Use of a Single-Strip Chromatography System to Assess the Lipophilic Component in Technetium-99m Exametazime Preparations

Denise I. Webber, A. Michael Zimmer, Monica C. Geyer, and Stewart M. Spies

Northwestern University Medical Center, Chicago, Illinois

Use of a single-strip miniaturized chromatography system to quantitate the lipophilic component in technetium-99m (^{99m}Tc) exametazime preparations was investigated. The chromatography system consisted of Whatman 17 strips and ethyl acetate as the solvent system. The operation parameters, accuracy and reliability of the single-strip chromatography system, were evaluated. Results of the study demonstrated that the single-strip chromatography system was accurate in assessing the lipophilic fraction in ^{99m}Tc exametazime preparations when compared to the three-strip chromatography method. The single strip system is rapid with a solvent developing time of ~2 min. As outlined, the single-strip chromatography system can easily be incorporated into the radiopharmaceutical quality control program of any nuclear medicine department.

Technetium-99m (^{99m}Tc) exametazime (hexamethyl propyleneamine oxime or HMPAO), commercially available as Ceretec® (Amersham Corp., Arlington Heights, IL), is used for detection of altered regional cerebral perfusion in stroke patients (*1*). With the addition of oxidant-free ^{99m}Tc pertechnetate to an exametazime vial containing stannous ion, a lipophilic technetium complex is formed. This lipophilic component is the active moiety necessary for optimum brain uptake of ^{99m}Tc exametazime. With time, the lipophilic complex is converted to a secondary hydrophilic form, which is unable to effectively cross the blood-brain barrier. The origin of this secondary form is not yet known, but complex conversion can be rapid. Therefore, reconstituted ^{99m}Tc exametazime preparations may only be used up to 30 min postpreparation (*1*).

The conventional chromatography quality control procedure determines the percentage of free pertechnetate, percentage of hydrolyzed reduced ^{99m}Tc, and percentage of lipophilic exametazine complex by means of three separate miniaturized chromatography systems (*1,2*). Levels of free pertechnetate are assessed using miniaturized strips of ITLC-SG (Gelman Instruments, Ann Arbor, MI) with 0.9% sodium chloride. With this system, free pertechnetate migrates close to or at the solvent front, while all other radiochemical components remain at the origin. Hydrolyzed reduced ^{99m}Tc levels are assessed using Whatman 31ET strips with 50% aqueous acetonitrile. With this chromatography system, hydrolyzed reduced ^{99m}Tc remains at the origin, while all other radiochemical components migrate from the origin. The radiolabeled lipophilic component is assessed using miniaturized ITLC-SG with methyl ethyl ketone. With this system, the lipophilic component and free pertechnetate migrate close to the solvent front and the hydrophilic and hydrolyzed reduced components remain at the origin. Calculated results of these three chromatography systems are combined for the summary of the radiochemical composition of ^{99m}Tc exametazime.

The Ceretec® package insert indicates that the conventional three-strip quality control procedure takes ~15 min to complete. Since the useful life of reconstituted ^{99m}Tc exametazime preparations is only 30 min, rapid quality control procedures are needed to effectively evaluate radiochemical purity. This study was undertaken to determine if a more rapid single-strip miniaturized chromatography system could accurately assess the radiochemical purity, specifically the lipophilic component, of ^{99m}Tc exametazime preparations.

METHODOLOGY

Based on previous experience (*3–7*), our laboratory investigated numerous chromatography strip solvent systems in order to determine if a single-strip method was viable. Specific chromatography systems were selected after it was determined that the developing solvent flow from the origin to the top of the 1 cm × 8 cm strips took less than 60 sec. Following experimental chromatographic evaluations, three chromatography systems were chosen for further evaluation. These included Whatman 31ET strips with ethyl acetate. Whatman

For reprints contact: A. Michael Zimmer, PhD, Section of Nuclear Medicine, Northwestern Memorial Hospital, 250 E Superior Street, Chicago, IL 60611.

17 strips with ethyl acetate, and Gelman ITLC-SG strips with ethyl acetate.

