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Original Article

Optimizing the Assessment of [99mTc]-SestaMIBI Radiochemical Purity: A Study on Stability in Syringe Storage

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ABSTRACT

Background: [99mTc]-SestaMIBI ([99mTc]Tc-MIBI), approved by the FDA in 1990, has become widely used for cardiac perfusion imaging. It is crucial to ensure this radiopharmaceutical's radiochemical purity (RCP), as it directly impacts the accuracy of patient diagnoses. The FDA has established standards mandating the verification of RCP before administering the tracer to patients. Maintaining consistent and reliable assessments within nuclear medicine departments remains a persistent challenge despite its importance.

Methods: In this research, we initially focused on evaluating various methods for determining RCP using thin layer chromatography (TLC) with different stationary and mobile phases, as well as column cartridges (Sep-Pak_C_{18}). We optimized the method and compared its efficacy against the standard technique. Subsequently, we assessed the stability of the radiopharmaceutical under 2 distinct storage conditions: syringes and vials.

Results: Our investigation of aluminum oxide (Al_2O_3), silica gel (SG), and Whatman paper TLCs yielded the most favorable results when ethanol, methyl ethyl ketone, and ethyl acetate were employed as solvents, respectively. For the Sep-Pak_C_{18} method, saline and ethanol solvents proved the most effective. Notably, RCP exceeded 90% up to 6 hours following radiopharmaceutical preparation, which aligns with pharmacopeial standards. These findings were consistent for both syringe and vial storage conditions.

Conclusions: In this research, we successfully developed and validated 3 unique methods for determining the RCP of [99mTc]Tc-MIBI. Among these, the Whatman FN1 method utilizing Ethyl Acetate as the solvent demonstrated the most promising results, offering a combination of speed, robustness, reproducibility, and cost-efficiency that renders it suitable for routine use in clinical settings. Furthermore, our findings indicate that provided no other quality control concerns are present, the radiopharmaceutical kit remains stable in terms of RCP, making it a viable option for patient injections. ***(Iranian Heart Journal 2025; 26(1): 67-80)***

KEYWORDS: Radiochemical purity, Quality control, [99mTc]Tc-SestaMIBI, Thin layer chromatography, Sep-Pak_C_{18}

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The preference for [99mTc]Tc-labeled compounds in nuclear medicine can be attributed to the favorable physical and radiation properties of [99mTc]Tc, as well as its accessibility in a sterile, pyrogen-free, and carrier-free form derived from [99Mo]Mo-[99mTc]Tc Generators. These advantages have led to the widespread use of [99mTc]Tc-labeled compounds, which account for nearly 80% of radiopharmaceuticals employed in nuclear medicine. As radiopharmaceuticals are designed for human use, they must undergo stringent quality control measures to ensure patient safety and efficacy. A crucial component of this process is assessing the radiochemical purity (RCP) of a radiopharmaceutical, which is defined as the ratio of radioactivity present in the desired chemical form to the total radioactivity within the compound. This parameter is vital in determining the suitability of a radiopharmaceutical for its intended purpose. [1] Radiochemical impurities can arise during various stages, including the production process and storage of radiopharmaceuticals. These impurities display distinct physiological characteristics compared to the intended chemical compound, which can complicate measurement interpretation and render the radiopharmaceutical ineffective. [2]

Thin layer chromatography (TLC) has become a widely utilized technique in the field of nuclear medicine for evaluating RCP. [3, 4] Radio-TLC, although featuring a relatively simple experimental setup, requires thorough validation to ensure its effectiveness. Despite this, radio-TLC offers a significant benefit over radio-HPLC, as it allows for the comprehensive detection of all administered radioactivity without the need for complex recovery procedures. [5] The core principle of this analytical method is based on the capillary-driven movement of a mobile phase (solvent) through a layer of adsorbent (stationary phase). For radiopharmaceutical analysis, techniques must be efficient and safe. TLC offers dependable separation characteristics, coupled with simple and rapid execution. The primary impurities present in 99mTc pharmaceutical formulations comprise free pertechnetate ([99mTc]TcO_4^-) and reduced, hydrolyzed technetium (HYD). These two 99mTc forms can be separated from pharmaceuticals labeled with 99mTc via a single TLC procedure. The selection of different mobile and stationary phases can influence the migration characteristics of free pertechnetate, whereas colloids usually remain static in most TLC systems as insoluble material at the origin.

[99mTc]-SestaMIBI ([99mTc]Tc-MIBI) is a lipophilic cationic complex that has been widely employed as a myocardial perfusion imaging agent, specifically for detecting myocardial ischemia and infarcts. [6,7] MIBI stands for methoxy isobutyl isonitrile. It is crucial to recognize that the clinical application of this radiopharmaceutical is contingent upon its adherence to high-quality standards and uniformity. As per the [99mTc]Tc-MIBI monograph, an RCP value of at least 90% is mandatory for its use in clinical settings. [8] The United States Pharmacopeia (USP) 2021 recommended the RCP test method, involving the use of a reverse-phase chromatographic plate and a solvent system comprising acetonitrile, methanol, 3.85% ammonium acetate, and tetrahydrofuran in a 4:3:2:1 ratio, can be challenging for busy nuclear medicine centers to implement9. Alternative approaches proposed in previous studies involve the application of 2 TLC systems, which can be time-consuming and potentially impractical for high-volume clinical settings. [8, 10, 11]

This study's primary objective was to investigate various stationary phases in different solvents to identify a rapid, cost-effective, and reliable method for determining the RCP of the [99mTc]Tc-MIBI kit, which can be easily implemented in nuclear medicine centers. Furthermore, the research examined

the RCP of [^{99m}Tc]Tc-MIBI stored in both syringes and vials, with the ultimate goal of recommending optimal storage conditions and usage practices to ensure high RCP radiopharmaceuticals for patient care in clinical settings.

