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An Update of Miniaturized Chromatography Procedures
for
Newer Radiopharmaceuticals

by:

A. Michael Zimmer, Ph.D.

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AN UPDATE OF MINIATURIZED CHROMATOGRAPHY
PROCEDURES FOR NEWER RADIOPHARMACEUTICALS

STATEMENT OF OBJECTIVES

The primary goal of this correspondence lesson is to increase the reader's knowledge and understanding of the clinical usefulness of miniaturized chromatography procedures for determining the radiochemical purity of existing radiopharmaceuticals. This lesson discusses quality control (QC) procedures for newer radiopharmaceuticals with updated information on the application and efficacy of these QC techniques.

Upon successful completion of this lesson, the reader should be able to:

1.   Define radiochemical purity and appropriate assessment methods to evaluate radiochemical purity.

2.   Describe miniaturized chromatography systems and common errors that can result when using miniaturized chromatography systems.

3.   Describe miniatured chromatography procedures for newer Tc-99m radiopharmaceuticals including:

   a.   Tc-99m Monoclonal Antibodies
   b.   Tc-99m Exametazime (Ceretec™)
   c.   Tc-99m Teboroxime (Cardiotec™)
   d.   Tc-99m Sestamibi (Cardiolite™)
   e.   Tc-99m Mertiatide (TechneScan Mag3™)
   f.   Tc-99m Tetrofosmin (Myoview™)
   g.   Tc-99m Bicisate (Neurolite™)
   h.   Tc-99m (V) DMSA

4.   Describe miniatured chromatography procedures for newer In-111 radiopharmaceuticals including:

   a.   In-111 Monoclonal Antibodies
   b.   In-111 Octreotide (Octreoscan™)

5.   Describe miniatured chromatography procedures for newer iodinated radiopharmaceuticals including:

   a.   Iodinated Monoclonal Antibodies
   b.   I-123 Iodoamphetamine

COURSE OUTLINE

AN UPDATE OF MINIATURIZED CHROMATOGRAPHY PROCEDURES FOR NEWER RADIOPHARMACEUTICALS

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INTRODUCTION

Because radiopharmaceuticals are intended for human administration, quality control procedures are essential in assuring the efficacy of these preparations. Although many quality control procedures are performed by manufacturers, radiopharmaceuticals are compounded in nuclear pharmacies and/or nuclear medicine departments using reagent kits. Thus, the ultimate responsibility for quality assurance of these radiopharmaceuticals lies with the radiopharmacist.

Radiopharmaceuticals, whether commercial or in-house preparations, must be subjected to physicochemical and biological testing. Physicochemical testing includes the examination and determination of the physical state, osmolality, pH, chemical purity, radionuclidic purity and radiochemical purity. Biological testing of radiopharmaceutical preparations include sterility and pyrogenicity testing.

Radiochemical purity is defined as the proportion of the total activity that is present in the specified chemical form. Numerous methodologies can be employed to assess the radiochemical purity of radiopharmaceuticals including thin layer chromatography, paper chromatography, gel permeation chromatography, high performance liquid chromatography (HPLC), and gel electrophoresis. Because time is critical in a nuclear pharmacy and/or nuclear medicine department, the emphasis of radiochemical quality control procedures must be on rapid, yet relatively easy procedures, in order to gain the maximum amount of information in a minimum amount of time. With this in mind, this review is written with an emphasis on rapid radiochemical quality control procedures for newer radiopharmaceuticals. The quality control procedures outlined in the text are fairly easy to use and have proven to be reliable in a hospital nuclear pharmacy setting.

MINIATURIZED CHROMATOGRAPHY PROCEDURES

Miniaturized chromatography procedures for determining the radiochemical purity of existing radiopharmaceuticals have been extensively decribed in the literature (1-6). With miniaturized systems, chromatography strips, consisting of various support media, are cut into small sizes (0.7 cm x 6 cm or 0.7 cm x 8 cm strips). Lines denoting the origin, cut line and solvent front are drawn on each strip. An illustration of a typical miniaturized chromatography strip is shown in Figure 1. For ease in counting, specific sections of the strips are sequentially numbered. The location of the cut line is dependent on the migration of the specific radiopharmaceutical. Chromatographic procedures include spotting the preparation on the origin line of the respective strips and developing the strips in the appropriate solvent system. Following solvent migration to the solvent front line, the strips are removed, cut and counted for activity using appropriate counting systems such as a well detector or dose calibrator.
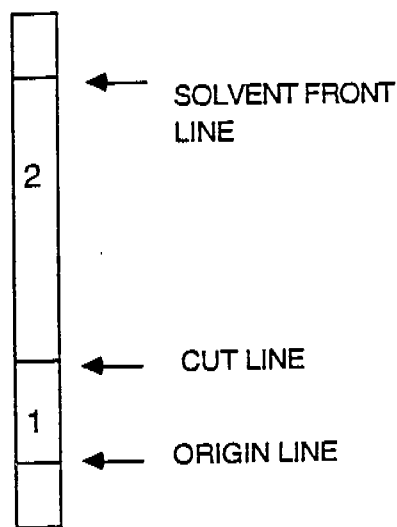

Figure 1. Typical miniaturized chromatography strips (0.7 cm x 6.0) showing origin, solvent front and cut lines.

Although miniaturized chromatography procedures have been used for many years with few problems, a few procedural errors can and do occur (7,8). Some of these are listed in Table 1. In our experience, the two most frequent errors include, (a) placing the radiopharmaceutical spot below the initial solvent level in the developing vials and (b) counting the strips incorrectly (placing the strip too close to the detector). Because procedural errors can result in grossly inaccurate assessments of radiochemical purity, careful technique must be utilized with these chromatographic procedures.

