

Rapid Quality Control Procedure for Technetium-99m-Bicisate

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Objective: The package insert for Neurolite® suggests a radiochemical purity (RCP) value of greater than 90% and a procedure for the RCP determination that uses a thin-layer chromatography (TLC) plate and a solvent system of ethyl acetate. This technique is very time-consuming, taking approximately 40 to 60 min to complete.

Methods: A new, convenient mini-paper chromatography (MPC) method has been developed for the RCP analysis of ^{99m}Tc-bicisate that utilizes precut (1.0 cm × 9.0 cm) Whatman Chr 17 paper as the stationary phase and ethyl acetate as the mobile phase. A blood collection tube (16 cm × 100 cm) was used as the developing chamber and did not require the pre-equilibration step for solvent saturation.

Results: Ten sets of triplicate measurements were obtained over a range of 72.1%–98.3%. Technetium-99m-bicisate had an *R_f* value of 0.8–1.0 in the proposed MPC method and this new method decreased the RCP analysis to 3–4 min. The recommended TLC method and the proposed MPC method correlate very closely with $r = 0.9987$ and a linear regression of $\text{TLC}\% = 0.96 \text{ MPC}\% + 3.63\%$. Overall the MPC method underestimates the RCP values obtained by the TLC method so that using the MPC's RCP values (without converting via the regression equation) leads to a conservative procedure. A ^{99m}Tc-bicisate kit may be rejected by the MPC method yet may pass with the TLC method. Therefore the MPC method will not create a false positive RCP result.

Conclusion: The MPC method provides a rapid and efficient RCP analysis of ^{99m}Tc-bicisate.

Key Words: technetium-99m-bicisate; radiochemical purity testing; thin-layer chromatography; mini-paper chromatography.

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Technetium-99m-bicisate, frequently known as ^{99m}Tc-ECD (N, N' 1, 2-ethylenediylbis-L-cysteine diethyl ester), is prepared with the Neurolite® kit (DuPont Merck Pharmaceutical Company, Billerica, MA) and sodium [^{99m}Tc] pertechnetate (Na ^{99m}TcO₄). This radiopharmaceutical was recently approved by the U.S. Food and Drug Administration for the

determination of regional patterns of blood perfusion in the brain (1). Technetium-99m-bicisate has been shown to have nearly ideal characteristics for SPECT brain imaging with high initial cerebral uptake. Technetium-99m-bicisate has slow clearance from the brain but clears rapidly from the rest of the body in both nonhuman primates and human subjects (2–5). When comparing ^{99m}Tc-bicisate with the only other ^{99m}Tc-labeled radiopharmaceutical (i.e., ^{99m}Tc-exametazime, commonly known as ^{99m}Tc-HMPAO) for brain perfusion study, the initial brain uptake of ^{99m}Tc-bicisate is about 6% (1) of the injected dose while initial uptake of ^{99m}Tc-exametazime is 3.5–7% (6). However, ^{99m}Tc-bicisate clears more rapidly from facial muscles and salivary glands producing a higher brain-to-background ratio than ^{99m}Tc-exametazime.

For highest radiochemical purity (RCP), ^{99m}Tc-exametazime requires the use of fresh eluate (i.e., <2 hr old) from a ^{99m}Tc generator which was previously eluted within 24 hr to reconstitute the kits (6). There are no such restrictions on ^{99m}Tc eluate for the preparation of ^{99m}Tc-bicisate (1). Technetium-99m-exametazime has rapid decomposition in vitro. Once reconstituted the radiopharmaceutical needs to be used within 30 min (6), whereas ^{99m}Tc-bicisate has a radiochemical stability of 6 hr post-preparation (1).

Both the package insert for ^{99m}Tc-bicisate and for ^{99m}Tc-exametazime recommend confirmation of RCP after radiolabeling (1,6). However, in determining RCP, ^{99m}Tc-exametazime requires a three-strip paper chromatography with three different developing solvents (6) whereas the RCP method for ^{99m}Tc-bicisate only requires a one-strip paper chromatography and one developing solvent (1).

