

Evaluation of the Hepatobiliary Excretion of Technetium-99m-MAG3 and Reconstitution Factors Affecting Radiochemical Purity

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Technetium-99-MAG3 is a renal tubular function agent. However, sporadic liver and gallbladder visualization have raised questions about kit stability, impurities and nonrenal routes of excretion. To address these issues, studies were conducted to optimize the labeling efficiency of the Technescan MAG3 kit and to evaluate the hepatobiliary excretion of the MAG3 complex.

Methods: Thirty-six vials of the commercial formulation of ^{99m}Tc -MAG3 were prepared according to manufacturer's instructions and evaluated for radiochemical purity using two methods: a combination of high-performance liquid chromatography and paper chromatography (HPLC/PC); and the manufacturer's miniature chromatography system (Sep-Pak procedure). **Results:** The labeling efficiency was significantly higher when the kit was reconstituted with 10 ml (96.6%) of saline versus 5 ml (91.4%) ($p < 0.01$). The radiochemical purity of the kits remained stable for up to 6 hr, but the purity determined by Sep-Pak averaged 2.5% higher than that determined by HPLC procedures ($p < 0.01$). Rat studies to evaluate renal and hepatobiliary elimination of MAG3 showed no difference in the %ID excreted into the urine by 60 min in all groups of animals studied. However, the %ID excreted into the bile was significantly higher for the kit formulation than the HPLC-purified MAG3, 9.9% versus 6.6% ($p = 0.0475$). **Conclusion:** The radiochemical purity of the Technescan MAG3 kit can be improved by reconstituting with larger volumes. In addition, the studies in rats suggest that fasting or kit impurities may be a contributing factor to increased hepatobiliary visualization in patient studies.

Key Words: technetium-99m-MAG3; hepatobiliary excretion; radiochemical purity

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Technetium-99m-mercaptoacetyltriglycine (MAG3) is a renal tubular function agent introduced to replace ^{131}I o-iodohippurate (OIH) (1-3). The ^{99m}Tc label and an easy

to prepare kit has facilitated its widespread clinical acceptance (4-19). Technetium-99m-MAG3 is primarily cleared by the kidneys by tubular secretion (17,20,21), although there is a very small glomerular filtration component (18). Recent conflicting reports, however, have suggested that ^{99m}Tc -MAG3 may also be cleared by the hepatobiliary system.

Hepatic uptake and excretion may interfere with diagnostic kidney images and invalidate measurements of renal clearance based on plasma sampling. In a study with HPLC-purified ^{99m}Tc -MAG3, Taylor et al. (22) reported that the complex had minimal accumulation in the liver and gallbladder with one of six patients demonstrating minimal biliary activity. Biodistribution studies of the ^{99m}Tc -MAG3 kit formulation in normal volunteers demonstrated that approximately 2% of the injected dose was eliminated through the hepatobiliary system, however, in three patients with severe renal failure, 10% of the dose was present in the gastrointestinal tract at 210 min (4).

Other investigators have also reported sporadic liver and gallbladder activity. Some of the apparent hepatic activity may be due to retention of ^{99m}Tc -MAG3 in the intravascular pool of the liver, however, this would not account for gallbladder visualization (8,14,23-26). Russell et al. (14) have reported occasional liver visualization on ^{99m}Tc -MAG3 images but not on OIH images. Jafri et al. (8) report that the ^{99m}Tc -MAG3 images showed slight hepatic uptake which was unrelated to the level of renal function. The majority of the published reports of sporadic liver and hepatobiliary activity have been associated with the commercial kit formulation of ^{99m}Tc -MAG3. These kits produce ^{99m}Tc -MAG3 in high yields, however, there are some radiolabeled impurities which may localize in the liver or gallbladder (31).

Technetium-99m-MAG3 has also been used to quantitate renal function. Although the plasma clearance of ^{99m}Tc -MAG3 ranges from 50% to 65% of that of OIH, there is good correlation between the two clearances (2-5,9-11,27-30). Simplified one- or two-plasma sample techniques have been developed to be used in a clinical setting, but the plasma clearance of an agent reflects clearance by all elim-

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ination pathways including uptake by the hepatocytes and hepatobiliary excretion. If there is significant accumulation by the hepatocyte with subsequent hepatobiliary elimination of ^{99m}Tc -MAG3, the plasma activity would decrease, the calculated clearance would increase and renal clearance would be overestimated.

