
Kit Preparation of Technetium-99m-Mercaptoacetyltriglycine: Analysis, Biodistribution and Comparison with Technetium-99m-DTPA in Patients with Impaired Renal Function

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Technetium-99m-mercaptoacetyltriglycine ($^{99m}\text{Tc-MAG}_3$) was prepared by a frozen solution method, enabling the preparation of kits yielding a product substantially free of lipophilic impurities (96% $^{99m}\text{Tc-MAG}_3$). However, biliary activity was not completely eliminated as HPLC-purified $^{99m}\text{Tc-MAG}_3$ was also excreted by that route. Sequential $^{99m}\text{Tc-DTPA}$ and $^{99m}\text{Tc-MAG}_3$ renal scans were performed in 15 patients with renal dysfunction, including renal transplant recipients. In all cases, the $^{99m}\text{Tc-MAG}_3$ kit preparation provided superior images to $^{99m}\text{Tc-DTPA}$ at all levels of renal function due to a higher target-to-background ratio and a plasma clearance twice as fast as $^{99m}\text{Tc-DTPA}$. Interpretation of delayed $^{99m}\text{Tc-MAG}_3$ images, however, was complicated by biliary excretion which will limit quantitative estimates of renal clearance. A $^{99m}\text{Tc-MAG}_3$ kit is likely to be of value in renal transplant assessment and in cases of significant renal impairment but would not appear to offer major advantages over $^{99m}\text{Tc-DTPA}$ in routine renal imaging.

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Several groups have now reported on the value of the new technetium-labeled renal radiopharmaceutical mercaptoacetyltriglycine (MAG_3) as a ^{99m}Tc replacement for iodine-131-orthoiodohippurate in experimental animals (1–4), human volunteers (5), and in patients with renal disease (6–10). High-performance liquid chromatography- (HPLC) purified $^{99m}\text{Tc-MAG}_3$ is cleared by tubular secretion and appears to have a renal extraction approaching that of hippuran (1,7). Better renal images should therefore be obtainable at a lower radiation dose than with glomerular agents such as

^{99m}Tc -diethylenetriaminepentaacetic acid (DTPA) in patients with renal impairment. Problems have arisen however in the development of a kit formulation which is clearly necessary for the practical clinical use of the agent.

In the present study, we report the development and bioanalysis of a kit preparation of $^{99m}\text{Tc-MAG}_3$. Second, in order to validate its routine use, we have compared this agent with our usual renal radiopharmaceutical, $^{99m}\text{Tc-DTPA}$, in patients with a spectrum of renal dysfunction.

METHODS

Kit preparation, analysis, and biodistribution of $^{99m}\text{Tc-MAG}_3$ are presented in the Appendix.

Toxicity Studies

Prior to patient use, MAG_3 kits were subjected to sub-acute toxicity testing in mice. This test included histologic examination of organs of mice injected with a total cumulative dose of 4930 times the human dose in ten equal increments over a 12-day period.

Clinical Studies

Approval for the project was granted by the Human Ethics Committee, Royal Adelaide Hospital. Table 1 documents clinical details of the nine non-transplant patients and six transplant recipients.

Imaging Procedure

The $^{99m}\text{Tc-MAG}_3$ and $^{99m}\text{Tc-DTPA}$ studies were undertaken on successive days on well-hydrated patients. A large field of view gamma camera (Siemens) was positioned behind the patient who was seated in a chair reclined at 20°, with the field of view including kidneys and part of the heart. After a rapid bolus injection of the radiopharmaceutical, data were collected in 2-sec frames by the computer (PDP-11/Gamma 11) for 20 frames, followed by 62 frames of 20-sec duration. Analog images were obtained at 3-sec intervals for 36 sec and then at 1-min intervals to 5 min, and 5-min intervals to 30 min. Delayed images were obtained up to 3 hr postinjection.

