Rapid Preparation and Quality Control of Technetium-99m MAG3™

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Technetium-99m mertiatide (^{99m}Tc-MAG3) is a newly approved radiopharmaceutical with a renal clearance similar to iodine-131 O-iodohippurate. The radiolabeling of ^{99m}Tc requires a 10-min heating period in a boiling water bath and an additional 8–10 min for determining radiochemical purity with a relatively expensive Sep-Pak C18 cartridge. A microwave oven heating method is an effective way to reduce the heating time (13 sec) while maintaining excellent labeling efficiency for ^{99m}Tc-MAG3 (average 99%). A two-strip mini-paper chromatography system with 1:1:2 chloroform/acetone/tetrahydrofuran and 0.9% NaCl as mobile phases is a simple and rapid (2–3 min) procedure for the routine quality control of ^{99m}Tc-MAG3. The preparation and quality control of ^{99m}Tc-MAG3 can be completed within 5 min with the combination of the microwave oven heating method and the mini-paper chromatography system.

The Food and Drug Administration recently approved the drug MAG3™ (Mallinckrodt Medical, Inc., St. Louis, MO) for the preparation of technetium-99m mertiatide (^{99m}Tc-MAG3). This diagnostic radiopharmaceutical has similar biological properties to iodine-131 O-iodohippurate [^{131}I]OIH (*1–3*) and therefore has been proposed to be a suitable replacement for [^{131}I]OIH. The radiolabeling of ^{99m}Tc-MAG3 involves a heating process due to the slow exchange rate between reduced ^{99m}Tc and the betiatide ligand (N-[N-[N-[(benzoylthio) acetyl] glycyl] glycyl] glycine) at room temperature (*4*). Consequently, the standard labeling method as stated in the package insert (*5*) requires a 10-min heating period in a rolling boiling water bath. A microwave oven heating method has been shown to be a useful technique to considerably reduce the heating time for the labeling of ^{99m}Tc-Sestamibi (*6,7*). Therefore, the first objective of this study was to evaluate the possibility of expediting the heating process by using a microwave oven for the preparation of ^{99m}Tc-MAG3.

Technetium-99m MAG3 is the second ^{99m}Tc labeled radiopharmaceutical with a package insert requirement that the radiochemical purity (RCP) of the reconstituted solution be checked prior to clinical use in patients (^{99m}Tc-exametazime also requires RCP determination [*8*]). The recommended method for determination of RCP of ^{99m}Tc-MAG3 involves the application of a reverse phase chromatography with a Waters Sep-Pak® C18 cartridge (SPC), part 51910 (Millipore Corporation, Milford, MA). Unlike the most commonly used paper chromatography (PC) and instant thin-layer chromatography method, the SPC method requires an activation process of the cartridge before it can be utilized for the RCP analysis. This complex cartridge analysis system is not only time-consuming, typically taking 8–10 min, but it is also more expensive, costing approximately $2.30 per cartridge. DuCret et al. (*9*) and Taylor et al. (*2*) have used the PC method with Whatman No. 3 or 3MM paper (Maidstone, England) as the stationary phase with acetone and distilled water or a 60/40 acetonitrile/water mixture as the mobile phases. In our laboratory we have measured the average time for developing the paper strip under those conditions and found it to be 15–20 min, making their PC methods undesirable (*2,9*). Therefore, the second objective of this investigation is to develop a simpler, cheaper, and more rapid radiochromatographic procedure for routine quality control of ^{99m}Tc-MAG3.

