UNM COLLEGE of PHARMACY

UNM RADIOPHARMACY 1973 2003 30 YEARS OF EXCELLENCE

.::VOLUME 16, LESSON 1::.

An Update of Miniaturized Chromatography procedures for newer Radiopharmaceuticals
Second Release

Continuing Education for Nuclear Pharmacists
And
Nuclear Medicine Professionals

By

A. Michael Zimmer, Ph.D.

The University of New Mexico Health Sciences Center, College of Pharmacy is accredited by the Accreditation Council for Pharmacy Education as a provider of continuing pharmacy education. Program No. 039-000-10-060-H04-P 1.5 Contact Hours or .15 CEUs. Initial release date: 11/24/2010

-- Intentionally left blank –

Instructions:

Upon purchase of this Lesson, you will have gained access to this lesson and the corresponding assessment via the following link <http://hsc.unm.edu/pharmacy/radiopharmacyCE/>

To receive a Statement of Credit you must:

1. Review the lesson content
2. Complete the assessment, submit answers online with 70% correct (you will have 2 chances to pass)
3. Complete the lesson evaluation

Once all requirements are met, a Statement of Credit will be available in your workspace. At any time you may "View the Certificate" and use the print command of your web browser to print the completion certificate for your records.

NOTE: Please be aware that we **cannot** provide you with the correct answers to questions you received wrong. This would violate the rules and regulations for accreditation by ACPE. We can however, tell you which question number(s) you received wrong. You may contact the CE Administrator to request this information.

Disclosure:

The Author does not hold a vested interest in or affiliation with any corporate organization offering financial support or grant monies for this continuing education activity, or any affiliation with an organization whose philosophy could potentially bias the presentation.

Foreword:

This lesson was originally published as Volume III, Number 5 in 1993. It is being released again at the request of subscribers looking for information and references about alternate (from the package insert) quality control procedures.

As with any alternate procedure, each site should test the proposed methods to auto-confirm the validity of the procedure. Validation should be conducted on material not intended for patients. It should be noted that alternative solvents may appear on federal, state or local hazardous materials listings. Use appropriate precautions for personnel safety and protection.

An Update of Miniaturized Chromatography Procedures for Newer Radiopharmaceuticals Second Release

By
A. Michael Zimmer, Ph.D.

Editor, CENP
Jeffrey Norenberg, MS, PharmD, BCNP, FASHP, FAPhA
UNM College of Pharmacy

Editorial Board
Stephen Dragotakes, RPh, BCNP, FAPhA
Michael Mosley, RPh, BCNP
Neil Petry, RPh, MS, BCNP, FAPhA
James Ponto, MS, RPh, BCNP, FAPhA
Tim Quinton, PharmD, BCNP, FAPhA
S. Duann Vanderslice, RPh, BCNP, FAPhA
John Yuen, PharmD, BCNP

Advisory Board
Dave Abbott, RPh, BCNP
Dave Engstrom, PharmD, BCNP
Mark Gurgone, BS, RPh
Vivian Loveless, PharmD, BCNP, FAPhA
Brigette Nelson, MS, PharmD, BCNP
Janet Robertson, BS, RPh, BCNP
Brantley Strickland, BCNP
Susan Lardner, BCNP
Christine Brown, BCNP

Director, CENP
Kristina Wittstrom, MS, RPh, BCNP, FAPhA
UNM College of Pharmacy

Administrator, CE & Web Publisher
Christina Muñoz, M.A.
UNM College of Pharmacy

While the advice and information in this publication are believed to be true and accurate at the time of press, the author(s), editors, or the publisher cannot accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, expressed or implied, with respect to the material contained herein.

Copyright 2010
University of New Mexico Health Sciences Center
Pharmacy Continuing Education

COURSE OUTLINE

AN UPDATE OF MINIATURIZED CHROMATOGRAPHY PROCEDURES FOR NEWER RADIOPHARMACEUTICALS

STATEMENT OF LEARNING 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™) and generic
    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 [n-lI1 radiopharmaceuticals including:
    a. Tn-111 (Octreoscan TM)

5. Describe miniatured chromatography procedures for newer iodinated radiopharmaceuticals including:
    a. Iodinated Monoclonal Antibodies

AN UPDATE OF MINIATURIZED CHROMATOGRAPHY PROCEDURES FOR NEWER RADIOPHARMACEUTICALS

A. Michael Zimmer, Ph.D.

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 described 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 8cm 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.