METHODS

Materials

In this study, the following materials and resources were utilized:

1. Sodium pertechnetate-99m ([^{99m}Tc]$NaTcO_4$) obtained from ^{99}Mo/^{99m}Tc generators provided by PARS ISOTOPE Co. in Tehran, Iran
2. Pars-MIBI cold kits sourced from PARS ISOTOPE Co
3. PTW-CURIMENTOR 4 medical device for radio-TLC analysis
4. TLC plates: Whatman paper (Merck), TLC silica gel (SG) 60 F254 (Sigma-Aldrich), TLC aluminum oxide (Al_2O_3) 60 F254, neutral (Merck)
5. Sep-Pak_C_{18} cartridges (Waters) for additional TLC analysis
6. Chemicals: ethanol, ethyl cetate, methanol, methyl ethyl ketone, and others, all purchased from Sigma-Aldrich manufacturer.

According to the manufacturer's guidelines, the radiopharmaceutical should be prepared as follows:

1. Inside a lead shield, add up to 3 milliliters of sterile and pyrogen-free sodium pertechnetate solution, eluted from a ^{99}Mo/^{99m}Tc generator to the [^{99m}Tc]Tc-MIBI vial.
2. Heat the mixture at 100 °C for 10 minutes to ensure proper radiolabeling.

As per the manufacturer's guidelines, the prepared radiopharmaceutical can be stored at a temperature range of 15 °C to 25 °C, and its stability is maintained for up to 6 hours. It is essential to follow the quality control procedures mentioned in the manufacturer's brochure before administering the radiopharmaceutical.

The process of preparing and analyzing the TLC strips involved the following steps:

1. Cutting different types of stationary phases into strips ranging from 1.5 cm to 10 cm in length
2. Filling the TLC chambers with 1–2 mL of the desired solvent
3. Using 2 mL syringes (25 gauge) to apply the sample onto the baseline of the TLC plate
4. Running the TLC and allowing the solvent to migrate approximately 90% of the way up the plate
5. Cutting the TLC strips into 1 cm sections once the process is complete
6. Measuring the radioactivity of each section using a dose calibrator.

The column was initially prepared by rinsing it with 10 mL of normal saline solution. The [^{99m}Tc]Tc-MIBI radiopharmaceutical sample was then injected into the column using a 2 mL syringe. A subsequent washing step was performed with an appropriate solvent (wash A) to eliminate technetium pertechnetate impurities. Following the initial washing step, a different solvent (wash B) was used to extract the [^{99m}Tc]Tc-MIBI from the column. After the extraction, the radioactivity of both the cartridge and the eluate from the column was measured for washes A and B using a dose calibrator.

The initial phase of the experiment involved assessing the accuracy of the RCP determination methods for [^{99m}Tc]Tc-MIBI radiopharmaceuticals, as outlined by the manufacturer. This was achieved by implementing quality control tests to evaluate the RCP of the [^{99m}Tc]Tc-MIBI kit. Two samples of the stationary phase were spot-labeled for each selected solvent. To

facilitate the identification of technetium pertechnetate impurity movement on the stationary phase, 2 distinct samples were labeled. One sample consisted of the [^{99m}Tc]Tc-MIBI radiopharmaceutical, while the other contained both [^{99m}Tc]Tc-MIBI radiopharmaceutical and technetium pertechnetate impurity as an internal standard.

An experimental procedure was conducted as described earlier to select a suitable solvent for determining the RCP of [^{99m}Tc]Tc-MIBI radiopharmaceuticals through chromatography. This process involved the utilization of paper, SG, and Al_2O3 stationary phases,

During this stage, the setup for the RCP determination test method for [^{99m}Tc]Tc-MIBI was established using a Sep-Pak_C_{18} cartridge column. The procedure outlined earlier was closely followed, ensuring adherence to the recommended guidelines and best practices.

Building upon the previous steps, suitable solvents were identified for paper, SG, and Al_2O_3 stationary phases, and an appropriate method for utilizing the cartridge column was chosen. The combination of Al_2O_3 stationary phase with ethanol solvent was designated as the standard method. Consequently, the results obtained from this standard method served as the basis for validating the other methods established in the previous step.

To conduct the validation process, 20 samples of prepared radiopharmaceuticals underwent testing using the standard method. Each method was repeated 3 to 6 times for each sample to ensure the statistical significance of the results.

The next stage of the study focused on comparing the stability of [^{99m}Tc]Tc-MIBI in 2 storage conditions: vials and syringes. Twenty [^{99m}Tc]Tc-MIBI kits were evaluated for stability up to 6 hours post-preparation using both the standard and optimized methods established earlier for determining RCP.

To conduct this assessment, a radiolabeled sample of [^{99m}Tc]Tc-MIBI was stored in either a vial or a syringe for 2 hours. Subsequently, quality control tests were performed using the specified methods.

Statistical Analysis

The statistical analysis was conducted using IBM SPSS Statistics 27 software. Two specific statistical tests, namely the paired-samples *t*-test and repeated measures, were employed to identify any significant differences between the various data groups.