MATERIALS AND METHODS

Preparation of ^{99m}Tc-MAG3 with Microwave Oven

Following the package insert instructions (*5*), 5 ml of ^{99m}Tc sodium pertechnetate solution containing 100 mCi (3,700 MBq) was added to each of five MAG3 vials. When required, 0.9% sodium chloride injection, USP was used to dilute the $^{99m}TcO_4^-$ solution to a final volume of 5 ml. After the addition of 2 ml filtered air, each vial was agitated for 10 sec to allow oxidization of excess stannous ions and thus prevent the progressive formation of ^{99m}Tc-labeled impurities. Before placing the vial in the microwave oven, approximately 15–20 ml of argon gas was removed from the vial using a 20 ml syringe until a vacuum was created. A styrofoam cap was placed over the metal seal on the vial to prevent sparking. The vial was then put in the microwave oven (Kenmore,

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Model No. 565.8962781, Sears, Roebuck and Co., Chicago, IL) and heated for 13 sec at 452.5 W. The RCP of ^{99m}Tc-MAG3 was determined by the recommended SPC method at 5 min, 30 min, 1 hr, 3 hr, 6 hr, and 24 hr after reconstitution (as described below).

Reference Method for Determination of RCP of ^{99m}Tc-MAG3

This method was used as a reference standard because it is recommended in the MAG3 package insert (*5*). The procedures involve three major steps:

1. *Preparation of SPC.* Prior to performing the reverse phase chromatography with SPC, a gradient of strongly to weakly polar solvents needed to be washed through the cartridge in order to activate SPC. The nonpolar C18 bonded phase was first solvated with 10 ml of 200 proof ethanol and was followed by flushing the cartridge with 10 ml of 1 mN HCl. The cartridge was then drained by pushing 5 ml of air through the cartridge with a syringe.
2. *Sample Analysis.* One-tenth ml of ^{99m}Tc-MAG3 was applied to the long end of the cartridge. The SPC was then eluted successively with 10 ml of 1 mN HCl and 1:1 ethanol/ and 10-ml/ 0.9% NaCl solution. The two fractions of sample eluates and cartridge were collected in a culture tube for counting.
3. *Counting.* The radioactivity of the first sample elution (hydrophilic ^{99m}Tc impurity plus a fraction of hydrolyzed-reduced [H-R] ^{99m}Tc), the second sample elution (^{99m}Tc-MAG3), and the cartridge (the remaining H-R ^{99m}Tc plus non-elutable impurities) were assayed in a dose calibrator. The percentages of ^{99m}Tc-MAG3, hydrophilic ^{99m}Tc species, and H-R ^{99m}Tc were calculated by dividing each fraction of radioactivity with the total activity of both sample liquid fractions and the cartridge.

Proposed Method for Determination of RCP of ^{99m}Tc-MAG3

The new quality control method consisted of a two-strip mini-paper chromatograph (MPC) system. Two precut paper strips (1 cm × 8.5 cm) of the Solvent Saturation Pads (Gelman Sciences, Ann Arbor, MI) were used as the stationary phases and were developed with chloroform ($CHCl_3$):acetone (CH_3COCH_3):tetrahydrofuran (THF) (1:1:2) (Solvent 1) and 0.9% NaCl (Solvent 2) as the mobile phases (Table 1). After applying 5 μl samples of ^{99m}Tc-MAG3 to the origin of the two paper strips, the strips were placed in two glass tubes (Venoject® blood collection tube, Terumo Medical Corporation, Elkton, MD), which contained the mobile phase solvents. The glass tubes were capped with a rubber stopper to provide a solvent-saturated atmosphere. The percentage of ^{99m}Tc-MAG3 was calculated by subtracting the percent of activity at the origin of Solvent 2 from the percent of activity at the origin of Solvent 1. The percent of activity on the top-half of the Solvent 1 strip contributed the percentage of hydrophilic ^{99m}Tc, whereas the percent of activity remaining at the origin of the Solvent 2 strip provided the percentage of H-R ^{99m}Tc.

TABLE 1. R_f Values of the Radiochemical Species in ^{99m}Tc-MAG3 Preparation Using MPC System

Solvent	R_f Values		
	^{99m}Tc-MAG3	Hydrophilic ^{99m}Tc	H-R ^{99m}Tc
$CHCl_3/CH_3COCH_3$/THF (1:1:2)	0	0.5–1.0	0
0.9% NaCl	0.5–1.0	0.5–1.0	0

The capability of the MPC system to detect hydrophilic ^{99m}Tc species and H-R ^{99m}Tc was tested by analyzing samples from ^{99m}Tc sodium pertechnetate and ^{99m}Tc sulfur colloid with the MPC method.

