# Technetium-99m MAG₃: Labeling Conditions and Quality Control

Dennis L. Nosco, Robert G. Wolfangel, Michael J. Bushman, Glenn D. Grummon, Mary E. Marmion, and David W. Pipes

*Mallinckrodt Medical Inc., St. Louis, Missouri*

*TechneScan MAG3™, a radiopharmaceutical kit developed by Mallinckrodt Medical Inc., St. Louis, MO, for use in the evaluation of renal tubular function, was tested under a variety of labeling conditions. The effects of varying time (0–30 min) and heating temperature (90°C–110°C), technetium-99m activity (0–150 mCi) and volume (4–10 ml), timing and volume (0–10 ml) of air addition to the kit, and age of generator eluate (0–12 hr) used in reconstitution were examined. Analysis of the results from the above experiments show that, when used within the parameters described in the package insert, the reconstituted TechneScan MAG3 kit gives acceptable radiochemical purity (i.e., greater than 90%) every time. Of special note, results from these studies indicate that ⁹⁹ᵐTc generator eluate which has stood for more than 6 hr after elution should not be used to label the kits. A novel quality control method using a C18 Sep Pak (Millipore Corp., Milford, MA) is described. This method is easy and quick to perform and gives excellent accuracy, with results similar to those obtained using high performance liquid chromatography.*

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In the search for a technetium-99m- ($^{99}$Tc) based renal tubular function imaging agent a number of candidates have been proposed for the replacement of radiolabeled orthoiodohippuric acid (1,2). The best $^{99m}$Tc-based agent encountered so far is disodium [N-[N-[N-(mercaptoacetyl)glycylglycyl]glycinato(5-)-N, N',N'',S] oxo[$^{99m}$Tc]technetate (V)] (3), (Fig. 1), also referred to as $^{99m}$Tc-MAG₃ or $^{99m}$Tc mertiatide, which was discovered by Alan Fritzberg and coworkers (4).

The original formulation of $^{99m}$Tc-MAG₃ developed by Fritzberg and coworkers was not suitable for routine radiopharmacy practice. Therefore, Mallinckrodt Medical, Inc. developed a stable, practical lyophilized kit formulation for $^{99m}$Tc-MAG₃. This kit formulation, referred to as TechneScan MAG3, contains sufficient reducing agent (stannous, 200 μg, as stannous chloride dihydrate) to ensure good shelf life of the cold kit (i.e., acceptable product purity is generated upon reconstitution even when the kit has been stored for two years), as well as good labeling over a wide range of

$^{99m}$Tc activities (5,6). Also included in the kit are sufficient amounts of the ligand (betiatide = S-benzoylmercaptoacetylglycylglycylglycine, 1 mg), transfer agent (sodium tartrate, 40 mg), and bulking agent (lactose, 20 mg) to provide good labeling yields and a good mixture which can be readily lyophilized.

After this formulation had been established, a number of parameters were evaluated with regard to the day-to-day use of the kit, including time and temperature of heating, $^{99m}$Tc activity and volume, timing and volume of air addition to the kit, and age of generator eluate before use. The results of these evaluations, as well as some others, are presented and discussed in detail in this paper (6).

## MATERIALS AND METHODS

Lyophilized TechneScan MAG3 kits were used in all studies. Unless otherwise noted, the instructions on the package insert were followed. Kits were reconstituted with 4–10 ml
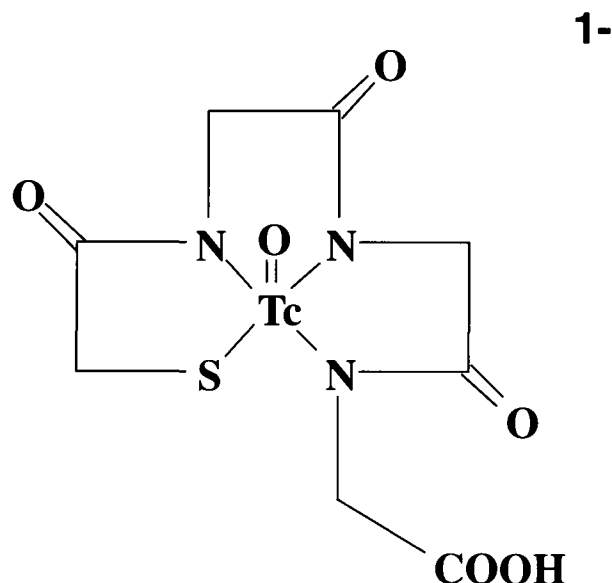
**FIG. 1.** Structure of Tc-MAG₃.

