

Rapid Miniaturized Chromatographic Quality-Control Procedures for Tc-99m Radiopharmaceuticals

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Our laboratory has adopted a complete miniaturized chromatography system for Tc-99m radiopharmaceuticals in order to improve upon the commercial systems currently available. Three distinct, separate, chromatographic procedures are used to determine the labeling efficiencies of Tc-99m-labeled sulfur colloid, MAA, stannous chloride, phytate, DMSA, DTPA, pyrophosphate, diphosphonate, methylene diphosphonate, polyphosphate, and glucoheptonate. The chromatographic systems include Whatman 31 ET paper and acetone, Gelman ITLC-SG and 0.9% sodium chloride, and Gelman ITLC-SA and acetone. The chromatographic strips are miniaturized (1 × 6 cm), colored-coded, marked, and numbered. All the chromatographic quality-control procedures are simple, rapid, and can easily be incorporated into the routine quality-control program of any nuclear medicine facility.

J Nucl Med 18: 1230–1233, 1977

Chromatographic quality-control procedures for Tc-99m radiopharmaceuticals are an important part of the total quality-control program in a nuclear medicine facility. Numerous chromatography procedures for Tc-99m agents have been mentioned in the literature—including Whatman No. 1 paper chromatography with 85% methanol (1); Gelman instant thin-layer chromatography silica gel (ITLC-SG) with 85% methanol (2); and Gelman ITLC-SG with acetone (3). Other thin-layer and paper-chromatography media and solvent systems for Tc-99m agents have also been investigated (4,5). In addition, a chromatography system has been introduced that not only determines free [^{99m}Tc] pertechnetate but also determines reduced Tc-99m not bound to the radiopharmaceutical (hydrolyzed reduced Tc-99m) (3–8). Recently, a miniaturized chromatography system has been described for specific Tc-99m radiopharmaceuticals (9). The purpose of the present study was to investigate a rapid miniaturized chromatography system that could be used easily for practically all Tc-99m radiopharmaceuticals.

MATERIALS AND METHODS

31 ET* chromatography paper, ITLC-SG† chromatography paper, and instant thin-layer chromatography silicic acid (ITLC-SA)† were cut into 1-cm × 6-cm strips. Pencil lines

were drawn across the paper at 1, 3, and 5 cm from the bottom of the strip and the resultant sections were labeled 1 and 2 with a pencil for the 31 ET paper and ITLC-SA paper, and 3 and 4 for the ITLC-SG paper. In order to identify each strip more readily, colored tape was added to the top of each type of chromatography paper. In addition, specific marking-pen lines (which move with the solvent and thus identify the solvent front) were drawn on the reverse sides of the chromatography strips. The completed strips are shown in Fig. 1.

Various Tc-99m radiopharmaceuticals—including pertechnetate, Tc-99m sulfur colloid, Tc-99m Sn MAA, Tc-99m Sn phytate, and Tc-99m stannous chloride—were each spotted on the bottom pencil line of 10 strips of 31 ET chromatography. The strips were promptly placed in 10-ml vials, to which approximately 1 ml of acetone was added and developed until the solvent front had migrated to the top pencil line (approximately 30 sec). The strips were

Received Dec. 14, 1976; revision accepted July 20, 1977.

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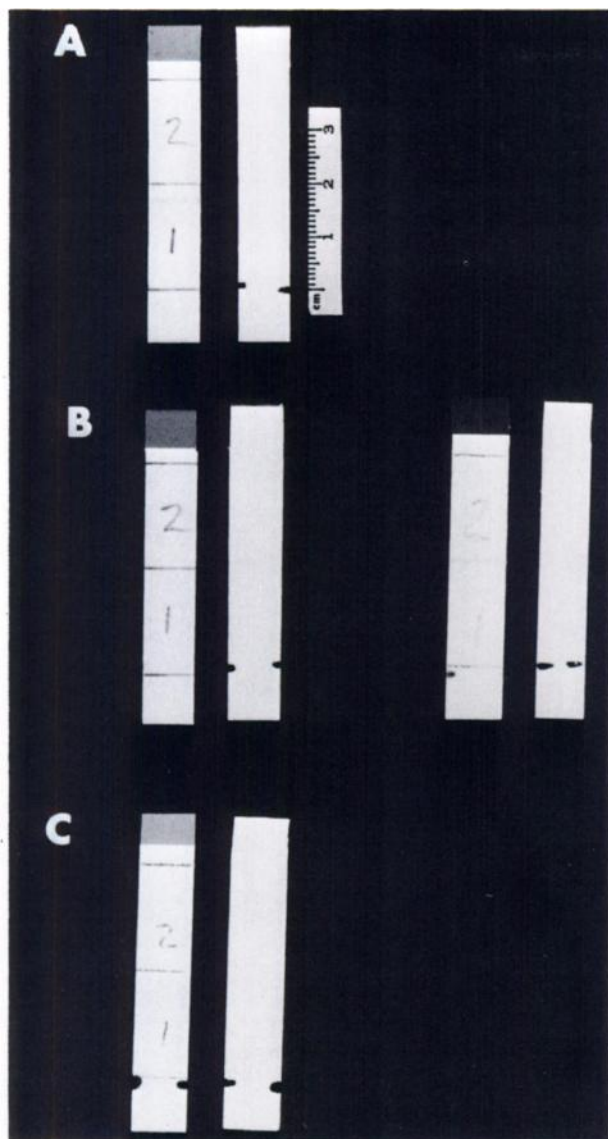


