Pitfalls in the Standard Radiochemical Purity Testing for Technetium-99m-Exametazime

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Objective: The purpose of this study was to examine discrepancies in the standard method for determining the radiochemical purity (RCP) of ^{99m}Tc-exametazime. The package insert for ^{99m}Tc-exametazime indicates that the entire RCP testing procedure requires approximately 15 min to complete, although the solvent-developing time for the three-paper chromatography system is described to be ≤100 sec. According to the package insert, all solvent-migrated radiochemical components moved up to relative front (R_f) 0.8–1.0; however, the suggested cut lines for three paper strips are all well below the stated R_f value.

Methods: Radiochromatogram and autoradiography techniques were used to determine and evaluate the radioactivity distribution of different radiochemical species on the strips. The times for solvent migration and the entire RCP testing procedure were measured.

Results: The mean times to run the three standard paper chromatography systems, namely, ITLC-SG/MEK, ITLC-SG/saline and Whatman/50% CH_3CN strips were 130.4 ± 9.0 sec, 86.7 ± 9.4 sec and 123.1 ± 6.1 sec, respectively (n = 55). Although our solvent-developing time was longer than the suggested ≤100 sec, the entire RCP analysis procedure (i.e., sample spotting, solvent migration, radioactivity measurement and RCP calculation) can be completed within 5–7 min. Both ITLC-SG/MEK and Whatman/50% CH_3CN systems showed significant streaking of radioactivity on the strips (i.e., 0.5–1.0 and 0.6–1.0, respectively) which does not agree with the recommended R_f 0.8–1.0.

Conclusion: The quality control information for ^{99m}Tc-exametazime should be modified in order to provide accurate information for the time required to perform the entire RCP procedure, the solvent-developing times for three strips and the R_f values for three chromatography systems.

Key Words: technetium-99m-exametazime; radiochemical purity; quality control

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Exametazime, which is also known as hexamethylpropylene amine oxine (HMPAO), is commercially available under the brand name Ceretec™ (Amersham Corporation, Arlington Heights, IL). After exametazime is labeled with ^{99m}Tc, a lipophilic ^{99m}Tc-exametazime (commonly known as ^{99m}Tc-HMPAO) is formed, and this radiopharmaceutical can be used for the detection of altered regional cerebral perfusion in the stroke patient (*1*).

Technetium-99m-exametazime is the first radiopharmaceutical on which radiochemical purity (RCP) testing must be performed and an RCP value of >80% obtained prior to patient administration (*1*). The requirement for the determination of RCP before injection is necessary due to the rapid decomposition of the primary (lipophilic) ^{99m}Tc-exametazime after reconstitution (*2,3*).

The standard method for RCP determination as stated in the package insert for ^{99m}Tc-exametazime (*1*) involves the use of three miniaturized paper chromatography systems for the complete definition of all three potential radiochemical impurities (i.e., free pertechnetate (free Tc), hydrolyzed-reduced ^{99m}Tc (HR Tc) and secondary ^{99m}Tc-exametazime complex). The three chromatography systems consist of:

1. ITLC-SG/MEK: an instant thin-layer chromatography strip impregnated with silica gel (ITLC-SG) (Gelman Sciences, Ann Arbor, MI) developed with methyl ethyl ketone (MEK), 99.5%+ high-pressure liquid chromatography (HPLC) grade (Aldrich Chemical Company, Milwaukee, WI).
2. ITLC-SG/saline: ITLC-SG developed with nonbacteriostatic 0.9% NaCl solution.
3. Whatman/50% CH_3CN: Whatman Grade 31ET Chr paper (Whatman LabSales, Hillsboro, OR) developed with 50% v/v aqueous acetonitrile (CH_3CN), the 50% CH_3CN was prepared by diluting the 99.9%+ HPLC grade CH_3CN (Aldrich Chemical Company, Milwaukee, WI) with nonbacteriostatic sterile water for injection, USP (Abbott Laboratories, North Chicago, IL) (Fig. 1).

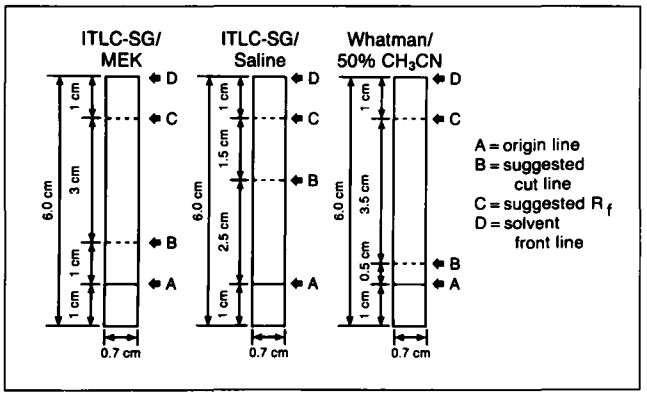

FIGURE 1. Standard three-strip chromatographic systems for ^{99m}Tc-exametazime RCP determination. R_f is the relative position that an individual radiochemical species moves in relation to the distance that the solvent from (S_f) moves and can be calculated by the following equation: R_f = distance from origin to spot center/ distance from origin to S_f.