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TABLE 1
Patient Data, Plasma Clearance, and Mean Cortical Transit Times For ^{99m}Tc -MAG₃ and ^{99m}Tc -DTPA in Patients with Renal Dysfunction

Diagnosis	Serum creatinine	Clearance (ml/min)		Mean cortical transit times (min)			
	(mmol/l)	MAG ₃	DTPA	MAG ₃		DTPA	
				R	L	R	L
Native kidneys							
Hypertension	0.07	236	83	0.9	1.3	1.4	1.4
Mesangio-capillary Glomerulonephritis	0.15	124	45	6.4	8.3	1.2	2.9
Renal sarcoidosis	0.20	62	37	4.0	4.0	1.8	2.3
Acute renal failure, Hypertension	0.27	ND*	ND	2.0	2.7	1.7	2.3
IgA glomerulonephritis	0.34	48	22	0.9	1.4	2.0	1.6
Acute renal failure	0.42	52	21	3.5	3.1	1.6	4.1
Analgesic nephropathy	0.45	31	18	1.8	2.3	1.7	2.7
Hereditary nephritis (Alport's)	0.78	16	11	2.4	1.4	1.2	2.9
Light-chain nephropathy	1.15	18	29	ND	ND	ND	ND
Transplant kidneys							
Stable function	0.12	229	ND	1.6		ND	
Stable function	0.17	120	41	1.9		1.1	
Obstruction	0.18	41	ND	3.0		ND	
Acute rejection	0.33	83	ND	2.3		ND	
Chronic vascular Rejection	0.50	46	44	1.4		0.8	
Acute tubular necrosis	dialysis	ND*	ND	ND		ND	

* ND = not done.

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Lateral views were obtained to correct differential function estimates for differences in kidney depth. The transplant recipients were scanned while lying supine with the gamma camera anterior to the transplanted kidney and bladder.

One patient was studied on successive days using HPLC-purified ^{99m}Tc -MAG₃ followed by the kit preparation of ^{99m}Tc -MAG₃. Whole-body scans were performed at identical times with regions defined over various organs to semiquantitatively compare extrarenal activity.

Data Analysis

Whole kidney and cortical regions of interest were carefully defined over both kidneys. Subtraction of the background region lateral and superior (for liver overlap) to the kidney allowed generation of renogram curves and differential renal function. Using deconvolution analysis of the time-activity curves from the kidney and left ventricle, transit times of tracer through whole kidney and cortex were determined from the impulse retention function. The mean of transit times from T minimum to T maximum for ^{99m}Tc -DTPA and ^{99m}Tc -MAG₃ were compared.

Statistical analysis was performed using the Student paired t-test.

Clearance Studies

Plasma clearance was based on a single injection, single-compartment model with plasma samples being drawn at 45 min and 120 min for ^{99m}Tc -MAG₃ and 1 and 3 hr for ^{99m}Tc -DTPA. Clearance was expressed as ml/meter² surface area \times 1.73. Mean radiopharmaceutical doses administered: ^{99m}Tc -

MAG₃ = 211 MBq (range 152-303); ^{99m}Tc -DTPA = 603 MBq (range 548-655).

RESULTS

Properties of ^{99m}Tc -MAG₃ Kits

Detailed results are presented in the Appendix. HPLC analysis of frozen HPLC-purified MAG₃ kits indicated that ^{99m}Tc -MAG₃ accounted for 96% of radioactivity with the remaining 4% comprising four predominantly lipophilic impurities. Investigation into the hepatobiliary secretion of the ^{99m}Tc -MAG₃ kit lead to the interesting finding that HPLC-purified ^{99m}Tc -MAG₃ itself was secreted into the bile of mice.

Toxicity Studies

No abnormalities were observed in any of the 15 tissues examined by a qualified veterinary pathologist when compared with a control group of mice.

