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Review - Human and Animal Health

Current Approach in Radiochemical Quality Control of the ^{99m}Tc-Radiopharmaceuticals: a mini-review

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HIGHLIGHTS

- ANVISA recommends the performance of quality control tests of ^{99m}Tc-radiophamaceuticals.
- Requirement of hospital radiopharmacy routine adaptation to ANVISA's recommendation.
- Requirement of the radiochemical purity (RCP) methods validation.
- Dose calibrator is reliable for RCP analysis in hospital radiopharmacies.

Abstract: To present optimized chromatographic systems for radiochemical purity (RCP) evaluation of ^{99m}Tc-eluate and ^{99m}Tc-radiopharmaceuticals, as well as to assess doses calibrator reliability for routine purposes in hospital radiopharmacies. RCP was determined by different systems and radioactivity was quantified by TLC-scanner, doses calibrator and gamma-counter. Suitable and optimized systems were presented for RCP analyses. No significant differences were observed between radioactivity counting devices and, thus, doses calibrator showed reliability for RCP determination in hospital radiopharmacies.

Keywords: ^{99m}Tc-radiopharmaceuticals; Radiochemical purity; Ascendant chromatography; Chromatographic system; Dose calibrator.

INTRODUCTION

Radiopharmacy is a pharmaceutical specialty concerning to development, production, manipulation, quality control, and dispensing of radiopharmaceuticals, which are specific molecules for imaging diagnosis and/or treatment in nuclear medicine [1]. In Brazil, radiopharmaceutical practices must be under the Brazilian Health Surveillance Agency (ANVISA) and the National Commission of Nuclear Energy (CNEN) guidelines.

Currently, most of the radiopharmaceuticals used in nuclear medicine for diagnosis purposes are freeze-dried kits for *in situ* radiolabelling with sodium pertechnetate ($Na^{99m}TcO_4^-$). Freeze-dried formulations aim to increase product stability, allowing a reliable process and easy radiopharmaceutical reconstitution [2].

Radiopharmaceuticals present peculiarities related to their production and quality control, due to the presence of radionuclide in its composition and to the administration via, which is mostly intravenous [1]. Therefore, they must be under the Good Manufacturing Production (GMP) rules, as established in the Collegiate Board of Directors Resolution (RDC) no. 63/2009 by ANVISA, along with specific quality control tests that must be performed [3].

In this context, ANVISA has published the RDC no. 38/2008 recommending as mandatory the performance of quality control tests of eluate from molybdenum-99-technetium-99 metastable (^{99}Mo-^{99m}Tc) generator, and of ^{99m}Tc-radiophamaceuticals, before administration into patients, according to manufacturer`s recommendation, pharmacopoeias, guidelines and scientific papers [4]. Prior to the RDC no. 38/2008, hospital radiopharmacies did not perform quality control assays of their products, which was an exclusive responsibility of the industrial radiopharmacy. After ANVISA`s regulations were published, hospital radiopharmacies had to adapt their routine to these new procedures, specially to the determination of ^{99m}Tc-radiophamaceuticals` radiochemical purity (RCP).

RCP is defined as the total radioactivity percentage present in the desired chemical form in a radiopharmaceutical, that is, bound ^{99m}Tc to the ligand of interest. Free ^{99m}Tc ($^{99m}TcO_4^-$) and hydrolyzed-reduced ^{99m}Tc ($^{99m}TcO_2$) are classified as radiochemical impurities [5]. In industrial radiopharmacy, RCP determination is performed in devices, such as gamma-counter and thin layer chromatography (TLC)-scanner, due to their high counting sensitivity [6]. On the other hand, in hospital radiopharmacy, doses calibrator is commonly used to measure chromatographic stripes, once it is usually available, despite of its lower sensitivity when compared to the formers.

Based in this scenario, specially concerning to RDC no. 38/2008 implementation in the Brazilian legislation and, therefore, the requirement of hospital radiopharmacy routine adaptation, this short note aims to present the most cost-benefit chromatographic systems for the RCP evaluation of ^{99m}Tc-eluate and ^{99m}Tc-radiopharmaceuticals, as well as to assess doses calibrator reliability for nuclear medicine routine.