This study was designed to (1) determine different activities and volumes which can be used to reconstitute the commercially available United States kit formulation and affect radiochemical purity; (2) determine the charge characteristics of the ionizable carboxylate group as an uncharged complex which may be reabsorbed from the tubular lumen; (3) use a rat model to quantitate the hepatobiliary excretion of ^{99m}Tc -MAG3 as well as identify the radiochemical form which is excreted into the urine and bile and evaluate situations which may affect the hepatobiliary excretion; and (4) evaluate the rate of hepatobiliary excretion of ^{99m}Tc -tartrate, an intermediate formed in the kit preparation of ^{99m}Tc -MAG3.

MATERIALS AND METHODS

Radio pharmaceutical Preparation

Preparation of the Kit Formulation of ^{99m}Tc -MAG3. Thirty-six vials of the commercial formulation of ^{99m}Tc -MAG3 (Technescan MAG3, Mallinckrodt Medical, St Louis, MO) were prepared according to the manufacturer's instructions [lot #096001A ($n = 13$) and #0962003A ($n = 23$)]. Sodium pertechnetate was obtained from $^{99}\text{Mo}/^{99m}\text{Tc}$ commercial generator systems (Mallinckrodt Medical or E.I. duPont, N. Billerica, MA). After eluting the generator, the desired activity 50 or 100 mCi, was diluted to either 5 or 10 ml using saline obtained from plastic (Abbott Labs, North Chicago, IL) or glass (Kendall McGaw Inc., Irving, CA) vials. After the addition of the sodium pertechnetate, 2 ml of filtered air was added to the vial, the vial was agitated vigorously for 15 sec then placed in a rolling water bath for 10 min.

Synthesis and Purification of ^{99m}Tc -MAG3. Approximately 40–50 mCi of ^{99m}Tc -sodium pertechnetate in 0.5 ml was obtained from a commercial $^{99m}\text{Tc}/^{99}\text{Mo}$ generator (Mallinckrodt Medical or E.I. duPont) and added to 20 mg sodium glucoheptonate and 0.006 mg of stannous chloride in 0.1 ml of water. The solution was allowed to incubate for approximately 5 min and then added to the S-benzoyl protected mercaptoacetyl triglycine (S-Bz MAG3) ligand. After incubation, the reaction vial was heated at 95°C for 15 min. The resulting solution was purified by high-performance liquid chromatography (HPLC) using a 5- μ , 4.6 \times 250-mm C-18 ODS reverse-phase column (Ultrasphere ODS Beckman Instruments, San Ramon, CA or Microsorb, Rainin Instruments, Woburn, MA) with a 1% ethanol/0.01 M sodium phosphate buffer at a pH of 6.9. The outflow from the tubing was coupled to a gamma scintillation detector, the output being directed to an integrator recorder (Hewlett-Packard HP 3390A, Wilmington, DE). Technetium-99m-MAG3 had a retention time of approximately 8 min and was collected as it eluted from the HPLC column. A sample of the purified material was evaluated by HPLC to verify the radiochemical purity of the collection.

Quality Control Testing of the Kit Formulation of ^{99m}Tc -MAG3. Two methods were used to evaluate the radiochemical purity of the kit formulation over a 6-hr period: (1) a combination of high-performance liquid chromatography and paper chromatog-

raphy (HPLC/PC procedure) and (2) the manufacturer's miniaturized chromatography system (Sep-Pak procedure).

HPLC analysis used a NovaPak C-18, 5- μ m, 3.9 \times 160-mm column (Waters Chromatography Division, Milford, MA) as the stationary phase and a gradient solvent system consisting of 0% or 8% tetrahydrofuran (THF)/0.01 M potassium phosphate buffer with 1 ml triethylamine at a pH of 5.0. The HPLC mobile phase was run at a flow rate of 1 ml/min and over a linear gradient, starting at 0% THF and increasing to 8% THF over 30 min. The outflow from the tubing was coupled to a Beckman Model 170 gamma scintillation detector which had lead attenuators placed between the crystal and the outflow tubing. Paper chromatography utilized Whatman 3MM paper (Maidstone, England) as the stationary phase with a 60:40 acetonitrile-to-water mixture as the mobile phase. The radiochemical purity results were assayed by a Canberra 35+ scintillation well counter (Canberra Instruments, Meriden, CT). All samples evaluated were within the linear range of the detector.