Table 1
Common Errors or Pitfalls Associated with Miniaturized Chromatography Systems

Source of Error	Result
Origin, where strip spotted, is below the initial solvent level in the developing vial.	Activity will distribute throughout the entire chromatgraphy strip resulting in inaccurate results. Spot new strip correctly.
Strips are counted too close to the NaI(Tl) well detector.	Dead time of crystal may be excessive resulting in gross overestimation of percent activity associated with the lower activity section of the strip. Increase distance of strips to detector, thus reducing dead time.
Strips are counted in dose calibrator	Insensitivity of dose calibrator may result in large errors when counting low activity strips. If possible spot more radiopharmaceutical activity on strip prior to developing
Chromatography strips and solvents are too old.	Migration pattern of radiopharmaceutical may be changed. Also streaking of activity may occur. These can lead to erroneous results. Use new solvents and dry strips prior to use.
Strips and/or solvents reversed.	Total inaccurate results may be obtained. Repeat entire QC procedure.
Radiopharmaceutical spot is dried prior to solvent development.	Oxidation of radiopharmaceutical may occur. Also binding of radiopharmaceutical with support media may result. Results in inaccurate assessment of radiochemical purity. Repeat entire QC procedure.
Strip is eluted past solvent front line.	If strip is eluted significantly past the solvent front line, the cut line must be changed to maintain the same Rf value.

QUALITY CONTROL OF NEWER Tc-99m RADIOPHARMACEUTICALS

Miniaturized chromatographic systems for newer Tc-99m radiopharmaceuticals are shown in Table 2. Specific chromatography systems outlined in Table 2 have been developed in various nuclear pharmacy or clinical nuclear medicine laboratories, or are modifications of the manufacturers' recommended quality control procedures. These systems were designed to be faster and/or easier to use than the specific manufacturers' chromatography systems. Specific chromatography procedures for newer Tc-99m radiopharmaceuticals are described below.

Tc-99m Monoclonal Antibodies

Our laboratory has developed a miniaturized chromatography system, consisting of ITLC-SG strips (0.7 x 6 cm) with 0.9% NaCl, to assess the radiochemical purity of Tc-99m labeled monoclonal antibodies and antibody fragments (9). With the chromatography system, free Tc-99m pertechnetate migrates with the solvent front ($R_f = 1.0$), whereas the radiolabeled monoclonal antibody remains at the origin ($R_f = 0.0$). A typical activity distribution profile of Tc-99m labeled monoclonal antibody and free Tc-99m pertechnetate on a developed chromatography strip is shown in Figure 2. It should be emphasized that the chromatography system outlined above does not distinguish radiolabeled dimers or radiolabeled aggregates from radiolabeled antibodies.

Table 2

Miniaturized Chromatography Procedures for Tc-99m Radiopharmaceuticals.

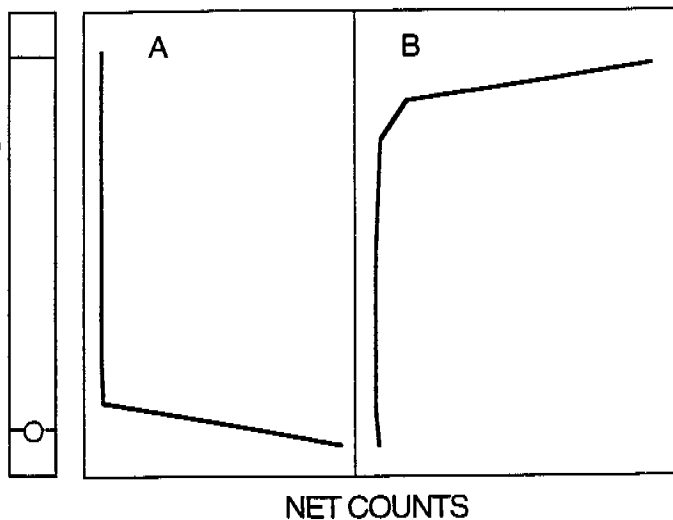| Compound	Support Media	Solvent	Radiochemical Impurities Detected	Ref
Tc-99m Monoclonal Antibodies	ITLC-SG	Normal Saline	Free Tc-99m Pertech	(9)
Tc-99m Exametazime (Single Strip)	Whatman 17	Ethyl Acetate	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced Tc-99m Lipophobic Fraction	(10)
Tc-99m Teboroxime	Whatman 31ET Whatman 31ET	Normal Saline Normal Saline/Acetone (1:1)	Soluble Tc-99m Impurities Tc-99m Hydrolyzed Reduced	(16)
Tc-99m Sestamibi (Single Strip)	Whatman 31ET	Ethyl Acetate	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced Tc-99m Polar Impurities	(18)
Tc-99m Mertiatide	Gelman Solvent Sat Pads Gelman Solvent Sat Pads	Chloroform/Acetone/Tetrahydrofuran (1:1:2) Normal Saline	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced	(19)
Tc-99m Tetrofosmin	ITLC-SG	Methylene chloride/acetone (65:35)	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced	(20)
Tc-99m Bicisate	Whatman 17	Ethyl Acetate	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced Tc-99m Lipophobic Fraction	(21)
Tc-99m (V) DMSA	Whatman 17 ITLC-SG	50% Aqueous Acetonitrile Methyl Ethyl Ketone	Free Tc-99m Pertech Tc-99m Hyrolyzed Reduced	

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Figure 2. Chromatography strip activity distribution of (A) Tc-99m monoclonal antibody and (B) Tc-99m pertechnetate in chromatography system consisting of ITLC-SG with 0.9% NaCl.
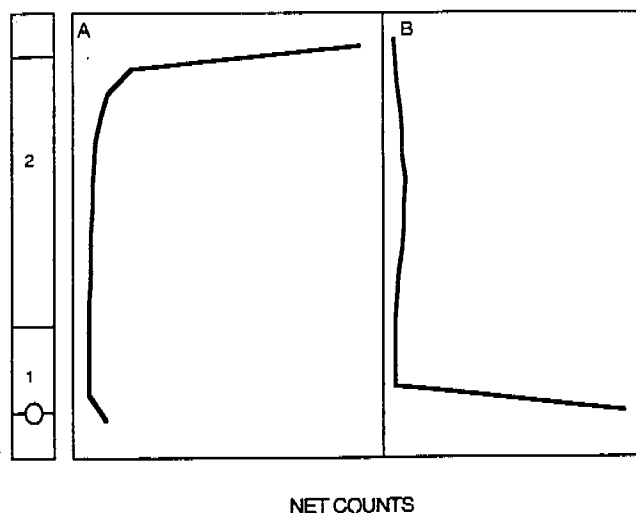

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Figure 3. Chromatography strip activity distribution of (A) Tc-99m exametazime and (B) Tc-99m pertechnetate in chromatography system consisting of Whatman 17 with ethyl acetate.

