Chapter 9

Quality Control Methods of ^{99m}Tc Pharmaceuticals

9

9.1 Determination of Radiochemical Purity

9.1.1 Thin-Layer Chromatography Methods

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Thin-layer chromatography (TLC) is commonly used for the determination of radiochemical purity in nuclear medicine. TLC was described as early as 1967 for testing radiopharmaceuticals (Hoye 1967). Since the introduction of TLC (Izmailov and Shraiber 1938; Stahl 1956), a variety of modifications and new applications have been reported (Fairbrother 1984).

The principle of this analytical method is that a mobile phase (solvent) moves along a layer of adsorbent (stationary phase) due to capillary forces. Depending on the distribution of components between the stationary and the mobile phase, a radioactive sample spotted onto the adsorbent will migrate with different velocities, and thus, impurities are separated. The distance each component of a sample migrates is expressed as the R_f value. The R_f is the relative migration of a component in relation to the solvent front (SF):

$$R_f = \frac{\text{Distance from origin of the component}}{\text{Distance of the SF}}$$

The R_f values range from 0–1. If a component migrates with the SF, the R_f is 1. If a component remains at the point of application (origin), the R_f is 0. For a given TLC system, which is defined by the mobile and the stationary phases, the R_f value of a pure chemical compound is specific and reproducible.

The main principles of separation are adsorption (electrostatic forces), partition (solubility), and ion exchange (charge). Information on the theoretical background of TLC is presented elsewhere (Miller 2004). Depending on the movement of the mobile phase, TLC may be ascending or descending; in the nuclear medicine laboratory, ascending TLC is the method of choice (Robbins 1983).

For the analysis of radiopharmaceuticals, techniques should be fast and safe. TLC offers reliable separation properties with easy and rapid performance. The applied sample remains quantitatively on the plate, and therefore, no losses of radioactivity during analysis occur. The commercially available ready-to-use stationary phases combine adsorbent with ionic or hydrophobic properties and are suited for separation of a variety of molecules using polar or nonpolar solvents. This chapter emphasizes methods for routine use in nuclear medicine and describes materials, techniques, and methods for quantification (Carpenter 1986; Hammermaier et al. 1986; Thoebald 1984).

9.1.1.1 ^{99m}Tc Species Separated by TLC

The main impurities in ^{99m}Tc pharmaceutical preparations are free pertechnetate ($^{99m}TcO_4^-$) and reduced, hydrolized technetium (colloidal ^{99m}Tc). These two ^{99m}Tc species may be separated from ^{99m}Tc pharmaceuticals by simple TLC procedures.

The migration properties of free pertechnetate may be influenced by the choice of different mobile and stationary phases. When silica gel or paper is used as stationary phase, the migration of free pertechnetate depends on the solubility of this anion in the solvent. In a polar solvent like saline, 80% methanol, acetone or 2-butanone (methyl ethyl ketone, or MEK) pertechnetate migrates with the SF ($R_f = 0.6–1.0$). If a nonpolar, lipophilic solvent (e.g., ethylacetate, chloroform) is used and the sample is dried (no water content), free pertechnetate remains at the origin. In addition, when using an anion-exchange material in for stationary phase (e.g., aluminum oxide), free pertechnetate will be retained at the start.

Colloids do not migrate in most TLC systems since insoluble material will stay at the origin. Changing the mobile or the stationary phase will not affect the migration properties of colloidal ^{99m}Tc. This is the reason why hydrolized ^{99m}Tc species are not determined in colloidal and particulate preparations (e.g., macroaggregated albumin [MAA]), microspheres, or high-molecular ^{99m}Tc species such as monoclonal antibodies. The major impurity recognized by the pharmacopeia in the case of these radiopharmaceuticals is free pertechnetate, moving with the SF (Robbins 1983; Zimmer and Pavel 1977).

