A Rapid Chromatographic Method for Quality Control of Technetium-99m-Bicisate

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The purpose of this work was to develop a simple and rapid method to determine the radiochemical purity of ^{99m}Tc-bicisate.

Methods: A rapid paper chromatographic (PC) method was developed to determine the radiochemical purity of ^{99m}Tc-bicisate and compare the results with those of the manufacturer's recommended method. The present PC method included Whatman 3MM paper as the solid phase and ethyl acetate as the solvent.

Results: The time for chromatography by this technique was 4–5 min compared to about 23 min by the manufacturer's method. The R_f value of ^{99m}Tc-bicisate (R_f = 0.9–1.0) was widely different from those of $^{99m}TcO_4^-$ and reduced ^{99m}Tc (R_f = 0.0 for both) so the chromatographic strip after development could be readily cut into two segments, in order to determine the labeling yield.

Conclusion: No significant difference in labeling yields was found between the present method and the manufacturer's method. The PC method using Whatman 3MM paper and ethyl acetate is a simple and fast technique to determine the radiochemical purity of ^{99m}Tc-bicisate and may be substituted for the manufacturer's recommended method to save time.

Key Words: technetium-99m-bicisate; quality control; chromatography methods

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Technetium-99m-bicisate (ethyl cysteinate dimer [ECD]; brand name Neurolite®, Du Pont Merck Pharmaceuticals, North Billerica, MA) is a stable lipophilic complex used in the detection of cerebrovascular abnormalities in patients (*1–3*). As with any radiopharmaceutical, ^{99m}Tc-ECD must have a high level of radiochemical purity (RCP) for better quality of images. Higher percentages of radiochemical impurities such as $^{99m}TcO_4^-$ and hydrolyzed ^{99m}Tc result in poor images and unnecessary radiation exposure to the patient. Therefore, the RCP of radiopharmaceuticals should be determined before administration to humans.

The manufacturer's recommended method for determining the RCP of ^{99m}Tc-bicisate consists of a thin layer chromatography using Baker-Flex silica-gel IB-F as the solid phase and ethyl acetate as the solvent (*4*). This method takes 22–27 min, which is a long time in busy institutions. We report an alternative rapid paper chromatographic (PC) method using Whatman 3MM paper and ethyl acetate, and compare the results with those of the manufacturer's method.

MATERIALS AND METHODS

Technetium-99m-pertechnetate was obtained from the ^{99}Mo-^{99m}Tc generator (Mallinckrodt Inc., St. Louis, MO) by eluting with physiologic saline. Technetium-99m-bicisate was prepared according to the manufacturer's instructions by adding 100 mCi of ^{99m}Tc-pertechnetate to the kit vial. Hydrolyzed ^{99m}Tc was prepared by dissolving stannous chloride dihydrate in concentrated hydrochloric acid followed by the addition of ^{99m}Tc-pertechnetate (*5*). The pH of the solution was adjusted between 5.5–5.8 with 6N NaOH, which gave hydrolyzed technetium.

In order to establish the relative migration of the three components, $^{99m}TcO_4^-$, reduced ^{99m}Tc and ^{99m}Tc-bicisate, in PC using Whatman 3MM paper and ethyl acetate, approximately 1–2 drops of each sample were placed 1 cm from the bottom of each 1 × 8 cm Whatman 3MM paper strip and dried for 2 min. The strips were developed by the ascending PC method using ethyl acetate as the solvent (*6*). The strips were cut in 1-cm segments which were then counted in a NaI(Tl) well counter (Canberra Industries Inc., Meriden, CT). The plot of activity of each strip versus the distance traveled by the solvent gave the R_f values of different components as follows:

$$R_f = \frac{\text{distance migrated by solute from origin}}{\text{distance migrated by solvent from origin}}. \quad \text{Eq. 1}$$

This PC method was performed for $^{99m}TcO_4^-$, reduced ^{99m}Tc and ^{99m}Tc-bicisate samples. After establishing the R_f values of the three components, the ^{99m}Tc-bicisate preparation was analyzed by the PC method using Whatman 3MM paper

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TABLE 1
R_f Values Determined by PC using Whatman 3MM Paper and Ethyl Acetate

Radiochemical species	R_f value
^{99m}Tc-bicisate	0.9–1.0
$^{99m}TcO_4^-$	0.0
Reduced ^{99m}Tc	0.0

and ethyl acetate to determine the RCP (i.e., the labeling yield) of ^{99m}Tc-bicisate. Twelve samples were analyzed as dry spots (i.e., sample dried for 2 min after spotting) and 13 samples as wet spots (i.e., immediately after spotting) to see any difference between the two techniques. The time for chromatographic development was noted for each sample. After development, each strip was cut into two segments at 4 cm above the origin end of the strip. The activities in the two segments were measured in a dose calibrator (Capintec CRC-12R, Capintec, Pittsburgh, PA). The RCP (%) was calculated as follows:

$$\text{RCP (\%)} = \frac{\text{Activity in top} \times 100}{\text{Activity in top + activity in bottom}}. \quad \text{Eq. 2}$$

The mean values of time of chromatography and RCP values and their s.d. were calculated from the data of all samples.

For comparison, the RCP of ^{99m}Tc-bicisate was also determined for 25 samples according to the manufacturer's recommended method which uses the Baker-Flex silica-gel IB-F strip and ethyl acetate. One to two drops of the sample were placed 2 cm from the bottom on a 7.5 × 2.5 cm Baker-Flex strip and dried for 2 min. The strips were developed in ethyl acetate and cut into two segments at 4.5 cm from the origin end of the strip. Then, the activities in the two segments were measured in the dose calibrator and the RCP (%) was determined by Equation 2. The mean and its s.d. were calculated from the data of 25 samples.