Tc-99m Exametazime (Ceretec™)

An excellent review of the manufacturer's recommended three-strip chromatography procedure is found in the literature (8). Our laboratory has developed a single-strip miniaturized chromatography system to separate the Tc-99m lipophillic fraction (R_f = 1.0) from other Tc-99m radiochemical impurities (R_f = 0.0) (10). The specific chromatography system consists of Whatman 17 paper (Whatman Chromatography Products, Clifton, NJ) with ethyl acetate as the solvent. A typical activity distribution profile of Tc-99m exametazime and free Tc-99m pertechnetate on a developed Whatman 17 chromatography strip is shown in Figure 3. The single-strip chromatography system is very rapid, taking less than one minute to complete, and is faster and easier to

use than the three strip chromatography method recommended by the manufacturer (11). With the single-strip system outlined above, it is important to place the strip in the solvent immediately after spotting; failure to do so will result in underestimating the Tc-99m lipophillic component. It should also be emphasized that the single-strip chromatography system separates the lipophillic fraction from all other radiochemical impurities and, therefore, quantitation of specific radiochemical impurities is not possible.

Tc-99m Teboroxime (Cardiotec™)

The chromatography system, as described in Table 2, is a miniaturized version of the system recommended by the manufacturer (12). By miniaturizing the system, strip developing time has been significantly reduced from approximately 20 minutes for the manufacturers' recommended strips to less than three minutes for the miniaturized strips. At the same time, maximal separation between Tc-99m impurities (pertechnetate and hydrolyzed reduced Tc) and Tc-99m teboroxime has been maintained. A chromatography strip activity distribution profile of a typical Tc-99m teboroxime preparation is shown in Figure 4. Migration of the radiopharmaceutical and free Tc-99m pertechnetate (R_f = 1.0) occurs in the chromatography system consisting of Whatman 31ET with acetone/saline (1:1). With this chromotagraphy system, hydrolyzed reduced Tc-99m remains at the origin (R_f = 0.0). Separation of free pertechnetate (R_f = 1.0) from Tc-99m teboroxime (R_f = 0.0) occurs in the chromatography system consisting of Whatman 31ET with saline. With this system, hydrolyzed reduced Tc-99m also remains at the origin.
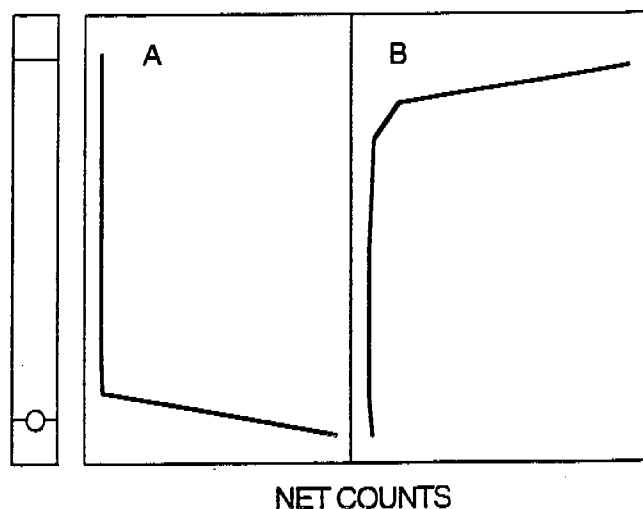

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Figure 4. Chromatography strip activity distribution of Tc-99m teboroxime in chromatography system consisting of (A) Whatman 31ET with normal saline and (B) Whatman 31ET with acetone/normal saline (1:1).

Tc-99m Sestamibi (Cardiolite™)

The recommended manufacturer's chromatographic quality control procedure (13) utilizes aluminum oxide thin layer chromatography plates. In addition, the manufacturer recommends a 15-minute radiopharmaceutical spot drying time prior to strip development. Because of these factors, the time needed to perform radiochemical purity evaluations of Tc-99m sestamibi is exceedingly long (30 minutes). As a result, a number of alternative rapid miniaturized chromatography procedures have been proposed and are listed in Table 3. This includes a two-strip method (14), which utilizes Gelman ITLC-SG (Gelman Instruments, Ann Arbor, MI) strips with normal saline and acetone as the respective solvents. With this system, free Tc-99m pertechetate and hyrolyzed reduced Tc-99m are separated from Tc-99m sestamibi. However, other radiochemical impurities may not be identified with this chromatography system. Our laboratory has investigated a single-strip chromatography system consisting of Whatman 31ET and ethyl acetate (16). A typical activity distribution of Tc-99m sestamibi on Whatman 31ET strips is shown in Figure 5. With this system, free Tc-99m pertechnetate and hydrolyzed reduced Tc-99m remain at the origin whereas Tc-99m sestamibi migrates from the origin with an R_f value of 0.6 to 1.0. With the single-strip chromatography system outlined above, possible polar radiochemical impurities would also remain at the origin. A comparison of the radiochemical purity of Tc-99m sestamibi preparations, using the miniaturized chromatography procedure described above and the conventional manufacturer's chromatography procedure, are shown in Table 4. Close correlations in labeling efficiencies of Tc-99m sestamibi were observed between the two chromatography systems evaluated.

product package insert, is relatively easy to use. However, possible technical errors can occur. Following radiopharmaceutical loading, the cartridge must be eluted slowly with ethanol/saline. If not eluted slowly, Tc-99m mertiatide will remain on the cartridge resulting in false estimations in radiochemical purity.
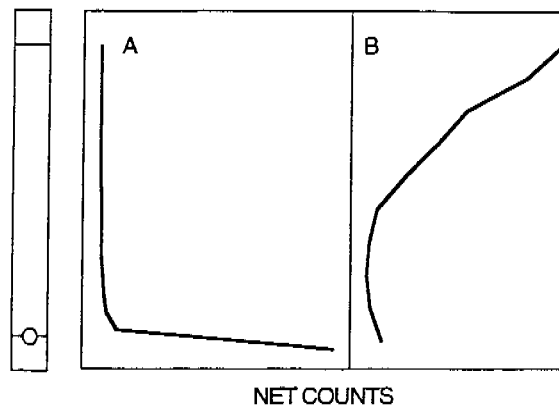

