Radiopharmacy

Common Errors Associated with Miniaturized Chromatography

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Miniaturized chromatography systems have been used over the years with few problems; yet it is imperative that a standard procedure be scrupulously followed. Variation in proper radiochromatographic technique can result in false radiopurity estimations. The two most frequent causes of these errors are simple procedural mistakes: (1) counting the strips too close to the gamma scintillation detector, thus exceeding count rate capabilities; and (2) spotting the origin below the initial solvent level in developing vials. Improper technique can result in large overestimations of impurity levels in Tc-99m-labeled radiopharmaceuticals.

Radiochemical purity of Tc-99m-labeled radiopharmaceuticals can be measured quickly and efficiently with miniaturized chromatographic systems (*1–4*). Since 1976, miniaturized systems have been increasing in popularity; they allow quality control procedures to be performed more easily and with less time. When a precise chromatographic procedure is followed, results are obtained rapidly and reproduced with minimal variation. On the other hand, improper technique will degrade a system's reliability by producing erroneous radiochemical evaluations.

Widespread use of miniaturized chromatography systems has simplified quality control programs in nuclear medicine. However, two problems have surfaced as the most frequent causes of error in using chromatographic techniques. We investigated these two problems: (1) count rate loss caused by detector distance on chromatography strip counting, and (2) improper solvent chamber levels on hydrolyzed reduced Tc-99m determinations. We also elaborate on the effects of these two parameters on chromatographic evaluations of Tc-99m-labeled radiopharmaceuticals using specific miniaturized chromatography systems.

Materials and Methods

In order to assess the effect of counting distance on instrument deadtime loss, four miniaturized Whatman 31 ET paper chromatography strips (1 cm × 6 cm) were spotted with 5 μl of [^{99m}Tc] pertechnetate (approximately 100 μCi). The strips were counted individually at five specific distances (20, 15, 10, and 5 cm, and at the surface) from a 2 × 2 in. NaI (Tl) well detector interfaced to a Tracor Northern multichannel analyzer. To determine the percent of counting loss for the varying distances, the chromatography strips were counted accordingly: (1) strip 1 and strip 2 were counted individually and together at the five predefined distances, (2) strips 1, 2, and 3 were counted individually and together at the five distances, and (3) strips 1, 2, 3, and 4 were counted individually and together at the five distances. Counting loss was determined using the paired sources method described in the literature (*5*).

The effect of distance on calculated levels of free pertechnetate was evaluated. A miniaturized 31 ET strip was spotted with Tc-99m-hydroxymethylene diphosphonate (HDP) and developed in acetone. The strip was cut at its center line and each section counted independently at the five distances from the scintillation detector. The percent of free pertechnetate was determined for each set of counts obtained at the varying distances.

To evaluate the effects of the initial solvent chamber levels, Tc-99m-HDP was spotted on silica gel-instant thin layer chromatography (ITLC-SG) strips. Two developing chambers containing distilled water were prepared as illustrated (Fig. 1). The level of the water in vial A (Fig. 1) is below the origin of the developing strip (proper solvent chamber level); the level in vial B is prepared so that the solvent would submerge the origin of a spotted strip (improper solvent chamber level). The strips were developed, cut into 0.6-cm sections, and counted.

Effects of solvent levels on the clinical evaluation of hydrolyzed-reduced Tc-99m (Tc-HR) were determined. Ten ITLC-SG strips were spotted with Tc-99m-HDP. Five strips were developed in vials with the solvent levels *below* the origin (proper technique), and five strips were developed in vials with solvent levels *above* the origin (improper technique). The strips were cut at the center line, counted, and calculated for

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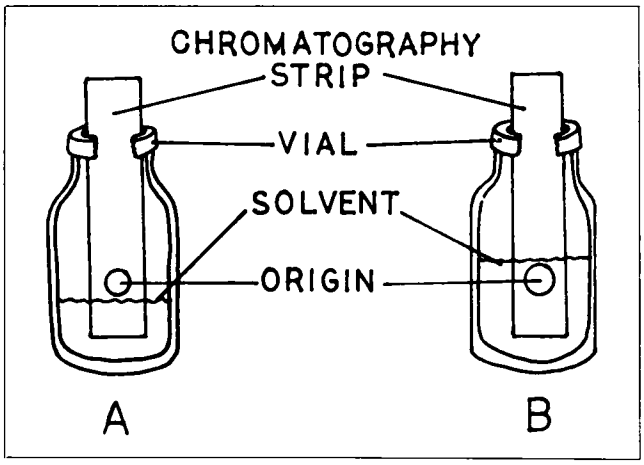

FIG. 1. Initial solvent conditions in miniaturized chromatography system: (A) Proper chromatographic procedure; solvent level below origin. (B) Improper chromatographic procedure; solvent level above origin.

the percent of Tc-HR. The data were statistically summarized by determining means and standard deviations.

Results

Figure 2 shows data obtained when the strips are counted at various distances from the scintillation detector. Percent counting loss at each distance was determined and compared to source-to-detector distance. Figure 2 also shows that percent counting loss must be accounted for in chromatographic analysis. Counting loss increases as distance between the strips and scintillation detector decreases. High activity in close proximity to the scintillation detector results in large deadtime loss and counts accumulated are grossly erroneous. Increasing the source-to-detector distance can solve the problem of deadtime in a counting set-up.

Strips counted too close to the detector exhibit false levels of calculated free pertechnetate in Tc-99m HDP preparations, as shown in Table 1. The level of [^{99m}Tc] pertechnetate was actually 6.5 to 68% for 20 cm and 0 cm, respectively. Clearly the calculations are a gross overestimation of the true impurity levels in the radiopharmaceutical. A sample's proximity to the scintillation detector must be considered when performing chromatographic counting procedures. This is especially true for detector instruments with no deadtime correction.

