ANALYTICAL PITFALLS WITH ^{99m}Tc-LABELED COMPOUNDS

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The rapid growth of the number of ^{99m}Tc-labeled compounds in nuclear medicine requires analytical techniques that can accurately identify various components in these products. In our opinion, the recent literature contains much misleading analytical data concerning the identification and purity of various technetium compounds. Generally in these papers only a single chromatographic system was used whereas bioassay was relied on heavily. We feel that perhaps this situation should be reversed and that analytical techniques should be emphasized. Multicomponent products are much more easily identified using chemical techniques instead of bioassay. Variation of the parameters to obtain a high yield is easier when the chemical components are known rather than the organ distribution. Finally, species differences do not overshadow the results in analytical techniques as they often do in bioassay.

Since the different chemical forms of technetium show different biological behavior, failure to identify and quantify these forms has resulted in misuse of the compound, claims of in vivo instability which are not accurate, incorrect internal radiation dose calculations, and general confusion and often abandonment of compounds which are extremely valuable if obtained in their pure form.

This study compares various analytical methods now in use to point out the advantages and disadvantages of each method in the hope that this will inspire the introduction of further analytical analysis in nuclear medicine.

METHODS AND MATERIALS

Commonly used ^{99m}Tc-labeled compounds were analyzed by anion exchange chromatography at pH 2–3, by paper chromatography with both nitrogen-purged 85% methanol and nitrogen-purged saline as solvents, and by gel chromatography. The first three are commonly used chromatographic methods and need no further explanation. The fourth, gel chromatography, was performed on a 0.9 × 35-cm column of Sephadex G25 eluted with nitrogen-purged saline (*1*). A standard apparatus is shown in Fig. 1. Table 1 contains the quantitative data for each sys-

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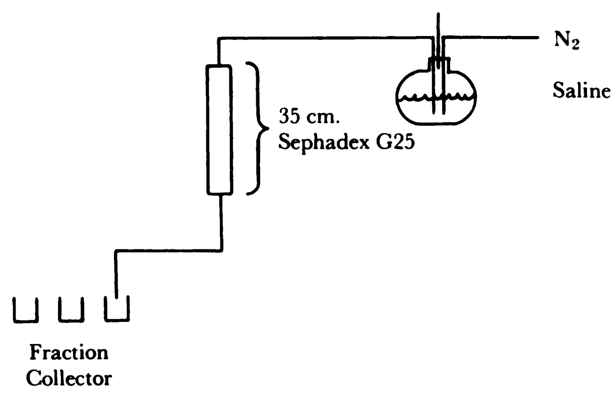

FIG. 1. Gel chromatography apparatus.

TABLE 1. QUANTITATIVE CHROMATOGRAPHIC DATA

	Tc(IV) HSA	Tc(IV) chelate	Hydrolyzed reduced Tc	TcO_4^-(VII)
Anion exchange	eluate	eluate	eluate	adsorbed
Whatman paper in 85% methanol (Rf)*	origin	origin	origin	0.5–0.6
Whatman paper in saline (Rf)	0.85–0.95	0.85–0.95	origin	0.65–0.80
Gel chromatography (Sephadex G25)	void vol†	12–20 ml	adsorbed	38–46 ml

* Rf is defined as the migration distance of the sample component divided by the migration distance of the solvent front.
† The void volume is the elution volume for a substance that is completely excluded from the gel.

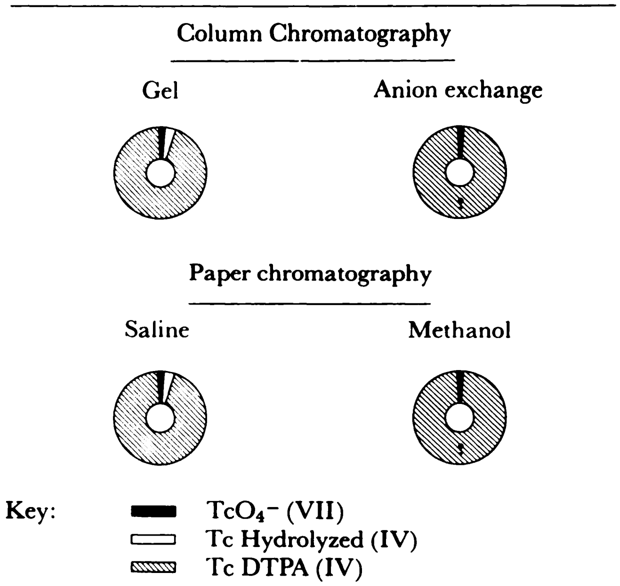

FIG. 2. Analysis of ^{99m}Tc-DTPA (stannous method).