FIG. 2. Labeling of Tc-MAG₃ kit.



FIG. 4. Schematic representation of Tc-MAG₃ Sep Pak quality control.
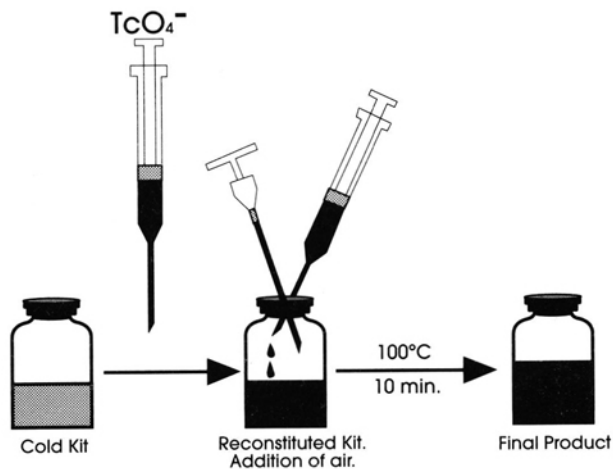
of saline containing $^{99m}$Tc (20–150 mCi) prepared by adding varying amounts (0.1–10 ml) of commercial generator eluate to the appropriate amount of 0.9% saline (0–9.9 ml). (Mallinckrodt Medical, Du Pont, Squibb, and Medi-Physics generators were eluted ~ every 24 hr; $^{99m}$Tc eluate was used within 6 hr of elution unless otherwise noted.) Various amounts of air (0–10 ml) were immediately drawn into the vial head-space through a sterile filter and the vial was then heated to 90°C–110°C in a water bath within a lead shield for varying amounts of time (0–30 min) (see Fig. 2 for reaction schematic).

The vial was then removed from the water bath, placed in a lead shield, allowed to cool to nearly room temperature and sampled at 0–2 hr and 6–8 hr postheating to determine radiochemical purity. Paper chromatography, analyzing for hydrolyzed, reduced Tc (HR-Tc), was done using Whatman 3MMCHR 1-in. chromatography strips (7). These strips were spotted with one drop of solution from the reconstituted kit, developed to 10 cm in 60/40 acetonitrile/saline mixture and air dried in an oven (110°C) before being analyzed using a radiochromatogram scanner. The radioactivity at the origin was assigned to be HR-Tc.

All soluble radiochemical products were determined using high performance liquid chromatography (HPLC) (Fig. 3)
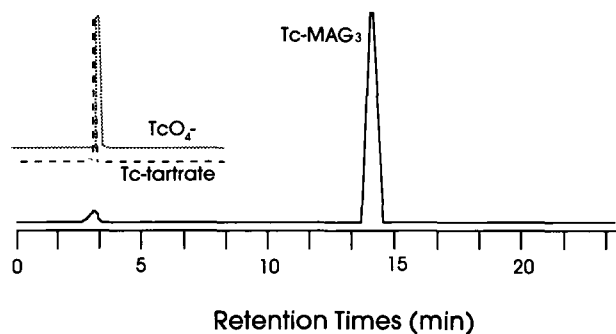


FIG. 3. HPLC scans of a reconstituted Tc-MAG₃ kit with overlayed Tc-tartrate and TcO₄⁻ scans.
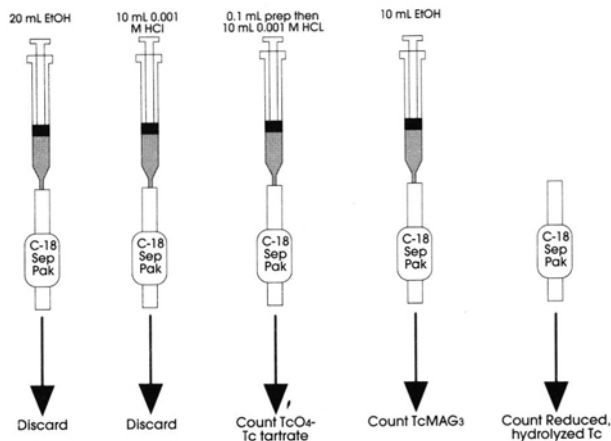
under the following HPLC conditions: a NovaPak C18 3.9 × 150-mm column; a linear gradient of 0–30 min, 90%A/10%B to 20%A/80%B (solvent A = 0.01 M potassium phosphate monobasic, 0.1% triethylamine in water, solvent B = 0.01 M potassium phosphate monobasic, 0.1% triethylamine in 20%THF/80% water). HPLC and paper chromatography purity results were compared to those obtained using a C18 Sep Pak (Millipore Corp., Milford, MA) following the procedure shown schematically in Figure 4.