According to the package insert of ^{99m}Tc-bicisate, the recommended method for RCP determination utilizes a single thin-layer chromatography (TLC) plate and a developing solvent of ethyl acetate (1). However, this method is very time-consuming since it requires solvent saturation in the developing tank (15–30 min), sample spot drying time (5–10 min) (7), solvent developing time (~15 min) and additional plate drying time (~2.5 min), totaling 40–60 min to complete. Due to the time constraints placed upon a busy nuclear pharmacy in which radiopharmaceuticals need to be prepared as quickly and as efficiently as possible, a faster

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method for RCP of ^{99m}Tc -bicisate would be advantageous. Therefore, the purpose of this study was to develop a rapid, yet efficient, method for routine RCP analysis of ^{99m}Tc -bicisate.

MATERIALS AND METHODS

Neurolite® Kit Formulation

The Neurolite kit formulation consists of two nonradioactive vials. Vial A contains 0.9 mg bicisate dihydrochloride ($\text{ECD} \cdot 2 \text{HCl}$), 0.36 mg disodium EDTA dihydrate as a transchelation agent, 24 mg mannitol as a bulking agent for the lyophilization process, 12–72 μg $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$ and a reducing agent. The pH of the content in vial A is 2.45–2.95 before lyophilization under N_2 (1). Vial B contains a buffer solution of 4.1 mg $\text{Na}_2\text{HPO}_4 \cdot 7 \text{H}_2\text{O}$, 0.46 mg $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and 1 ml sterile water for injection (1). Each vial is sterile and nonpyrogenic. The buffer solution (pH 7.2–8.0) in vial B functions as a pH adjustment for $\text{Na}^{99m}\text{TcO}_4$. Vial B is stored under air. Both vials A and B should be stored at 15–25°C and vial A should be protected from light (1).

Preparation of ^{99m}Tc -Bicisate

According to the manufacturer's instructions, for the preparation of ^{99m}Tc -bicisate (1), 3.70 GBq (100 mCi) of sterile nonpyrogenic $\text{Na}^{99m}\text{TcO}_4$ in approximately 2.0 ml was initially added to vial B. Three milliliters of 0.9% NaCl was injected into vial A and shaken for a few seconds for the contents to dissolve. Within 30 sec, 1.0 ml of vial A was transferred to vial B. The contents of vial B are swirled and allowed to stand at room temperature (i.e., 15–30°C) for 30 min. RCP should be checked after the 30-min incubation time.

TLC Method for RCP Analysis of ^{99m}Tc -Bicisate. The recommended TLC procedure involves the use of a 2.5 cm \times 7.5 cm Bakerflex silica gel IB-F (Baker #4463-03, J T Baker, Inc., Phillipsburg, NJ) as the stationary system and high-performance liquid chromatography (HPLC) grade ethyl acetate as the mobile phase.

The RCP analysis was performed in accordance with the package insert of ^{99m}Tc -bicisate (1). Fresh ethyl acetate was poured into a chromatographic developing jar to the depth of 3–4 mm. The tank was covered and allowed 15–30 min for solvent equilibration. In order to obtain reproducible RCP results, the manufacturer's instructions for the TLC procedure requires the headspace in the chromatographic tank be pre-equilibrated with ethyl acetate. Faint pencil lines were drawn on the TLC strip at 2.0 cm, 4.5 cm and 7.0 cm, to indicate the origin, cut line and solvent front, respectively (Fig. 1). Approximately 5 μl of the ^{99m}Tc -bicisate sample was placed at the center of the origin line and allowed to dry for 5–10 min. The plate was then placed in the pre-equilibrated TLC tank and developed to the solvent front. The TLC plate was removed and dried in a well-ventilated area. The developed TLC plate was cut at the 4.5-cm mark and each individual half was counted using a dose calibrator. With the TLC system, ^{99m}Tc -bicisate has an R_f (relative

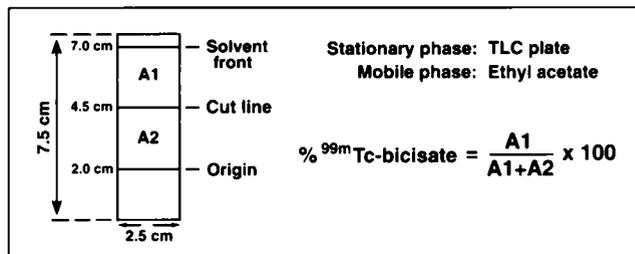


FIGURE 1. Schematic diagram of the standard TLC method for the RCP determination of ^{99m}Tc -bicisate.

front) value of 0.9 ± 0.1 and the other radiochemical impurities, hydrolyzed-reduced ^{99m}Tc (H-R ^{99m}Tc), free ^{99m}Tc , ^{99m}Tc -EDTA and ^{99m}Tc (IV) ECD, remain at the origin (i.e., $R_f = 0.0$) (1). RCP was calculated using the formula listed in Figure 1.