In order to evaluate the migration of ^{99m}Tc exametazime and ^{99m}Tc pertechnetate in the chromatography systems outlined above, specific strips were spotted with ~2 μl of test material and immediately placed in 20-ml glass scintillation vials (Kimball Glass, Toledo, OH), containing 0.6–1.0 ml of ethyl acetate (Analytical Reagent Grade, Mallinckrodt Chemical Works, St. Louis, MO). After solvent migration, the developed strips were cut in 15 equal segments beginning at the origin and ending at the solvent front. The sections were then counted for activity and the results graphically expressed.

From the experimental procedure outlined above, the most promising chromatography system, namely Whatman 17 strips with ethyl acetate, was compared to the conventional chromatography system outlined by the manufacturer (*1*). The radiochemical purity of a ^{99m}Tc exametazime preparation was analyzed by each of the two specific chromatography systems at 5 min and 30 min, then at 1, 2, 4, and 6 hr after formulation. For each specific chromatography system and time period, four replicate evaluations were performed. The data were summarized by calculating means and s.d. The purpose of this comparison was to determine if the single-strip ethyl acetate systems would parallel the results obtained using the conventional three-strip solvent chromatography system.

The effect of spotting time, that is, the time between spotting the radiopharmaceutical and placing the strip in the respective solvent, was evaluated using Whatman 17 strips with ethyl acetate. A ^{99m}Tc exametazime preparation was spotted on Whatman 17 strips and the spots dried for 0, 15, 30, 60, and 120 sec prior to solvent development. For each specific drying time, four replicate strips were spotted and developed. The data was statistically summarized by calculating means and s.d.

The accuracy and reliability of the single-strip chromatography system (Whatman 17 strips with ethyl acetate) in assessing the radiochemical purity of ^{99m}Tc exametazime was further evaluated. The lipophilic component of six specific ^{99m}Tc exametazime preparations was determined at 5 min after preparation using the chromatography method outlined above, and the results were compared to the conventional chromatography system. For each specific preparation, four replicate samples were evaluated and the data summarized as outlined above.

RESULTS

The activity distribution of ^{99m}Tc exametazime and free ^{99m}Tc pertechnetate in the three specific single-strip chromatography systems (Whatman 31ET strips with ethyl acetate, Whatman 17 strips with ethyl acetate, Gelman ITLC-SG strips with ethyl acetate) are graphically expressed in Figures 1 and 2, respectively. Undesirable migration of free pertechnetate and the incomplete separation of ^{99m}Tc exametazime using Gelman ITLC-SG strips and ethyl acetate made this system unsuitable for further study. The Whatman 17 strips with ethyl acetate chromatography system was chosen for further study because maximal separation of ^{99m}Tc exametazime from free pertechnetate and minimal streaking of activity were observed.

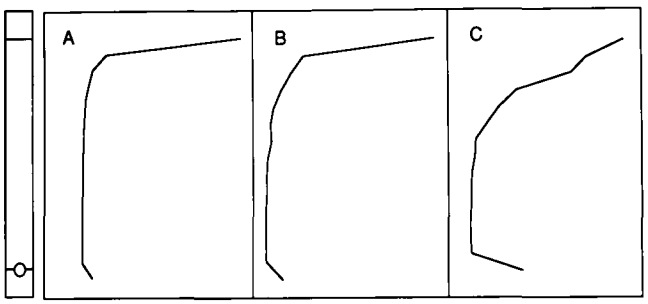

FIG. 1. Chromatography strip activity distribution of technetium-99m exametazime in chromatography systems consisting of (A) Whatman 17 strips with ethyl acetate; (B) Whatman 31ET strips with ethyl acetate; (C) Gelman ITLC-SG strips with ethyl acetate.

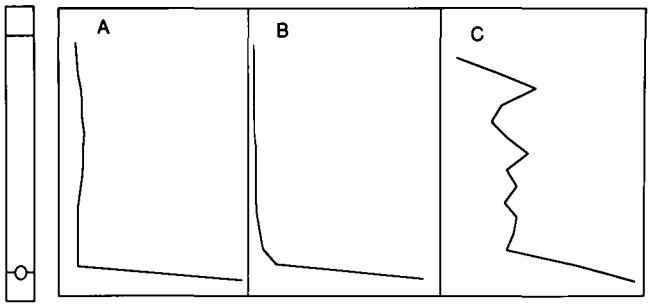

FIG. 2. Chromatography strip activity distribution of technetium-99m pertechnetate in chromatography systems consisting of (A) Whatman 17 strips with ethyl acetate; (B) Whatman 31ET strips with ethyl acetate; (C) Gelman ITLC-SG strips with ethyl acetate.