Radiochemical purity testing utilizing the manufacturer's recommended miniaturized chromatography system was performed simultaneously with the HPLC/PC procedure. A C-18 Sep-Pak cartridge (Waters Chromatography, Milford, MA) was activated by eluting 10 ml of 100% ethanol through the column followed by 10 ml of 0.001 N HCl. The cartridge was then drained by flushing it with 5 ml of air. A 0.1-ml sample of the reconstituted kit was loaded onto the top of the cartridge, 10 ml of 0.001 N HCl was eluted through the column with the eluant collected into a test tube, this was followed by eluting the column with a 1:1 mixture of 100% ethanol:0.9% sodium chloride solution which was also collected into a test tube. The Sep-Pak cartridge was then placed into a test tube and all of the samples were assayed in a dose calibrator (Capintec Radioisotope Calibrator, Model CRC-7, Ramsey, NJ) to determine the activity in each fraction.

Preparation of the Kit Formulation of ^{99m}Tc -Tartrate. Technetium-99m-sodium pertechnetate, 0.5 ml of (10–20 mCi), from a commercial $^{99m}\text{Tc}/^{99}\text{Mo}$ generator was added to a stannous tartrate kit (RhoMed Inc., Albuquerque, NM). The solution was allowed to incubate for approximately 30 min. The ^{99m}Tc -tartrate was initially purified on the NovaPak C-18 column using the 0% to 8% THF gradient buffer system. The ^{99m}Tc -tartrate had a retention time of 2–3 min with ^{99m}Tc -sodium pertechnetate having a retention time of approximately 9–10 min. The ^{99m}Tc -tartrate was collected as it eluted from the column and was repurified prior to animal studies by injecting the collected material onto a C-18 ODS reverse-phase column; using a 1% ethanol/0.01 M phosphate buffer at a pH 6.9. The retention time of the ^{99m}Tc -tartrate using the 1% ethanol/0.01 M phosphate buffer was 2–3 min and was collected as it eluted from the HPLC column.

Charge Characteristics of the Ionizable Carboxylate Group

The x-ray crystal structure of ^{99m}Tc -MAG3 has shown that the metal is complexed through one sulfur and three amide nitrogens and contains a free carboxylate group (Fig. 1) (15). The charge properties of the ionizable carboxylate group of ^{99m}Tc -MAG3 were evaluated by anion exchange HPLC. Technetium-99m-MAG3 was initially prepared and purified by reverse phase HPLC using a 1% ethanol/0.01 M sodium phosphate buffer. The purified ^{99m}Tc -MAG3 was subsequently injected onto an anion exchange column (Beckman SAX column, Beckman Instruments, San Ramon, CA) which used a mobile phase consisting of a 0.01 M sodium phosphate/0.01 M sodium sulfate at pH values ranging

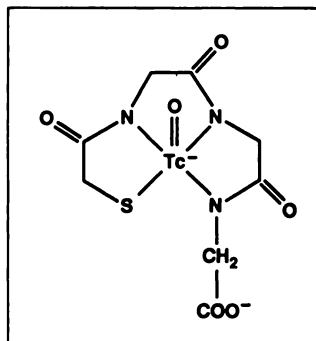


FIGURE 1. Crystal structure of ^{99m}Tc -MAG3.

from 2.40 to 6.08 (2.40, 2.50, 2.59, 2.81, 3.00, 3.21, 3.41, 3.84, 4.37, 4.68, 5.65 and 6.08).

Urine and Hepatobiliary Excretion of ^{99m}Tc -MAG3 and ^{99m}Tc -Tartrate. Male Sprague-Dawley rats weighing between 250 and 350 g were used for these experiments. The animals were anesthetized with ketamine HCl 100 mg/kg body weight and supplemented as needed. The anesthetized rats were placed on a heated surgical table, a tracheostomy was performed, the bladder was cannulated with heat-flared PE 50 tubing, the jugular vein was cannulated with two PE 50 catheters and the bile duct was cannulated with PE 10 tubing which was coupled to PE 50 tubing. Core temperature was continuously monitored and maintained throughout all surgical procedures and for the duration of the study. To maintain adequate hydration, saline was continuously infused (4 ml/hr) through one of the jugular vein catheters. After the animals had stabilized, the control rats received a 2–6-mCi bolus injection of the HPLC-purified ($n = 6$) or the kit formulation ($n = 5$) of ^{99m}Tc -MAG3 administered intravenously through the second jugular line followed by a 0.5-ml normal saline flush. The activity in the syringe was measured immediately prior to and following the injection of the ^{99m}Tc -MAG3 to determine the net activity injected. Following injection, bile and urine samples were collected at 10-min intervals for 1 hr with all samples being assayed in a dose calibrator.