FIG. 1. Chromatography strips used. (A) Whatman 31 ET; (B) Whatman ET and ITLC-SG; (C) ITLC-SA.

dried and cut into eight equal segments: four below the center line and four above. Each segment was then counted for activity.

Tc-99m Sn DMSA was spotted on the bottom pencil line of ten ITLC-SA paper strips, developed in acetone (approximately 45 sec), sectioned and counted similarly.

A two-strip miniaturized chromatography system was used for Tc-99m Sn diphosphonate, Tc-99m Sn pyrophosphate, Tc-99m Sn glucoheptonate, and Tc-99m Sn DTPA. Ten determinations were performed on each agent. Each determination consisted of spotting the specific radiopharmaceutical on both 31 ET paper strips and ITLC-SG paper strips on the bottom pencil line. The 31 ET strips were immediately placed in a vial containing approximately 1 ml of acetone and developed until the solvent front migrated to the top pencil line (about 30 sec). The ITLC-SG strips were simultaneously placed in a vial containing approximately 1 ml of 0.9% NaCl and developed until the solvent front reached the top pencil line (about 45 sec). Both strips were then removed, cut into four segments above and four below the center line, and each segment counted for activity using gamma scintillation spectrometry.

For each study, the sum of the net segment counts was determined (ten individual observations for each segment). The total counts for a particular segment were then expressed as a percentage of the summed counts of all the segments.

RESULTS

The results for determining free pertechnetate using the 31 ET and acetone chromatography system are given in Table 1. As the data show, free pertechnetate migrates close to the acetone solvent front ($R_f = 0.9$), whereas the specific Tc-99m radiopharmaceutical (Tc-99m-labeled sulfur colloid, MAA, stannous chloride, or phytate) remains at the origin ($R_f = 0.0$). From the data, it is quite evident that if the strips were cut at the center pencil line, separating sections 1 and 2, the specific radiopharmaceutical would be located in section 1 and free pertechnetate in section 2. A simplified procedure was formulated for these specific radiopharmaceuticals and is listed in Table 2. It is currently used in our routine quality-control program.

The results for determining free pertechnetate in Tc-99m Sn DMSA preparations using the ITLC-SA paper and ace-

TABLE 1. ACTIVITY DISTRIBUTION (% STRIP COUNTS) FOR SPECIFIC Tc-99m RADIOPHARMACEUTICALS USING SINGLE-STRIP MINIATURIZED CHROMATOGRAPHY PROCEDURE

Chromatography strip and solvent	Strip section	Pertechnetate	Radiopharmaceutical (% of total activity)			
			Tc-99m sulfur colloid	Tc-99m Sn MAA	Tc-99m stannous chloride	Tc-99m Sn phytate
31 ET paper and acetone	1 (origin)	0.6	97.7	97.9	99.6	99.2
	2	0.4	0.1	0.5	0.2	0.2
	3	0.4	0.1	0.2	0.0	0.1
	4	0.6	0.1	0.2	0.0	0.0
	5	2.4	0.4	0.4	0.0	0.0
	6	30.4	0.7	0.4	0.1	0.2
	7	38.0	0.5	0.3	0.0	0.3
	8 (solvent front)	27.2	0.3	0.1	0.1	0.0

TABLE 3. ACTIVITY DISTRIBUTION (% STRIP COUNTS) FOR Tc-99m Sn DMSA USING SINGLE-STRIP MINIATURIZED CHROMATOGRAPHY PROCEDURE