The comparison of the lipophilic component in a ^{99m}Tc exametazime preparation using the conventional three-strip chromatography system and the Whatman 17 single-strip chromatography system is shown in Figure 3 and Table 1. Lipophilic component analysis was performed up to 6 hr after preparation. As indicated by Figure 3, a close correlation in the lipophilic component of a ^{99m}Tc exametazime preparation was observed between the two respective chromatography systems. These results indicated that the single-strip method was accurate in assessing the lipophilic component when compared to the conventional chromatography system. It should be noted that the close correlation between chromatography systems spanned a wide range of lipophilic fraction assessments (50% to 94%).

The effect of spot drying on the results of lipophilic assessment in ^{99m}Tc exametazime preparations using Whatman 17 paper is found in Figure 4. As spot drying times increased, levels of lipophilic components decreased. These data indicate that either some oxidation and/or a conversion process is occurring in the lipophilic component, or an interactive process between the radiopharmaceutical and the chromatography paper is occurring. In order to minimize these processes, it is

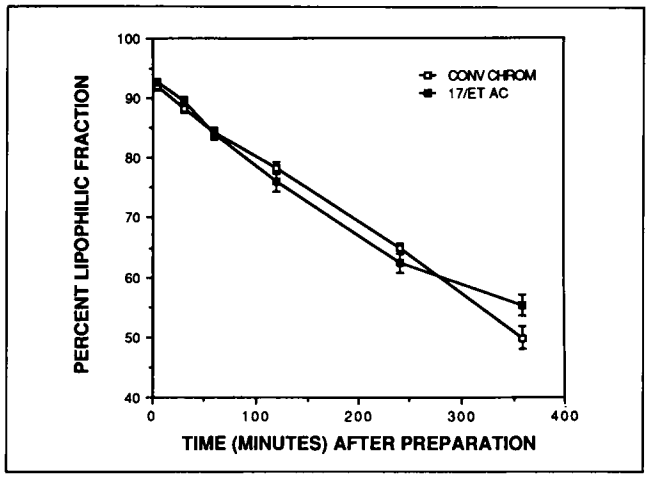

FIG. 3. Comparison of conventional three-strip chromatography system to single-strip chromatography system in evaluating lipophilic component in technetium-99m exametazime preparations.

TABLE 1. Lipophilic Component in Technetium-99m Exametazime Preparations Using Conventional Three-Strip and Whatman 17 Single-Strip Chromatography Systems

	Lipophilic Component ($\bar{m}$% ± s.d.)	
Preparation	Three-Strip System	Single-Strip System
1	90.2 ± 0.3*	90.3 ± 0.3*
2	92.8 ± 0.8	88.6 ± 1.4
3	94.8 ± 0.7	96.4 ± 0.4
4	87.8 ± 1.5	94.6 ± 0.1
5	92.9 ± 0.7	91.7 ± 0.5
6	93.1 ± 0.4	94.2 ± 0.2

* N = 4.

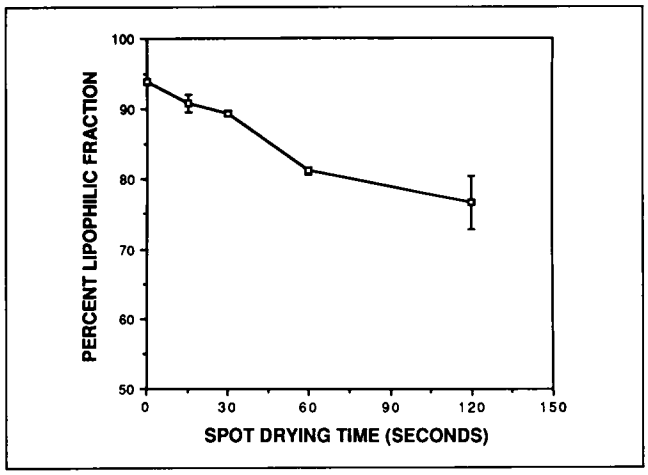

FIG. 4. Effect of radiopharmaceutical drying time, after spotting on Whatman 17 chromatography strips, on lipophilic fraction of ^{99m}Tc exametazime preparations.

mandatory that the chromatography strip be placed in the solvent immediately after radiopharmaceutical spotting.