To evaluate the excretion of ^{99m}Tc -tartrate, a third group of rats ($n = 5$) received a 2–10- μCi bolus injection which was administered through the second jugular line. A significantly lower dose of ^{99m}Tc -tartrate was used because of the dual HPLC purification steps required to isolate the complex. After injection, the line was flushed with 0.5 ml of normal saline. Urine and bile samples were collected at 10-min intervals and the activity excreted into the urine and bile was determined by counting them in an automated gamma well counter (Tracor Analytic Model 1197 Automated Gamma System, Des Plaines, IL) along with a 1:100 dilution of the injected dose. All samples were background subtracted and decay corrected to the time of dose administration.

Chromatographic Analysis of Urine and Bile

Zero to 10-min urine and bile samples were collected following the administration of HPLC-purified and the reconstituted kit formulation of ^{99m}Tc -MAG3. The urine and bile samples were evaluated by HPLC using a Ultrasphere ODS or Microsorb C-18 column and a 1% ethanol/0.01 M sodium phosphate buffer. The urine and bile were also mixed with equal activities of the purified material and then injected onto the HPLC column. Urine and bile samples obtained after the injection of the kit formulation of ^{99m}Tc -MAG3 were also evaluated by HPLC using the NovaPak C-18 column and the 0%–8% THF gradient solvent system. The kit formulation was evaluated on the NovaPak column. This sys-

tem has been shown to separate kit impurities (9) which the Ultrasphere or Microsorb C-18 columns with a 1% ethanol/0.01 M sodium phosphate buffer could not.

Hepatic Elimination Study

To determine the mechanism of hepatobiliary elimination for ^{99m}Tc -MAG3, a group of rats were surgically prepared as previously described. Prior to the bolus injection of HPLC-purified ^{99m}Tc -MAG3 (2–6 mCi), indocyanine green (ICG) was infused at a rate of 0.4 mg/kg body weight/min for 30 min (32). After the 30-min infusion period, a bolus injection of ^{99m}Tc -MAG3 was administered through the second jugular line while maintaining the ICG infusion. Urine and bile were collected at 10-min intervals for 60 min and assayed in a dose calibrator.

Hepatobiliary Elimination of ^{99m}Tc -MAG3 in Fasting Rats and Rats Administered Protein Bound ^{99m}Tc -MAG3

Two groups of rats were studied to determine what effects fasting and a prior incubation with plasma proteins would have on the hepatobiliary excretion of ^{99m}Tc -MAG3. One group of rats was fasted for 24 hr prior to surgical preparation. Following surgery, a bolus injection of HPLC-purified ^{99m}Tc -MAG3 was administered followed by bile and urine collections at 10-min intervals for 60 min. In the second group of animals, HPLC-purified ^{99m}Tc -MAG3 was incubated with plasma from a donor rat for 10–60 min. Immediately prior to administration, the percent of ^{99m}Tc -MAG3 that was plasma protein bound was determined utilizing an ultrafiltration technique (Centrifree Micropartition System, Amicon Corp., Danvers, MA) (1,17,21). After injection, bile and urine samples were collected at 10-min intervals for 60 min.

Statistical Analysis

Statistical analysis was performed using a Student's t-test where differences giving a $p < 0.05$ were considered significant.

RESULTS

HPLC Preparation of ^{99m}Tc -MAG3

The radiolabeling of ^{99m}Tc -MAG3 by transchelation from ^{99m}Tc -glucoheptonate typically produced a labeling efficiency of greater than 80%. After HPLC separation, the collected material was greater than 99.9% radiochemically pure.