Formulation of High, Low, and Intermediate RCP Levels of ^{99m}Tc-MAG3

In order to evaluate the accuracy of the proposed MPC system, ^{99m}Tc-MAG3 preparations with intermediate RCP levels ranging from 80%–86% and 87%–94% were chosen to compare the results of the new MPC procedure to those of the reference SPC procedure. The intermediate RCP ranges are critical given the ≥90% RCP specified in the package insert (*5*). Comparison studies were also undertaken to evaluate the overall accuracy performance of our new MPC system in determining both low RCP level (67%–78%) and high RCP level (95%–99%).

The formulation of the intermediate RCP values of ^{99m}Tc-MAG3 involved the preparation of two ^{99m}Tc-MAG3 kits: one of high RCP (normal preparation) and one of low RCP (as described below). The RCP of each preparation was first determined by the reference SPC method and then various volumes of each labeled vial were mixed to generate solutions of intermediate RCP. Both the reference SPC procedure and the new MPC procedure were applied to measure the intermediate RCP values simultaneously.

1. *High RCP Preparation.* The high RCP preparation was prepared by adding maximum allowable $^{99m}TcO_4^-$ activity of 100 mCi (3,700 MBq) to a MAG3 kit according to the package insert directions (*5*). The RCP level was measured by the reference SPC method (RCP should be on the order of 95% or higher ^{99m}Tc-MAG3).
2. *Low RCP Preparation.* A MAG3 kit was reconstituted with 100 mCi (3,700 MBq) ^{99m}Tc sodium pertechnetate. For this preparation, the ^{99m}Tc-MAG3 vial was allowed to sit at room temperature for 1–3 min and then was transferred to a precooled lead pig sitting in a crushed ice bath. The measurement of the RCP was performed with the reference SPC procedure. It is important to maintain the labeled vial at a cold temperature to minimize further changes in the measured RCP. At room temperature, the RCP continues to increase (*4*), therefore making it difficult to prepare solutions of the antic-

ipated intermediate RCP accurately. Under the ice-cold conditions, the RCP should be less than 50%.

3. *Solution with Intermediate RCP.* The following equations were applied to calculate the volumes of high and low RCP vials required to prepare the mixtures of intermediate RCP.

$$Vol_{lo} = \frac{a}{1 + a}, \quad Vol_{hi} = 1 - Vol_{lo}$$

where

$$a = \frac{[RCP_{hi}\ (b/c)] - [RCP_{int}\ (b/c)]}{RCP_{int} - RCP_{lo}},$$

Vol_{lo} is the volume (ml) of low RCP solution needed for the intermediate RCP solution, Vol_{hi} is the volume (ml) of high RCP solution needed for the intermediate RCP solution, RCP_{hi} is the measured RCP of the vial prepared in step 1, RCP_{int} is the desired RCP of intermediate solution (step 3), RCP_{lo} is the measured RCP of the vial prepared in step 2 + 1%, b is the amount of ^{99m}Tc activity (mCi) added to the kit in step 2, and c is the amount of ^{99m}Tc activity (mCi) added to the kit in step 1.

The calculated volumes of Vol_{hi} and Vol_{lo} were combined to obtain the intermediate RCP solution. Due to the gradual increase in RCP in the low RCP kit, the actual RCP of the intermediate RCP solution needed to be measured by the reference SPC method and the new MPC method simultaneously.