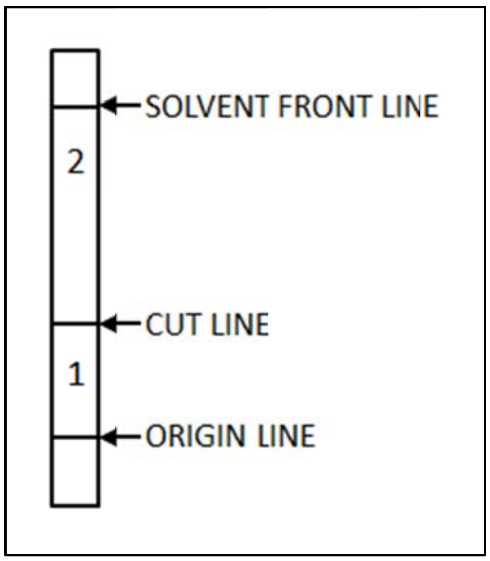

Figure 1. Typical miniaturized chromatography strips showing origin, solvent front and cut lilies.

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 Error 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 chromatography strip resulting in inaccurate results. Spot new strip correctly.
Strips are counted too close to the NaI(TI) 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 cute line must be changed to maintain the same R_f value.

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% NaCI, 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 31 ET Whatman 31 ET	Normal Saline Normal Saline/Acetone (1:1)	Soluble Tc-99m Impurities Tc-99m Hydrolyzed Reduced	
Tc-99m Sestamibi (Single Strip)	Whatman 31ET	Ethyl Acetate	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced Tc-99m Polar Impurities	(16)
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	(18)
Tc-99m Tetrofosmin	ITLC-SG	Methylene chloride/acetone (65:35)	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced	(19)
Tc-99m Bicisate	Whatman 17	Ethyl Acetate	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced Tc-99m Lipophobic Fraction	(20)
Tc-99m (V) DMSA	Whatman 17 ITLC-SG	50% Aqueous Acetonitrile Methyl Ethyl Ketone	Free Tc-99m Pertech Tc-99m Hydrolyzed Reduced	(21)

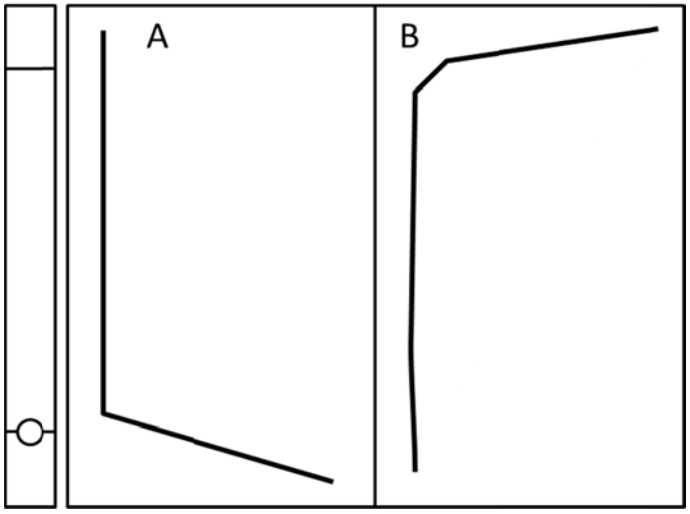

Figure 2. Chromatography strip activity distribution of (A) Tc-99m antibody and (B) Tc-99m pertechnetate in chromatography system consisting of ITLC-SG with 0.9% NaCl.

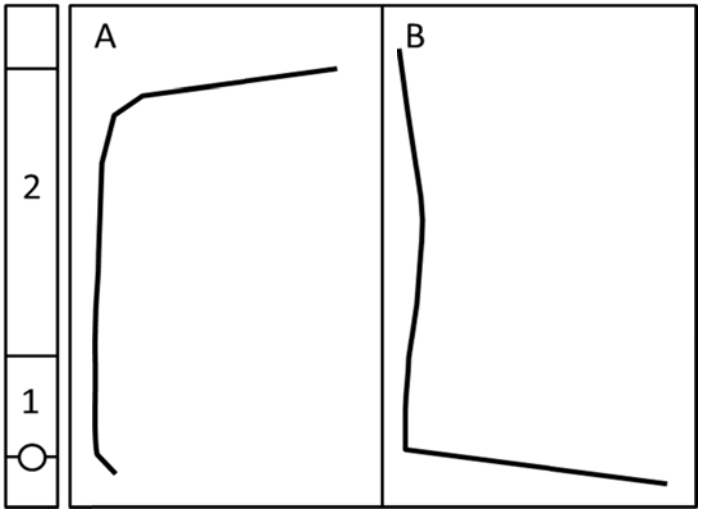

Figure 3. Chromatography strip activity distribution of (A) Tc-99m exametazime and (8) Tc-99m pertechnetate in chromatography system consisting of Whatman 17 with ethyl acetate.

