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ALTERNATIVE METHODS FOR RADIOCHEMICAL PURITY TESTING IN RADIOPHARMACEUTICALS

Ideli M. de Oliveira, Patrícia de A. Martins, José L. da Silva, Marcelo P. S. Ramos, José A. S. Lima, Priscilla B. Pujatti, Neuza T. O. Fukumori, Margareth M. N. Matsuda

Instituto de Pesquisas Energéticas e Nucleares - IPEN/CNEN-SP
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
idelimorais@yahoo.com.br

ABSTRACT

The radiochemical purity (RCP) testing is as prerequisite for radiopharmaceuticals before the administration to the patient. Because time is critical in nuclear medicine, emphasis should be given to the radiochemical quality control procedures, in order to obtain the maximum amount of information in the minimum period of time. Radiochemical purity is defined as the proportion of the total radioactivity in the product that is present in the specified chemical form. Usually, the RCP is evaluated by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The most widely used technique for RCP determination in radiopharmaceutical preparations is TLC-aluminium (TLC-Al), instant thin layer chromatography-silica gel (ITLC-SG) and paper chromatography (PC). Indeed, many of the pharmacopeial methods use these techniques. The purpose of the present study was to evaluate different chromatographic systems for RCP in ^{67}Ga-Citrate, ^{111}In-Octreotide, ^{177}Lu-DOTATATE and ^{153}Sm-HA. PC was performed with 3MM/1MM Whatman plates, TCL-Al sheets from Merck and ITLC-SG sheets from Pall Corporation and Varian Inc. The mobile phases were 0.16 mol.L^{-1} sodium acetate, 0.9% sodium chloride (p/v), 0.1 mol.L^{-1} sodium citrate buffer, 0.2 mol.L^{-1} EDTA, methanol:0.4 mol.L^{-1} ammonium acetate (1:1) mixture, and pyridine:ethanol:water (1:2:4) mixture. The samples were placed on plates in triplicate and immediately put into pre-saturated chambers with the mobile phase. After the chromatographic separation, the plates were dried and cut into 7, 10 or 12 segments and each one was separately measured in a gamma counter during 0.20 minutes (set on the radioisotope window). The results in the gamma counter were expressed in counts per minute (cpm). The chromatographic systems for ^{177}Lu-DOTATATE and ^{153}Sm-HA gave the best performances in 0.1 mol L^{-1} sodium citrate buffer/TLC-Al and 0.9% (p/v) sodium chloride/Whatman 1, respectively. ^{111}In-Octreotide showed better results in 0.2 mol L^{-1} EDTA/TLC-Al. Two systems were evaluated for ^{67}Ga-Citrate, 3MM Whatman PC with 0.16 mol.L^{-1} sodium acetate and pyridine:ethanol:water (1:2:4) mixture. The best performance was obtained with 0.16 mol.L^{-1} sodium acetate. The established limits of RCP vary from 95% to 97% and all the results showed to be in accordance with the specifications for each analyzed product.

1. INTRODUCTION

The radiochemical purity (RCP) is defined as the proportion of the total radioactivity of the radionuclide present in the stated chemical form. Impurities may arise during preparation and storage of radiopharmaceuticals. Radiochemical impurities in a radiopharmaceutical preparation would rarely produce a serious toxic reaction but may lead to a serious error in diagnosis. These impurities may degrade image quality, increase absorbed radiation dose, or localize in areas other than those intended, ultimately giving incomplete or incorrect information [1].

The determination of RCP requires the use of methods to separate different labeled chemical species which may be present in the radiopharmaceutical preparation. Numerous

methodologies can be employed to assess the RCP of radiopharmaceuticals including thin layer chromatography (TLC), paper chromatography (PC), gel permeation chromatography, high performance liquid chromatography (HPLC) and gel electrophoresis. Because time is critical in a nuclear medicine department, the emphasis of radiochemical quality control should be sensitivity, reproducibility, capacity of separating all possible components without changing the sample composition, and preferably being convenient to achieve the maximum amount of information in the minimum period of time [2].