Proposed Method for RCP Analysis of ^{99m}Tc -Bicisate. The proposed mini-paper chromatography (MPC) method involves the use of precut (1.0 cm \times 9.0 cm) Whatman Chr 17 chromatographic paper (Whatman LabSales, Hillsboro, OR) as the stationary phase, ethyl acetate as the mobile phase and Venoject® blood collection tube (16 cm \times 100 cm) (Sherwood Medical, St. Louis, MO) as the developing tube.

This MPC procedure was performed by placing HPLC grade ethyl acetate in the bottom of a Venoject® blood collection tube to a depth of approximately 1 cm. The tube was then capped with the rubber stopper creating a closed solvent-saturated atmosphere. Faint pencil lines were drawn on precut MPC paper at 2.0 cm (i.e., origin) and 5.5 cm (i.e., cut line) from the bottom of the strip (Fig. 2). Approximately 5 μl of the final solution of ^{99m}Tc -bicisate was placed at the origin. The strip was immediately placed in the glass tube and the tube was recapped. The ethyl acetate was then allowed to develop to the top of the strip. After the strip was developed, it was cut at the 5.5-cm pencil line (Fig. 2) and each half was immediately measured with a dose calibrator. There was no need to allow the strip to dry, as in the standard TLC method. The R_f value of the bound ^{99m}Tc -bicisate with the MPC method was 1.00 and the R_f value for

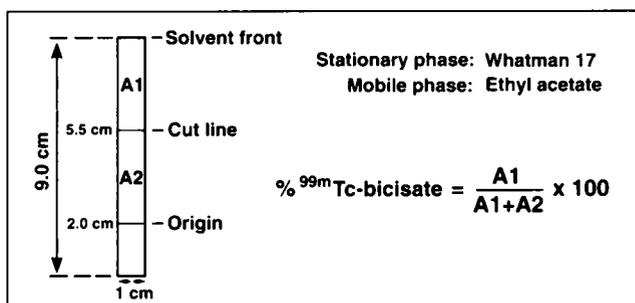


FIGURE 2. Schematic diagram of the proposed MPC method for the RCP determination of ^{99m}Tc -bicisate.

TABLE 1
Comparison of TLC and MPC Analysis for RCP of ^{99m}Tc -Bicisate*

TLC	MPC
98.3 ± 0.2	96.5 ± 0.2
97.9 ± 0.3	97.1 ± 0.2
97.9 ± 0.1	96.9 ± 0.5†
97.7 ± 0.2	97.2 ± 0.1
96.2 ± 0.6	96.3 ± 0.5
92.0 ± 0.1	91.7 ± 0.3
91.1 ± 0.1	90.8 ± 0.4
86.4 ± 0.3	86.0 ± 0.2
81.3 ± 0.3	80.7 ± 0.2
72.9 ± 0.2	72.1 ± 0.3

*mean ± s.d. of triplicate measurements

†n = 4

the radiochemical impurities such as H-R ^{99m}Tc and free ^{99m}Tc was 0.0. The RCP was determined by the same equation stated in the TLC method (1) (Fig. 2).

Comparison of Radioactivity Distribution Patterns of TLC and MPC Methods

Autoradiochromatogram (ARG). Radioactive distribution patterns of ^{99m}Tc -bicisate in the TLC method (1) and in the proposed MPC method were obtained by ARG method. After the solvent migration process, the chromatographic strips were allowed to dry. The dried strips were then placed on x-ray film (20.3 cm × 25.4 cm) (Kodak Ektascan™ MC film, EMC-1, Eastman Kodak Company, Rochester, NY) in a film cassette (Picker Source One Cassette, Picker International, Highland Heights, OH) for 10–15 min. The x-ray film was then developed.

Radiochromatogram. Radiochromatograms were also obtained for comparison of radioactive distribution patterns of ^{99m}Tc -bicisate. The dried, developed chromatographic strips were placed on the radiochromatogram scanner (Radiomatic VISTA™ Model 100, Radiomatic Instruments and Chemical Co., Inc., Meriden, CT) for the measurement of radioactivity distribution.