TABLE 1. Effect of Detector Distance on Chromatography Strip Counting

Distance from detector (cm)	Calculated free [^{99m}Tc] pertechnetate (%)
20	6.5
15	6.9
10	7.7
5	10.7
0	68.0

Figure 3 shows the effect of solvent levels on activity distribution along a chromatography strip (ITCL-SG and distilled water). It correlates directly with Fig. 1, which depicts the initial solvent levels in the developing vials. Vial A in Fig. 1 shows the proper solvent level; the liquid is below the origin and activity will migrate with the solvent front. Figure 3(A) exemplifies this notion of activity migration since virtually all of the counts are at the solvent front. On the other hand, vial B in Fig. 1 shows an improper solvent level; the origin is initially submerged in the solvent. Therefore, the majority of activity does not migrate with the solvent front but remains at the origin resulting in gross overestimations of hydrolyzed-reduced levels for Tc-99m radiopharmaceuticals.

Table 2 clearly illustrates the effect of solvent levels on evaluating hydrolyzed-reduced levels in spotted radiopharmaceuticals. With the proper solvent level (below the spot), Tc-HR levels of 0.5% were observed. However, with the improper solvent level (above the spot), erroneously high Tc-HR levels of 87.7% were observed. In addition, a great deal of variability in the results was observed with the improper solvent level as is shown by the relatively high standard deviation (14.1%).

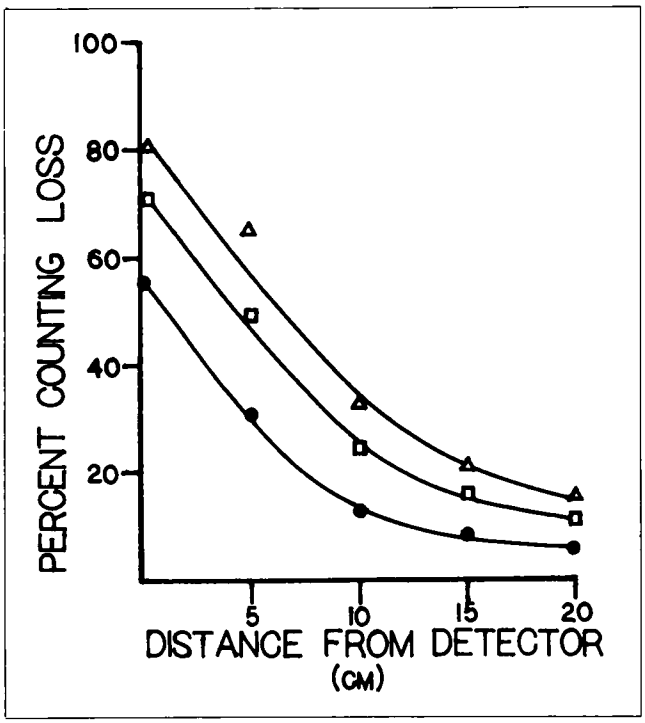

FIG. 2. Effect of detector distance on percent counting loss: ● = 200 μCi; □ = 300 μCi; △ = 400 μCi.

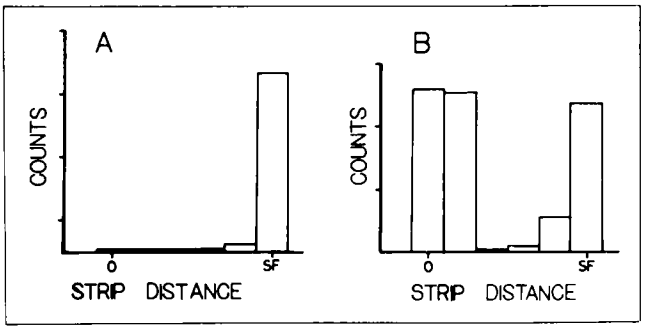

FIG. 3. Activity distribution of Tc-99m-HDP on ITLC-SG with distilled water as solvent: (A) Initial solvent level below origin. (B) Initial solvent level above origin.

TABLE 2. Effect of Initial Solvent Chamber Levels on Tc-HR Determinations in Tc-99m HDP Preparations

Initial solvent conditions	Percent Tc-99m HR (mean ± standard deviation)*
Solvent chamber level below origin	0.5 ± 0.1
Solvent chamber level above origin	87.7 ± 14.1

*n = 5.

Discussion

We believe the two problems outlined in this article are the most frequently encountered. The source-to-detector distance and the initial solvent conditions are two parameters that directly affect chromatographic evaluation of radiopharmaceuticals. Gross overestimations of both free [^{99m}Tc] pertechnetate and Tc-HR in preparations can result from improper radiochromatographic technique—namely, counting strips too close to the detector and submerging the origin below the solvent in developing vials.

We used 31 ET paper with acetone and ITLC-SG with distilled water to evaluate free pertechnetate and Tc-HR, respectively. A recent report (*6*) indicates spot drying before development eliminates false high levels of free pertechnetate; in this study, Gelman ITLC-SG with acetone was used to evaluate free pertechnetate. Another report (*7*) has noted that with ITLC-SG, water saturation of the chromatographic strips can occur, causing migration of the Tc-99m radiopharmaceuticals from the origin, which results in overestimation of free pertechnetate levels. To alleviate this problem, 31 ET paper in acetone should be used. This system is designed to be developed immediately while retaining maximal separation between free and bound Tc-99m. There are additional problems associated with spot drying prior to solvent development. Overdrying can produce a significant free pertechnetate artifact and the drying procedure markedly increases the time necessary to perform the chromatographic evaluation.

The objective of miniaturized chromatography systems is to perform quality control as quickly as possible while still retaining accuracy.

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