Clinical Data

Table 1 and Figure 1 show the comparable ^{99m}Tc -MAG₃ and ^{99m}Tc -DTPA plasma clearance data correlated with renal function in 10 patients. The mean volume of distribution of ^{99m}Tc -MAG₃ (9881 ± 4401 ml) was somewhat less than that of ^{99m}Tc -DTPA, ($12,358 \pm 4220$ ml), presumably reflecting a higher level of protein binding (1,2,3). Mean clearance of ^{99m}Tc -MAG₃ (75 ± 67 ml/min) was twice as fast as

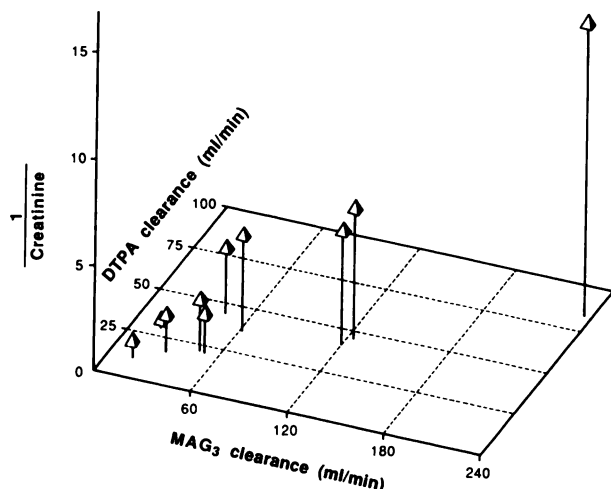


FIGURE 1
Correlation between inverse serum creatinine, ^{99m}Tc -MAG₃ clearance and ^{99m}Tc -DTPA clearance. Pearson correlation coefficients all >0.9 , $p < 0.0003$.

^{99m}Tc -DTPA, (35 ± 20 ml/min) consistent with predominantly tubular excretion of the agent.

Table 1 also compares the cortical transit times of ^{99m}Tc -MAG₃ and ^{99m}Tc -DTPA derived from deconvolution of the renogram. Transit of ^{99m}Tc -MAG₃ was generally slower ($p < 0.05$) than that of ^{99m}Tc -DTPA at all levels of renal function.

In all cases, ^{99m}Tc -MAG₃ provided superior images at significantly lower doses than DTPA due to a higher signal-to-noise ratio. This was particularly apparent in cases of severe renal impairment (Fig. 2) and was most dramatically shown in a case of primary nonfunction in a transplant recipient where ^{99m}Tc -DTPA scanning failed to image the kidney at all (Fig. 3). Graft biopsy on the same day as the ^{99m}Tc -MAG₃ scan excluded significant rejection and confirmed acute tubular necrosis. Diuresis and improvement in renal function occurred within the next 2 to 3 days in this patient.

In the small number of transplant recipients, the specific question of the value of ^{99m}Tc -MAG₃ in predicting rejection events could not be adequately addressed. However, in one patient, acute rejection was

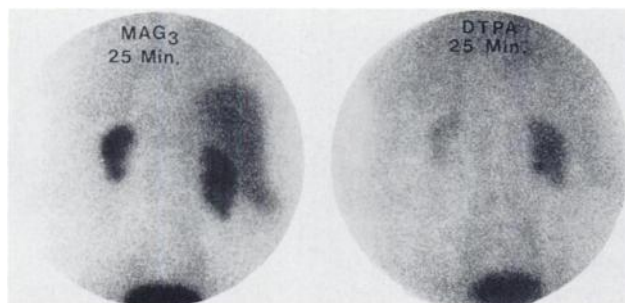


FIGURE 2
Comparable images using ^{99m}Tc -MAG₃ and ^{99m}Tc -DTPA in a patient with serum creatinine 0.34 mmol/l.

accompanied by a plateaued renogram curve and an increased cortical transit time ($T_{\text{mean}} = 2.3$ min). After treatment with OKT3, a pan T-cell monoclonal antibody, the renogram returned towards normal and cortical transit time decreased ($T_{\text{mean}} = 1.5$ min) in association with a fall in serum creatinine levels (0.33 mmol/l to 0.20 mmol/l).