9.1.1.2 Stationary Phases

Standard TLC Materials. Standard TLC plates are available as glass plates, and as plastic or aluminum foils covered with the stationary phase. Aluminum or plastic foils have the advantage that they are easily cut into pieces for measurements of radioactivity. A broad range of stationary phases is commercially available including silica gel, reversed-phase silica, aluminum oxide, synthetic resins (ion-exchange chromatography), and cellulose (partition chromatography). The length of plates (foils) may vary between 10 and 20 cm, although miniaturized systems have been introduced (< 5 cm). Generally, the developing distance will depend on the number of components in a sample and the relative retention properties in a system as well as the time used for migration.

For determination of the radiochemical purity of ^{99m}Tc pharmaceuticals, methods using standard TLC materials have been described (Zimmer and Pavel 1977). Reversed-phase materials offer advantages with respect to more polar solvents that are miscible with the aqueous medium of the sample (Carpenter 1986). Alumina plates are used to separate anionic ^{99m}Tc-pertechnetate from neutral or positively charged complexes.

The main limitation of standard TLC techniques is the time required for analysis. Due to the particle size (20 μm) of adsorbent materials, the developing time is usually > 30 min. This is too long considering additional time for measurements and quantification.

The main advantage of standard TLC materials lies in the comparably high resolution, exemplified by the separation of two ^{99m}Tc-dimercaptosuccinic acid (DMSA) complexes in one TLC system: The trivalent ^{99m}Tc-DMSA complex is separated as an impurity in a preparation of pentavalent ^{99m}Tc-DMSA (Westera et al. 1985).

High-Performance TLC (HPTLC). HPTLC materials have a smaller average particle size (5 vs 20 μm) and a narrower particle size distribution when compared with conventional materials. Actually, the development of the chromatogram is faster and the time for analysis shorter. However, HPTLC materials are preferable for more complex separations; their use for determinations of the radiochemical purity in nuclear medicine is limited.

Instant TLC (ITLC). ITLC materials are the most frequently used stationary phases in nuclear medicine. ITLC methods fulfill the need for rapid and accurate analysis of the radiochemical purity of radiopharmaceuticals and have thus been accepted by the European Pharmacopeia.

ITLC plates are made of fiberglass sheets, impregnated with an adsorbent, usually silica gel (e.g., SG). Due to the fine mesh material, the migration properties are increased many-fold by the TLC materials. The time for the development of any chromatogram may be reduced to <5 min, without affecting the separation of radiochemical impurities.

Although ITLC materials are more expensive, fiberglass sheets offer high economy, since the flexible material may be cut to any size. Chromatographic systems utilizing these materials have been described (Frier and Hesslewood 1980; Theobald 1994; Zimmer and Pavel 1977).

The separation properties of silica gel 60 depend to a certain extent on the "activated" state of the adsorbent, which is related to the water content. However, when using organic solvents, it is recommended to dry the ITLC plate before use by heating for 10–20 min at 110 °C (Frier M and Hesslewood 1980).

The most common impurities in radiopharmaceuticals remain at the origin ($R_f=0$) or migrate with the SF ($R_f=0.8$–1.0). In order to separate and quantify two (or more) impurities, two (or more) analytical systems are generally used. A typical procedure, using different solvents, is shown in Fig. 9.1.1.1. Using an organic solvent (MEK) for separation, colloidal forms and the Tc complex remain at the origin, and free pertechnetate migrates with the SF. This system is suited to quantify free ^{99m}Tc-pertechnetate. In saline, free pertechnetate and the ^{99m}Tc complex migrate with the SF; while reduced, hydrolyzed ^{99m}Tc remains at the start. Using saline, the colloidal ^{99m}Tc species is quantified. With some experience, the whole procedure may be performed in <15 min, before application of the radiopharmaceutical to patients. Attention must be paid when ITLC strips are marked to indicate the spotting area or SF, since the material is fragile and easily damaged, which may affect results.

Silica Gel. The chemical properties are based on siloxane (Si–O–Si) and silanol (Si–OH). Polar groups are responsible for the interaction of the adsorbent with water and with the sample to be analyzed. Silica stationary phases (3–8 μm) have been produced for ITLC as silica gel (ITLC-SG) and silicic acid (ITLC-SA). ITLC-SG is the most frequently used adsorbent for routine radiochemical purity determinations (Table 9.1.1.1).