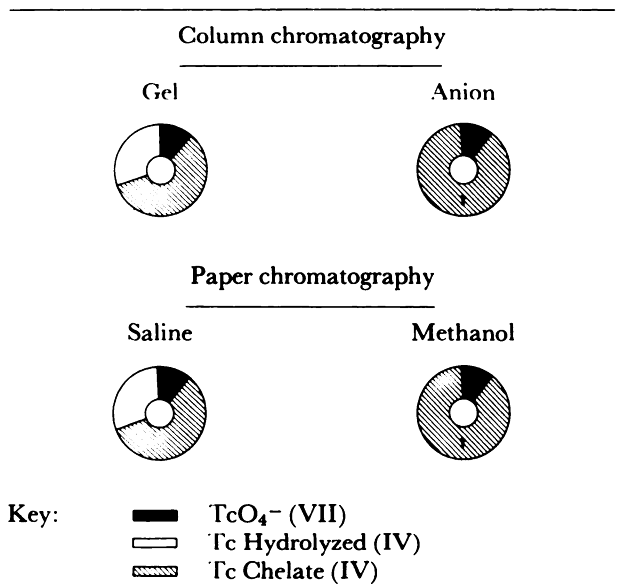

FIG. 3. Analysis of ^{99m}Tc-DTPA (Renotec).

tem. We hope to show in a subsequent publication (*2*) that it is indeed the Tc(IV) state which is present in ^{99m}Tc-chelates.

RESULTS

We have compared the analytical data for technetium-diethylenetriaminepentaacetic acid (Tc-DTPA) prepared by two methods and for technetium-human serum albumin (Tc-HSA) prepared at two pH values. Figure 2 shows the analytical data for Tc-DTPA prepared by the stannous method developed in our laboratory (*3*). As can be seen the gel and saline systems show three components, TcO_4^-, Tc-DTPA, and hydrolyzed Tc whereas the methanol and anion systems show only two, TcO_4^- and presumably Tc-DTPA. These data are to be compared with the data for another preparation of Tc-DTPA which is commercially available under the trade name Renotec. As can be seen in Fig. 3, analysis by either methanol or the anion system would indicate that both preparations are similar, i.e., both contain mostly Tc-DTPA. The gel and saline systems, however, show that the two products differ greatly in that the hydrolyzed technetium peak is greatly enhanced in the case of Renotec. This difference is corroborated by the biological distributions for the two preparations which are shown in Fig. 4. As can be seen, the urinary excretion is much less for Renotec than for Tc-DTPA. However, using either the methanol or anion exchange system, one would suspect the compounds to be identical.

The second set of examples involves the preparation of Tc-HSA using a low pH preparation and a high pH preparation according to procedures pub-

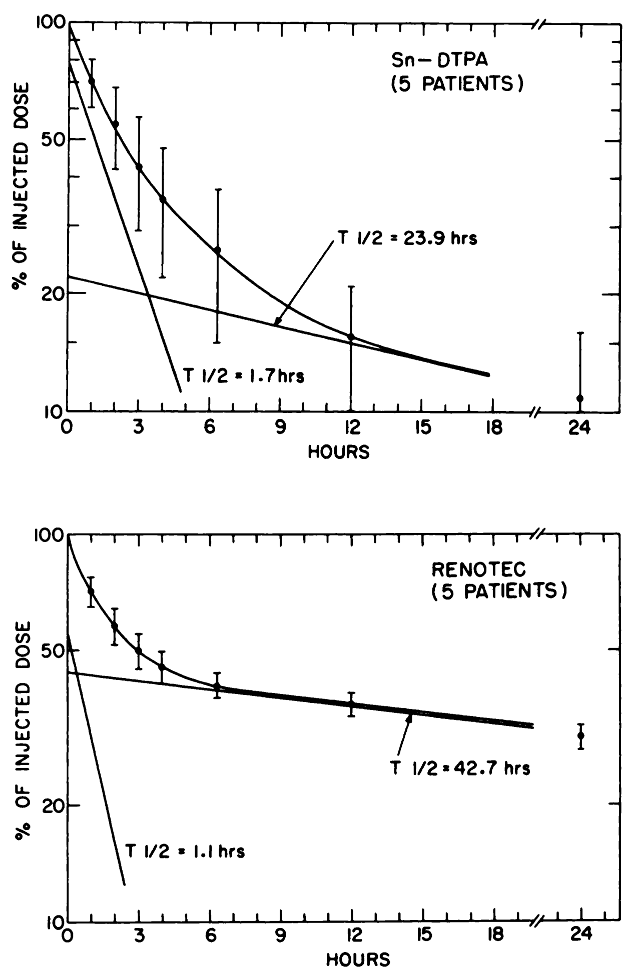

FIG. 4. Total-body retention of DTPA preparations.