Radiochemical Purity of the Kit Formulation

The radiochemical purity at 30 min and 6 hr for the commercial kit formulation evaluated by HPLC/PC and the Sep-Pak procedures are listed in Table 1. The results for 36 vials reconstituted using saline obtained from plastic ($n = 16$) and glass ($n = 20$) are listed. There was no significant difference when comparing the radiochemical purity of kits reconstituted with pertechnetate diluted with saline from plastic or glass vials or between different generator manufacturers. Kits were radiolabeled with either 100.2 ± 5.6 mCi or 54.1 ± 2.3 mCi of pertechnetate in 5 ml of saline or 102.7 ± 2.6 mCi or 53.0 ± 2.4 mCi of pertechnetate in 10 ml of saline. The radiochemical purity determined by HPLC/PC was significantly higher at 30 min when the kits were reconstituted with 10 ml of saline (96.6% compared to 91.4% with 5 ml of saline ($p < 0.01$)).

TABLE 1
Radiochemical Purity Results for ^{99m}Tc Technescan MAG3 at 30 Minutes and 6 Hours Postreconstitution Using 5 and 10 ml of Saline from Glass and Plastic Containers*

Radiochemical purity determined by:	5-ml volume	10-ml volume
HPLC/PC		
30 min	91.4 (± 3.5)	96.6 (± 1.5)
6 hr	90.8 (± 1.5)	96.5 (± 1.2)
Sep Pak		
30 min	94.5 (± 2.7)	98.5 (± 1.3)
6 hr	94.4 (± 2.2)	98.0 (± 1.1)

*Purity was determined using HPLC/PC and Sep-Pak techniques. Mean (± 1 s.d.)

Similar results were obtained at 6 hr postreconstitution. In a recent study, Nosco et al. (38) reported that reconstitution volumes of less than 4 ml generally produce a radiochemical purity of less than 90%. Utilizing saline from plastic vials produced a radiochemical purity at 30 min ranging from 89.2% to 97.3% by HPLC/PC and 92.7% to 99.1% using the Sep-Pak procedure. Similarly, the kits reconstituted with saline from glass vials produced an initial radiochemical purity ranging from 89.5% to 97.2% by HPLC/PC and 93.7% to 99.2% by Sep-Pak procedures. The radiochemical purity determined by Sep-Pak averaged 2.5% higher than the radiochemical determined by the HPLC/PC procedures ($p < 0.01$). Regardless of the method used for radiochemical purity evaluation the kits remained stable for the 6-hr testing period.

pKa of the Ionizable Carboxylate Group

To determine the pKa of the ionizable carboxylate group, the pH of the mobile phase was plotted as a function of retention time with the inflection point or the pKa value of the curve occurring at a pH of 4.27. Utilizing similar methods, this value was similar to the pKa value determined for ^{99m}Tc CO₂DADS (33).

Renal and Hepatobiliary Elimination of ^{99m}Tc -MAG3

The results of the renal and hepatobiliary elimination of ^{99m}Tc -MAG3 for the rat studies are listed in Table 2. The

percent of the injected dose excreted into the urine at 60 min for the kit formulation and the HPLC-purified ^{99m}Tc -MAG3 was 85.0% and 84.1%, respectively (Fig. 2), which were not significantly different. However, the 9.9% of the injected dose excreted into the bile following the kit formulation was significantly greater than the 6.6% for the HPLC-purified ^{99m}Tc -MAG3 ($p = 0.0475$) (Fig. 3). The radiochemical purity of the kit formulation used for these studies was 95.6% as determined by HPLC/PC and 97.0% as determined by Sep-Pak.

HPLC evaluation of the kit formulation of ^{99m}Tc -MAG3 demonstrated multiple radioactive components in the kit formulation; these same peaks were also found in the urine and bile samples when evaluated by HPLC. Blocking the anionic transport system of the liver by infusing the rats with indocyanine green prior to injecting ^{99m}Tc -MAG3 significantly decreased the amount excreted into the bile (2.1%) ($p = 0.00536$) (Fig. 3). Fasting the rats for 24 hr prior to ^{99m}Tc -MAG3 injection significantly increased the amount of the injected dose excreted into the bile (11.7%) over control animals ($p = 0.00905$) (Fig. 3). Pre-incubation of ^{99m}Tc -MAG3 with donor rat plasma resulted in an average protein binding of $82.7\% \pm 8.0\%$ which was similar to literature values (17,21) and there was no significant difference in the percent bound when incubated for 10–60 min. This prebinding of ^{99m}Tc -MAG3 did not change the percent of the injected dose which was excreted into the bile (Fig. 3). In all cases, over 50% of the total biliary activity was excreted into the bile within the first 10 min (Table 2). There was no difference in the urinary excretion of HPLC-purified ^{99m}Tc -MAG3 in control rats compared to rats infused with indocyanine green, fasting rats and rats administered plasma protein-bound ^{99m}Tc -MAG3 (Fig. 2).