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Figure 5. Chromatography strip activity distribution of (A) Tc-99m pertechnetate and (B) Tc-99m sestamibi in chromatography system consisting of Whatman 31ET with ethyl acetate.
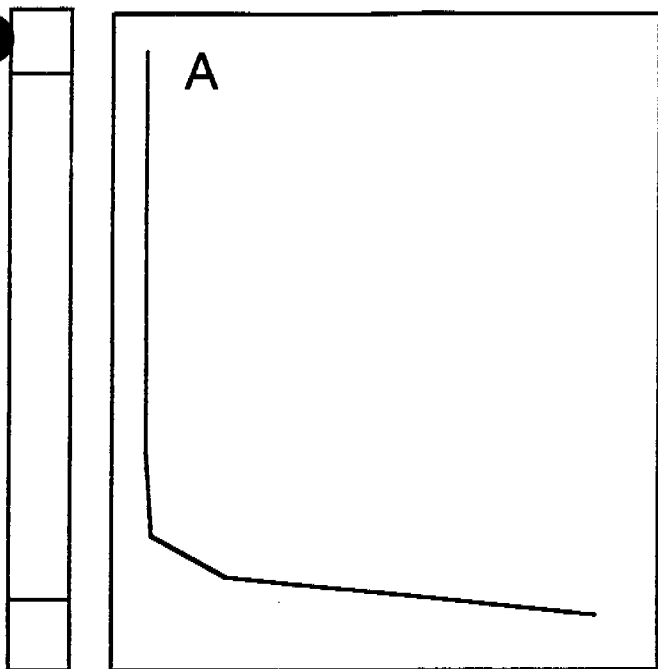
Table 4
Radiochemical Purity Evaluations of Tc-99m Sestamibi Using Conventional and Miniaturized Chromatography Systems *

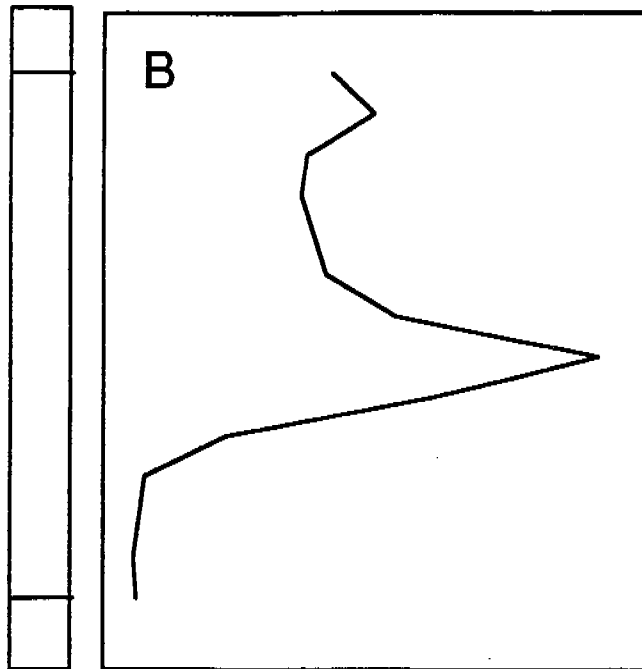| Preparation	Radiochemical Purity (%)	
	Conventional Chrom System	Miniaturized Chrom System **
1	97.5	96.7
2	97.3	96.8
3	97.4	96.0
4	97.0	96.3
5	97.0	96.7
6	97.1	96.3
7	97.6	96.1
8	98.4	98.9
9	95.5	94.2
10	96.6	95.5
11	98.0	99.6
12	98.3	97.3
13	98.7	98.5

* *The miniaturized chromatography system has not been validated at reduced radiochemical levels*
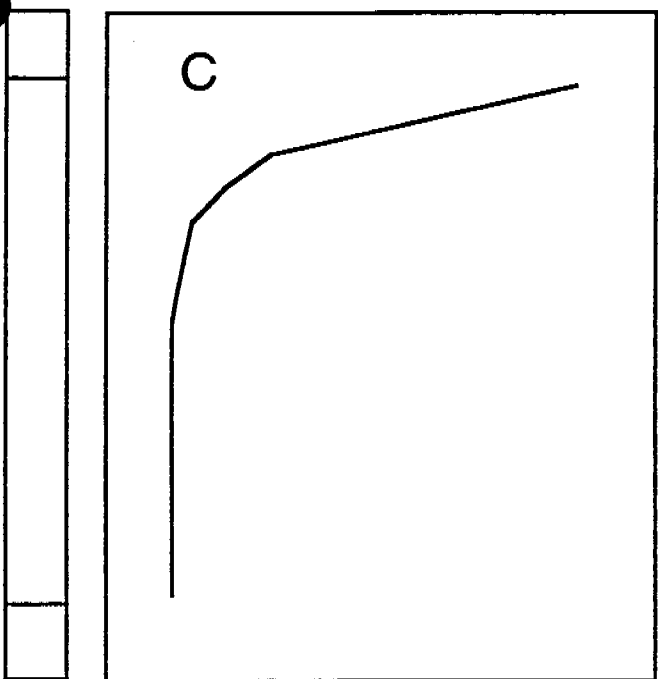
** *Whatman 31ET with ethyl acetate*

A miniaturized chromatography procedure to evaluate the radiochemical purity of Tc-99m mertiatide has been published (18). The procedure utilizes two different chromatography systems. System 1, which separates free Tc-99m pertechnetate (R_f = 0.5 to 1.0) from Tc-99m MAG3 (R_f = 0.0) and hydrolyzed reduced Tc-99m (R_f = 0.0), consists of Gelman Solvent Saturation Pads (0.7 x 8 cm) with chloroform: acetone: tetrahydrofuran (1:1:2) as the solvent system. System 2 separates hydrolyzed reduced Tc-99m (R_f = 0.0) from free Tc-99m pertechnetate (R_f = 1.0) and Tc-99m MAG3 (R_f = 1.0) and consists of Gelman Solvent Saturation Pads (0.7 x 8 cm) with 0.9% sodium Ccloride as the solvent system. Typical chromatography strip activity distributions of Tc-99m MAG3 and Tc-99m pertechnetate in both chromatography systems are shown in Figure 6.