RP Phases. The surface properties of silanol may be modified by reaction with alkylating agents that bind to the surface of porous silica gel 60.

Hydrophobic modification includes binding of nonpolar groups such as dimethyl (RP-2), octyl (RP-8), octadecyl (RP-18), and phenyl. The hydrophobic properties increase within the different groups from RP-2 to RP-18, and with the degree of saturation of the hydroxyl groups that has been attained.

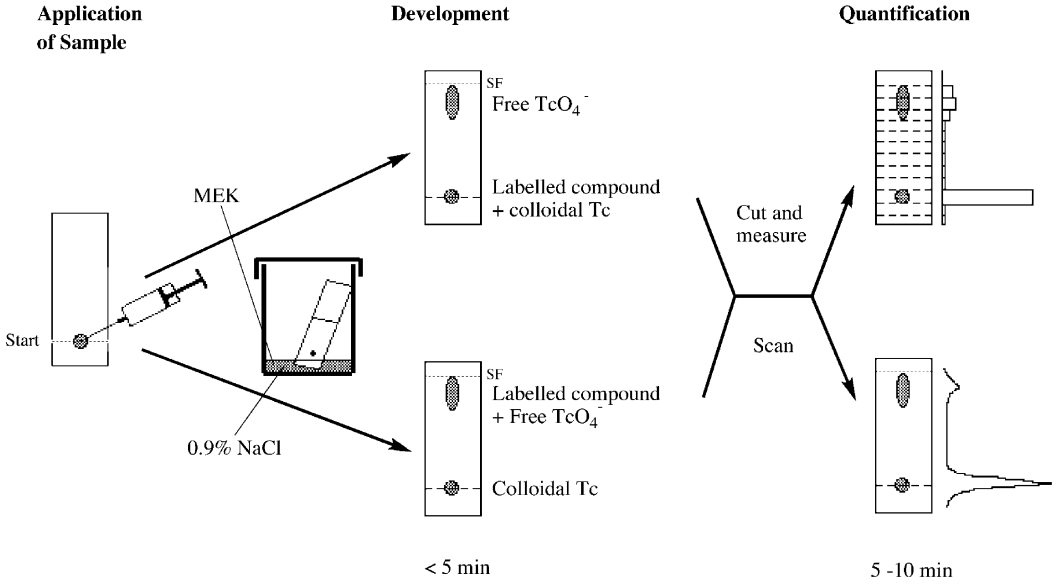

Fig. 9.1.1.1. Determination of the radiochemical purity of a radiopharmaceutical by instant thin-layer chromatography (ITLC) using two different solvents, methyl ethyl ketone (MEK) and saline

Table 9.1.1.1. Materials for thin-layer chromatography (*TLC*)

Layer type	Format (cm)	Ordering no.	Quantity
TLC glass plates (layer thickness 0.2 and 0.25 mm)			
Silica gel 60 F_{254}	20×20	Merck 1.05715.000	25 plates
Silica gel 60 F_{254}	5×10	Merck 1.05789.000	25 plates
Silica gel 60 F_{254}	2.5×7.5	Merck 1.15327.000	100 plates
RP-18 F_{254}	20×20	Merck 1.15389.000	25 plates
RP-18 F_{254}	5×20	Merck 1.15683.000	50 plates
RP-18 F_{254}	5×10	Merck 1.15685.000	25 plates
HPTLC Si 60 F_{254}	5×5	Merck 1.05635.000	100 plates
HPTLC LiChrospher Si 60 F_{254}	20×10	Merck 1.15445.000	25 plates
TLC aluminum sheets (layer thickness 0.2 mm)			
Silica gel 60	20×20	Merck 1.05553.0001	25 sheets
Silica gel 60 F_{254}	5×10	Merck 1.16834.0001	50 sheets
Silica gel 60 F_{254}	5×7.5	Merck 1.05549.0001	20 sheets
Aluminum oxide 60 F_{254} neutral	20×20	Merck 1.05550.0001	25 sheets
Aluminum oxide 150 F_{254} neutral	20×20	Merck 1.05551.0001	25 sheets
HPTLC LiChrospher Si 60 F_{254}	20×20	Merck 1.05586.0001	25 sheets
TLC plastic sheets (layer thickness 0.2 mm)			
Silica gel 60 F_{254}	20×20	Merck 1.05735.0001	25 sheets
Aluminum oxide 60 F_{254}	20×20	Merck 1.05581.0001	25 sheets
Baker-flex silica gel 1B-F	20×20	J. T. Baker 4463-04	25 sheets
Baker-flex silica gel 1B-F	2.5×7.5	J. T. Baker 4463-02	200 sheets
Baker-flex aluminum oxide 1B-F	20×20	J. T. Baker 4467-04	25 sheets
Baker-flex aluminum oxide 1B-F	2.5×7.5	J. T. Baker 4467-02	200 sheets
ITLC fiberglass sheets (layer thickness 0.2 mm)			
Silica gel (ITLC-SG)	20×20	Pall Gelman 61886	25 sheets
Silica gel (ITLC-SG)	5×20	Pall Gelman 61885	50 sheets
Silicic acid gel (ITLC-SA)	20×20	Pall Gelman 51432	25 sheets