In the RCP analysis, a small volume of the sample solution is applied to a chromatographic plate, about 1.0-1.5 cm from the base. The plate is then put into a chamber containing a suitable solvent. The solvent moves on the plate by capillary action and the components of the sample migrate at different rates due to their affinity to the stationary phase and/or solubility in the solvent. By changing the solvent, the separation measured by the retention factor (Rf) value, can be adjusted. The Rf value is determined by the relation between the distance traveled by the product and the total distance of the solvent front. The Rf values are not physical constants once they are variable depending on the solvent used and the chromatographic plate [3].

The separation of the compounds is based on the competition of the solute and the mobile phase for binding sites on the stationary phase. If normal phase as paper or silica gel are used as stationary phase, the more polar compound has a stronger interaction with the paper or silica gel while a less polar compound moves along the plate, resulting in a higher Rf value [3].

PC and TLC have the advantages of a fast run, good separation, different characteristics of stationary phases and the combination with a proper radioactivity detection system makes them suitable for most of the radiochemical analysis of radiopharmaceutical compounds [2]. According to the European Pharmacopoeia, ITLC-SG impregnated in glass fiber plates, a special kind of TLC plate produced by Pall Corporation, is one of the most used stationary phases [4]. It offers the advantage of a rapid development and ease of cutting. During 2008, Pall Corporation stopped manufacturing ITLC-SG and the development of alternative chromatography systems were peremptory [5].

The purpose of the present study was to evaluate the RCP of ^{67}Ga-Citrate, ^{111}In-Octreotide, ^{177}Lu-DOTATATE and ^{153}Sm-HA in different chromatographic systems.

2. EXPERIMENTAL

2.1. Materials and Reagents

Samples from different batches of ^{67}Ga-Citrate (gallium-67 citrate); ^{111}In-Octreotide (pentate-indium-111); ^{177}Lu-DOTATATE [DOTA (1,4,7,10-tetraazacyclododecane-*N*-,*N*-,*N*-, *N*-tetraacetic-acid)D-Phe1-Tyr3-octreotate] and ^{153}Sm-HA (hydroxiapatite-samarium-153) were produced at IPEN-CNEN/SP. $^{111}InCl_3$ and $^{177}LuCl_3$ were from MDS Nordion (Vancouver, Canada) and $^{153}SmCl_3$ was produced in the IEA-R1 reactor at IPEN-CNEN/SP. Ammonium acetate, citric acid, EDTA, ethanol, methanol, pyridine, sodium acetate, sodium chloride and sodium citrate were analytical grade (Merck).

ITLC-SG sheets were supplied by Pall Corporation and Agilent, USA; TLC-Al plates were from Merck, Germany and PC were from Whatman 3MM/1MM (W3MM/W1MM), England.

2.2. Radiochemical Purity Control

Tab. 1 describes the mobile and stationary phases used to perform the % RCP control of ^{67}Ga-citrate, ^{111}In-Octreotide, ^{177}Lu-Dotatate and ^{153}Sm-HA.

Table 1. Mobile and stationary phases for RCP analysis of radiopharmaceuticals.

PRODUCT	MOBILE PHASE	STATIONARY PHASE
^{67}Ga-Citrate	0.16 mol L^{-1} sodium acetate pH = 4.95	W3MM (9 x 1 cm)
	Pyridine:ethanol:water (1:2:4)	W3MM (9 x 1 cm)
^{111}In-Octreotide	0.1 mol L^{-1} sodium citrate buffer pH = 5.5	ITLC-SG, TLC-Al, W3MM and W1MM (14.5 x 1.5 cm)
	0.2 mol L^{-1} EDTA pH 5.0	TLC-Al (14.5 x 1.5 cm)
^{177}Lu-DOTATATE	Methanol:0.4 mol L^{-1}ammonium acetate (1:1)	ITLC-SG, TLC-Al, W3MM and W1MM (12.5 x 1.5 cm)
	0.1 mol L^{-1} sodium citrate buffer pH = 5.5	ITLC-SG, TLC-Al, W3MM and W1MM (14.5 x 1.5 cm)
^{153}Sm-HA	0.9% (p/v) NaCl	ITLC-SG, TLC-Al, W3MM and W1MM (12.5 x 1.5 cm)

The plates were activated before use by heating at 70 ºC for 10 minutes. Samples were applied with a glass capillary in triplicate plates and immediately put into pre-saturated chambers with the mobile phase.