DISCUSSION

The current conventional quality control method used to assess radiochemical purity of ^{99m}Tc exametazime (Amersham Corp., Arlington Heights, IL) utilizes three different chromatography strip and solvent systems. This recommended three-strip method is tedious and time-consuming. Our laboratory has investigated a single-strip chromatography system to evaluate the lipophilic component in ^{99m}Tc exametazime preparations. This system consists of miniaturized Whatman 17 chromatography strips (1 cm × 8 cm) with ethyl acetate as the solvent system. A typical miniaturized strip is shown in Figure 5. The origin and solvent front lines are drawn 1 cm from the bottom and top of the strip, respectively. The cut line is drawn 2.5 cm from the bottom of the strip. The chromatographic procedure used in our laboratory is shown in Table 2. It should be stressed that the strip must be placed in the solvent immediately after spotting. Any delay can result in an underestimation of the ^{99m}Tc-labeled lipophilic fraction.

The single-strip miniaturized chromatography procedure is very rapid. Total solvent developing time is ~2 min. The chromatography procedure is also accurate in assessing the

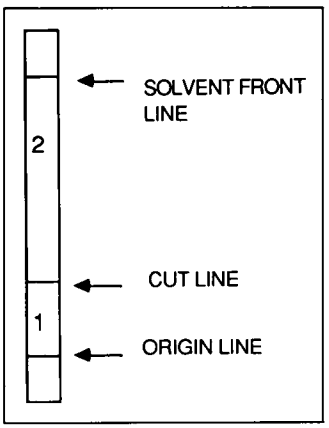

FIG. 5. Typical Whatman 17 chromatography strip (0.7 cm × 8.0 cm) showing origin, solvent front, and cut lines.

TABLE 2. Single-Strip Chromatographic Procedure for Evaluating the Lipophilic Component in Technetium-99m Exametazime Preparations

1. Place approximately 0.6–1.0 ml of ethyl acetate in a glass scintillation vial.
2. Spot ^{99m}Tc exametazime preparation at origin of miniaturized Whatman 17 chromatography strip.
3. Immediately place strip in solvent and allow solvent migration to solvent front line.
4. Cut strip at cut line, which is located at Rf = 0.25, dividing strip into Sections 1 and 2.
5. Count each Section for radioactivity. Obtain a background count.
6. Percent lipophilic fraction:

$$= \left[\frac{\text{(net counts Section 2)}}{\text{(net cts Section 1) + (net cts Section 2)}}\right]$$

lipophilic component in ^{99m}Tc exametazime preparations when compared to the conventional three-strip chromatography procedure. It should be noted that the single-strip system quantitates the lipophilic component only. If additional information regarding the nature of the radiochemical impurities is needed, the three-strip chromatography procedure must be utilized.

REFERENCES

1. Ceretec Package Insert. Amersham Corporation, Arlington Heights, IL, October 1989.
2. Karesh SM. Technetium 99m-exametazime: pitfalls in preparation and quality control. *J Nucl Med Technol* 1989;17:215–218.
3. Zimmer AM, Pavel DG. Rapid miniaturized chromatographic quality control procedures for Tc-99m radiopharmaceuticals. *J Nucl Med* 1977;18:1230–1233.
4. Zimmer AM, Pavel DG. Rapid miniaturized chromatographic quality control procedures for iodinated radiopharmaceuticals. *Am J Hosp Pharmacy* 1978;35:426–428.
5. Zimmer AM, Majewski W, Spies SM. Rapid miniaturized chromatography for Tc-99m IDA agents: comparison with gel chromatography. *Eur J Nucl Med* 1982;7:88–91.
6. Kazikiewicz JM, Zimmer AM, Spies SM, et al. Rapid miniaturized chromatography procedures for iodinated monoclonal antibodies: comparison to gel exclusion chromatography. *J Nucl Med Technol* 1987;15:129–132.
7. Webber DI, Zimmer AM, Spies SM. Common errors associated with miniaturized chromatography. *J Nucl Med Technol* 1983;11:66–68.