Table 3
Miniaturized Chromatography Procedures for Tc-99m Sestamibi

System	Support Media	Solvent	References
1	ITLC-SG ITLC-SG	Normal Saline Acetone	(14)
2	Gelman Solvent Sat Pads	Chloroform/tetrahydrofuran (1:1)	(15)
3	Whatman 31ET	Ethyl Acetate	(16)

Tc-99m Mertiatide (Technescan Mag3™)

The manufacturer's quality control procedure for Tc-99m mertiatide preparations utilizes solid phase extraction (17). With this technique, the radiolabeled sample is applied to a solid adsorbent (Sep-Pak C18 cartridges, Waters Chromatography, Milford, MA) and various radiochemical components are selectively eluted in a step-wise manner using appropriate solvents, including 0.001 N HCl and ethanol/saline (1:1). The technique, as outlined by the manufacturer's

Figure 6. Chromatography strip activity distribution of (A) Tc-99m Mertiatide and (B) Tc-99m pertechnetate in chromatography system consisting of Gelman Solvent Saturation Pads with chloroform:acetone:tetrahyrofuran (1:1:2). Chromatography strip activity distribution of (C) Tc-99m Mertiatide and (D) Tc-99m pertechnetate in chromatography system consisting of Gelman Solvent Saturation Pads with 0.9% NaCl.

Tc-99m Tetrofosmin (Myoview™)

Northwestern University Medical School laboratory has miniaturized the chromatography system recommended by the manufacturer for Tc-99m tetrofosmin (19). The miniaturized chromatography system consists of a single Gelman ITLC-SG strip (1 x 10 cm) and methylene chloride:acetone (65:35 v/v) as the solvent system. With the outlined chromatography system, free Tc-99m pertechnetate migrates with the solvent front ($R_f = 1.0$), Tc-99m tetrofosmin migrates with an R_f value of 0.5 and hydrolyzed reduced Tc-99m remains at the origin ($R_f = 0.0$). A chromatography strip activity distribution of a typical Tc-99m tetrofosmin and Tc-99m pertechnetate are shown in Figure 7. Besides origin and solvent front lines, two cut lines are drawn at an R_f value of 0.2 and 0.8, respectively. After solvent development, the strip is cut into 3 sections: the lower section contains hydrolyzed reduced Tc-99m; the middle section contains Tc-99m tetrofosmin; the upper section contains free Tc-99m pertechnetate.
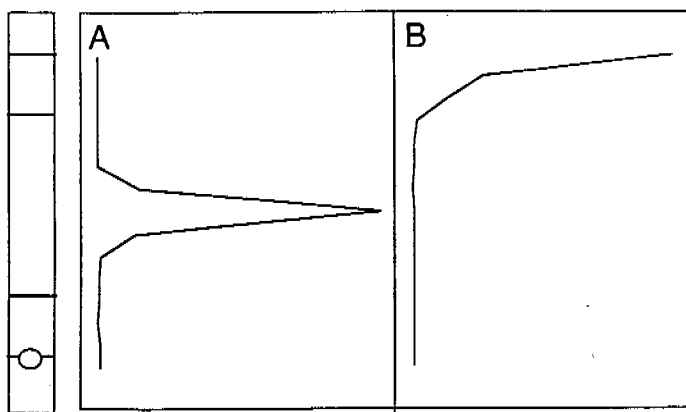

Figure 7. Chromatography strip activity distribution of (A) Tc-99m tetrofosmin and (B) Tc-99m pertechnetate in chromatography system consisting of ITLC-SG with methylene chloride:acetone (65:35).

Several important issues must be addressed when using the specific chromatography system outlined above including radiopharmaceutical spot size and exact solvent ratios. Radiopharmaceutical spot size can affect the migration of Tc-99m tetrofosmin (Figure 8a). The chromatography procedure utilizes a spot size of 5 ul. Increasing radiopharmaceutical spot size from 5 ul increases the R_f of Tc-99m tetrofosmin, whereas decreasing the spot size from 5 ul has the reverse effect. In addition, the solvent ratios utilized must be exact. Slightly increasing the acetone concentration in the solvent system increases the migration of Tc-99m tetrofosmin, while slighly decreasing the acetone concentration has the reverse effect (Figure 8b).

Tc-99m Bicisate (Neurolite™)

The miniaturized chromatography system (20) used to assess the radiochemical purity of Tc-99m bicisate is the same as the one used to assess labeling efficiency of Tc-99m exametazime (10). The chromatography system consists of Whatman 17 chromatography strips (0.7 x 8 cm) and ethyl acetate as the solvent. Using this system, Tc-99m bicisate migrates with an R_f value of 0.8-1.0 whereas free Tc-99m pertechnetate and hydrolyzed reduced Tc-99m remain at the origin ($R_f = 0.0$). The chromatography procedure is rapid, taking approximately one to two minutes for solvent development, as opposed to the manufacturer's recommended chromatography procedure, which takes approximately 40 to 60 minutes to complete (20).

Tc-99m (V) DMSA

A miniaturized chromatography procedure to assess the radiochemical purity of Tc-99m (V) DMSA has been developed (21). The chromatography procedure utilizes two chromatography systems. One system, which consists of Whatman 17 chromatography strips (1 x 9 cm) and 50% v/v aqueous acetonitrile, separates free Tc-99m pertechnetate ($R_f = 1.0$) and Tc-99m (V) DMSA ($R_f = 0.6-0.9$) from hydrolyzed reduced Tc-99m ($R_f = 0.0$). The other chromatography system, which separates free Tc-99m pertechnetate ($R_f = 1.0$) from Tc-99m (V) DMSA ($R_f = 0.0-0.2$) and hydrolyzed reduced Tc-99m ($R_f = 0.0$), consists of ITLC-SG strips (1 x 9 cm) and methyl ethyl ketone.

QUALITY CONTROL OF NEWER In-111 RADIOPHARMACEUTICALS

Miniaturized chromatography systems for newer In-111 labeled radiopharmaceuticals are listed in Table 5. These include In-111 monoclonal antibodies, such as In-111 satumomab (Oncoscint) and In-111 antimyosin (Myoscint), and In-111 octreotide (Octreoscan).

Table 5

Miniaturized Chromatography Procedures for In-111 Radiopharmaceuticals.