Technetium-99m-Tartrate: In Vitro and In Vivo Results

Technetium-99m-tartrate was a minor component found in the kit formulation when evaluated by HPLC. The radio-labeling efficiency of the ^{99m}Tc -tartrate kit formulation typically produced the complex in greater than 95% yields. Intravenous administration of the HPLC-purified ^{99m}Tc -tartrate in five rats resulted in $43.8\% \pm 14.9\%$ (mean ± 1 s.d.) of the injected dose being excreted into the urine in 60 min with only $1.3\% \pm 0.4\%$ (mean ± 1 s.d.) of the injected

TABLE 2
Summary of Rat Studies Comparing the Cumulative Percent of the Dose Excreted Into the Urine and Bile in 60 Minutes

	(n)	Urine (60 min)	Bile (60 min)	Bile (10 min)
HPLC-purified ^{99m}Tc -MAG3	(6)	84.1 (± 4.4)	6.6 (± 2.9)	3.7 (± 2.0)
Technescan MAG3 kit formulation	(5)	85.0 (± 2.3)	9.9 (± 1.6)	5.1 (± 1.2)
Infusion of ICG	(6)	88.1 (± 4.4)	2.1 (± 1.0)	1.1 (± 0.6)
Fasting	(6)	80.4 (± 2.1)	11.7 (± 2.6)	6.1 (± 1.5)
Plasma protein-bound MAG3	(8)	87.6 (± 6.9)	6.6 (± 2.4)	3.8 (± 1.6)

Mean (± 1 s.d.)

dose being excreted into the bile in the same time period. Within the first 10 min, the average excretion into the bile was only $0.2\% \pm 0.1\%$ (mean \pm 1 s.d.) of the injected dose.

Metabolism Study of ^{99m}Tc -MAG3

HPLC tracings for the crude ^{99m}Tc -MAG3 preparation labeled utilizing a transchelation reaction from ^{99m}Tc -glucoheptonate, the purified ^{99m}Tc -MAG3, along with urine and bile samples are also illustrated in Figure 4. There was a single major peak which eluted from the HPLC column after injection of the urine and bile samples (Figs. 4C D) using a C-18 ODS reverse-phase column with a 1% ethanol/0.01 M sodium phosphate buffer. In the bile sample, there were two earlier eluting peaks but the total activity associated with these peaks was less than 0.1%. Figures 4E-F show the results of mixing urine and bile with equal activities of HPLC-purified ^{99m}Tc -MAG3. In these samples there was a single major peak which eluted from the HPLC column which corresponded to ^{99m}Tc -MAG3.

DISCUSSION

The S-benzoyl-protected MAG3 ligand can be radiolabeled with ^{99m}Tc by a transchelation reaction in excellent yields. Optimal kit labeling occurred when the kit was reconstituted in larger volumes (10 ml) producing an average radiochemical purity of greater than 97% by HPLC/PC and greater than 99% by Sep-Pak. A comparison of saline obtained from glass or plastic vials was evaluated as both types of vials are routinely used. Plastic containers have been reported to be less inert than glass and have the disadvantage of two-way permeability of gasses and may leach materials into the vial contents (39,40). In spite of these potential limitations, there was no significant difference in the kit radiolabeling when using saline obtained from either plastic or glass containers. There was a significant difference in the radiochemical purity when evaluated by HPLC/PC or by Sep-Pak, with the Sep-Pak reporting