Accuracy and Consistency Testing Between TLC and MPC Methods

For the purpose of evaluating overall performance and accuracy of the proposed MPC method, ^{99m}Tc -bicisate preparations involving RCP levels at 91–93%, 86–88%, 80–82% and 70–75% were obtained by adding an additional volume of $\text{Na}^{99m}\text{TcO}_4$ to the initial preparation of ^{99m}Tc -bicisate. Each of the preset RCP levels was confirmed with the reference TLC method. Triplicate RCP measurements of each ^{99m}Tc -bicisate sample, in different RCP ranges with both the TLC and the MPC method, were obtained in order to evaluate the consistency of the proposed MPC method (Table 1).

Statistical Analysis

We have modeled the RCP measurement as a mean value with a normally distributed deviation of constant variance.

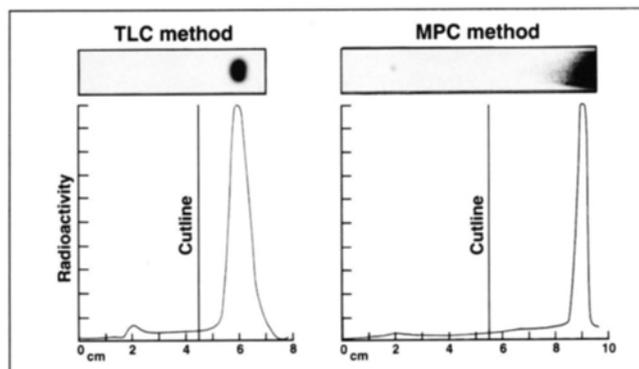


FIGURE 3. ARGs (top) and radiochromatogram scans (bottom) for the TLC method (left) and the MPC method (right) of an acceptable ^{99m}Tc -bicisate preparation with RCP value of 96.5%.

Using this model, we computed a reasonable safety limit for the measured RCP values since a measured RCP value may be either above or below the true (mean) RCP value. If one wished to be confident that the true RCP value is above 90%, it is necessary to have the observed RCP value to be greater than a certain cut-off RCP value greater than 90%.

We have also modeled the calibration between the RCP values of the TLC and MPC methods in terms of linear regression. The linear regression analysis used the mean of three replicated TLC measurements as the response versus the individual MPC's RCP value as a predictor.

RESULTS AND DISCUSSION

According to the package insert for ^{99m}Tc -bicisate, the RCP value of the ^{99m}Tc -bicisate preparation should be $\geq 90\%$ (1). RCP testing should be performed to ensure the radio-pharmaceutical is free of radiochemical impurities that may degrade image quality, interfere with the diagnostic interpretation and expose the patient to unnecessary radiation. Also, RCP testing is highly recommended in the *Nuclear Pharmacy Practice Standards* (8).

As stated previously, the average RCP analysis of ^{99m}Tc -bicisate using the recommended TLC method takes 40–60 min to perform, and multiple steps (i.e., solvent saturation, sample spot drying, solvent developing and TLC plate drying) (1,7) which are time-consuming and cumbersome. The new MPC method decreased the time of RCP analysis of ^{99m}Tc -bicisate to 3–4 min and did not require the solvent saturation time and spot drying times. Therefore, the new MPC method makes RCP analysis of ^{99m}Tc -bicisate very efficient and less cumbersome.

As seen by the ARG and radiochromatograms (Fig. 3), at an RCP value of 96.5% both the TLC and MPC methods show a clean separation between the origin and the solvent front. A clear separation between the origin and the solvent front was also seen at an unacceptable RCP value (i.e., 85.0%) (Fig. 4). On these two examples, both the TLC and MPC methods demonstrated that radiochemical impurities stayed at the origin of the chromatographic strips, whereas

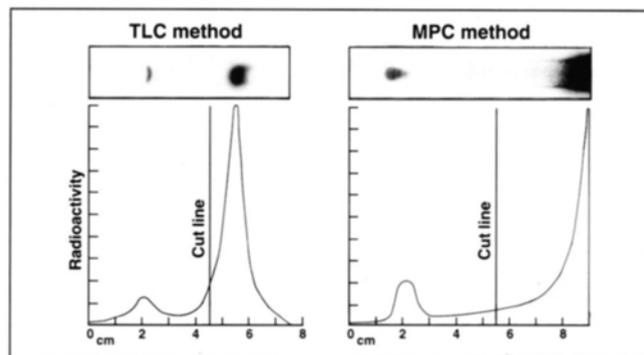


FIGURE 4. ARGs (top) and radiochromatogram scans (bottom) for the TLC method (left) and MPC method (right) of an unacceptable ^{99m}Tc -bicisate preparation with RCP value of 85.0%.

the bound ^{99m}Tc -bicisate migrates to the top of the strips. The fact that there was no streaking in the middle of the MPC chromatographic paper strip, as one would see if the paper chromatographic method was not effective, indicated that the MPC method was equivalent to the TLC method.