RESULTS AND DISCUSSION

The Optimal Protocol for Assessing RCP

During this stage, the RCP measurements of [^{99m}Tc]Tc-MIBI were assessed using the stationary phases and solvents specified by the radiopharmaceutical manufacturer. It was observed that the combination of the Al_2O_3 stationary phase with ethanol provided the most effective separation of the radiopharmaceutical from radiochemical impurities. These findings align with those of other studies, where this method has also been acknowledged as the standard approach for RCP determination in [^{99m}Tc]Tc-MIBI kits. [3,12,13] Despite the effectiveness of the Al_2O_3 stationary phase and ethanol combination for RCP determination, the lengthy testing time of 40 minutes for a 10 cm TLC strip renders it impractical for routine use in nuclear medicine centers. [13] On the other hand, the SG stationary phase with a saline solvent and the Whatman paper stationary phase with methanol were unable to achieve adequate separation of [^{99m}Tc]Tc-MIBI from radiochemical impurities such as sodium pertechnetate and [^{99m}Tc]Tc-HYD. [14] Our experimental findings revealed that TLC Whatman in methanol was unable to separate

[99mTc]Tc-MIBI from free pertechnetate in the sample, which contradicts the claims made by Faria et al [15] in 2015. According to their study, when Whatman paper and methanol are used as the stationary phase and mobile phase, respectively, [99mTc]TcO_4^- remains at the origin, while [99mTc]Tc-MIBI migrates to the solvent front. In contrast to the findings on TLC Whatman with methanol, our study demonstrated that when using TLC-SG with a saline solvent, [99mTc]Tc-MIBI was retained at the baseline. This method failed to effectively separate the radiopharmaceutical from [99mTc]Tc-HYD (Fig. 1). Hassanpour et al [10] proposed 2 TLC methods for assessing the RCP of [99mTc]Tc-MIBI, utilizing Whatman and SG as stationary phases in conjunction with methanol and saline solvents, respectively. Betul Tasdelen [8] introduced another approach for determining the RCP of [99mTc]Tc-MIBI using ITLC-SG in a dual solvent system consisting of acetone and saline. This method aimed to provide enhanced resolution between [99mTc]Tc-MIBI and its radiochemical impurities, ensuring accurate RCP determination.

Cooper et al [11] advocated for a 2-strip method as an alternative to the manufacturer's recommended one-strip approach.

The United States Pharmacopeia (USP) 2021 provides guidelines for the RCP test of [99mTc]Tc-MIBI in the section titled "Technetium Tc 99m SestaMIBI Injection." The recommended approach involves using a reverse-phase chromatographic plate and a solvent system comprising a fresh mixture of acetonitrile, methanol, 3.85% ammonium acetate, and tetrahydrofuran in a 4:3:2:1 ratio. Although this method is intended to ensure accurate RCP determination for [99mTc]Tc-MIBI kits, its complexity can pose challenges for nuclear medicine centers with high patient volumes and limited resources, making it difficult to implement in such settings. [9] Considering the limitations associated with existing methods for determining RCP in [99mTc]Tc-MIBI kits, there is a clear demand for an alternative approach that addresses these shortcomings. Ideally, this new method should be rapid, valid, cost-effective, and easy to manage within the constraints of a busy nuclear medicine center.

To determine the most effective RCP test method for [99mTc]Tc-MIBI kits, various TLC stationary phases were evaluated, including TLC-Al_2O_3, TLC-Whatman FN1, TLC-SG, and Sep-Pak_C_{18}, in conjunction with different solvents. To accurately identify the position of Na [99mTc]TcO_4 on the TLC strips, technetium pertechnetate impurity from the $^{99}Mo/^{99m}Tc$ generator eluate was added as an additional spot on the TLCs. This systematic evaluation of RCP test methods aimed to establish the most reliable and efficient approach for assessing RCP in [99mTc]Tc-MIBI kits. It is interesting to note that Daci et al [16] reported contradictory findings to our study, as they did not achieve satisfactory separation using TLC-SG in a methyl ethyl ketone solvent. This highlights the variability in experimental outcomes and the importance of conducting thorough investigations when determining the most effective methods for RCP assessments.

Despite these discrepancies, the current study found that ethyl acetate and methyl ethyl ketone were the most efficient solvents for TLC-Whatman and TLC-SG, respectively (Fig. 2).

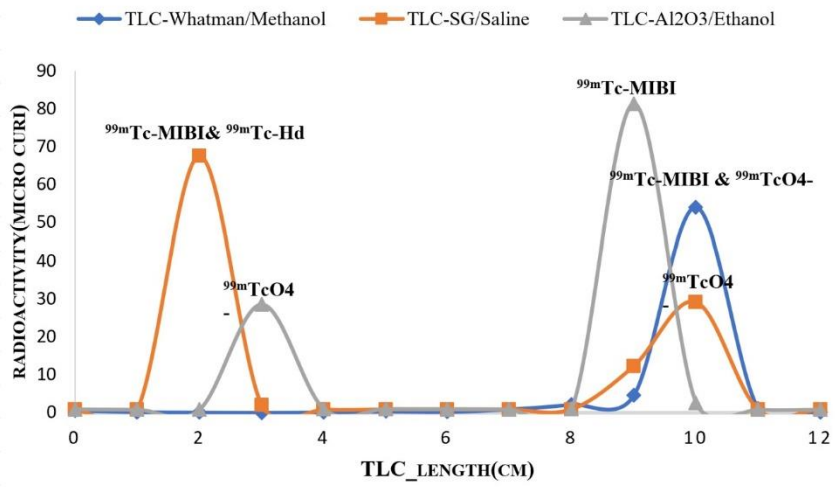

Figure 1: The image showcases the evaluation of the RCP for [^{99m}Tc]Tc-MIBI and the internal standard [^{99m}Tc]TcO_4^- using TLC-Whatman, TLC-SG, and TLC-Al_2O_3 in different solvents.
[^{99m}Tc]Tc-MIBI:[^{99m}Tc]-SestaMIBI, RCP: radiochemical purity, TLC: thin layer chromatography, SG: silica gel, Al_2O_3: Aluminium oxide