MATERIAL AND METHODS

To achieve those goals, we have revised chromatographic systems previously described in the literature. These methods were reproduced in our research laboratory in order to compare technical parameters, like reproducibility, effectiveness, cost, speed and easiness of execution. We have made slight modifications in some systems, compared to the respective references available, like changes in both stationary/mobile phases and chromatographic strip`s cutoff, intending routine improvement. It is important to highlight that each radiopharmaceutical, in this work, was radiolabeled with $^{99m}TcO_4^-$ in four different batches and RCP analyses were performed by ascendant chromatography in triplicate. Strips' radioactivities were quantified in the following sequence: TLC-scanner (AR-2000, Eckert & Ziegler®, Germany), dose calibrator (CRC-25R, Capintec®, USA), and automatic gamma-counter (Wizard 3' 2480, Perkin Elmer®, USA).

RESULTS AND DISCUSSION

Ascendant chromatographic parameters, chromatographic systems, cutoff and R_f, for RCP analyses of ^{99m}Tc-eluate and the evaluated ^{99m}Tc-radiopharmaceuticals are described in **Table 1**.

Table 1. Chromatographic systems for RCP analysis of ^{99m}Tc-eluate and ^{99m}Tc-radiopharmaceuticals.

Radiopharmaceutical (Rpharma)	Chromatographic system		Cutoff	Retention factor (R_f)			Ref.
	Stationary phase*	Mobile phase		Rpharma	TcO_4^-	TcO_2	
Sodium Pertechnetate $Na^{99m}TcO_4$	W3MM paper	85% Methanol	2 cm from the origin	---	0.7-0.8	0.0	[7]
^{99m}Tc-Sestamibi (^{99m}Tc-MIBI-TEC)	W3MM paper	Acetone	Half	0.9-1.0	0.9-1.0	0.0-0.1	a
		0.9% Sodium Chloride	Half	0.0-0.1	0.9-1.0	0.0-0.1	
^{99m}Tc-Disofenin (^{99m}Tc-DISI-TEC)	W3MM paper	Acetone	2 cm from the origin	0.9-1.0	0.9-1.0	0.0-0.1	a
	TLC-SG (Al) plate	20% Sodium Chloride	2 cm from the front	0.0-0.1	0.9-1.0	0.0-0.1	
^{99m}Tc-Medronate (^{99m}Tc-MDP-TEC)	W3MM paper	Acetone	Half	0.0-0.1	0.9-1.0	0.0-0.1	b
		0.9% Sodium Chloride	Half	0.9-1.0	0.9-1.0	0.0-0.1	
^{99m}Tc-Macrosalb (^{99m}Tc-PUL-TEC)	W3MM paper	85% Methanol	2 cm from the front	0.0-0.1	0.9-1.0	---	b

Cont.

^{99m}Tc-Phytate (^{99m}Tc-FITA-TEC)	W3MM paper	85% Methanol	Half	0.0-0.1	0.9-1.0	---	b
^{99m}Tc-Pentetate (^{99m}Tc-DTPA-TEC)	W3MM paper	Acetone	Half	0.0-0.1	0.9-1.0	0.0-0.1	b
		0.9% Sodium Chloride	Half	0.9-1.0	0.9-1.0	0.0-0.1	
^{99m}Tc-Succimer (^{99m}Tc-DMSA-TEC)	W3MM paper / TLC-SG (Al) plate	Acetone	Half	0.0-0.1	0.9-1.0	0.0-0.1	b
		0.9% Sodium Chloride	Half	0.9-1.0	0.9-1.0	0.0-0.1	
^{99m}Tc-Bicisate (^{99m}Tc-ECD-TEC)	TLC-SG (Al) plate	Ethyl acetate	Half	0.9-1.0	0.0-0.1	0.0-0.1	[7,8]
^{99m}Tc-Dextran (^{99m}Tc-DEX (70 / 500)-TEC)	W3MM paper	Acetone	Half	0.0-0.1	0.9-1.0	---	b
^{99m}Tc-Pyrophosphate (^{99m}Tc-PIRO-TEC)	W3MM paper	Acetone	Half	0.0-0.1	0.9-1.0	0.0-0.1	b
		0.9% Sodium Chloride	Half	0.9-1.0	0.9-1.0	0.0-0.1	
^{99m}Tc-Colloidal TIN (^{99m}Tc-TIN-TEC)	W3MM paper	Acetone	Half	0.0-0.1	0.9-1.0	---	b

*Chromatographic strips with 8 cm, between application point and solvent front, for mobile phase development.