Despite better images in general from ^{99m}Tc -MAG₃, the hepatobiliary excretion of the complex, and possibly the contaminants within the kit, makes interpretation of delayed images difficult, e.g., when required for assessing renal obstruction. Figure 4 demonstrates the significant bowel activity present on an anterior image of the abdomen 2 hr after injection of ^{99m}Tc -MAG₃. The degree of liver and bowel activity, however, do not appear to be closely related to the level of renal function and in our hands was also independent of the time between boiling the ^{99m}Tc -MAG₃ kit and injecting the patient, in contrast to the results reported by other workers (8). A semiquantitative organ distribution study of ^{99m}Tc -MAG₃ using gamma camera estimates in a patient with significantly impaired renal function showed little difference in gallbladder, liver and gut activity when HPLC-purified ^{99m}Tc -MAG₃ was compared with the kit preparation of ^{99m}Tc -MAG₃. In combination with the animal data on biliary excretion of ^{99m}Tc -MAG₃ (see Appendix), these findings suggest that the hepatobiliary activity was mainly related to the ^{99m}Tc -MAG₃ complex itself, rather than to the kit impurity.

DISCUSSION

The reports of previous workers (5,6,9) describing the potential value of ^{99m}Tc -MAG₃ for the investigation of renal disease led us to investigate methods of preparing a stable kit prior to using the product in a clinical comparison with ^{99m}Tc -DTPA. Initial attempts met with failure due to short shelf-life and the appearance of unacceptable quantities of impurities on HPLC analysis. However, HPLC purification of the ligand enabled the preparation of frozen solution kits yielding a product with much reduced labeled lipophilic impurity content. Even then, there was a sporadic appearance of products containing high levels of labeled impurities which are likely to result from hydrolysis of MAG₃ under transient alkaline conditions as the ligand was being dissolved. Care taken to prevent alkaline conditions has enabled the preparation of a kit with satisfactory biodistribution data.

Several groups have now compared the clinical and experimental performance of ^{99m}Tc -MAG₃ with radioiodinated hippuran (1-9). All report favorably on the possibility of ^{99m}Tc -MAG₃ being a hippuran replacement, although there are reservations about its value in the quantification of effective renal plasma flow (7). As ^{99m}Tc -DTPA is currently the most commonly used

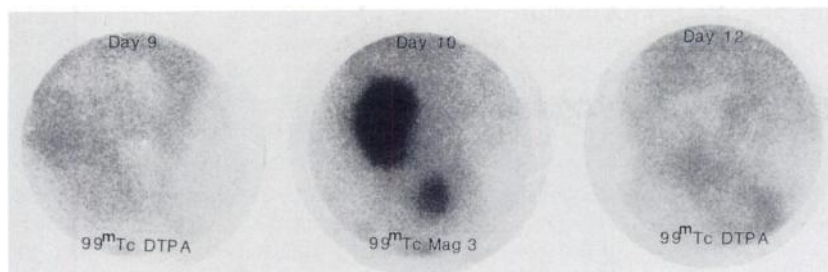


FIGURE 3
Imaging of a transplanted kidney with primary nonfunction using either ^{99m}Tc -MAG₃ or ^{99m}Tc -DTPA.

renal radiopharmaceutical in our country, it seemed appropriate to examine the question of whether ^{99m}Tc -MAG₃ could replace ^{99m}Tc -DTPA in the routine clinical situation. The patient group studied exhibited a spectrum of renal impairment and renal pathology. In every case, the quality of analog images obtained with ^{99m}Tc -MAG₃ was clearly superior to ^{99m}Tc -DTPA with the real advantage being seen in the cases of severe renal impairment. In one case of primary nonfunction of the transplanted kidney, the graft could only be visualized with ^{99m}Tc -MAG₃. In the post-transplant period, the ability to accurately define the whole kidney and cortical regions of interest is clearly of critical importance if quantitative measurements of perfusion or transit times are to be used to predict rejection events. Encouraged by the association of a decrease in mean cortical transit time of ^{99m}Tc -MAG₃ with reversal of acute rejection by the anti-T cell monoclonal, OKT3 in a transplant recipient, we are now embarking on a large scale project investigating the value of cortical transit times of ^{99m}Tc -MAG₃ in predicting rejection in the immediate post-transplant situation.