In some experiments, a time delay was inserted between reconstitution of the kit with $^{99m}$Tc-labeled saline and the addition of 2 ml of air. After the air was added to the reconstituted kit, the kit was immediately placed in a boiling water bath for 10 min. In other experiments, a time delay was inserted between the addition of air and the heating of the kit in the water bath. In both types of experiments, the directions in the package insert were followed for all other parts of the procedure.

A number of experiments were performed in which generator eluate from a molybdenum (Mo)/Tc generator was allowed to stand for 0, 6, and 12 hr after elution. This eluate (50–140 mCi of $^{99m}$Tc diluted to a volume of 5 ml with 0.9% saline) was then used to label kits following the directions in the package insert.

Reference samples of Tc-tartrate were prepared by adding 1 ml of $^{99m}$Tc-labeled saline to 3 ml of deaerated water containing 40 mg of sodium tartrate (pH = 7.5) and 200 μg of stannous chloride dihydrate. This mixture was heated under an argon atmosphere in a sealed vial at 100°C for 10 min, and after cooling, the vial was sampled.

## RESULTS AND DISCUSSION

Reconstitution of the kits with $^{99m}$Tc-labeled saline (<100 mCi of $^{99m}$Tc, eluate less than 6-hr old), followed by addition of 2 ml of air and immediate heating at 100°C for 10 min, leads to a reproducible radiochemical purity of greater than 90%, as measured by a combination of HPLC and paper chromatography, or by Sep Pak quality control.
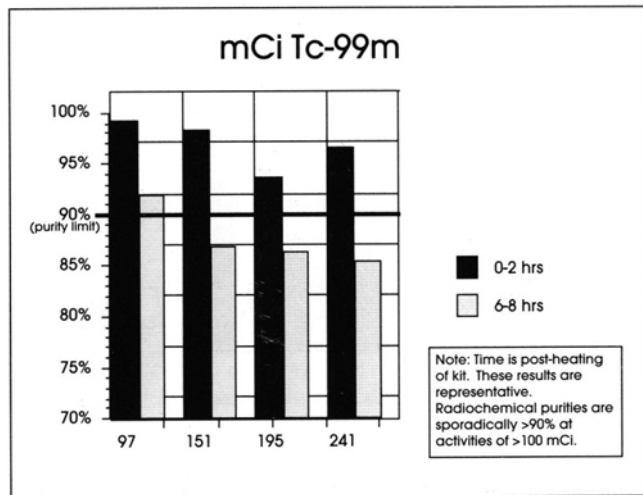
**FIG. 5.** Radiochemical purity of reconstituted Tc-MAG$_3$ kits as a function of $^{99m}$Tc activity. Kits reconstituted with 4 ml of generator eluate from a Mallinckrodt generator.



**FIG. 7.** Radiochemical purity of reconstituted Tc-MAG$_3$ kits as a function of heating time. Kits reconstituted with 4 ml of generator eluate from a Mallinckrodt generator with $^{99m}$Tc activities of 69–96 mCi.

The main purpose of our study was to demonstrate how the various labeling parameters can affect the radiochemical purity. Figure 5 shows how the radiochemical purity of the reconstituted kit decreases with increasing $^{99m}$Tc activity. This is especially notable at the 6–8 hr timepoint where the major impurities are pertechnetate (referred to as free Tc) and Tc-tartrate. Although it is sometimes possible to obtain a good radiochemical purity immediately after labeling when reconstituting the kit with *more* than 100 mCi of $^{99m}$Tc, the good results are sporadic, and unacceptable purity almost always results as the preparation stands post-heating. Reconstitution volumes of less than 4 ml are not recommended since the resultant radiochemical purity is usually <90% (Fig. 6).