After migration of the mobile phase up to 1 cm from the top, the plates were dried, cut into 7, 10 or 12 pieces and each segment had the radioactivity measured in a gamma counter (Perkin Elmer Gamma Counter) during 0.20 minutes using each radioisotope energy window. The gamma counter data were expressed in counts per minute (cpm).

The impurities were calculated by expressing the percentage of the activity corresponding to the Rf of the impurity in relation to the total radioactivity on the plate and the RCP was found by subtracting the impurities percentage from 100.

3. RESULTS AND DISCUSSION

In 2008, the interruption of ITLC-SG plate manufacturing was a serious event for the quality control of radiopharmaceuticals produced in the entire world, as it was the stationary phase specified for many routine methods for determination of RCP. In 2010, Varian Inc which was acquired by Agilent started the manufacturing of ITLC-SG.

In this work, some mobile phases and stationary phases, including ITLC-SG plates from Agilent, were evaluated to determine the RCP of ^{111}In-Octreotide, ^{177}Lu-Dotatate and ^{153}Sm-HA. The RPC control described in the United States Pharmacopeia (USP) for ^{67}Ga-citrate which recommends 0.16 mol L^{-1} sodium acetate was also compared with a mixture of pyridine, ethanol and water [6].

The R_f values of the radiopharmaceuticals and their impurities in each chromatographic system were determined and are related on Tab. 2 and 3, respectively.

Table 2: Rf values of radiopharmaceuticals in different chromatographic systems

Product	Mobile phase	Stationary phase				
		ITLC-SG Pall	ITLC-SG Agilent	TLC-Al	W3MM	W1MM
^{67}Ga-Citrate	0.16 mol L^{-1} sodium acetate	NA	NA	NA	0.8 – 0.9	NA
	Pyridine : ethanol : water (1:2:4)	NA	NA	NA	0.8 – 0.9	NA
^{177}Lu-DOTATATE	Methanol : 0.4 mol L^{-1} ammonium acetate (1:1)	NA	NA	0.0	0.4 – 0.8	0.4 – 0.8
	0.1 mol L^{-1} sodium citrate buffer pH = 5.5	0.0 – 0.2	NA	0.0	0.3 – 0.7	0.3 – 0.7
^{111}In-Octreotide		0.0 – 0.3	0.0 – 0.2	0.0 – 0.2	0.3 – 0.5	0.4 – 0.7
	0.2 mol L^{-1} EDTA	NA	NA	0.0 – 0.3	NA	NA
^{153}Sm-HA	0.9% (p/v) NaCl	0.0	0.0	0.0	0.0	0.0

NA – Not Applicable

Table 3: R_f values of the impurities in different chromatographic systems.

Impurity	Mobile phase	Stationary phase				
		ITLC-SG Pall	ITLC-SG Agilent	TLC-Al	W3MM	W1MM
^{67}GaCl	0.16 mol L^{-1} sodium acetate	NA	NA	NA	0.0	NA
	Pyridine : ethanol : water (1:2:4)	NA	NA	NA	0.0	NA
^{177}LuCl$_3$	Methanol : 0.4 mol L^{-1} ammonium acetate (1:1)	NA	NA	0.0	0.0 – 0.2	0.0 – 0.2
	0.1 mol L^{-1} sodium citrate buffer pH = 5.5	0.8 – 1.0	NA	0.8 – 1.0	0.8 – 1.0	0.8 – 1.0
^{111}InCl$_3$		0.8 – 1.0	0.8 – 1.0	0.8 – 1.0	0.8 – 1.0	0.8 – 1.0
	0.2 mol L^{-1} EDTA	NA	NA	0.6 – 0.9	NA	NA
^{153}SmCl$_3$	0.9% (p/v) NaCl	1.0	1.0	1.0	1.0	1.0

The RCP and standard deviations (SD) of ^{67}Ga-citrate, ^{111}In-Octreotide, ^{177}Lu-Dotatate and ^{153}Sm-HA, expressed as percentage, are shown in Tab. 4.