Clearance of ^{99m}Tc -MAG₃ from the plasma was significantly faster than DTPA at all levels of renal function, as would be expected from an agent primarily removed by renal tubular secretion. The clear clinical advantage is that the failing kidney can be visualized in situations where the glomerular agent, ^{99m}Tc -DTPA,

provides very poor background/target ratios. Deconvolution of the renogram yielded mean cortical transit times for ^{99m}Tc -MAG₃ that were somewhat slower than for ^{99m}Tc -DTPA, which may reflect the added time for active tubular cell transport versus glomerular filtration.

The appearance of biliary excreted activity in patients with poor renal function is a complicating factor in the interpretation of ^{99m}Tc -MAG₃ scans (2). Although three of the four commonly labeled impurities appearing in MAG₃ kits were shown to have a considerable biliary excreted component [confirming the nonquantitative work of Brandau, et al. (11)], it is unlikely that these impurities are responsible for the biliary activity seen in the clinical situation described above since their total concentration in the injected dose was always <4%. This is supported by the fact that the small quantity of radioactivity appearing in the bile of mice injected with HPLC-purified ^{99m}Tc -MAG₃ is due to ^{99m}Tc -MAG₃ itself.

In conclusion, it would appear that ^{99m}Tc -MAG₃ will offer significant advantages over ^{99m}Tc -DTPA in monitoring postrenal transplant events. In the failing native kidney, ^{99m}Tc -MAG₃ will provide clearer images than ^{99m}Tc -DTPA. However, hepatobiliary secretion may complicate interpretation of delayed images. In addition, the ability to measure glomerular filtration rate as an accurate and well accepted measure of global renal function will be lost if ^{99m}Tc -MAG₃ replaces ^{99m}Tc -DTPA. We would, therefore, suggest that ^{99m}Tc -MAG₃ is a valuable addition to the list of renal radiopharmaceuticals but is unlikely to generally replace ^{99m}Tc -DTPA.

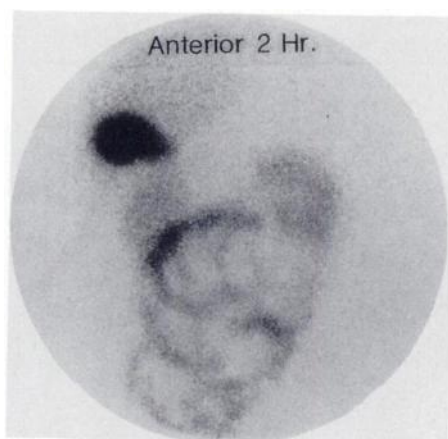


FIGURE 4
Biliary and bowel activity present on the anterior abdominal image 2 hr after injection of ^{99m}Tc -MAG₃.

APPENDIX: KIT PREPARATION, ANALYSIS, AND BIODISTRIBUTION OF ^{99m}Tc -MAG₃

Preparation and HPLC Purification of Benzoyl-MAG₃ (Bz-MAG₃) Ligand

Bz-MAG₃ ligand was synthesized according to the method of Fritzberg et al. (1). m.p. 197°C (lit 195-196°C (1)); Elemental analysis: Found: C 48.9%, H 4.90%, N 11.2%, S 9.1%. Calculated for C₁₅H₁₇N₃O₆S: C 49.0%, H 4.67%, N 11.4%, S 8.7%.

A C₁₈ Resolve column (Waters Assoc., Milford, MA) (8 × 100 mm) in a radial compression module (Waters Assoc.) was flushed with 50% methanol, followed by sterile ethanol: 0.01 M phosphate buffer (5:95), pH 6.5. Bz-MAG₃ (30-35 mg) was

dissolved in 1.5 ml buffer with warming to 50°C and the cautious addition of 1 M NaOH; the pH was not allowed to rise above 5.0. After passage through a 0.2-micron pore diameter filter, the MAG_3 solution was loaded onto the column via a U6K injector (Waters Assoc.). The column was eluted at a flow rate of 1.0 ml/min and the eluate collected in 2-ml fractions in sterile containers.