The results obtained upon heating the reconstituted kits for varying times are shown in Figure 7. Although labeling appears to be essentially complete at 6 min, heating for 10 min assures good radiochemical purity. Heating beyond 10 min does not seem to adversely affect the purity of the
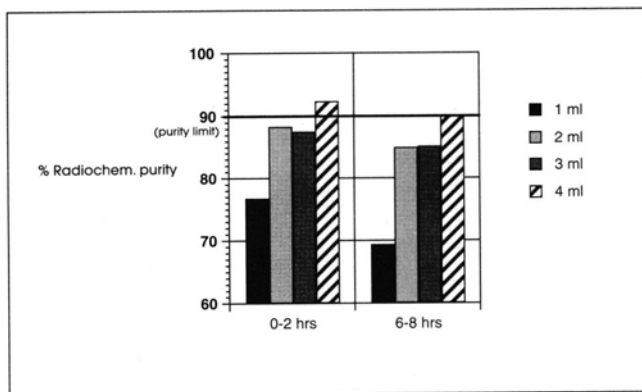
product. Heating for 10 min at *slightly* lower or higher temperatures (simulating preparation of the labeled kit at higher altitudes or when using hard water in the water bath, respectively) also does not seem to adversely affect the purity of the product (Table 1).

Extensive work was done to determine the effects of adding air to the kit before heating (Fig. 8). The sensitivity of the radiochemical purity of the kit to the amount of air added was more pronounced when generator eluates were used from manufacturers other than Mallinckrodt. The deleterious effects were most pronounced when a Squibb generator was used. Addition of a minimum of 2 ml of air gives acceptable radiochemical yields with all kits, although the addition of more than 2 ml of air does not seem to decrease the purity of the product. Figure 9 shows that as the amount of $^{99m}$Tc added to the kit is increased the radiochemical purity decreases. Once again, this effect is most pronounced with the Squibb generator.

Figures 10 and 11 demonstrate that even with large volumes of generator eluate or generator eluate obtained from a



**FIG. 6.** Radiochemical purity of reconstituted Tc-MAG$_3$ kits as a function of reconstitution volume. Kits reconstituted with 1 ml of generator eluate plus 0, 1, 2, and 3 ml of saline.
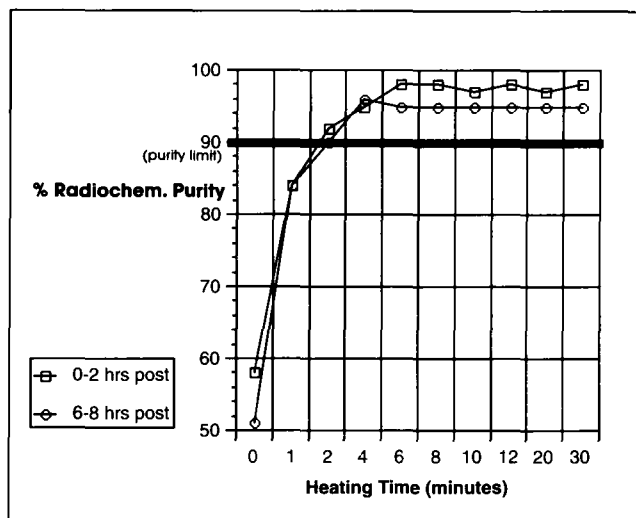
**TABLE 1. Radiochemical Purity of Reconstituted Tc-MAG$_3$ Kits as a Function of Heating Temperature**

| Heating Temperature (°C) | % Radiochemical purity | |
| | 0–2 hr post-heating | 6–8 hr post-heating |
| --- | --- | --- |
| 90 | 97 | 96 |
| 100 | 98 | 95 |
| 110 | 95 | 95 |