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 a 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

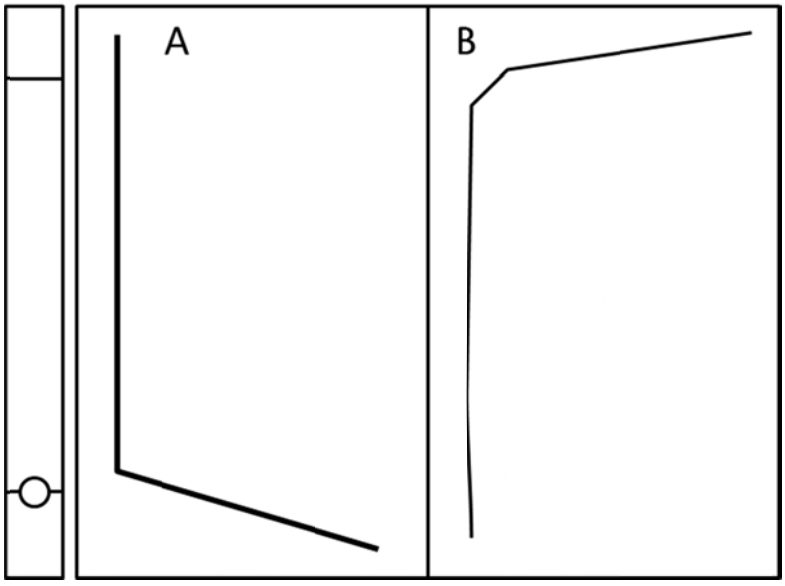

Figure 4. Chromatography strip activity distribution of Tc-99m teboroxime in chromatography system consisting of (A) Whatman 3IET with normal saline and (8) Whatman 3IET with acetone/normal saline (1:1)

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.

Tc-99m Sestamibi (Cardiolite™)

The recommended manufacturer's chromatographic quality control procedure (13) utilizes aluminum oxide think 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 evaluation 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 list 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 3IET and ethyl acetate (16). A typical activity distribution of Tc-99m sestamibi on Whatman 3IET 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

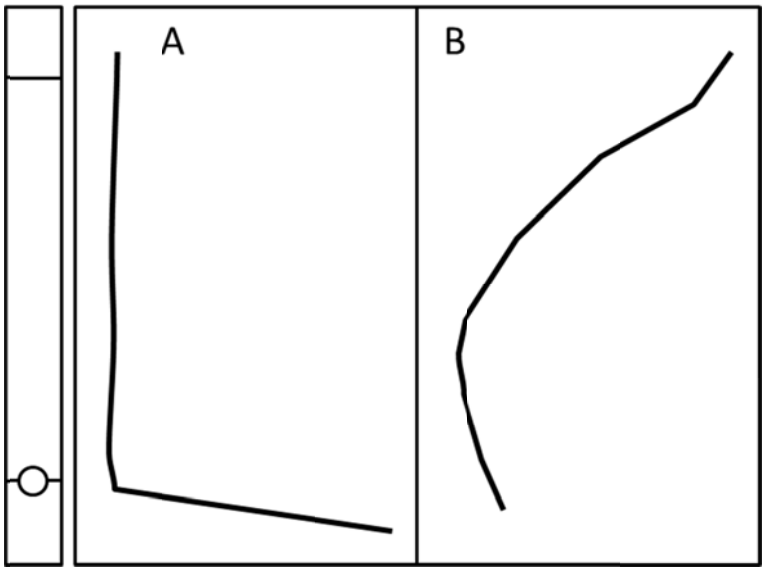

Figure 5. Chromatography strip activity distribution of (A) Tc-99m pertechnetate and (B) Tc-99m sestamibi in chromatography system consisting of Whatman 31ET with ethyl acetate.

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.

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 Acetone	(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 CI8 cartridges, Waters Chromatography, Milford, MA) and various radiochemical components are selectively eluted in a step-wise manner using appropriate solvents, including 0.001 N HCI and ethanol/saline (1: 1). The technique, as outlined by the manufacturer's 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.