Compound	Support Media	Solvent	Rf Values	Ref
In-111 Monoclonal Antibodies (DTPA challenge)	ITLC-SG	Normal Saline	In-111 MOAB= 0.0 In-111 DTPA= 1.0	(22)
In-111 Octreotide (DTPA Challenge)	ITLC-SG	Normal Saline	In-111 Octreotide= 0.0 In-111 DTPA= 1.0	

Table 6

Miniaturized Chromatography Procedures for Iodinated Radiopharmaceuticals.

Compound	Support Media	Solvent	Rf Values	Ref
Iodine	ITLC-SG	Acetone	Iodide= 1.0 Iodate= 0.0 Periodate= 0.0	(26)
Iodinated Monoclonal Antibodies	ITLC-SG	Normal Saline	Iodinated Antibody= 0.0 Free iodide= 1.0	(24)
I-123 Iodoamphet-amine (IMP)	ITLC-SA	10% NaCl	Unbound I-123= 1.0 I-123 IMP = 1.0	(25)

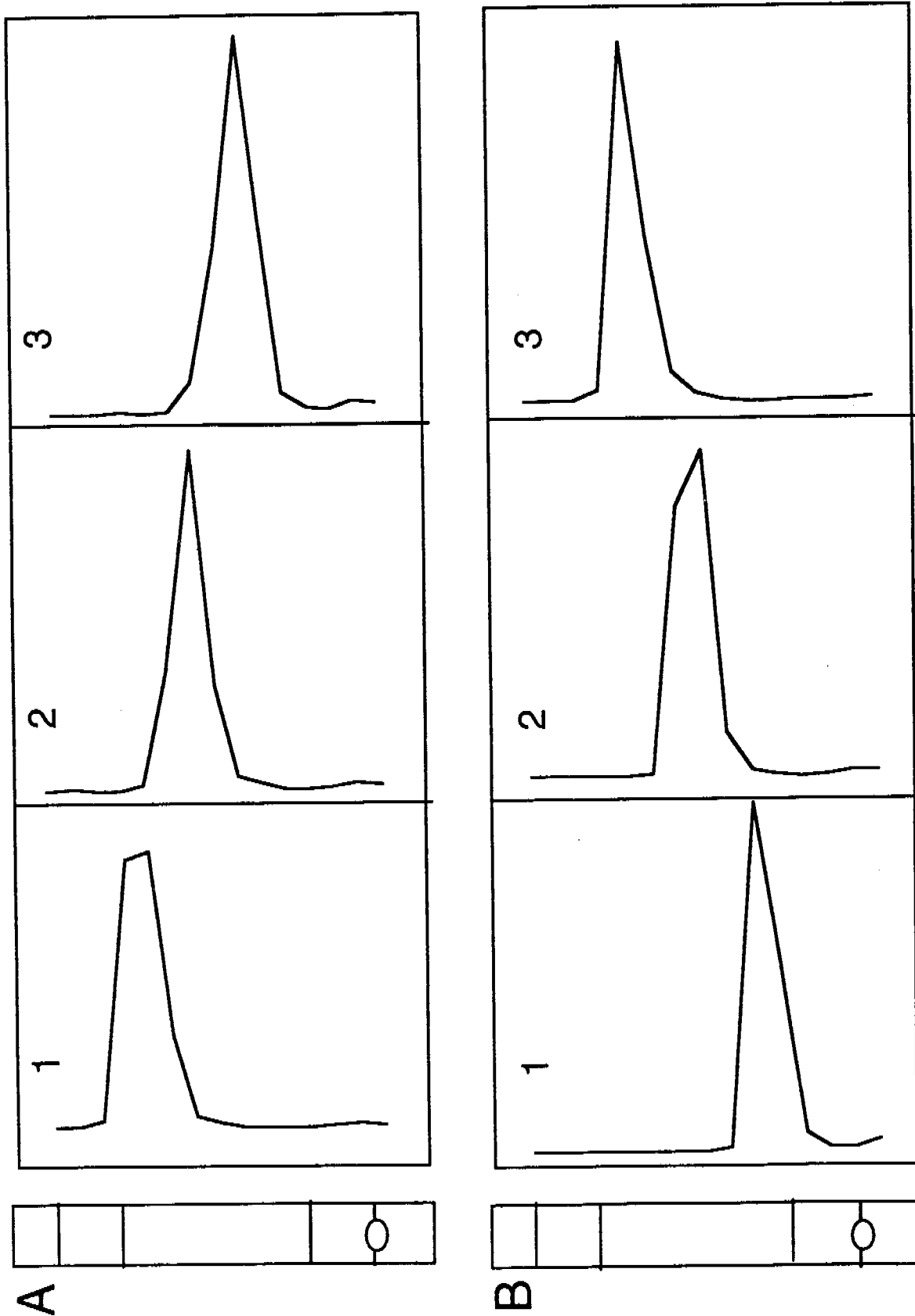Figure 8. (A) Effect of altering methylene chloride:acetone solvent ratios, from (1) 60:40, (2) 65:35 and (3) 70:30, on the migration of Tc-99m tetrofosmin using ITLC-SG chromatography strips. (B) Effect of varying radiopharmaceutical spot size, ranging from (1) 2.5 ul, (2) 5.0 ul and (3) 10.0 ul, on the migration of Tc-99m tetrofosmin using ITLC-SG chromatography strips with methylene chloride:acetone (65:35).

In-111 Monoclonal Antibodies

The miniaturized chromatography system for In-111 monoclonal antibodies includes Gelman ITLC-SG strips (0.7 x 6 cm) with 0.9% sodium chloride (22). Prior to chromatography spotting, an aliquot of the In-111 antibody preparation is challenged with DTPA (0.05 M), in order to complex unbound or weakly bound In-111. This is achieved by mixing 50 ul of In-111 labeled antibody with 25 ul of 0.05 M DTPA for one minute. Following radiopharmaceutical spotting and solvent migration, In-111 DTPA chelate migrates with the solvent front (R_f = 1.0) whereas In-111 antibody remains at the origin (R_f = 0.0). A chromatography strip activity distribution of In-111 DTPA chelate and In-111 antimyosin antibody is found in Figure 9.
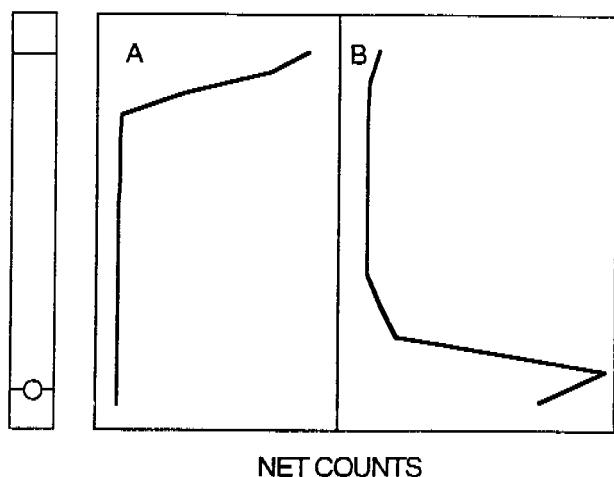

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Figure 9. Typical chromatography strip activity distribution of (A) In-111 DTPA and (B) In-111 labeled monoclonal antibody (Myoscint) in chromatography system consisting of ITLC-SG with normal saline.