RESULTS

Preparation of ^{99m}Tc-MAG3 with Microwave Oven

Five MAG3 vials were used to evaluate the feasibility of using the microwave oven to replace the boiling water bath in our preparation of ^{99m}Tc-MAG3. Five milliliters of ^{99m}Tc activity ranging from 100.5 mCi (3,718.5 MBq) to 107.5 mCi (3,977.5 MBq) were added to each kit. The labeling efficiencies (% of primary complex) measured by the recommended SPC method were 99.5 ± 0.1% (5 min), 99.2 ± 0.4% (30 min), 99.4 ± 0.2% (1 hr), 99.3 ± 0.1% (3 hr), 99.0 ± 0.2% (6 hr), and 96.8 ± 0.3% (24 hr) (Fig. 1). The overall average RCP value of the five ^{99m}Tc-MAG3 kits was 98.9 ± 1.0% (n = 30) during the 24-hr evaluation period.

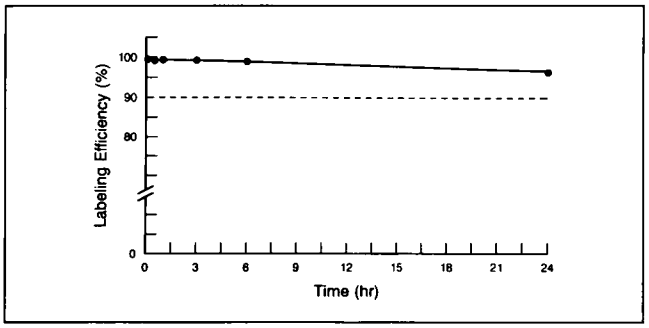

FIG. 1. Plot of labeling efficiency of ^{99m}Tc-MAG3 prepared with microwave oven heating method. Each point is the mean ± s.d. for five kits. The dotted line represents the minimum acceptance level of RCP (90%).

Comparison of the New MPC Method and the Reference SPC Method

The average time for performing the RCP determination with the recommended SPC procedure (preparation of SPC, sample analysis and sample counting) was 8–10 min whereas it took 2–3 min to complete the RCP measurement with the new MPC procedure (developing and counting two paper strips).

The results from analyzing the samples of $Na^{99m}TcO_4$ (n = 6) and ^{99m}Tc sulfur colloid (n = 6) with the MPC method indicated that the free ^{99m}Tc migrated to the top half of the strip in 1:1:2 $CHCl_3/CH_3COCH_3$/THF (R_f = 0.5–1) and moved with 0.9% NaCl to R_f = 1, whereas radioactivity of ^{99m}Tc sulfur colloid remained at the origin (R_f = 0) in both solvent systems.

Comparison of the results of MPC and SPC methods show no significant difference for the determinations of low RCP levels (67%–78%), intermediate RCP levels (80%–86% and 87%–94%), and high RCP levels (95%–99%) (Fig. 2 and Table 2). The overall differences of the RCP values measured by both techniques were very small in the intermediate ranges (difference = 0.9 ± 0.6%, n = 33). Among the 17 preparations of ^{99m}Tc-MAG3 with RCP values in the range of 87%–94%, RCPs determined by the standard SPC procedure indicated that eight preparations were higher than the RCP acceptance lower limit (90% RCP), whereas the RCPs of the other nine

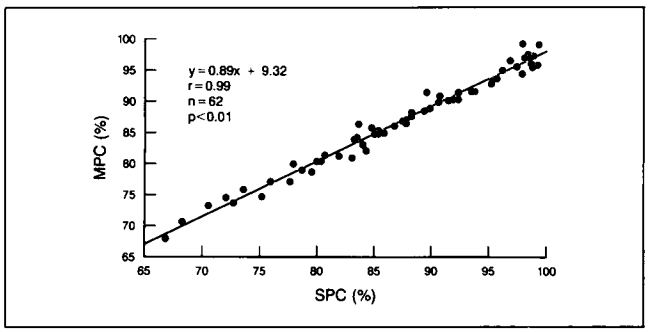

FIG. 2. Radiochromatographic analysis comparison between SPC and MPC methods in the measurement of RCP (67%–99%) of ^{99m}Tc-MAG3.