There are two major results from our statistical analysis of the MPC-TLC data. The first is that the standard TLC method is not perfect; the second is that the proposed MPC method compares favorably to the recommended TLC method. The replicated measurements show a consistent variation between the two methods, and deviations from the means have a symmetric distribution which is reasonably close to Gaussian. For the recommended TLC method, confidence limits were computed assuming a normal distribution with mean of 90 and standard deviation of 0.26%, as estimated with the within-sample variation. A cut-off of 91% seems reasonable (Table 2).

The statistical analysis revealed that the RCP results from both the TLC and MPC methods correlated very closely ($r = 0.9987$) for the overall RCP values in the range of 72–97% ($n = 18$) with a linear regression of $\text{TLC \%} = 0.96 \text{ MPC \%} + 3.63\%$ (Fig. 5). Based on this regression and the confidence levels from Table 2, it is quite safe to use 90% as a cut-off value for the proposed MPC method. That is, if the RCP value from the proposed MPC method is 90% or more, the probability is 0.9969 that the mean RCP value from the standard TLC method is 90% or more.

The regression model gave an excellent fit to the RCP levels below 97% ($r = 0.9987$, $n = 18$) (Fig. 5). However, as

TABLE 2
Confidence Limits for TLC and MPC Methods

	RCP values (%)			
	90.0	90.4	90.6	91.0
Confidence level for TLC method	0.5000	0.9393	0.9899	0.9999
Confidence level for MPC method	0.9969	1.0000	1.0000	1.0000

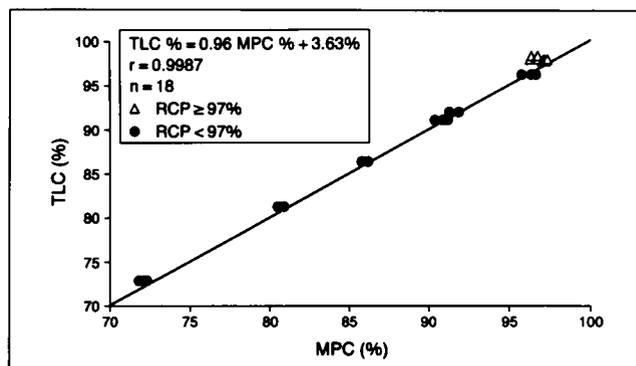


FIGURE 5. Linear regression analysis of the RCP measurements of ^{99m}Tc -bicisate by the MPC and TLC methods.

can be seen in the residual plot (Fig. 6), there is an apparent change in the relationship between the TLC and MPC methods in the very high RCP range (97–98%). Nevertheless, the RCP values obtained by the MPC method at the high range were still an underestimate (Table 1).

Our results demonstrated that RCP values determined by the MPC method underestimated the values obtained by the TLC method (Table 1). Therefore, using the MPC value (without converting via the regression equation) leads to a conservative result. In other words, a ^{99m}Tc -bicisate kit may be rejected by the proposed MPC method yet would pass with the standard TLC method. This means that the proposed MPC method will not create a false positive result of RCP measurement.

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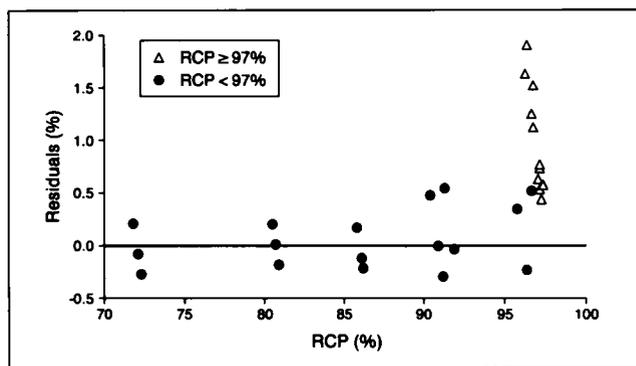


FIGURE 6. Residual plot for the RCP measurement of ^{99m}Tc -bicisate by the MPC and TLC methods.

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