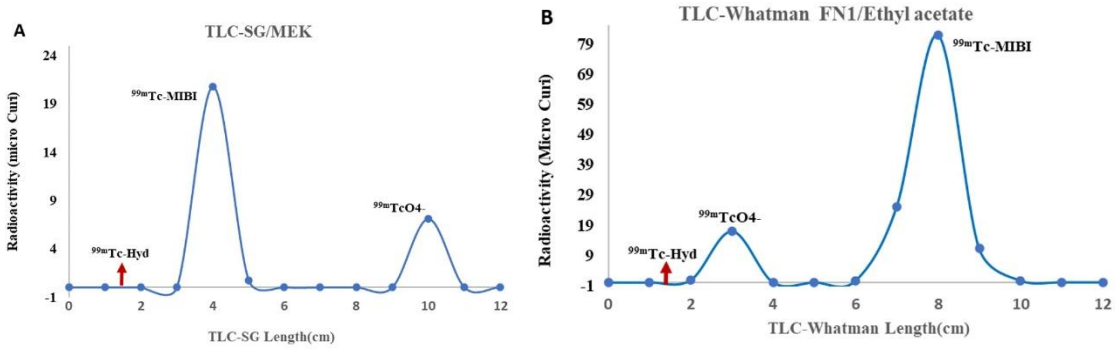

Figure 2: The images illustrate (A) TLC-SG in methyl ethyl ketone and (B) the TLC-Whatman FN1 paper in ethyl acetate for the [^{99m}Tc]Tc-MIBI sample with Na[^{99m}Tc]TcO_4 as the internal standard.
[^{99m}Tc]Tc-MIBI:[^{99m}Tc]-SestaMIBI, RCP: radiochemical purity, TLC: thin layer chromatography, SG: silica gel

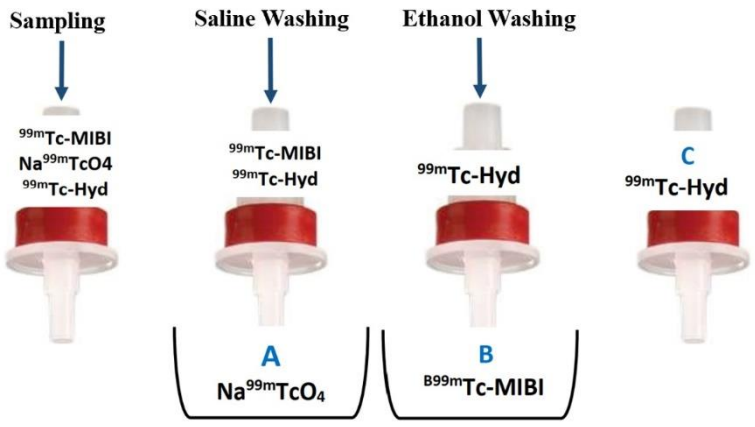

Figure 3: The image shows the steps for performing the RCP testing of [^{99m}Tc]Tc-MIBI using the Sep-Pak_C_{18} column.
RCP: radiochemical purity, [^{99m}Tc]Tc-MIBI:[^{99m}Tc]-SestaMIBI

As depicted in the figure, both chromatographic systems effectively achieved the separation of radiochemical impurity and radiopharmaceutical. The successful separation can be attributed to the equilibrium established between the stationary and mobile phases, which is a fundamental principle in all forms of chromatography.

In the case of TLC-SG, the highly polar stationary phase, when combined with the aprotic and polar methyl ethyl ketone solvent, facilitated the effective separation of radiochemical impurities ($[^{99m}Tc]TcO_4^-$ and [99mTc]Tc-HYD) from [99mTc]Tc-MIBI (Fig. 2 A).

Paper chromatography utilizes the water trapped within the cellulose filter paper as the stationary phase, and the separation of compounds in the sample relies on the partitioning of these compounds between the stationary and mobile phases. In this context, the paper chromatography system, when employed with the dipolar aprotic ethyl acetate solvent, demonstrated its capability to effectively separate impurities from [99mTc]Tc-MIBI (Fig. 2B). The $[^{99m}Tc]TcO_4^-$ species carries a negative charge, which contributes to its high polarity. This high polarity is further influenced by the presence of negatively charged oxygen atoms within the compound. As a result, $[^{99m}Tc]TcO_4^-$ exhibits strong hydrophilic properties. [6] Due to its high solubility in polar solvents, such as water, $[^{99m}Tc]TcO_4^-$ remains at the origin of the TLC in the Whatman FN1 chromatography system. This is because the water present in the cellulose filter paper serves as the stationary phase, and the strong hydrophilic properties of $[^{99m}Tc]TcO_4^-$ favor its interaction with this polar environment.

MIBI's cationic and lipophilic nature makes it less soluble in polar solvents, such as water. Consequently, during chromatography, MIBI preferentially partitions into the less polar mobile phase, allowing it to move toward the end of the chromatography paper (Fig. 2B). This behavior enables the separation of MIBI from radiochemical impurities with distinct polarity and solubility characteristics.

To determine the RCP of MIBI using either chromatographic system, the chromatography papers can be cut into sections corresponding to the separated components. The radioactivity of each section can then be measured, indicating the relative abundance of [99mTc]Tc-MIBI and any impurities present. By comparing the radioactivity of the [99mTc]Tc-MIBI section to the total radioactivity of all sections, the RCP of the [99mTc]Tc-MIBI sample can be calculated.

TLC relies on a complex interplay of physicochemical factors that govern the separation and retention of analyte molecules. The size, shape, and charge of the molecules, along with the polarity of the solvent and stationary phase, significantly impact the chromatographic process. Moreover, the interactions between these components further influence the outcome of TLC analysis.

Achieving optimal separation and retention in TLC necessitates the careful optimization of experimental conditions to balance these factors. Various parameters, such as temperature, humidity, and mobile phase composition, can be adjusted to fine-tune the chromatographic system.