TABLE 2. Comparison Between SPC* and MPC† Analytic Systems in Determining RCP

RCP range (%)	n	Difference‡ (%)
67–78	11	1.6 ± 0.9
80–86	16	0.9 ± 0.7
87–94	17	1.0 ± 0.6
95–99	18	1.5 ± 0.9

* Sep-Pak® C18 cartridge system.

† Mini-paper chromatography. (1) 1:1:2 $CHCl_3/CH_3COCH_3$/THF. (2) 0.9% NaCl.

‡ Difference (%) = SPC (%) − MPC (%). All values are expressed as $\bar{x}$ ± s.d.

solutions were below the 90% limit. The numbers of acceptance and rejection in the same preparations (RCP levels of 87%–94%) were matched by the new MPC method with only one exception (89.6% by SPC versus 91.6% by MPC).

DISCUSSION

The radiolabeling of ^{99m}Tc-MAG3 with a microwave oven significantly reduces the heating period (13 sec) and provides excellent labeling efficiency (98.9 ± 1.0%, n = 30). The small standard deviations of the mean labeling efficiencies and the high RCP values throughout the 24-hr period for the five ^{99m}Tc-MAG3 kits prepared with a microwave oven (Fig. 1) are good indications that a microwave oven can be utilized to prepare ^{99m}Tc-MAG3. However, there are several technical variables related to the usage of the microwave oven that need to be considered and resolved.

1. The heating period is related to the liquid volume in the vial, the geometric location of the vial in the microwave oven, and the type of microwave oven. One has to determine the optimum operation conditions (e.g., heating time and wattage) by trial and error. In order to keep the vial in the same position inside the microwave oven, a plastic vial holder can be mounted in the center of the tray.
2. Any residual gas volume left in the head space of the vial may cause an explosion due to excess steam pressure built up inside the vial during the microwave heating process. It is very important to make certain that a vacuum condition is achieved before the vial is placed in the microwave oven.
3. The metal cap on the ^{99m}Tc-MAG3 vial should be covered with styrofoam to avoid sparking.
4. It may not be necessary to lead shield the microwave oven since the heating time is very short (13 sec). However, the ALARA (as low as reasonably achievable) concept should still apply during the entire process: use a grip tong to manipulate the labeled vial and keep a distance away from the microwave oven while it is in use.

The introduction of ^{99m}Tc-MAG3 has resulted in a second required radiochromatographic RCP procedure for a ^{99m}Tc-labeled radiopharmaceutical and the first description of using the SPC method for RCP determination (*5*). The SPC can provide a more complete chromatographic separation of the ^{99m}Tc-labeled complexes. However, the complex procedure, costly cartridge, and lengthy process make the SPC method less than ideal for routine use in a busy nuclear medicine department or commercial nuclear pharmacy.

Another disadvantage associated with the application of SPC in the radiochromatographic analysis of ^{99m}Tc-MAG3 is the increased radiation exposure for the person performing the procedure. The SPC method requires 0.1 ml of ^{99m}Tc-MAG3 preparation to be loaded into the cartridge (*5*), which may consist of ^{99m}Tc radioactivity between 0.5 mCi (18.5 MBq) and 2.5 mCi (92.5 MBq) per application. The proposed MPC system utilizes two-strip PC, which only requires two 5-μl samples (50 μCi–250 μCi; 1.9 MBq–9.3 MBq) for the RCP analysis and it takes less than 3 min to complete the RCP determination. The RCP values determined by our MPC system were closely correlated with the recommended SPC method (Fig. 2). The RCP measurements using the two techniques were very close in the crucial intermediate ranges, 80%–86% (difference = 0.9 ± 0.7%, n = 16) and 87%–94% (1.0 ± 0.6%, n = 17) (Table 2).

In conclusion, we suggest that the combined usage of a microwave oven heating method and the expedient MPC testing system will provide a fast and reliable method for the preparation and quality control of ^{99m}Tc-MAG3.

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