The UV absorption spectrum (Unicam Instruments SP800A, Cambridge, England) was measured in the range 200-300 m μ . The most concentrated fractions were combined, taking care to exclude any containing a band with maximum absorption at 225 m μ (Bz-MAG₃ has absorption maxima at 243 and 267 m μ).

Preparation of MAG_3 Kits

The theoretical number of kits utilizing 1.0 mg Bz-MAG₃ per kit was calculated and a stannous pyrophosphate solution containing 2.0 mg tetrasodium pyrophosphate decahydrate and 34 μ g anhydrous stannous chloride per kit (pH 5.0) was prepared and added to the nitrogen-purged Bz-MAG₃ solution. The pH was readjusted to 5.0 and the solution diluted to a Bz-MAG₃ concentration of 1.0 mg/ml then 1.0 ml dispensed into nitrogen-flushed rubber-closed vials which were immediately frozen at -20°C and stored at this temperature.

Labeling and Stability of Kits

Technetium-99m-pertechnetate (0.1-2.0 ml, 1.0-2.0 GBq) was added to the MAG_3 kit, which was then heated in a boiling water bath for 10 min. HPLC was used to determine labeling efficiency of the MAG_3 kit and presence of radiochemical impurities. Integration of each peak and of the entire chromatogram enabled the proportion of each component to be calculated.

The HPLC analysis of $^{99\text{m}}\text{Tc-MAG}_3$ prepared from frozen HPLC-purified MAG_3 kits showed a mean value of 95.6% \pm 1.2% (n = 8) of the radioactivity was in the form of $^{99\text{m}}\text{Tc-MAG}_3$ with four other main radiochemical impurities identified as A, B, C, and D. The biologic behavior of the kit was very similar to HPLC-separated $^{99\text{m}}\text{Tc-MAG}_3$ (Table A-1), with 94% of the injected dose appearing in the urine of mice at 1 hr.

The stability of the labeled product was determined by

HPLC analysis within 30 min of preparation and again 6 hr later. Biodistribution data in mice at 1 hr postinjection were also obtained at these time points. Table A-1 shows that there was negligible deterioration in performance over a 6-hr working day.

The shelf-life of the frozen kits was determined by HPLC analysis and biodistribution studies at various intervals of time after preparation. The kits generally performed adequately after 4 mo storage at -20°C, although occasional batches with reduced stability have been encountered.

Biologic Behavior of $^{99\text{m}}\text{Tc-MAG}_3$ and Labeled Impurities

Technetium-99m-Mag₃ (500 MBq/ml) was prepared and passed through the HPLC column as described above. Peaks due to impurities were collected in separate containers and the biodistribution in sets of three female Balb/c mice determined. The mice were injected with 0.2 ml containing ~40 kBq activity and dissected at 1 hr postinjection.

The biodistribution of the four impurities A, B, C, and D is shown in Table A-2 with peaks B, C, and D particularly showing relatively high levels of hepatobiliary excretion. However, these impurities accounted for an extremely small fraction of the kit activity (up to 4%).

The main peak containing pure $^{99\text{m}}\text{Tc-MAG}_3$ (as determined by biodistribution study and re-injection through the HPLC column) was injected into three mice (100 MBq/mouse). At 45 min, the mice were dissected and the bile aspirated from the gallbladders and pooled, diluted with 0.4 ml N₂ flushed saline, membrane filtered, then passed through the HPLC column.

HPLC chromatograms of HPLC-purified $^{99\text{m}}\text{Tc-MAG}_3$ and the bile of mice injected with this product indicate that $^{99\text{m}}\text{Tc-MAG}_3$ is excreted via the hepatobiliary system to a small extent.