Sep-Pak_C_{18} protocol

The following protocol outlines the use of the Sep-Pak_C_{18} cartridge as a stationary phase for determining the RCP of [99mTc]Tc-MIBI:

1. **Step 0: Moisturizing Sep-Pak_C_{18}** - Add 10 mL of saline to the cartridge to moisten the sorbent, ensuring proper sample flow.
2. **Step 1: Sampling** - Load 50 to 100 µCi of prepared [99mTc]Tc-MIBI onto the column.
3. **Step 2: Washing with saline** - Wash the cartridge with 10 mL saline followed by a 10 mL air flush to remove $[^{99m}Tc]TcO_4^-$ impurities.

4. **Step 3: Washing with ethanol** - Elute [^{99m}Tc]Tc-MIBI from the column using 15 mL of ethanol, followed by a 10 mL of air flush. The HYD impurity will remain in the column (Fig. 3).
5. **Step 4: Radioactivity measurement** - Measure the radioactivity of the saline eluate (A), ethanol eluate (B), and the cartridge in a dose calibrator.
6. **Step 5: RCP calculation** - Determine the RCP using the formula RCP = B / (A + B + C) × 100.

The Sep-Pak_C_{18} protocol proved effective in accurately separating radiochemical impurities. Sep-Pak cartridges with C_{18} hydrophobic reversed phase are designed to retain nonpolar compounds and particles. [17,18] [^{99m}Tc]Tc-MIBI, being a nonpolar compound, was retained within the Sep-Pak_C_{18} column due to the hydrophobic interactions between [^{99m}Tc]Tc-MIBI and the stationary phase. On the other hand, the polar impurity [^{99m}Tc]TcO_4^- was successfully washed out of the column using saline in step 2, as it exhibited minimal interactions with the hydrophobic reversed phase.

To validate the accuracy of this separation method, a known quantity of [^{99m}Tc]TcO_4^- was intentionally added as the primary impurity in the column. The separation process was then monitored, and the results demonstrated that all [^{99m}Tc]TcO_4^- was effectively removed during step 2, while the entire amount of [^{99m}Tc]Tc-MIBI remained in the column.

Cosnir et al [19] reported similar findings regarding the effective separation of ^{99m}TcO4- using Sep-Pak_C_{18} in determining the RCP of ^{99m}Tc-labelled human serum albumin nanocolloid. Their results further support the use of Sep-Pak_C_{18} as a reliable method for radiochemical impurity separation in nuclear medicine applications.

Given the successful demonstration of the Sep-Pak_C_{18} protocol for accurate and efficient separation, it was selected for further evaluation in the study.

3.2. Validation of New Protocols for RCP Assessment Using Al_2O_3 and Ethanol

To evaluate the performance of 3 TLC methods and cartridge chromatography for determining RCP in [^{99m}Tc]Tc-MIBI kits, results from analyzing 20 [^{99m}Tc]Tc-MIBI kits were presented in graphs. The RCP values obtained using each new method were compared with those obtained using the standard TLC-Al_2O_3/Ethanol method. For accurate comparison, the standard TLC-Al_2O_3/Ethanol method was repeated at least 3 times for each sample. The graphical analysis revealed that the RCP values obtained using Whatman FN1 with Ethyl Acetate solvent were consistently lower compared to those obtained with the standard TLC-Al_2O_3/Ethanol method. Statistical analysis further highlighted significant differences in the average RCP in most cases (12 out of 20 kits).

Despite these differences, it is essential to consider their clinical relevance. As the observed variations in RCP were not substantial and remained within the acceptable range for all kits, these differences may not significantly impact clinical applications. Therefore, while statistically significant differences were identified, they may be considered negligible in the context of ensuring the overall safety and efficacy of radiopharmaceuticals used in nuclear medicine procedures. The consistently lower RCP values obtained using the Whatman paper method suggest that this technique does not have a positive bias. This observation can instill confidence in clinicians regarding the reliability and accuracy of the RCP determination. In cases where the RCP values are within the acceptable range, healthcare professionals can confidently utilize the radiopharmaceutical in nuclear medicine procedures.

The lower RCP levels observed with the Whatman paper method may be attributed to the unique characteristics of this stationary phase. The rapid dispersion of the water-based spotting solvent on the surface of the Whatman paper during spotting could potentially influence the separation efficiency and contribute to the lower RCP values obtained using this method.

The broader spreading of spots on the Whatman paper compared to the Al_2O_3 stationary phase may result in decreased separation efficiency. This reduced separation can lead to an apparent decrease in RCP when using the Whatman paper for chromatographic analysis. The average RCP values obtained using the TLC-Whatman (Fig. 4) and TLC-SG (Fig. 5) methods were generally lower than those obtained using the standard TLC-Al_2O_3/ethanol method. Notably, the average RCP values from TLC-SG were closer to those from the Whatman paper method, exhibiting less variability. Although statistical differences were observed (5 out of 20 kits), these differences were not considered clinically significant.

Based on these findings, it can be concluded that TLC-SG, when used with methyl ethyl ketone as the solvent, is a viable and validated method for determining the RCP of [^{99m}Tc]Tc-MIBI kits in clinical settings.

The lower average RCP values obtained using this method, compared to the standard method, suggest that it does not have a positive bias. This lack of bias implies that the method is unlikely to yield falsely high RCP results. Consequently, when this method presents RCP values within the acceptable range, healthcare professionals can confidently rely on these results and employ this method for determining RCP in clinical settings.