However, there are some questions and inconsistencies in the chromatography information as stated in the package insert (*1*).

The package insert indicates that the entire RCP testing procedure requires ~15 min to complete, although the development time for three paper chromatography strips is described in the same package insert to be ~100 sec (i.e., ITLC-SG/MEK: ~45 sec, ITLC-SG/saline: ~45 sec and Whatman/50% CH_3CN: ~100 sec) (*1*). It is questionable that the other steps for the RCP testing (i.e., spotting sample, cutting strips, counting radioactivity and calculating RCP %) would take more than 13 min (i.e., 15 min–100 sec).

According to the package insert (*1*), all solvent-migrated radiochemical species move to R_f (relative front) 0.8–1.0; however, the suggested cut lines for three strips are all well below the stated R_f value of 0.8–1.0 (Fig. 1). When the strips are cut at the designated cut line as suggested by the manufacturer (Fig. 1) (*1*), the location of the cut lines for ITLC-SG/MEK, ITLC-SG/saline, and Whatman/50% CH_3CN are measured at R_f 0.2, R_f 0.5 and R_f 0.1, respectively. If various radiochemical components were clearly separated by the solvent and migrated to the narrow R_f range of 0.8–1.0 as stated in the package insert (*1*), there should be no need to cut the strips at such low points (i.e., R_f 0.2, R_f 0.5 and R_f 0.1).

The purpose of this study was to examine these two discrepancies in the standard three-strip chromatographic systems for the RCP determination of ^{99m}Tc-exametazime.

MATERIALS AND METHODS

Technetium-99m-exametazime was prepared from a freeze-dried Ceretec™ kit according to the package insert (*1*). The percent of the primary ^{99m}Tc-exametazime, the secondary ^{99m}Tc-exametazime complex, free Tc and HR Tc were measured by the standard three-strip chromatographic method (*1*). A ^{99m}Tc-exametazime sample for the RCP determination was withdrawn from the vial with a 1-ml syringe with 27.5-gauge needle (Monoject® Tuberculin syringe, Sherwood Medical Company, St. Louis, MO).

After 5-μl samples of ^{99m}Tc-exametazime preparation were applied to the origin of the three strips, each of the three paper strips was immediately placed in a Venoject® blood collection tube with red stopper (16 × 100 mm, 10 ml) (Sherwood Medical Company, St. Louis, MO) which contained 0.3–0.4 ml of the developing solvent. The Venoject® tube (16 × 100 mm) is slightly larger than the glass test tube (12 × 75 mm) specified in the Ceretec™ package insert (*1*). A closed solvent-saturated, even-vapor atmosphere could be created by capping the Venoject® glass tube with the rubber stopper that comes with the tube before and during the solvent development. The times required to run the three different chromatography systems (i.e., the time necessary for each solvent to migrate from the bottom of the strip to S_f) and the total RCP testing time (i.e., sample spotting, chromatography, solvent migration, cutting the strips, counting the strips and performing the RCP calculations) were measured.

In order to evaluate the radiochromatographic migration patterns and to determine the R_f values of different radiochemical species of ^{99m}Tc-exametazime, the autoradiography (ARG) technique was used initially. After completion of the solvent-migration process, the three paper strips were air dried for ~10–15 min. The dry strips were then exposed to an x-ray film (Kodak Ektascan B film, EB-1, Eastman Kodak Company, Rochester, NY) for ~15 min. The ARG method was also utilized to visualize the distribution patterns and to determine the R_f values of ^{99m}Tc-pertechnetate in the same three-strip radiochromatographic systems for ^{99m}Tc-exametazime, especially the ITLC-SG/saline system in which only free ^{99m}Tc-pertechnetate migrates to the top of the strip (*1*).

The actual radioactivity distribution on each chromatographic paper strip was accomplished after the ARG process by cutting off 1-cm segments from each strip of both ITLC-SG/MEK and ITLC-SG/saline systems (i.e., a total of six segments) and 0.5-cm segments from the Whatman/50% CH_3CN (i.e., total of 12 segments). Each separate segment was then counted to measure the radioactivity.

RESULTS AND DISCUSSION

The mean times required to run ITLC-SG/MEK, ITLC-SG/saline and Whatman/50% CH_3CN strips were 130.4 ± 9.0 sec, 86.7 ± 9.4 sec and 123.1 ± 6.1 sec, respectively (n = 55). Our solvent-developing times are longer than the suggested times (i.e., ITLC-SG/MEK: ~45 sec, ITLC-SG/saline: ~45 sec and Whatman/50% CH_3CN: ~100 sec) (*1*). We do not know the reason why our developing times for both ITLC-SG systems were longer than the times stated in the package insert (*1*). Each strip was cut to the same size, as specified by the manufacturer, and the ITLC-SG chromatography paper was obtained from the suggested supplier (i.e., Gelman Sciences, Ann Arbor, MI).