Table 4

Radiochemical Purity Evaluations of Tc-99m Sestamibi Using Conventional and miniaturized Chromatography Systems*		
Radiochemical Purity (%)		
Preparation	**Conventional Chrom System**	**Miniaturized Chrom System ****
1	97.5	96.8
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 I, 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.

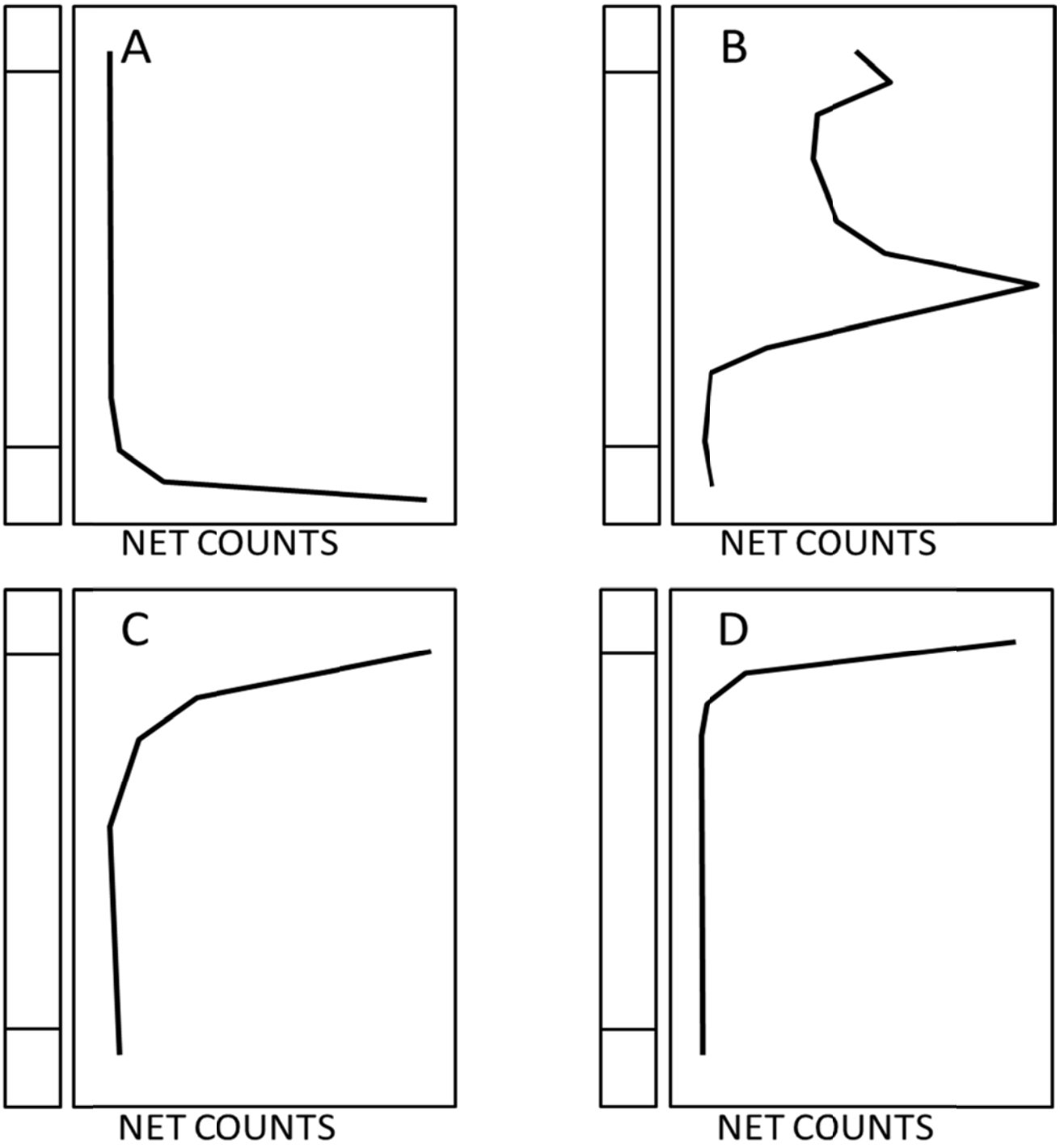

Figure 6. Chromatography strip activity distribution of (A) Tc-99m Mertiatide and (B) Tc-99m pertechnetate in chromatography system consisting of Gelman Solvent Saturation Pads with chloroform:acetone:tetrahydrofuran: (1:1:2). Chromatography strip activity distribution of (C) Tc-99m Mertiatide and (D)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

Tc-99m TetroCosmin (MyoviewTM)

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.