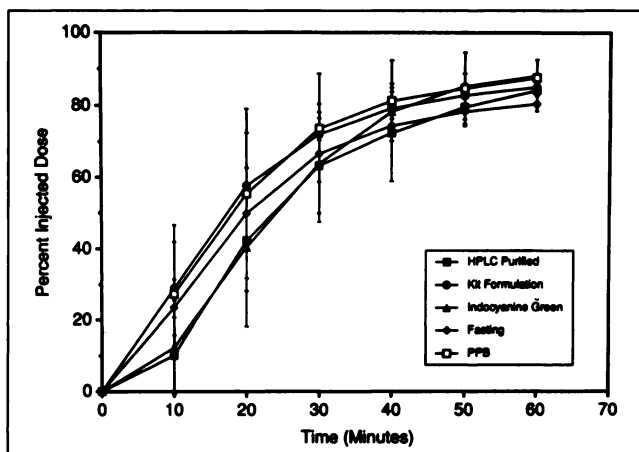


FIGURE 2. Cumulative urinary excretion of the HPLC-purified ^{99m}Tc -MAG3, the kit formulation of ^{99m}Tc -MAG3, rats infused with indocyanine green 30 min prior to injection of HPLC-purified ^{99m}Tc -MAG3. Rats fasted for 24 hr and rats administered plasma protein-bound ^{99m}Tc -MAG3.

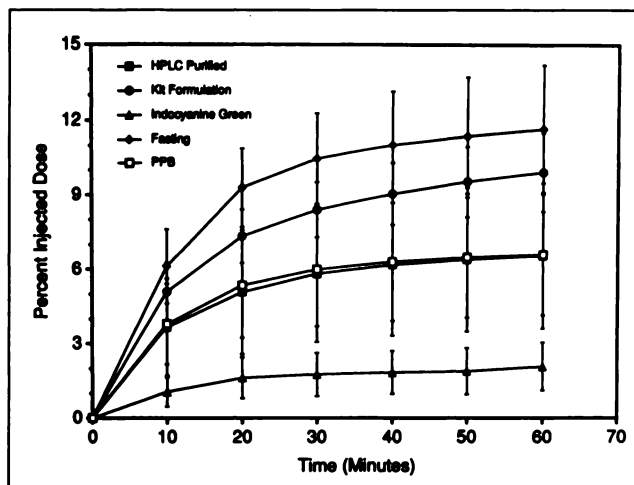


FIGURE 3. Cumulative biliary excretion of the HPLC-purified ^{99m}Tc -MAG3, the kit formulation of ^{99m}Tc -MAG3, rats infused with indocyanine green 30 min prior to injection of HPLC-purified ^{99m}Tc -MAG3. Rats fasted for 24 hr and rats administered plasma protein-bound ^{99m}Tc -MAG3.

higher radiochemical yields. Regardless of the method of quality control used, or the activity or volume added within the manufacturer's guidelines, the kit formulation was found to be stable over the recommended 6-hr shelf-life, which is in agreement with other reports (19,38).

Technetium-99-MAG3 has a free carboxylate group which extends from the third amide nitrogen (Fig. 1) (15). The complex has been reported to be negatively charged by electrophoresis (31). This study confirms that the ^{99m}Tc complex is negatively charged and dianionic at physiological pH, with the pKa of the ionizable carboxylate group being 4.27. Urine pH may be as low as 4.5 (37), therefore some of the ^{99m}Tc -MAG3 complex in the urine may be in the monoanionic form. The monoanionic form may have different passive reabsorption properties than the dianionic form; however, we would expect the dianionic form to predominate under all conditions with minimal reabsorption occurring for either charged species.

In control rats injected with HPLC-purified ^{99m}Tc -MAG3, 84.1% of the injected dose was excreted into the urine by 60 min and 6.6% was excreted into the bile. The ^{99m}Tc -MAG3 complex was eliminated unchanged when excreted by either the kidneys or the liver. There was no significant difference in the cumulative 60-min urine excretions in any of the animal groups. There were differences, however, in the amount of hepatobiliary elimination. There was a significantly higher percent ($p = 0.047$) of the injected dose excreted into the bile for the kit formulation of ^{99m}Tc -MAG3 when compared to HPLC-purified ^{99m}Tc -MAG3. The HPLC-purified MAG3 was greater than 99.9% radiochemically pure whereas the kit formulation reconstituted with 10 ml of saline was 95.6% radiochemically pure by HPLC. This increase in hepatobiliary elimination of the ^{99m}Tc activity was associated with the excretion of radio-labeled kit impurities.