RESULTS

The R_f values of TcO_4^-, hydrolyzed ^{99m}Tc and ^{99m}Tc-bicisate for both the Whatman 3MM paper plus ethyl acetate and Baker-Flex silica-gel IB-F plus ethyl acetate systems are presented in Table 1. In both systems, $^{99m}TcO_4^-$ and hydrolyzed ^{99m}Tc each has $R_f = 0$, while ^{99m}Tc-bicisate has $R_f = 0.9–1.0$ (Fig. 1). Such a difference in R_f values between ^{99m}Tc-bicisate and other radiochemical impurities makes it convenient to cut the strips into only two segments for determining the RCP.

The times to develop the chromatograms by the two methods are shown in Table 2. The time taken by our method (4.94 ± 0.44 min) is much shorter than that by the manufacturer's method (24.20 ± 0.96 min). For nuclear medicine departments that formulate their own radiopharmaceuticals and have to determine the labeling yield of each radiopharmaceutical, a time saving of almost 19 min by our method is highly desirable.

The RCP values obtained by the two methods are given in Table 2. There is no difference in labeling yields as determined

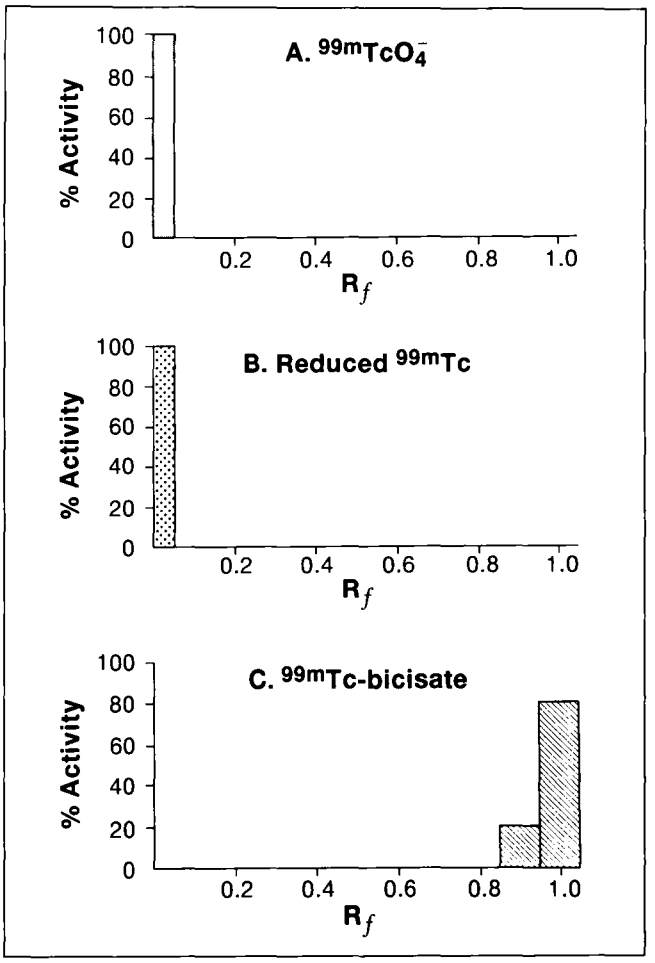

FIGURE 1. The chromatograms of (A) $^{99m}TcO_4^-$, (B) hydrolyzed ^{99m}Tc and (C) ^{99m}Tc-bicisate using Whatman 3MM paper and ethyl acetate.

by our method versus the manufacturer's method. The labeling yields determined by using dry spots versus wet spots are shown in Table 3. No significant difference is observed in labeling yields between the two techniques.

DISCUSSION

The chromatographic analysis of ^{99m}Tc radiopharmaceuticals is essential for the determination of the RCP of the

TABLE 2
Comparison of Chromatography Time and Percent RCP of Technetium-99m-Bicisate between the Manufacturer's Method and the Present Method

Method (n)	Time to complete (min)	RCP (%)
Baker-Flex silica-gel IB-F (25) (manufacturer)	24.2 ± 1.47	97.99 ± 1.31
Whatman 3MM (25) (present)	4.94 ± 0.44	98.52 ± 0.80

n = number of samples.

TABLE 3
Effect on the Percent RCP of Technetium-99m Bicisate of Drying or not Drying the Sample after Spotting on Whatman 3MM Paper

Spot (n)	RCP (%)
Dry (12)	98.48 ± 0.85
Wet (13)	98.55 ± 0.79

n = number of samples.

radiopharmaceutical so that highly pure radiopharmaceuticals are administered to humans for better images. The PC method should be as simple and less time consuming as possible. The Baker-Flex silica-gel IB-F plates are somewhat inconvenient to handle in that flakes come off when they are cut. The time to develop the chromatograms using these plates is significantly longer. In contrast, our method of PC using Whatman 3MM paper and ethyl acetate is convenient to perform and requires a short time of only 4–5 min to complete the chromatogram.

CONCLUSION

The PC technique using Whatman 3MM paper and ethyl acetate is a simple, efficient and rapid method for the determination of the radiochemical purity of ^{99m}Tc-bicisate and may be substituted for the current method recommended by the manufacturer.

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