Table 4: %RCP of ^{67}Ga-citrate, ^{111}In-Octreotide, ^{177}Lu-Dotatate and ^{153}Sm-HA in different chromatographic systems

Radiopharmaceutical	Mobile phase	Stationary phase				
		ITLC-SG Pall	ITLC-SG Agilent	TLC-Al	W3MM	W1MM
^{67}Ga-Citrate	0.16 mol L^{-1} sodium acetate	NA	NA	NA	98.15±0.87	NA
	Pyridine : ethanol : water (1:2:4)	NA	NA	NA	96.20±0.98	NA
^{177}Lu-DOTATATE	Methanol : 0.4 mol L^{-1} ammonium acetate (1:1)	NA	NA	NA	97.82±0.06	98.19±0.90
	0.1 mol L^{-1} sodium citrate buffer pH = 5.5	99.69±0.28	NA	99.67±0.36	95.50±2.07	95.29±3.21
^{111}In-Octreotide		99.39±0.38	97.70±0.57	99.86±0.12	97.73±0.96	93.60±3.35
	0.2 mol L^{-1} EDTA	NA	NA	99.56±0.01	NA	NA
^{153}Sm-HA	0.9% (p/v) NaCl	99.98±0.02	99.97±0.03	99.94±0.08	99.90±0.16	99.97±0.04

Using W3MM, the % RCP of ^{67}Ga-Citrate in 0.16 mol L^{-1} sodium citrate was 2% higher than in pyridine, ethanol and water mixture (Tab. 4). Sodium citrate solution efficiently separated the impurity from the product and the analysis was faster compared to the pyridine solvent.

The analysis time of ^{111}In-Octreotide was quite similar using ITLC-SG plate from Pall or Agilent and 0.1 mol L^{-1} sodium citrate solvent but the resolution using ITLC-SG plate from Agilent resulted in RCP 2% higher (Tab. 4). TLC-Al and 0.1 mol L^{-1} sodium citrate or 0.2 mol L^{-1} EDTA showed resolution similar to ITLC-SG plate from Agilent, even though the analysis time was more than 1 hour. The radioactivity profile with TLC-Al and 0.2 mol L^{-1} EDTA presented better results for ^{111}In-Octreotide (Tab. 4).

Rf values of ^{177}Lu-DOTATATE and $^{177}LuCl_3$ were the same in TLC-Al and methanol:0.4 mol L^{-1} ammonium acetate (1:1), showing that this system is not appropriate. The analysis of $^{177}LuCl_3$ with methanol:0.4 mol L^{-1} ammonium acetate (1:1) and ITLC-SG from Agilent showed a broad peak close to the origin whereas with ITLC-SG from Pall the corresponding peak was sharp. Albeit ITLC-SG from Pall and TLC-Al with 0.1 mol L^{-1} sodium citrate presented similar results, TLC-Al showed a better resolution. The best chromatographic profile was TLC-Al/0.1 mol.L^{-1} sodium citrate buffer, which are the current method at IPEN.

The poor resolution between the impurity and product peaks using W3MM and W1MM was the main factor that influenced the results of % RCP for ^{111}In-Octreotide and ^{177}Lu-DOTATATE (Tab. 4).

The radioactivity profile in the RCP analysis of ^{153}Sm-HA using ITLC-SG from Pall and Agilent, TLC-Al, W1MM or W3MM was very similar. In W1MM and 0.9% NaCl, the chromatographic separation was faster than other systems.The particulate nature provided product Rf = 0 and good resolution between the product and impurity peaks in all systems.

4. CONCLUSIONS

The results showed that different chromatographic systems can be used to determine the % RCP of radiopharmaceuticals. Despite some variations, the specifications were achieved for all the studied products, showing that there are options other than the compendial methods, since they should be validated.

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