100 ± 1 mCi of $^{99m}$Tc in 4 ml generator eluate from a Mallinckrodt generator was used with each kit.
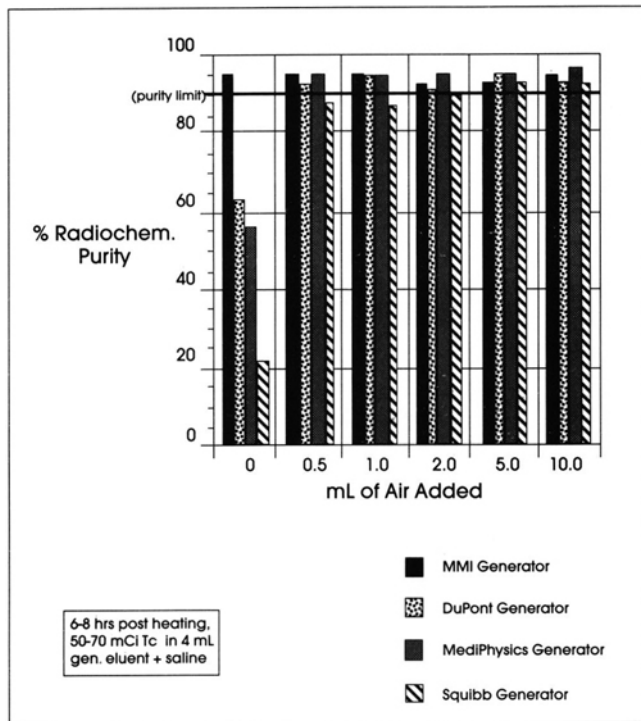
FIG. 8. Radiochemical purity of reconstituted Tc-MAG₃ kits as a function of volume of air added when using generators from different manufacturers.
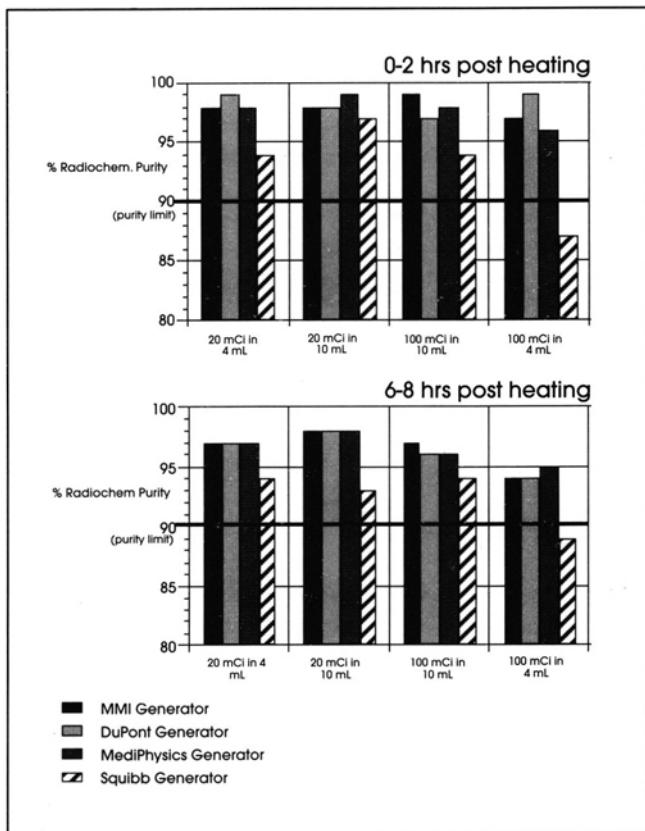


FIG. 9. Radiochemical purity of reconstituted Tc-MAG₃ kits using generators from different manufacturers.
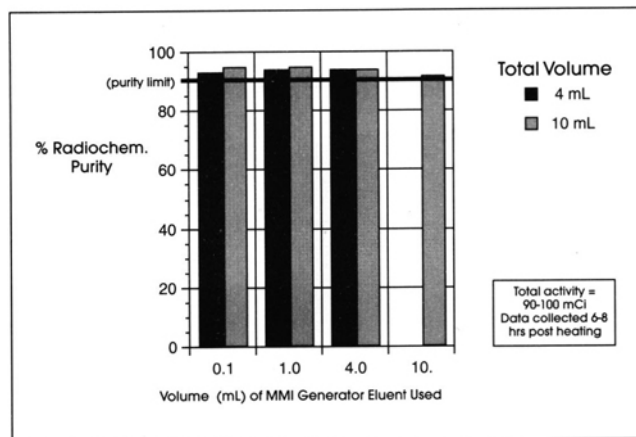


FIG. 10. Radiochemical purity of reconstituted Tc-MAG₃ kits using increasing volumes of generator eluate.

generator that had not been eluted for 72 hr (i.e., containing increased levels of $^{99m}Tc$ and radiolysis products, as would be seen in a generator that had not been eluted over a weekend), the kit still gives acceptable radiochemical purities (>90%) when the package insert instructions are followed.