Chromatography strip and solvent	Strip section	Radiopharmaceutical (% of total activity)	
		Pertech- netate	Tc-99m Sn DMSA
Silicic acid instant thin-layer chromatography and acetone	1 (origin)	0.0	71.6
	2	0.0	20.6
	3	0.0	5.2
	4	0.0	1.0
	5	9.2	0.8
	6	46.2	0.5
	7	32.8	0.2
	8 (solvent front)	11.6	0.1

tone chromatography system are shown in Table 3. Technetium-99m Sn DMSA remains at the origin ($R_f = 0.0$), whereas free pertechnetate migrates close to the solvent front ($R_f = 0.75$). Separation of the radiochemical components is again achieved by cutting the strip at the center pencil line. The same simplified procedure, as described in Table 2, is now used in our routine quality-control program.

The results of the chromatographic technique involving two distinct chromatography systems for specific Tc-99m radiopharmaceuticals are shown in Table 4. Free pertechnetate migrates close to the acetone solvent front on 31 ET paper ($R_f = 0.9$) leaving the specific Tc-99m radiopharmaceutical and hydrolyzed reduced Tc-99m (Tc-HR) at

the origin. With ITLC-SG paper and 0.9% NaCl, Tc-HR remains at the origin, whereas free pertechnetate and the specific Tc-99m radiopharmaceutical migrate with the 0.9% NaCl solvent front. Good separation was observed between the various radiochemical components, thus allowing the sectioning of each strip at the center line. The protocol in use for these specific radiopharmaceuticals is listed in Table 5 and is currently used in our daily radiopharmaceutical quality-control program.

DISCUSSION

We have basically divided the chromatographic evaluation of Tc-99m radiopharmaceuticals into two basic techniques: (a) a procedure involving single chromatography strips, which determine free pertechnetate, and (b) a procedure

TABLE 2. DETERMINATION OF FREE PERTECHNETATE IN Tc-99m-LABELED MAA, PHYTATE, SULFUR COLLOID, STANNOUS CHLORIDE, AND DMSA

1. Place approximately 1 ml acetone into a 10-ml glass vial.
2. Spot radiopharmaceutical on bottom pencil line of Whatman 31 ET chromatography paper (Gelman ITLC-SA for Tc-99m Sn DMSA).
3. Immediately place strip in vial containing acetone, and develop until solvent front migrates to top pencil line.
4. Cut strip at central pencil line, producing sections 1 and 2.
5. Count each section for activity (per unit time) using a gamma counter, and subtract background.
6. % free pertechnetate

$$= \left[\frac{(\text{net cts sect. 2})}{(\text{net cts sect. 1}) + (\text{net cts sect. 2})} \right] \times 100.$$

TABLE 4. ACTIVITY DISTRIBUTION (% STRIP COUNTS) FOR SPECIFIC Tc-99m RADIOPHARMACEUTICALS USING TWO-STRIP MINIATURIZED CHROMATOGRAPHY PROCEDURE

Chromatography strip and solvent	Strip section	Radiopharmaceutical (% of total activity)						
		Pertech- netate	Tc-99m Sn DTPA	Tc-99m Sn pyrophos- phate	Tc-99m Sn diphos- phonate	Tc-99m Sn methylene diphos- phonate	Tc-99m Sn polyphos- phate	Tc-99m Sn gluco- heptonate
31 ET paper and acetone	1 (origin)	0.6	97.3	77.3	97.9	82.0	84.9	98.3
	2	0.4	0.4	1.4	1.9	13.4	12.1	1.3
	3	0.4	0.0	0.1	0.0	4.1	2.6	0.1
	4	0.6	0.0	0.2	0.0	0.1	0.1	0.0
	5	2.4	0.0	2.4	0.0	0.1	0.1	0.1
	6	30.4	0.4	8.7	0.0	0.1	0.1	0.1
	7	38.0	0.9	6.5	0.1	0.1	0.0	0.1
	8 (solvent front)	27.2	1.0	3.4	0.0	0.1	0.1	0.0
Silica gel instant thin-layer chromatography and 0.9% NaCl	1 (origin)	0.0	0.6	0.5	0.3	0.1	0.6	0.2
	2	0.0	0.0	0.3	0.2	0.1	0.1	0.1
	3	0.0	0.0	0.2	0.3	0.1	0.2	0.0
	4	0.0	0.0	0.3	0.5	0.1	0.2	0.0
	5	0.0	0.2	1.0	0.7	0.2	0.4	0.1
	6	0.3	1.0	11.6	1.9	0.4	1.1	0.4
	7	15.7	26.8	29.9	10.9	1.9	6.0	2.5
	8 (solvent front)	83.9	71.3	56.1	85.2	96.9	91.3	96.7