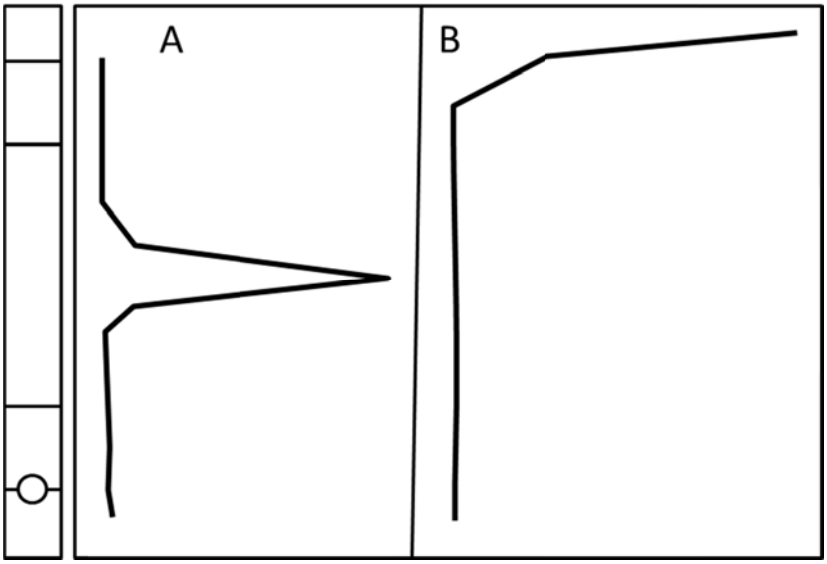

Figure 7. Chromatography strip activity distribution of (A) Tc-99m tetrofosmin and (B) Tc-99m pertechnetate in chromatography system consisting of ITLC-SG with methylene chloride:acetone (65:35).

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 slightly 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 Rr value of 0.8-1.0 whereas free Tc-99m pertechnetate and hydrolyzed reduced Tc-99m remain at the origin (Rr = 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 (l 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 Monocional 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 Monocional Antibodies	ITLC-SG	Normal Saline	Iodinated Antibody = 0.0 Free iodide = 1.0	(24)
I-123 Iodoamphetamine (IMP)	ITLC-SA	10% NaCl	Unbound I-123 = 1.0 I-123 IMP = 1.0	(25)