Figure 12 shows the results of two experiments to measure the effects of delay in completing certain steps in the labeling process. We measured the effect of delaying addition of air to a reconstituted kit and the effect in delaying the heating step after air had been added to the reconstituted kit. As can be seen in Figure 12, in both cases, the radiochemical purity decreased as the time between steps increased.

Figure 13 shows how the labeling yield is affected if generator eluate is used more than 6 hr after it is eluted from the generator. The radiochemical purity is almost always less than the minimum of 90% if 12-hr-old generator eluate is used.

Figures 5–13 all show that failure to follow the labeling procedures on the package insert can result in lower (though generally still greater than 90%) radiochemical yields. In

FIG. 11. Performance of Tc-MAG₃ kits when reconstituted with Mallinckrodt generator eluate from generators that had been eluted 24 and 72 hr previously.
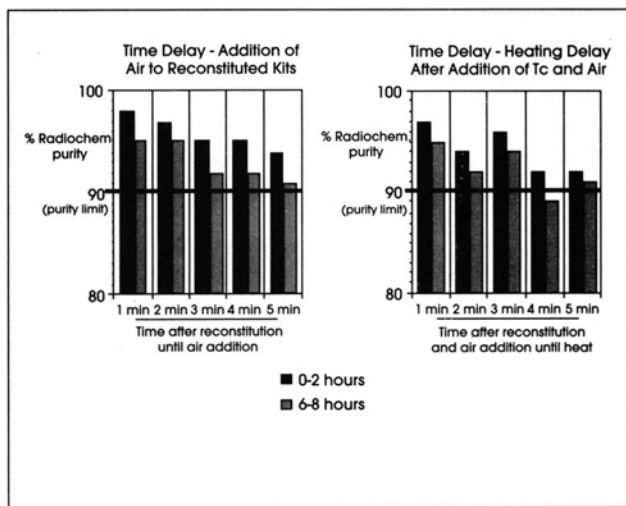
FIG. 12. Effect of delay of addition of air or heating on % radiochemical purity of reconstituted Tc-MAG$_3$ preparations. All experiments were carried out using 96 mCi ± 4 mCi of $^{99m}$Tc in 4 ml of saline from a Mallinckrodt generator. All experiments were carried out as described on the package insert, except for the delay in addition of air or delay in heating the reconstituted kit. Vials were sampled at 0–2 hr and 6–8 hr post-heating.

some instances, where additive effects from high activity levels (Figs. 5), different generators (Figs. 8 and 9), and failure to follow package insert instructions are seen, radiochemical purities of less than the minimum of 90% may be obtained.

The Sep Pak quality control for reconstituted Tc-MAG$_3$ kits was chosen because of its accuracy as well as its ease of use. Figure 14 illustrates saline-developed paper chromatography of a Tc-tartrate preparation, the precursor complex to Tc-MAG$_3$ (8). Tc-tartrate and free Tc represent the major impurities in the reconstituted kit. Figure 14 shows that the Tc-tartrate peak smears on a saline-eluted paper chromatography system. In fact, using a variety of common nontoxic solvent systems and a variety of paper and instant thin layer chromatography (ITLC) substrates (over 100 solvent/strip systems were tested at Mallinckrodt), no distinct separation was obtained between Tc-tartrate and Tc-MAG$_3$, usually due
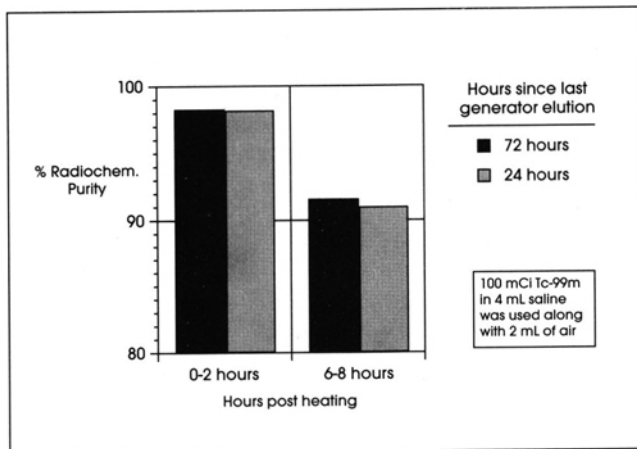