Alkaline Hydrolysis of Bz-MAG₃

It was observed that concentrated solutions of Bz-MAG₃ turn yellow and develop a distinctive thiol odor at alkaline pH. To investigate this effect of pH on labeling and biodistribution studies, the hydrolysis was repeated using HPLC-purified Bz-MAG₃ (2.0 mg/ml) at pH 12.0, following which

TABLE A-1
Biodistribution of $^{99\text{m}}\text{Tc-MAG}_3$ Preparations in Female Balb/c Mice at 1 Hr Postinjection^a

	HPLC separated	30 min [†]	Frozen kit 6 h [‡]	4 mo [§]
Blood [†]	0.1 (0.1-0.1)	0.3 (0.2-0.3)	0.3 (0.2-0.4)	0.4 (0.3-0.5)
Liver	0.1 (0.1-0.1)	0.3 (0.3-0.3)	0.2 (0.1-0.2)	0.2 (0.2-0.2)
Gallbladder [†]	3.6 (2.6-4.7)	2.9 (1.1-4.5)	2.7 (0.9-3.9)	4.1 (3.8-4.1)
Stomach	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.1 (0.0-0.3)
Intestine	1.4 (1.2-1.5)	1.8 (1.7-1.9)	2.5 (1.9-2.8)	1.9 (1.6-2.1)
Kidneys	0.1 (0.1-0.2)	0.3 (0.3-0.4)	0.2 (0.2-0.2)	0.3 (0.2-0.3)
Urine	95.5 (95.1-96.1)	94.0 (92.5-95.5)	91.1 (88.7-92.5)	94.2 (93.2-94.9)
Carcass	1.7 (1.4-2.2)	3.2 (1.8-4.7)	2.6 (1.9-3.6)	1.2 (1.1-1.2)
Recovery	99.5 (99.6-100.4)	101.6 (100.4-101.9)	97.1 (96.5-98.2)	98.8 (97.3-99.8)

^a % Dose/organ, mean and range (n = 3).

[†] % Dose/g.

[‡] Time after labeling.

[§] Time after preparation.

TABLE A-2
Biodistribution of ^{99m}Tc-labeled Impurities in Female Balb/c Mice at 1 Hr Postinjection*

	Peak A	Peak B	Peak C	Peak D	Hydrolysed MAG ₃
Blood†	2.7 (2.6–2.9)	2.2 (1.7–2.6)	8.2 (5.5–10.9)	11.4 (10.0–12.9)	5.2 (4.4–5.7)
Liver	2.0 (1.9–2.0)	3.3 (3.0–3.7)	4.6 (3.7–5.6)	5.2 (4.3–6.3)	2.0 (1.9–2.2)
Gallbladder†	5.2 (4.4–5.9)	73.9 (48.2–112.2)	224.6 (126.8–322.4)	76.1 (55.1–97.0)	28.6 (24.1–36.2)
Stomach	0.7 (0.6–0.8)	0.2 (0.2–0.3)	1.2 (0.5–2.6)	0.7 (0.6–0.8)	0.3 (0.3–0.4)
Intestines	4.2 (4.1–4.3)	27.1 (25.0–30.5)	26.9 (25.7–28.1)	27.4 (25.7–29.8)	14.4 (13.4–15.8)
Kidneys	6.2 (5.7–6.7)	1.7 (1.6–1.8)	8.2 (7.8–8.7)	11.7 (9.4–13.6)	6.3 (6.1–6.5)
Urine	53.0 (52.2–53.8)	54.0 (48.2–60.1)	11.6 (8.9–16.2)	7.3 (5.6–9.7)	49.4 (47.4–50.6)
Carcass	31.4 (31.2–31.6)	11.0 (10.6–11.5)	42.6 (33.7–49.8)	40.2 (37.0–41.6)	2.3 (20.3–24.3)
Recovery	100.4 (98.6–102.2)	100.5 (98.2–103.5)	102.2 (98.4–109.0)	101.2 (100.5–101.8)	99.6 (97.6–100.7)

* % Dose, mean and range (n = 3).

† % Dose/g.

kits were prepared containing the hydrolysis product from 1.0 mg Bz-MAG₃, 34 µg SnCl₂ and 2.0 mg sodium pyrophosphate.

Following the normal labeling procedure, HPLC examination of the hydrolyzed product showed the presence of some ^{99m}Tc-MAG₃ (32.8%) together with seven labeled impurities, including appreciable quantities of all labeled impurities seen previously. The biodistribution of this preparation is shown in Table A-2.

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