W3MM: Whatman 3MM; TLC-SG (Al): Thin Layer Chromatography – Silica gel (Aluminum).

[7]European Pharmacopoeia 7.0; [8]United States Pharmacopoeia 33.

[a]Authors` suggestion; [b]Manufacturer`s recommendation (IPEN-CNEN/SP).

Radiopharmaceuticals presented in **Table 1** refer to the most commonly used in nuclear medicine services for radiolabeling with ^{99m}Tc, and also ^{99m}Tc-eluate. The purposed chromatographic systems were selected taking into account some technical aspects.

We have selected the chromatographic systems considering stationary and mobile phases, besides time of chromatographic development. Concerning to stationary phases, the majority of the chosen systems employ 3MM chromatography paper, once it is the cheapest chromatographic strip compared to the others available for ascendant chromatography. When 3MM chromatography paper was not suitable, thin layer chromatography-silica gel aluminum plates - TLC-SG (Al) - were selected. In terms of mobile phases, we have taken into account good availability, purchase control by authorities, waste disposal with lower cost, easy preparation, low risk and toxicity to the professional. It was also prioritized shorter chromatographic developments, in order to speed up radiopharmaceutical dispensing for patients.

Furthermore, strip`s cutoffs were validated by means of TLC-scanner, which is gold standard for chromatographic strips counting [6]. When possible, cutoff in the half of the stationary phase was prioritized for standardize counting in doses calibrator.

In the specific case of ^{99m}Tc-Bicisate (^{99m}Tc-ECD-TEC), a single chromatographic system was selected considering practical aspects, previously described. Beyond that, for

^{99m}Tc-Sestamibi (^{99m}Tc-MIBI-TEC) and ^{99m}Tc-Disofenin (^{99m}Tc-DISI-TEC), we have proposed and validated new methods, also considering an optimized and operative routine.

Some radiopharmaceuticals, like ^{99m}Tc-Colloidal TIN (^{99m}Tc-TIN-TEC), ^{99m}Tc-Macrosalb (^{99m}Tc-PUL-TEC), ^{99m}Tc-Phytate (^{99m}Tc-FITA-TEC) and ^{99m}Tc-Dextran (^{99m}Tc-DEX (70 / 500)-TEC), present colloidal features in their composition and, thus, may not be separated from $^{99m}TcO_2$. Therefore, a single system was selected, in order to determine only the amount of $^{99m}TcO_4^-$.

TLC-scanner and automatic gamma-counter are both more sensitive devices than dose calibrator [6]. However, the latter is most commonly used in the routine activities of hospital radiopharmacy and, then, it is usually available to measure chromatographic strips for RCP analysis [1]. Thus, in order to confirm the effectiveness of dose calibrator as a reliable counting instrument for RCP assays, the radiopharmaceuticals ^{99m}Tc-eluate, ^{99m}Tc-Pentetate and ^{99m}Tc-Macrosalb were evaluated in the previously mentioned radioactivity counting devices. Our results are summarized in **Table 2** and compared to TLC-scanner data (**Figure 1**).

Table 2. Comparative RCP data obtained by different radioactivity counting devices.

Radiopharmaceutical	**Radiochemical Purity (RCP)**		
	Dose calibrator	**TLC-scanner**	**Gamma-counter**
Sodium Pertechnetate ($Na^{99m}TcO_4$)	99.85 ± 0.14	99.22 ± 0.46	98.93 ± 0.71
^{99m}Tc-Pentetate (^{99m}Tc-DTPA-TEC)	96.36 ± 2.70	96.78 ± 0.51	98.89 ± 0.31
^{99m}Tc-Macrosalb (^{99m}Tc-PUL-TEC)	98.63 ± 1.78	99.30 ± 0.69	99.19 ± 0.73

Values are expressed as “mean ± standard deviation” (n = 4). No significant differences were observed between counting methods (p-values > 0.05). Data were compared by Analysis of Variance (ANOVA) followed by Tukey multiple comparisons test (Prism software, version 5.0).