The graphical analysis in Figure 6 indicates that the average RCP obtained using the cartridge method is closer to the averages from the standard method when compared to the previous 2 graphs. In some instances, the cartridge method produces slightly higher average RCP values than the standard method. This suggests that the cartridge method provides results that are either comparable to or, in some cases, marginally higher than the standard method for determining RCP. Statistical analysis showed insignificant differences in RCP values (3 out of 20 kits) between the cartridge method and the standard method. This indicates that the cartridge method is a valid alternative for determining the RCP of [^{99m}Tc]Tc-MIBI kits when used with the specified protocol. Consequently, it can be reliably employed in clinical settings to ensure the quality and safety of radiopharmaceuticals for patient use. A study published in 1989 reported that utilizing Sep-Pak_C_{18} resulted in high and inaccurate RCP values for [^{99m}Tc]Tc-MIBI kits. [13] Another study claimed that the use of a C_{18} nonpolar cartridge column tends to overestimate the RCP of this radiopharmaceutical, whereas a polar alumina column provides results that are more accurate and closer to the true values. [20]

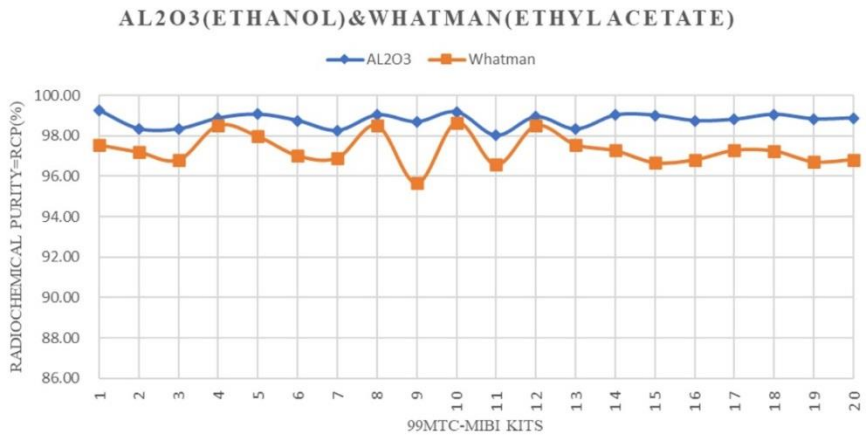

Figure 4: The image provides a comparison graph of RCP using the Al_2O_3 standard method and the TLC-Whatman paper with the ethyl acetate solvent for 20 kits.
TLC: thin layer chromatography, RCP: radiochemical purity, [^{99m}Tc]Tc-MIBI:[^{99m}Tc]-SestaMIBI, Al_2O_3: Aluminium oxide

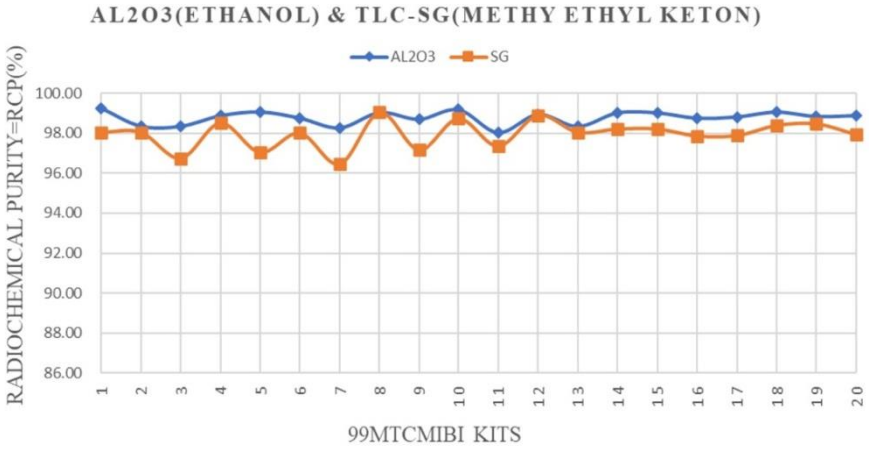

Figure 5: The image shows a comparison graph of RCP using the Al_2O_3 standard method and TLC-SG with the methyl ethyl ketone solvent for 20 [^{99m}Tc]Tc-MIBI kits.
RCP: radiochemical purity, TLC: thin layer chromatography, Al_2O_3: Aluminium oxide, SG: silica gel

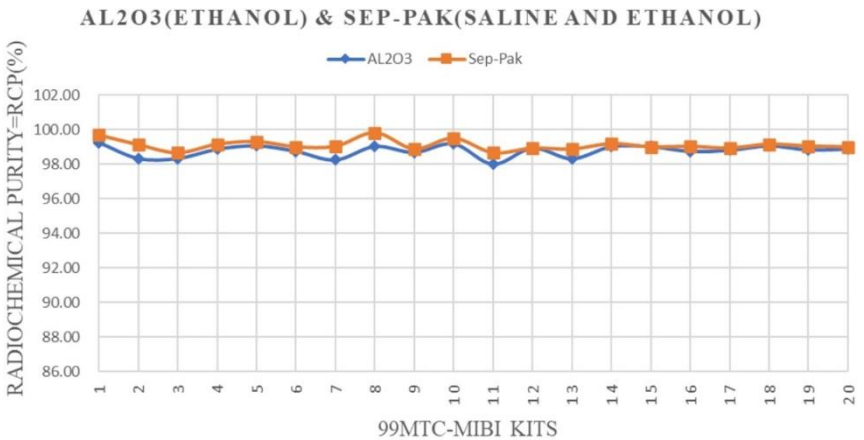

Figure 6: The image showcases a comparison graph of RCP using the Al_2O_3 standard method and the Sep-Pak_C_{18} cartridge with saline and ethanol solvents for 20 kits.
RCP: radiochemical purity, Al_2O_3: Aluminium oxide