TABLE 5. PROCEDURE 2: DETERMINING FREE PERTECHNETATE AND HYDROLYZED REDUCED Tc-99m IN Tc-99m-LABELED DTPA, GLUCOHEPTONATE, DIPHOSPHONATE, METHYLENE DIPHOSPHONATE, PYROPHOSPHATE, AND POLYPHOSPHATE

1. Place approximately 1 ml of acetone into one 10-ml glass vial and 1 ml of 0.9% NaCl into an identical vial.
2. Spot radiopharmaceutical on bottom pencil line of Whatman 31 ET paper chromatography and Gelman ITLC-SG chromatography strips.
3. Develop 31 ET paper strip in acetone solvent, and ITLC-SG strip in 0.9% NaCl solvent, until solvent front migrates to top pencil line.
4. Cut strips at center pencil line into sections 1, 2, 3, and 4.
5. Count all sections for activity (per unit time) using a gamma counter and subtract backgrounds.

% free pertechnetate

$$= \left[\frac{(\text{net cts sect. 2})}{(\text{net cts sect. 2}) + (\text{net cts sect. 1})} \right] \times 100.$$

% hydrolyzed reduced Tc-99m

$$= \left[\frac{(\text{net cts sect. 3})}{(\text{net cts sect. 3}) + (\text{net cts sect. 4})} \right] \times 100.$$

% labeling radiopharmaceutical

$$= 100 - \left[\frac{\% \text{ free}}{\text{pertechnetate}} \right] - \left[\frac{\% \text{ hydrolyzed}}{\text{reduced Tc-99m}} \right].$$

involving two chromatography strips, which determines free pertechnetate and Tc-HR. An ideal situation would be the determination of Tc-HR for all Tc-99m radiopharmaceuticals, but such a determination is difficult to perform on some radiopharmaceuticals with paper and/or thin-layer chromatography. Particulate radiopharmaceuticals will remain at the origin, as will Tc-HR, and no differentiation can be achieved (3). A rapid way to determine unbound Tc-99m in particulate radiopharmaceuticals (greater than 0.2 μm) is to filter the suspension through a 0.2- μm millipore filter, in which case an indication of Tc-HR is achieved in conjunction with the chromatographic determination of free pertechnetate (10,11). An appropriate alternative to determine unbound Tc-99m is centrifugation of particulates with repeat washings.

The chromatography strips are initially marked with pencil lines because these remain stationary with the solvents used. Marking-pen lines, which move with the solvent front, are placed on the back of the strip to ensure that no interaction occurs between the spotted radiopharmaceuticals and the marker. The strips are also numbered and color-coded to simplify the entire chromatographic procedure. The chromatography strip is immediately placed in the solvent with the radiopharmaceutical spot still wet to reduce the degree of radiopharmaceutical oxidation which yields free pertechnetate. Oxidation is further reduced by the rapid solvent development of the miniaturized chromatography strips.

Our miniaturized chromatography procedure is extremely rapid, easy to perform (see Tables 2 and 5), and can be

used for practically all Tc-99m radiopharmaceuticals. Labeling or tagging efficiencies can usually be determined within 3–5 min after spotting. We use these chromatography systems, as outlined with minimal interference within the normal routine of the nuclear-medicine department.

Compared with the most commonly used commercial chromatography kit, our chromatographic procedure is more rapid, with a development time of 30–45 sec, as opposed to one of about 4 min for the commercial kit. The shortened development time considerably reduces the time needed to perform the entire chromatographic procedure, and it also reduces the amount of oxidation to free TcO_4^- that can occur in the spotted radiopharmaceutical. The commercial kit also appears to give unusually high values for the hydrolyzed reduced Tc-99m fraction in Tc-99m pyrophosphate (9). The quality-control procedure used in this study can also be used to evaluate a total of 11 Tc-99m radiopharmaceuticals, whereas the commercial kit can evaluate only seven.

FOOTNOTES

* Whatman Chromatography Products, Clifton, N.J.

† Gelman Instrument Company, Ann Arbor, Mich.

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