In-111 Octreotide (Octreoscan™)

The manufacturer's quality control procedure for In-111 octreotide preparations involves solid phase extraction (23) using Sep-Pak C18 cartridges (Waters Chromatography, Milford, MA). The laboratory at Northwestern University Medical School has adapted the miniaturized chromatography procedure outlined above for In-111 monoclonal antibodies to evaluate the radiochemical purity of In-111 octreotide. The system utilizes ITLC-SG strips (0.7 x 6 cm) strips with 0.9% sodium chloride as the solvent system. Prior to radio-pharmaceutical spotting, an aliquot of In-111 octreotide is challenged with DTPA (0.05 M), as outlined above. Following solvent migration, In-111 octreotide remains at the origin (R_f = 0.0) while unbound and/or weakly bound In-111, as a DTPA chelate, migrates with the solvent front (R_f = 1.0). A typical activity distribution

of In-111 Octreotide and In-111 DTPA on a developed ITLC-SG strip is shown in Figure 10.
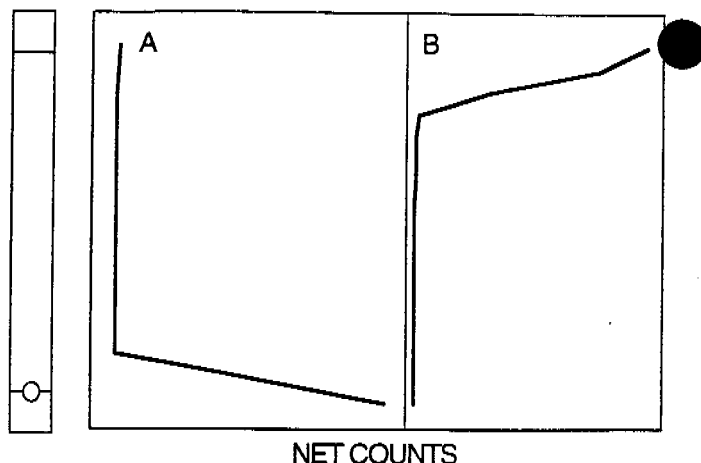

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Figure 10. Typical chromatography strip activity distribution of (A) In-111 labeled octreotide and (B) In-111 DTPA in chromatography system consisting of ITLC-SG with normal saline.

QUALITY CONTROL OF NEWER IODINATED RADIOPHARMACEUTICALS

Miniaturized chromatography systems for iodinated radiopharmaceuticals are listed in Table 6. The table includes the support media, solvent system and the migration of various radiochemical species.

Iodinated Monoclonal Antibodies

The miniaturized chromatography procedure to evaluate the radiochemical purity of iodinated monoclonal antibodies (24) is the same as that utilized for Tc-99m monoclonal antibodies. The procedure utilizes miniaturized ITLC-SG strips (0.7 x 6 cm) and 0.9% sodium chloride as the solvent system. Using this system, free iodide migrates with the solvent front (R_f = 1.0) while the iodinated monoclonal antibody remains at the origin (R_f = 0.0).
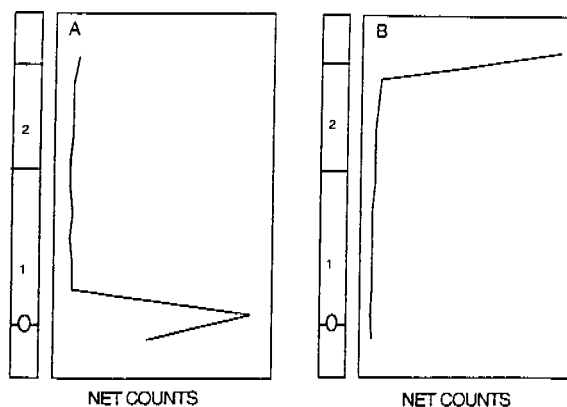

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Figure 11. Chromatography strip activity distribution of (A) I-123 Iodoamphetamine and (B) I-123 sodium iodide in chromatography system consisting of ITLC-SA with 10% sodium chloride.

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I-123 Iodoamphetamine (IMP)

Northwestern University Medical School laboratory has investigated a miniaturized chromatography system, utilizing ITLC-SA (0.7 x 6 cm) with 10% NaCl, to assess the radiochemical purity of I-123 iodoamphetamine (25). With this chromatography system, unbound I-123 migrates with the solvent front ($R_f = 1.0$) and I-123 iodoamphetamine remains at the origin ($R_f = 0.0$). The strip activity distribution of I-123 idoamphetamine and I-123 sodium iodide on ITLC-SA paper eluted with 10% NaCl is shown in Figure 11.

CONCLUSIONS

Due to the inherent instability of radiopharmaceutical preparations, it is essential to perform quality control procedures to insure the efficacy of these products. This is especially true of radiopharmaceuticals formulated in a nuclear pharmacy and/or nuclear medicine department. With these preparations, if certain levels of purity are not obtained, the radiopharmaceutical preparation cannot be clinically utilized. This lesson has reviewed the use of rapid, yet accurate, chromatography systems and procedures to evaluate the radiochemical purity of radiopharmaceuticals in a short time period. Some of the chromatography systems are modifications of the manufacturer's described systems, whereas others are newly developed systems which have been extensively tested.