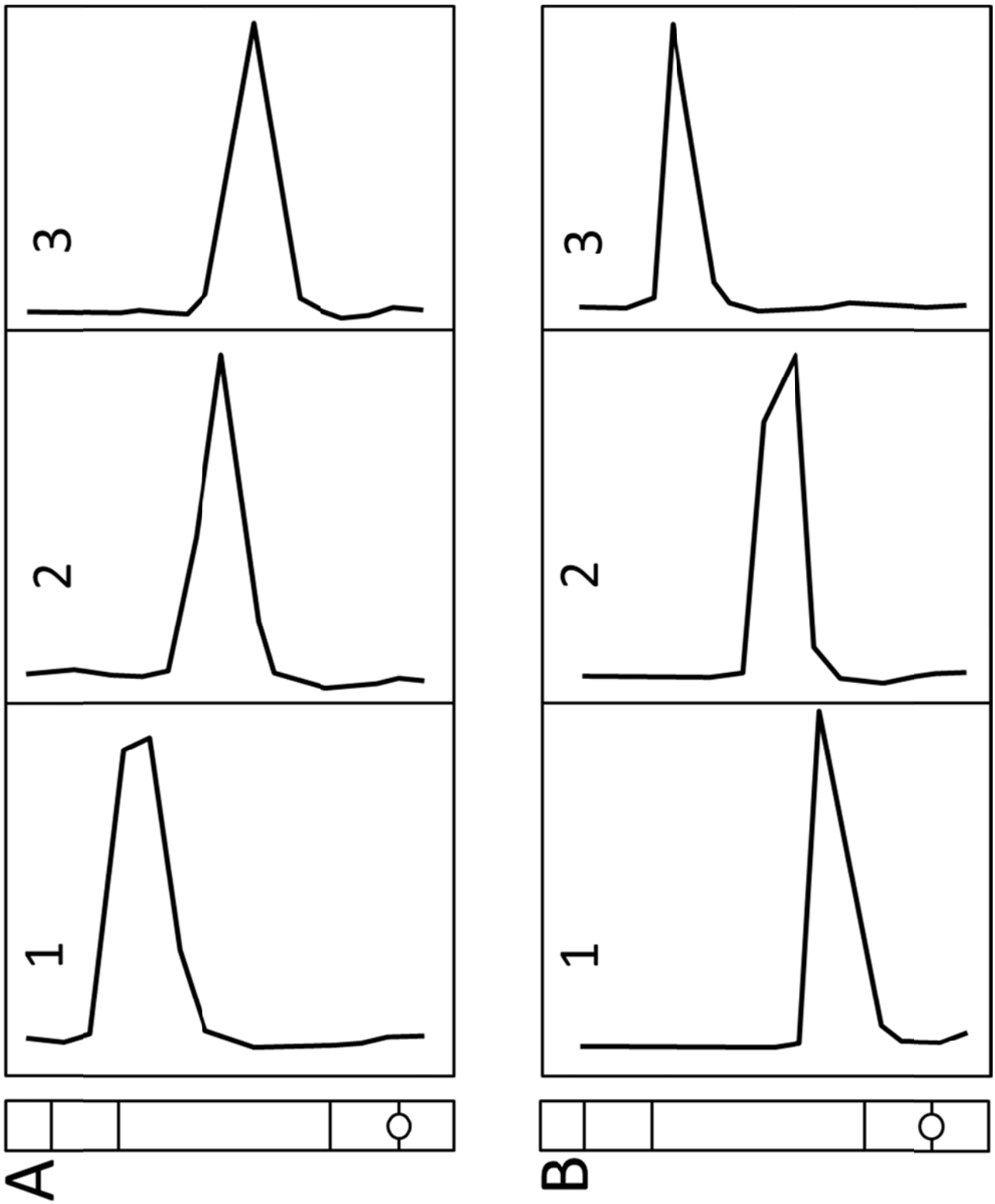

Figure 8. (A) Effect of altering methylene chloride:acetone solvent ratios, from (1) 60:40, (2) 65:35 and (3) 70:30, on the migration of Tc-99m tetrofosmin using ITLC-SG chromatography strips. (8) Effect of varying radiopharmaceutical spot size, ranging from (1) 2.5 ul, (2) 5.0 u1 and (3) 10.0 ul, on the migration of Tc-99m tetrofosmin using ITLC-SG chromatography strips with methylene chloride:acetone (65:35).

In-111 Monoclonal Antibodies

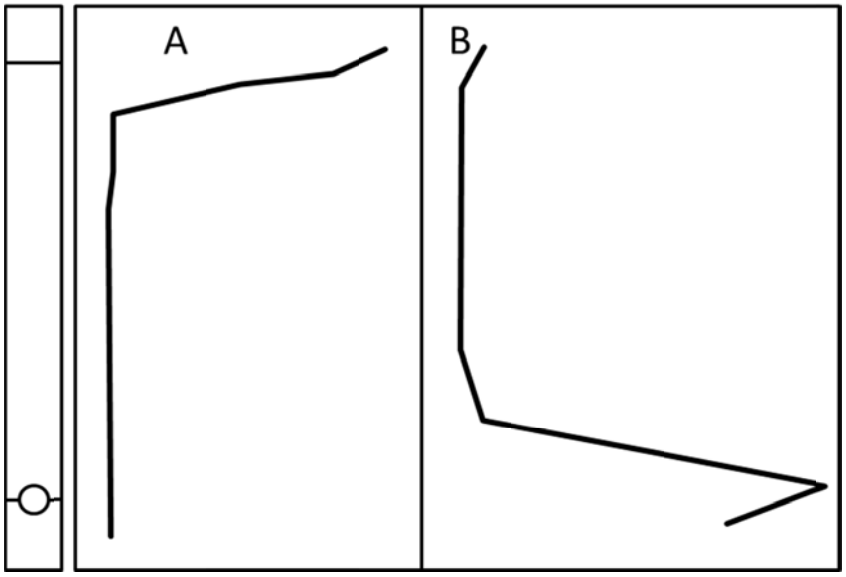

Figure 9. Typical chromatography strip activity distribution of (A) In-111 DTPA and (8) In-111 labeled monoclonal antibody (Myoscint) in chromatography system consisting of ITLC-SG with normal saline.

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 DTP A (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-11 1 DTPA chelate and In-111 antimyosin antibody is found in Figure 9.