Table 1: Comparisons of the 4 Methods Utilized in the Present Study to Determine the RCP of [99mTc]Tc-MIBI Concerning Cost, Duration, and Validity

Radiochemical Purity Methods	Cost of the Test	Duration of the Test	Validity of the Test
TLC-Al_2O_3/ethanol	High	40 min	Valid
TLC-SG/methyl ethyl ketone	High	20 min	Valid
TLC-Whatman/ethyl acetate	Low	10 min	Valid
Sep-Pak_C_{18}	High	5 min	Valid

[99mTc]Tc-MIBI:[99mTc]-SestaMIBI, RCP: radiochemical purity, TLC: thin layer chromatography, Al_2O_3: Aluminium oxide, SG: silica gel

As presented in Table 1, all 3 investigated methods were validated for measuring the RCP of [99mTc]Tc-MIBI kits when their respective protocols were followed. Regarding cost-effectiveness, the method employing the Whatman paper and the ethyl acetate solvent proved to be the most affordable option. In terms of speed and ease of use, both the cartridge column and Whatman paper methods demonstrated advantages over other methods.

Consequently, the combination of Whatman paper with Ethyl Acetate solvent was selected for the next phase of the study, alongside the standard TLC-Al_2O_3 method using Ethanol solvent.

3.3. Comparing Stability of [99mTc]Tc-MIBI in Vial and Syringe Storage

For investigating the changes in RCP of [99mTc]Tc-MIBI under 2 storage conditions – in a vial and in a syringe over time – the paper chromatography method using ethyl acetate solvent was chosen as a cost-effective and rapid option. This method was employed alongside the standard TLC-Al_2O_3 method utilizing ethanol solvent to ensure reliable and accurate results.

To provide a thorough assessment of [99mTc]Tc-MIBI's stability under various storage conditions, the study employed 2 validated methods for determining RCP: paper chromatography using ethyl acetate solvent and the standard TLC-Al_2O_3 method with ethanol solvent.

RCP test results were obtained at 8 AM, immediately after radiopharmaceutical preparation, and at 2-hour intervals (10 AM, 12 PM, and 2 PM) following preparation (Fig. 7-10).

The results obtained for the 20 [99mTc]Tc-MIBI kits were subjected to a paired sample *t*-test statistical analysis using SPSS software. This analysis aimed to compare the 2 storage methods, namely syringe and vial, and determine if there was a significant difference in RCP between them. A P value < 0.05 was used as the threshold to identify statistically significant differences in RCP values between the 2 storage methods. The statistical analysis revealed no significant difference in RCP values between the 2 storage methods (syringe and vial) at 8, 10, and 12 hours after preparation. However, a more notable difference was observed at 6 hours (2 PM), although the RCP values at this time point remained within the acceptable range according to the US Pharmacopeia (RCP above 90% is suitable for patient injection).

Considering these findings, it can be concluded that MIBI can be stored in either a syringe or vial for up to 6 hours post-preparation while maintaining an acceptable RCP level for administration to patients.

The data dispersion observed when comparing RCP measurement methods, TLC-Al_2O_3 and Whatman paper chromatography, suggests higher data variability with the Whatman paper chromatography method. This increased dispersion may be attributed to the thin solid phase of the Whatman paper, which can result in a more extensive spreading of the sampled spot at the origin.

No previous studies were found that compared the stability of [99mTc]Tc-MIBI between syringes and vials over time.

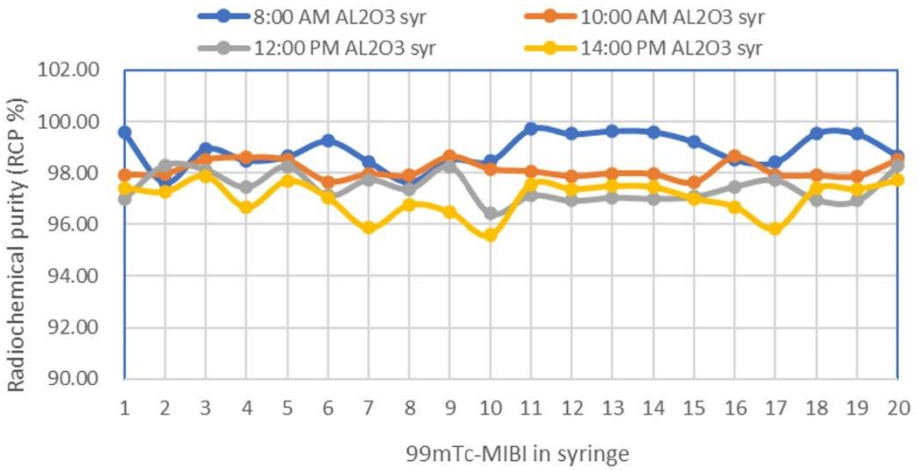

Figure 7: The image illustrates the RCP of [^{99m}Tc]Tc-MIBI stored in the syringe using Al_2O_3/ethanol in 2-hour time intervals. [^{99m}Tc]Tc-MIBI:[^{99m}Tc]-SestaMIBI, RCP: radiochemical purity, Al_2O_3: Aluminium oxide