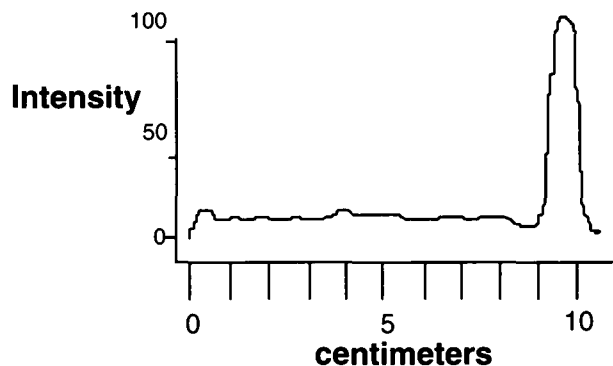FIG. 13. Effect on radiochemical purity of reconstituted Tc-MAG$_3$ kits based on eluate age.



FIG. 14. Radiochromatogram scan of saline-eluted paper chromatography of Tc-tartrate preparation.

to smearing of the type shown in Figure 14. This smearing is most likely due to decomposition of the metastable Tc-tartrate during the development of the strip.

In contrast to the paper/TLC results, the data in Table 2 clearly show that Tc-tartrate is removed from the Sep-Pak, along with the free Tc, in the 0.001 M HCl wash. Thus, Tc-tartrate, which can be confused with RHTc or Tc-MAG$_3$ if paper chromatography is used, is easily distinguished from RHTc and Tc-MAG$_3$ if the Sep Pak is used. Even when some separation is obtained for Tc-tartrate using paper or ITLC methods, classic cut-and-count analysis that works well for very good (>92%) or very bad (<85%) preparations may overestimate the purity of Tc-MAG$_3$ for preparations with a radiochemical purity of ~90%. (HPLC analysis can be used to distinguish Tc-tartrate from pertechnetate since there is a difference of 0.5 min in retention time between the two species, as seen in Fig. 3.)

### TABLE 2. Selected Comparisons Between Sep Pak and HPLC Quality Control Methods

| Component | Percentage of Radioactivity Found | |
| --- | --- | --- |
| | HPLC (retention time) | Sep Pak (fraction) |
| **Prep. 1: Tc-tartrate** | | |
| Tc-tartrate | 100 (1.2 min) | 100 (HCl wash) |
| **Prep. 2: Diluted generator eluate** | | |
| pertechnetate | 100 (1.8 min) | 100 (HCl wash) |
| **Prep. 3: 6% pertechnetate-spiked reconstituted Tc-MAG$_3$ kit** | | |
| Tc-tartrate + pertechnetate | 7 | 6.6 (HCl) |
| Tc-MAG$_3$ | 93 (15 min) | 92 (ethanol) |
| **Prep. 4: 1% RHTc-spiked reconstituted Tc-MAG$_3$ kit** | | |
| RHTc | 0.7* | 0.5 (Sep Pak) |
| Tc-MAG$_3$ | 97 | 98 (ethanol) |

*Determined by 60/40 acetonitrile/water paper chromatography.

## CONCLUSION

TechneScan MAG3 has been formulated to ensure good, reproducible labeling over a wide range of $^{99m}$Tc activities and volumes of generator eluate, with or without additional saline. It has been found that within the specified range of labeling parameters, a radiochemical purity of >90% is always obtained. If the kit is used outside the parameters listed in the package insert (e.g., >100 mCi of $^{99m}$Tc, <2 ml of air, or <4 ml of generator eluate plus diluent), a radiochemical purity below the lower limit quoted on the package insert (90%) may result. Most importantly, the use of generator eluate that has been allowed to stand for more than 6 hr also results in a reproducibly unacceptable radiochemical purity (Fig. 13).

Accurate, reproducible, and practical quality control of radiochemical purity by the use of a Sep Pak procedure has been demonstrated for the first time. In the development of new radiopharmaceuticals, this procedure offers an alternative to the cut-and-count ITLC and paper chromatographic methods that are now routinely used.

The above studies clearly show that the TechneScan MAG$_3$ kit is easily and efficiently labeled when the instructions on the package insert are followed. Moreover, the purity of the resulting reconstituted kit can be readily and accurately determined using a quick and convenient Sep-Pak quality control procedure.

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8. It has been proposed that the Tc-MAG$_3$ forms via a ligand exchange reaction involving the Tc tartrate complex. See Reference 4.