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QUESTIONS

1. Radiochemical purity is defined as the proportion of activity that is present in the specified _____ form.

   A. radionuclidic
   B. chemical
   C. biological
   D. none of the above

2. Miniaturized chromatography systems consist of which of the following:

   A. miniaturized strips
   B. miniaturized solvent volumes
   C. large strips
   D. sealed developing chambers

3. The two most common errors associated with miniaturized chromatography systems include (1) incorrect placement of radiopharmaceutical spot on chromatography strip and (2) _____.

   A. incorrect solvent developing time
   B. incorrect solvent used
   C. incorrect chromatography strips used
   D. incorrect counting of the developed chromatography strips

4. When assessing the radiochemical purity of Tc-99m monoclonal antibodies using ITLC-SG with saline, where does free Tc-99m pertechnetate migrate?

   A. remains at the origin
   B. migrates with the solvent front
   C. migrates with an R_f value of about 0.5
   D. none of the above.

5. When assessing the radiochemical purity of In-111 monoclonal antibodies, DTPA is added prior to radiopharmaceutical spotting in order to:

   A. allow a smooth migration of In-111 monoclonal antibody
   B. complex any unbound or loosely bound In-111
   C. adjust the pH of the radiopharmaceutical spot size
   D. none of the above

6. When using a single strip method to evaluate the radiochemical purity of Tc-99m Exametazime, the _____ component is separated from all radiochemical impurities:

   A. Tc-99m pertechnetate
   B. hydrolyzed reduced Tc-99m
   C. lipophillic component
   D. lipophobic component

7. The single-strip chromatographic evaluation of Tc-99m Sestamibi includes Whatman 31ET paper and _____ as the solvent.

   A. ethyl acetate
   B. normal saline
   C. acetone
   D. acetonitrile

8. When utilizing the miniaturized chroma-tography system for Tc-99m Tetrofosmin, if the ratio of the solvent system (methylene chloride:acetone) is changed from the standard 65:35 ratio, the result is:

   A. slow migration of the solvent on the chromatography strip
   B. slower migration of free Tc-99m pertechnetate
   C. altered migration of Tc-99m tetrofosmin
   D. no migration of Tc-99m radiochemical impurities

9. The miniaturized chromatography system used to evaluate the radiochemical purity of Tc-99m Bicisate is the same system as that used for

_____.

A. Tc-99m Tetrofosmin
B. Tc-99m Exametazime
C. Tc-99m Sestamibi
D. Tc-99m monoclonal antibodies

10. The miniaturized chromatography system used to evaluate the radiochemical purity of both In-111 monoclonal antibodies and In-111 Octreotide consist of:

A. ITLC-SG with normal saline and DTPA challenge
B. ITLC-SG with acetone, DTPA challenge
C. ITLC-SA with acetone, DTPA challenge
D. Whatman 31ET with normal saline, DTPA challenge

11. The miniaturized chromagraphy system used to evaluate the radiochemical purity of I-123 Iodoamphetamine consists of ITLC-SA with _____ as the solvent system.

A. normal saline
B. distilled water
C. 10% sodium chloride
D. 20% sodium chloride

12. Radiopharmaceutical spot drying on the chromatography strip prior to solvent development can result in inaccurate assessment of radiochemical purity due to:

A. possible oxidation of the radiopharmaceutical
B. spot size enlarging on the strip
C. spot migration on the strip
D. all of the above

13. Chromatography strip counting in a dose calibrator may lead to large errors when counting low activity strips because:

A. geometry variations in dose calibrator
B. attenuation of activity by strip
C. wrong setting on dose calibrator may be used
D. insensitivity of dose calibrator

14. What is the support media utilized for Tc-99m Mertiatide?
A. ITLC-SG
B. Whatman 31ET
C. Whatman 17
D. Gelman Solvent Saturation Pads

15. What is the general size the miniaturized chromatography strips?
A. 0.7 x 6 cm
B. 1 x 8 cm
C. 1 x 10 cm
D. 1 x 6 cm

16. Miniaturized chromatography strips have specific lines drawn on them. These lines consist of the origin line, solvent front line and _____ line.

A. developing
B. cut
C. target
D. none of the above

17. The purpose of the origin line is to indicate:

A. the line on which the radiopharmaceutical is spotted
B. the line where the solvent cannot exceed
C. the line where the strip is cut after developing
D. the line that marks the beginning of the strip

18. When counting, if the strips are placed too closely to the well detector, inaccurate results are obtained because:

A. the detector is too insensitive
B. the counting window may not be proper
C. the counting efficiency may increase
D. the dead time of the detector may cause signifcant errors

19. The single-strip method to evaluate the radiochemical purity of Tc-99m Exametazime consists of _____ with ethyl acetate as the solvent.

A. Whatman 17
B. Whatman 31ET
C. ITLC-SG
D. ITLC-SA

20. Migration of Tc-99m Teboroxime occurs when using which miniaturized chromatography system?

   A.   Whatman 31ET with saline
   B.   Whatman 31ET with acetone
   C.   Whatman 31ET with acetone:saline (1:1)
   D.   ITLC-SG with acetone:saline (1:1)

21. What is the major problem encountered when using the manufacturers recommended chromatography procedure to evaluate the radiochemical purity of Tc-99m Sestamibi?

   A.   chromatography system is not accurate
   B.   chromatography procedure is exceedingly long
   C.   chromatography solvent is difficult to obtain
   D.   chromatography strips are too difficult to cut

22. The manufacturers quality control procedure for Tc-99m Mertiatide utilizes:

   A.   conventional thin layer chromatography
   B.   miniaturized chromatography
   C.   high pressure liquid chromatography
   D.   solid phase extraction

23. With the recommended miniaturized chromatography system, to what location do Tc-99m monoclonal antibodies migrate?

   A.   solvent front
   B.   with an $R_f = 0.5$
   C.   remains at the origin
   D.   none of the above

24. With the recommended miniaturized chromatography system, separation of Tc-99m monoclonal antibodies from _____ is not possible.

   A.   radiolabeled dimers
   B.   radiolabeled aggregates
   C.   hydrolyzed reduced Tc-99m
   D.   all of the above

25. What is the major advantage to using miniaturized chromatography system to evaluate the radiochemical purity of radiopharmaceuticals?

   A.   short time needed to perform quality control procedures
   B.   accurate assessment of radiochemical purity
   C.   well defined chromatography procedures available
   D.   none of the above