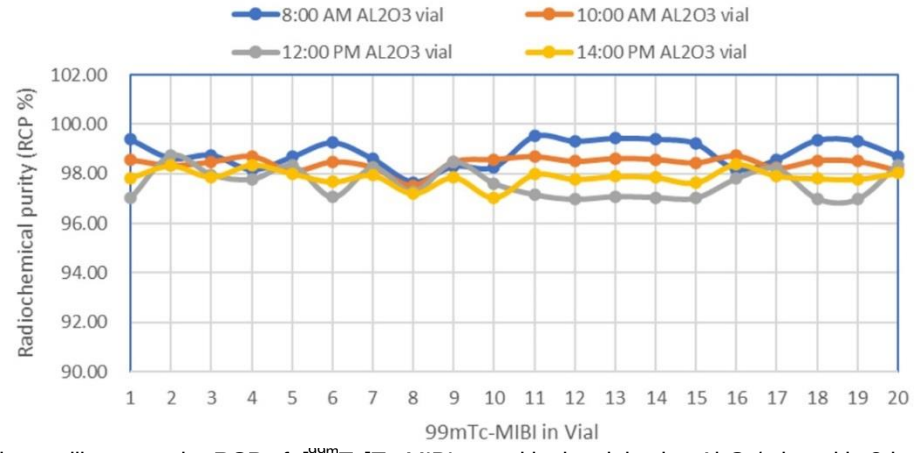

Figure 8: The image illustrates the RCP of [^{99m}Tc]Tc-MIBI stored in the vial using Al_2O_3/ethanol in 2-hour time intervals. [^{99m}Tc]Tc-MIBI:[^{99m}Tc]-SestaMIBI, RCP: radiochemical purity, Al_2O_3: Aluminium oxide

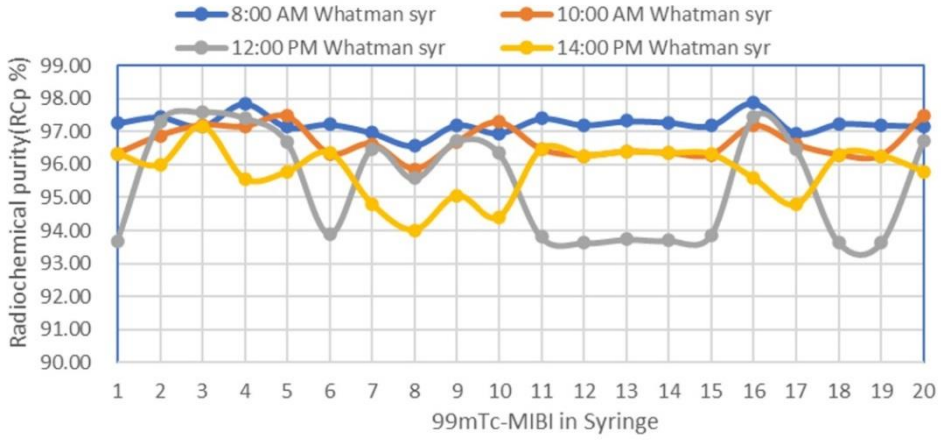

Figure 9: The image illustrates the RCP of [^{99m}Tc]Tc-MIBI stored in the syringe using Whatman/ethyl acetate in 2-hour time intervals.
RCP: radiochemical purity, [^{99m}Tc]Tc-MIBI:[^{99m}Tc]-SestaMIBI

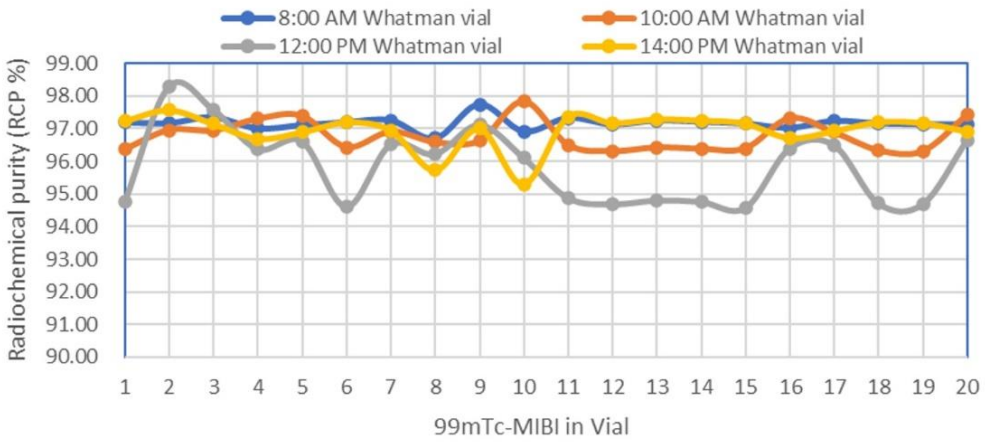

Figure 10: The image showcases the RCP of [99mTc]Tc-MIBI stored in the vial using Whatman/ethyl acetate in 2-hour time intervals.
RCP: radiochemical purity, [99mTc]Tc-MIBI:[99mTc]-SestaMIBI

CONCLUSIONS

In this study, 3 different methods for determining the RCP of [99mTc]Tc-MIBI radiopharmaceutical were developed and evaluated: TLC-Al_2O_3/ethanol, Whatman FN1 in ethyl acetate, and Sep-Pak_C_{18} cartridge. All 3 methods were successfully validated using the standard TLC-Al_2O_3/Ethanol method for each sample.
The Whatman FN1 in Ethyl Acetate method proved to be a fast, robust, reproducible, and cost-efficient option for routine use in clinical settings. Additionally, the Sep-Pak_C_{18} cartridge method, when applied using the introduced protocol, demonstrated its reliability and speed for RCP measurements.
An investigation into radiochemical stability under 2 storage conditions, syringe and vial, revealed that [99mTc]Tc-MIBI remains stable for up to 6 hours post-preparation. It is important to note that the reported stability for [99mTc]Tc-MIBI is solely based on the RCP aspect, and quality control assessments should also consider other factors, such as sterility and pyrogenicity testing.

Conflict of Interest: The authors of this research confirm that they have no financial interests or personal relationships that could have potentially influenced the findings presented in this paper. This declaration ensures that the research was conducted objectively and without bias.

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