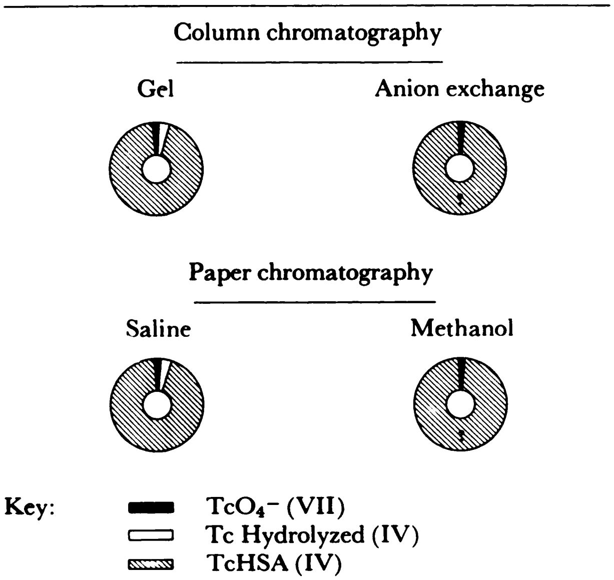

FIG. 5. Analysis of ^{99m}Tc-HSA (low pH method).

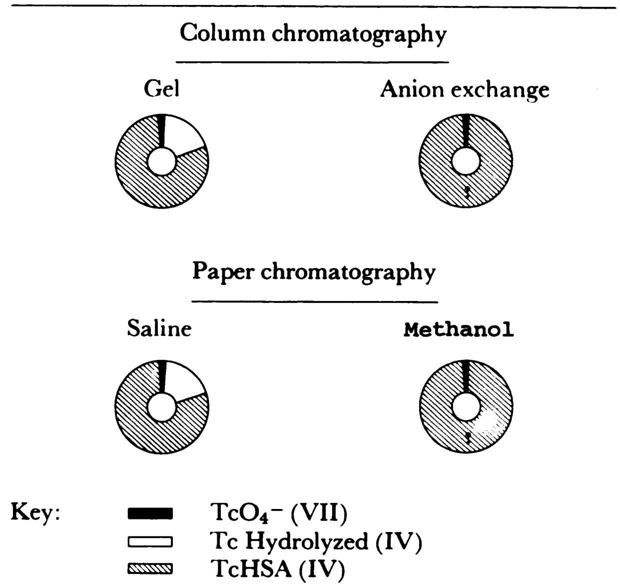

FIG. 6. Analysis of ^{99m}Tc-HSA (high pH method).

lished by us recently (*4*). Both compounds were prepared by adding TcO_4^- to a solution of stannous chloride and HSA at the appropriate pH. Figure 5 shows the analytical data for the preparation using the low pH procedure. Again the gel and saline systems show three components; TcO_4^-, Tc-HSA, and hydrolyzed technetium, whereas the methanol and anion systems show only two: TcO_4^- and presumably pure Tc-HSA. Figure 6 shows the analytical data for the preparation in which the TcO_4^- is added at pH 6. Here again the methanol and anion systems show the preparation to be comparable to the low pH procedure. However, the gel and the saline systems show three components, TcO_4^-, Tc-HSA, and hydrolyzed technetium. Reviewing the gel and saline data from the two preparations we see that these two preparations are quite different in the quantity of Tc-HSA and hydrolyzed technetium. These analytical data are consistent with the biological data obtained by us. The Tc-HSA prepared at high pH has considerable liver uptake whereas the Tc-HSA prepared at low pH has a normal blood pool distribution, reaffirming the purity of the product as obtained in the analytical analysis.

We feel that as nuclear medicine continues to grow so will the confusion concerning the biological distribution one would expect with a certain compound unless a multiple analytical approach is incorporated. We therefore strongly encourage the increased use of analytical procedures in nuclear medicine.

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REFERENCES

1. ECKELMAN W, MEINKEN G, RICHARDS P: The chemical state of ^{99m}Tc in biomedical products. *J Nucl Med* 12: 596–600, 1970

2. ECKELMAN W, MEINKEN G, RICHARDS P: The chemical state of ^{99m}Tc in biomedical products II. The chelation of reduced technetium with DTPA. Submitted to *J Nucl Med*

3. ECKELMAN W, RICHARDS P: Instant ^{99m}Tc-DTPA. *J Nucl Med* 11: 761, 1970

4. ECKELMAN W, MEINKEN G, RICHARDS P: ^{99m}Tc-human serum albumin. *J Nucl Med* 11: 707–710, 1971