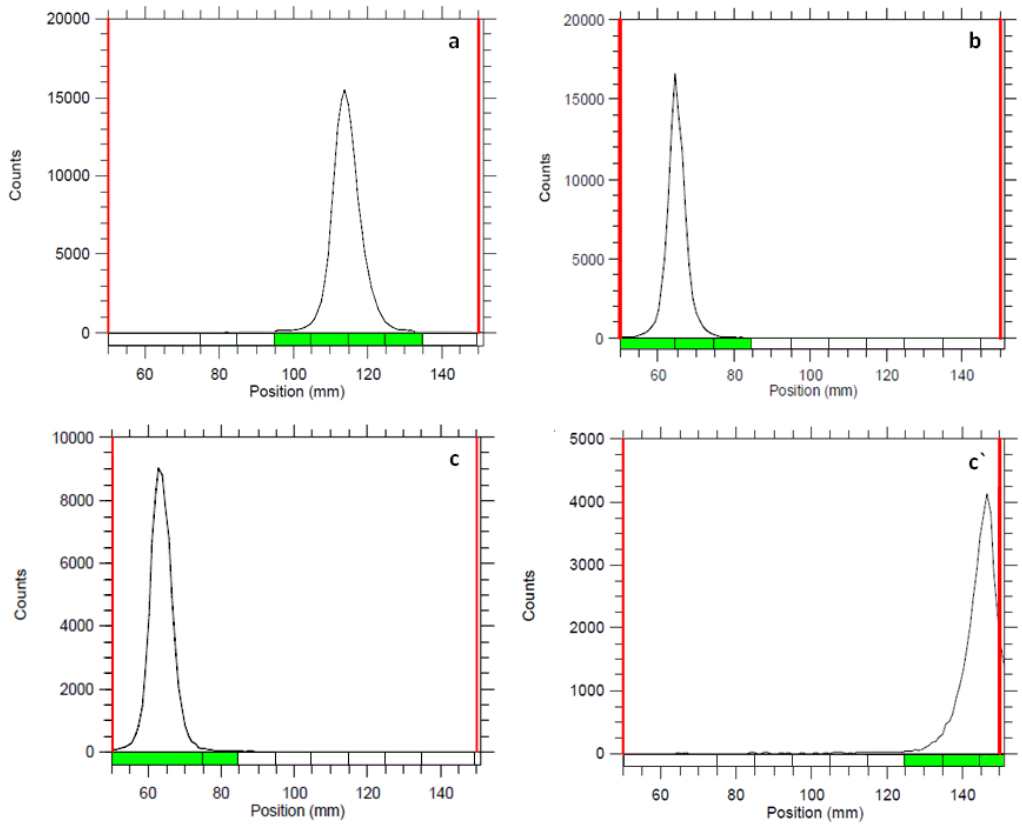

Figure 1. Radiochromatographic profiles obtained by TLC-scanner: (**a**) $Na^{99m}TcO_4$ (W3MM / 85% MeOH); (**b**) ^{99m}Tc-MAA (W3MM / 85% MeOH); (**c**) ^{99m}Tc-DTPA (W3MM / Acetone); (**c'**) ^{99m}Tc-DTPA (W3MM / 0.9% NaCl).

The RCP data were similar for all counting devices, with no statistical significant differences between means (*p*-values > 0.05). Therefore, our results indicate that dose calibrator can be used in routine activities for RCP evaluation, although it is less sensitive than TLC-scanner and automatic gamma-counter. Furthermore, it is important to point out that dose calibrator is already available for other purposes in a hospital radiopharmacy, and also it is cheaper and easier to use when compared to the others.

CONCLUSION

In summary, considering RDC no. 38/2008 and, thus, the requirement of hospital radiopharmacy routine adequacy, this mini-review presented optimized chromatographic systems for RCP evaluation of ^{99m}Tc-eluate and the most commonly used ^{99m}Tc-radiopharmaceuticals. Additionally, we have confirmed that dose calibrator is reliable for routine purposes and can be used for RCP analysis daily, providing adequacy of hospital radiopharmacy activities to the ANVISA`s regulation. Finally, it is important to emphasize the requirement of the RCP methods validation, as well as of any slight modifications in the chromatographic systems, such as stationary/mobile phase exchange or chromatographic strips size.

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Conflicts of Interest: The authors declare no conflict of interest.

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