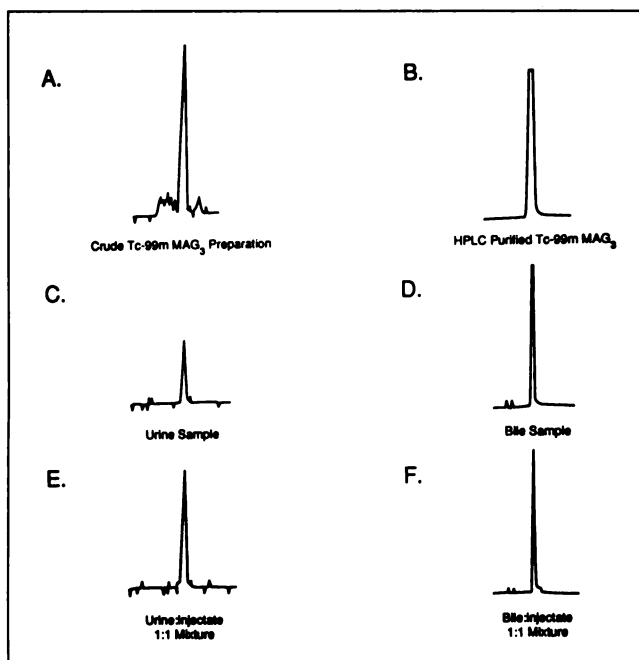


FIGURE 4. HPLC tracing of (A) the crude preparation of ^{99m}Tc -MAG3, (B) HPLC-purified ^{99m}Tc -MAG3, (C) Urine radioactivity, (D) Bile radioactivity, (E) Urine radioactivity mixed with equal activities of the HPLC-purified ^{99m}Tc -MAG3 and (F) Bile radioactivity mixed with equal activities of HPLC-purified ^{99m}Tc -MAG3.

Since ^{99m}Tc -MAG3 is dianionic at physiologic pH, it is likely to be transported by the hepatocytes using the anionic transport system. This active transport system also transports bromosulphophthalein and indocyanine green (32). The infusion of indocyanine green prior to the injection of HPLC-purified ^{99m}Tc -MAG3 significantly decreased ($p = 0.0055$) its biliary excretion, compared to control animals. This finding supports the hypothesis that ^{99m}Tc -MAG3 utilizes the anionic transport system.

Fasting the rats significantly increased the percent of the injected dose of ^{99m}Tc -MAG3 which was excreted through the hepatobiliary system ($p = 0.0091$). This finding was similar to that reported by Tyler et al. (34) who observed that fasting patients administered ^{99m}Tc -glucoheptonate had a higher incidence of gallbladder visualization. Alternatively, ^{99m}Tc -MAG3 is a modified tetrapeptide and as such, in protein-poor diets or in starvation, there may be an increase in mobilization to the liver similar to that reported for other amino acids (35,36). However, this liver uptake may be competitively inhibited by other peptides in the nonfasting state, thereby reducing the hepatobiliary excretion of ^{99m}Tc -MAG3.

The plasma protein binding of ^{99m}Tc -MAG3 has been reported to range from 79% to 90% with the major route of elimination occurring through the kidneys. The difference in the selectivity of ^{99m}Tc -MAG3 by the kidney or the liver is probably related to differences in its affinity for the anionic transport proteins found in the two organs. If transport by the hepatocytes requires the compound to be free in the Space of Disse before it is bound to the anionic

transport site, then pre-incubation of ^{99m}Tc -MAG3 with proteins would potentially minimize the amount transported and thus minimize hepatobiliary excretion (41,42). There was however, no significant difference in the amount excreted into the bile when compared to the control animals. This finding suggests that hepatocyte extraction does not require a compound to be free, or that binding to plasma proteins is not sufficiently strong to prevent dissociation of the ^{99m}Tc -MAG3 complex as it passes through the liver, or that binding to plasma proteins is almost instantaneous.

Since ^{99m}Tc -tartrate is an intermediate and minor radiochemical impurity formed in the commercial kit preparation, we evaluated its renal and hepatobiliary excretion in rats. Technetium-99m-tartrate did not show any significant excretion by the hepatobiliary system which suggests that its presence in the commercial kit formulation would not contribute significantly to gallbladder activity.

In conclusion, reconstituting with 10 ml of saline optimized the labeling efficiency of Technescan MAG3, the commercially available MAG3 kit. In rats, both ^{99m}Tc -MAG3 and kit impurities were excreted through the hepatobiliary system. Technetium-99m-MAG3 is actively transported and excreted via the liver using the anionic transport system with fasting increasing the hepatobiliary excretion. These factors may all contribute to the sporadic liver and gallbladder visualization which may occur during ^{99m}Tc -MAG3 renal studies.

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