The entire RCP analysis including all three chromatography systems (i.e., sample spot, solvent migration, radioactivity measurement and RCP calculation) was completed within 5–7 min (376.3 ± 31.0 sec, n = 12), as opposed to ~15 min as stated in the package insert (*1*).

The % primary, lipophilic ^{99m}Tc-exametazime complex can be easily calculated by subtracting the percent on the top of the ITLC-SG/saline strip from the percent on the top of the ITLC-SG/MEK strip (Table 1), whereas HR Tc can be determined with the Whatman/50% CH_3CN system in which HR Tc remains at the origin (*1*). Since the Whatman/50% CH_3CN system separates only the HR Tc from the other radiochemical species in a ^{99m}Tc-exametazime preparation and the measurement of % HR Tc does not contribute to the determination of primary ^{99m}Tc-exametazime complex, we believe this testing system should not be required for RCP analysis of ^{99m}Tc-exametazime. If the Whatman/50% CH_3CN was not performed, the total RCP testing time could be reduced to 5–6 min (318.7 ± 18.9 sec, n = 12).

Based upon the ARG studies performed on ^{99m}Tc-exametazime and ^{99m}Tc-pertechnetate, the measured R_f values for the solvent-migrated radiochemical species in both ITLC-SG/MEK and Whatman/50% CH_3CN systems were different from the suggested R_f values (Table 1). Only the measured R_f value for free Tc in the ITLC-SG/saline system agrees with the recommended R_f (i.e., 0.8–1.0) (Table 1). Figure 2 demonstrates that both the ITLC-SG/MEK and Whatman/50% CH_3CN systems showed a certain degree of migration (i.e., streaking) of radioactivity on the strip, in particular the ITLC-SG/MEK system in which a significant amount of radioactivity streaked up from R_f 0.5–1.0 (Table 1).

The actual radioactivity distribution on the three-strip paper chromatography systems was evaluated on seven samples of ^{99m}Tc-exametazime with RCP values ranging from 33.2%–96.1%. Our findings showed that if the ITLC-SG/MEK strips were cut at the stated R_f value of 0.8 (i.e., 4.0 cm above the origin) rather than the recommended cut line at 1.0 cm from the origin (i.e., R_f 0.2), there would be an RCP difference of 8.6% ± 3.2% (n = 7). Since the ITLC-SG/MEK system is crucial in the determination of % primary, lipophilic ^{99m}Tc-exametazime complex, a correct R_f range for this system is absolutely necessary for an accurate RCP measurement.

According to our ARG results, the R_f range for the ITLC-SG/MEK system should be 0.5–1.0 (Table 1). Because of the problems of migrational tailing on both ITLC-SG/MEK and Whatman/50% CH_3CN systems, the locations of the suggested cut lines are much lower than the suggested and measured R_f values to overcome the aforementioned miscalculated RCP %. Although the ITLC-SG/saline system clearly separates free Tc and shows no streaking problem, the cut line is still suggested to locate at R_f 0.5 below measured R_f value (i.e., R_f 0.8–1.0) (Table 1).

TABLE 1
R_f Values for Three-Strip Radiochromatography Systems of ^{99m}Tc-Exametazime

Strip/solvent	Radiochemical species	Suggested R_f	Measured R_f
ITLC-SG/MEK	Primary*, Free Tc	0.8–1.0	0.5–1.0
ITLC-SG/saline	Free Tc	0.8–1.0	0.8–1.0
Whatman/50% CH_3CN	Primary*, Secondary†, Free Tc	0.8–1.0	0.6–1.0

*Primary = lipophilic ^{99m}Tc-exametazime complex.
†Secondary = secondary ^{99m}Tc-exametazime complex.

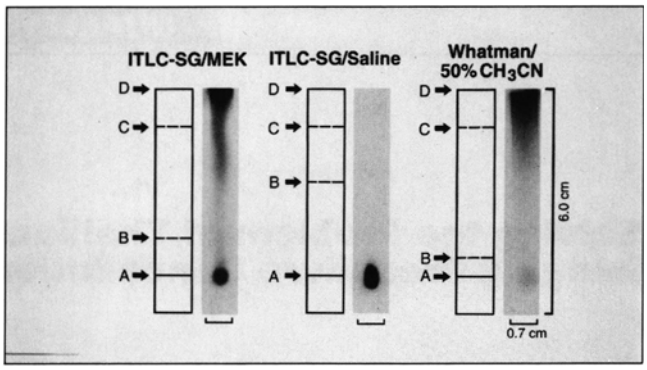

FIGURE 2. Radioactivity distribution patterns produced by ARG technique in the standard three-strip miniaturized radiochromatography systems for ^{99m}Tc-exametazime. A = origin, B = suggested cut line, C = suggested R_f, and D = S_f.

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