In-111 Octreotide (Octreoscan™)

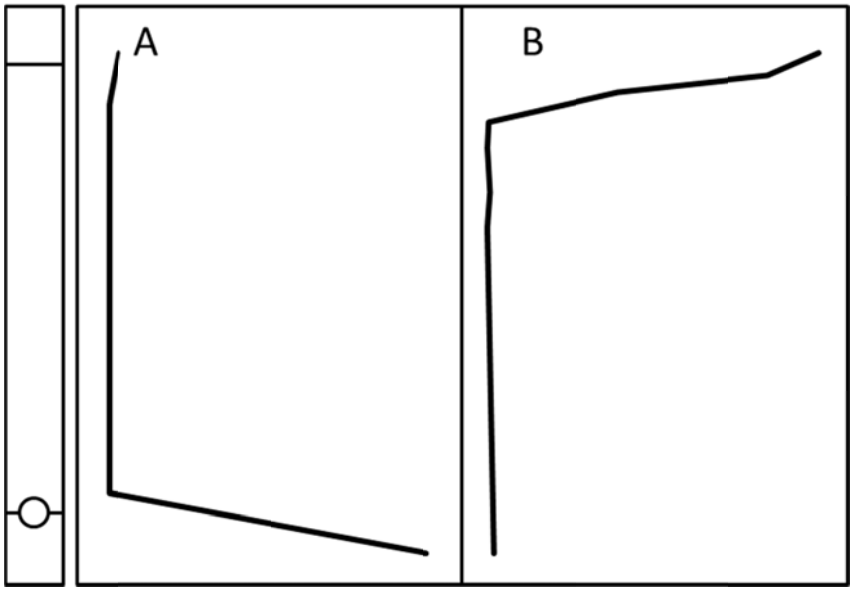

Figure 10. Typical chromatography strip activity distribution of (A) In-111 labeled octreotide and (8) In-111 DTPA in chromatography system consisting of ITLC-SG with normal saline.

The manufacturer's quality control procedure for In-111 octreotide preparations involves solid phase extraction (23) using Sep-Pak CIS 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 radiopharmaceutical 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.

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).

1-123 Iodoamphetamine (IMP)

Northwestern University Medical School laboratory has investigated a miniaturized chromatography system, utilizing ITLC-SA (0.7 x 6 cm) with 10% NaC1, to assess the radiochemical purity of 1-123 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 1-123 iodoamphetamine and I-123 sodium iodide on ITLC-SA paper eluted with 10% NaCl is shown in Figure 11.

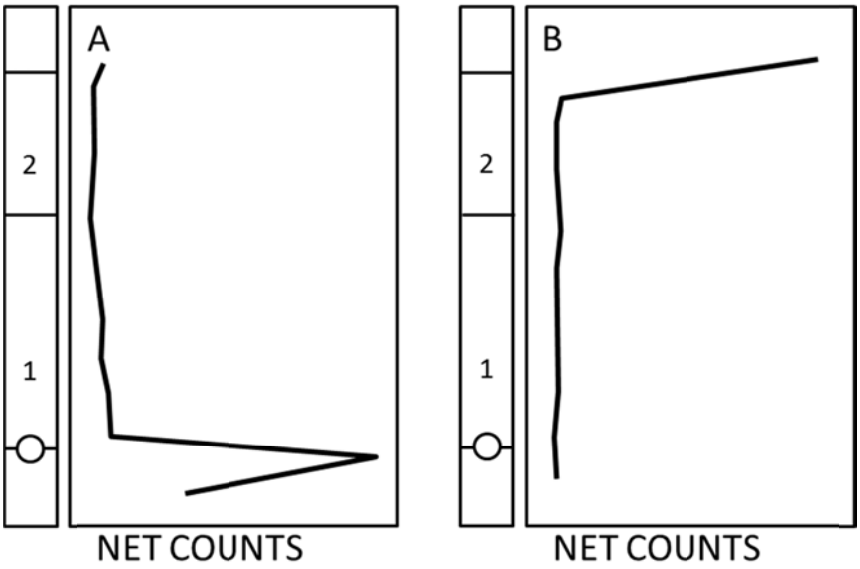

Figure 11. Chromatography strip activity distribution of (A) I-123 Iodoamphetamine and (B) I-123 iodide in chromatography system consisting of ITLC-SA with 10% sodium chloride.

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.

REFERENCES

1. Colombetti LG, Moerlien S, Patel GC, et al. Rapid determination of oxidation state of unbound 99mTc labeled radiopharmaceuticals. J Nucl Med 1976; 17:805- 809.

2. Zimmer AM. Pavel DG. Rapid miniaturized chromatographic quality control procedures for Tc-99m radiopharmaceuticals. J Nucl Med 1977;18:1230-1233.

