98.29 ±0.53	0.41 ± 0.09	1.31 ± 0.49
	60	98.28 ± 0.47	0.36 ± 0.10	1.36 ± 0.39
	240	97.40 ± 0.51	1.20 ± 0.39	1.40 ± 0.24
Whatman 1MM	30	98.36 ± 0.43	0.48 ± 0.22	1.17 ± 0.24
	60	98.76 ± 0.29	0.28 ± 0.26	0.96 ± 0.04
	240	98.34 ± 0.35	0.44 ± 0.11	1.22 ± 0.31
Whatman 3MM	30	98.56 ± 0.29	0.13 ± 0.03	1.32 ± 0.29
	60	98.70 ± 0.31	0.22 ± 0.06	1.09 ± 0.32
	240	98.25 ± 0.33	0.23 ± 0.15	1.52 ± 0.22

Table 3. Comparison of % radiochemical purity of ^{99m}Tc-ECD by ITLC-SG and PC

Stationary phase	Time after labeling (min)	% Radiochemical purity	% $^{99m}TcO_2$ (R_f=0) Acetone
ITLC-SG	30	98.34 ± 0.26	1.60 ± 0.26
	60	98.19 ± 0.11	1.78 ± 0.11
	240	97.47 ± 0.74	2.50 ± 0.75
Whatman 1MM	30	95.15 ± 1.66	4.78 ± 1.65
	60	95.24 ±0.45	4.73 ± 0.95
	240	94.68 ± 1.66	5.30 ± 1.66
Whatman 3MM	30	96.75 ± 0.16	3.17 ± 0.17
	60	96.19 ± 0.43	3.76 ± 0.43
	240	95.69 ± 0.33	4.28 ± 0.34

Table 4. Comparison of % radiochemical purity of ^{99m}Tc-HSA by ITLC-SG and PC

Stationary phase	Time after labeling (min)	% Radiochemical purity	% $^{99m}TcO_4$ (R_f=1) Methanol 85%
ITLC-SG	30	98.59 ± 0.39	1.42 ± 0.39
	60	98.70 ± 0.29	1.30 ± 0.29
	240	98.55 ± 0.52	1.45 ± 0.52
Whatman 1MM	30	99.90 ± 0.03	0.10 ± 0.03
	60	99.93 ± 0.01	0.08 ± 0.01
	240	99.87 ± 0.05	0.13 ± 0.05

Table 5. Comparison of % radiochemical purity of ^{99m}Tc-MIBI by ITLC-SG and PC.

Stationary phase	Time after labeling (min)	% Radiochemical purity	% ($^{99m}TcO_4$ + $^{99m}TcO_2$) (R_f=1) Methanol: ethyl acetate
ITLC-SG	30	98.53 ± 0.18	1.48 ± 0.18
	60	98.28 ± 0.14	1.73 ± 0.14
	240	98.65 ± 0.32	1.35 ± 0.32
Whatman 1MM	30	97.88 ± 0.09	2.12 ± 0.10
	60	97.38 ± 0.28	2.65 ± 0.28
	240	97.92 ± 0.15	2.08 ± 0.15
Whatman 3MM	30	98.99 ± 0.12	1.01 ± 0.12
	60	98.96 ± 0.13	1.04 ± 0.13
	240	98.82 ± 0.14	1.18 ± 0.14

Table 6. Comparison of % radiochemical purity of ^{99m}Tc-PYP by ITLC-SG and PC.

Stationary phase	Labeling time (min)	% Radiochemical purity	% $^{99m}TcO_2$ (R_f=0) NaCl 0.9%
ITLC-SG	30	98.57 ± 0.01	1.05 ± 0.09
	60	98.53 ± 0.18	1.08 ± 0.13
	240	98.12 ± 0.34	1.52 ± 0.30
Whatman 1MM	30	95.66 ± 0.74	4.00 ± 0.76
	60	95.54 ± 0.27	4.09 ± 0.31
	240	93.87 ± 0.89	5.78 ± 0.80

ITLC-SG or TLC and PC have been performed due to the low cost and short time of analysis in the determination of pertechnetate ($^{99m}TcO_4^-$) and technetium dioxide ($^{99m}TcO_2$) [5]. PC is easy to perform and the separation varies from a few minutes to some hours depending upon the compounds to be separated as well the properties of the solvent and absorbent and the operating conditions for the system. Using ITLC supports, development times can be reduced to less than 10 minutes with some solvents [4].

Although the radioactivity profile of the separation of the species on both stationary phases was satisfactory, the difference in results for % $^{99m}TcO_4^-$ and $^{99m}TcO_2$ was up to 4.2 % using PC for ECD and PYP. ITLC supports gave better resolution than conventional PC supports for these products. Otherwise, for HSA, the % $^{99m}TcO_4^-$ was up to 1 % using ITLC-SG plates.

In PC, different compounds in the sample mixture run at different rates due to differences of solubility in the solvent, and due to differences in their adsorption to the fibers in the paper. Because the paper is made of cellulose, a polar substance, the compounds within the mixture run farther if they are less polar. The more polar substances bind to the cellulose paper more strongly and therefore do not run as quickly [9].

In ECD analysis, comparing RP and ITLC-SG stationary phases for determination of $^{99m}TcO_4^-$, $^{99m}TcO_2$ and other impurities, it was observed that the RP sheet length as described in the USP was not sufficient for a good separation of the product and the impurities and more studies should be done.

All the values of radiochemical purity were above 93%. According to USP and IPEN specifications, the LR radiochemistry purity must be superior to 90%.

4. CONCLUSIONS

The results showed that the differences in the radiochemical purity determined by PC and ITLC were not significant, except for ^{99m} Tc-ECD and ^{99m} Tc-PYP were up to 4.2%. However, both methods can be used in the quality control of radiopharmaceuticals.

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