3. Zimmer AM, Majewski W, Spies SM. Rapid miniaturized chromatography for Tc-99m IDA agents: Comparison to gel chromatography. Eur J Nucl Med 1082;7:88-91.

4. Zimmer AM. Quality control of radiopharmaceuticals. Atomic Products Corporation. 1980.

5. Robbins PJ. Chromatography of technetium-99m radiopharmaceuticals--a practical guide. New York. Society of Nuclear Medicine, 1984.

6. Zimmer AM, Pavel DG. Rapid miniaturized chromatographic quality control procedures for iodinatedradiopharmaceuticals. Am J Hosp Pharmacy 1978;35:426-428.

7. Webber DI, Zimmer AM, Spies SM. Common errors associated with miniaturized chromatography. J Nucl Med Technol 1989;11:66-68.

8. Karesh SM. Technetium-99m-exametazime. Pitfalls in preparation and quality control, J Nucl Med Technol 1989l;17:215-218.

9. Webber DI, Zimmer AM, Kazikiewiez JM, et al. Rapid miniaturized chromatography systems for quality control of radiolabeled monoclonal antibodies. J Nucl Med Technol 1990;18:141

10. Webber DI, Zimmer AM, Spies SM, et al. Evaluation of a single-strip chromatography quality control procedure to assess radiochemical purity of technetium-99m Cretec. J Nucl Med Technol 1992;20:29-32.

11. Ceretec package insert. Amersham Corporation, Arlington Heights, IL, October 1989.

12. Cardiotec package insert. Squibb Diagnostics, New Brunswick, NJ, 199

13. Cardiolite package insert. E.I. DuPont de Nemours & Co. Billerica, MA, December, 1990.

14. Proulx A, Ballinger JR, Gulenchyn KY. Routine determination of radiochemical purity of Tc-99m MIBI. Appl Radioat Isot 1989;40:95-97.

15. Hung JC, Wilson ME, Brown ML et al. Rapid Quality Control method for Technetium-99m 3-methoxy isobutyl isonitrile (Tc-99m Sestamibi). J Nucl Med 1991;32:1835.

16. Zimmer, A.M., Spies, S.M. Quality Control Procedures for Newer Radiopharmaceuticals. J Nucl Med Technol 1991; 19:210-215.

17. TechneScan MAG3 package insert. Mallinckrodt Medical Inc., St Louis, MO, June 1990.

18. Hung JC, Wilson ME, Brown ML. Rapid preparation and quality control of Technetium-99m MAG3. J Nucl Med Technol 1991:19:176-179.

19. Geyer MC, Spies WG, Hendel RC, Spies SM, Zimmer AM. Chromatographic evaluation of Tc-99m tetrofosmin preparations: comparison of miniaturized and standard chromatography systems. J Nucl Med Technol 1994;22:113

20. Budde PA, Hung JC. Rapid quality control procedures for Tc-99m bicisate. J Nucl Med Technol 1988;15:717-720.

21. Hollar BS, Hung JC. Preparation and quality control of Tc-99m (V) DMSA. J Nucl Med Technol 1994;22:114.

22. Zimmer AM, Kazikiewicz JM, Spies SM. Rapid miniaturized chromatography for In-111 labeled monoclonal antibodies. J Nucl Med Technol 1994;22:114.

23. OctreoScan package insert. Mallinckrodt Medical Inc., St Louis, MO, June 1994

24. Kazikiewicz JM, Zimmer AM, Spies SM, et al. Rapid miniaturized chromatography procedures for iodinated monoclonal antibodies: comparison to gel exclusion chromatography. J Nucl Med Technol 1987;15:129-132.

25. Geyer M, Zimmer AM, Spies SM, et al. Rapid miniaturized chromatography for I-123 iodoamphetamine: comparison to gel and anion exchange chromatography. J Nucl Med Technol 1989;17:117.

26. Zimmer AM, Kazikiewicz JM, Rosen St. Chromatographic evaluation of the radiochemical purity of NaI-131: effect on monoclonal antibody labeling. Nucl Med Biol 1987;14:533-534.

ASSESSMENT 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 system 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. Lilophobic 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. The miniaturized chromatography system use 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

9. The miniaturized chromagraphy system used to evaluation 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

10. 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

11. 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

12. What is the general size of 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

13. When counting, if the strips are p laced 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 significant errors

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

    a. Whatman 17
    b. Whatman 31ET
    c. ITLC-SG
    d. ITLC-SA

15. 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