HPTLC high-performance thin-layer chromatography, *ITLC* instant thin-layer chromatography

RP phases offer higher selectivity for separation of nonpolar compounds or molecules with nonpolar groups. In certain cases, highly polar, ionic compounds may also be separated due to selective retention on these modified RP materials. Partially modified silica gel is also available as RP-18 alumina sheets.

Silica gel may also be modified with hydrophilic groups that are attached using short-chain nonpolar spacers. Hydrophilic modifications with polar groups include amino, cyano, and diol functionalities. Amino-SG offers weakly basic ion-exchange properties; diol-SG considerably less affinity for water when compared with unmodified SG-60. These HPTLC materials offer increased selectivity for complex separations of biomolecules and drugs.

Aluminum Oxide. Some separations of radiopharmaceuticals are based on adsorption chromatography with aluminum-coated plates. Aluminum oxide (Al_2O_3) has polar properties; it is also a weak anion exchange material. The pH adjusted adsorbent is available in three pH ranges: neutral (pH 7.0–8.0), basic (pH 9.0–10.0), and acidic (pH 4.0–4.5). Standardized products include aluminum oxide 60, 90, and 150. Plates are also available for UV detection.

Table 9.1.1.2. Materials for paper chromatography

Specification	Format (mm)	Ordering no.	Quantity
Whatman 1	100×300	3001845	100 sheets
Whatman 1	200×200	3001861	100 sheets
Whatman 3MM	50×250	30306122	50 sheets
Whatman 3MM	100×130	30306123	50 sheets
Whatman 3MM	150×150	3030286	100 sheets
Whatman 3MM	200×200	3030861	100 sheets
Whatman 31 ET	460×570	3031915	25 sheets

Cellulose. This organic material consists of polymerized glucose fibers (400–500 molecules) in nature and also as a synthetic product (40–200 glucose molecules). Cellulose interacts with water and serves as a stationary phase for the separation of polar substances by paper chromatography. As a powder, it is used as an adsorbent for TLC. Separation of polar substances by paper chromatography is described in the *European Pharmacopeia* for identification of ^{99m}Tc-pertechnetate (Council of Europe 1982).

Paper. Paper was the first material used for chromatography of radioactive compounds (Dickey 1953). Paper materials show low-resolution properties; however, since paper is robust and easy to cut, paper chromatography is still used and recommended for many applications. The mechanism of separation is probably different; nevertheless, paper chromatography is used "ascending" or "descending", like ITLC. Likewise, a developing distance of 8–10 cm is usually sufficient for the separation of free pertechnetate and colloidal impurities. The developing time might be slightly increased, but usually finished in < 10 min, if small sized paper strips are used.

Whatman 3MM is the material of choice for determination of the radiochemical purity by partition chromatography. (For information on materials, Tables 9.1.1.1 and 9.1.1.2.)

9.1.1.3 Mobile Phases

The saline/MEK system is applied for the analysis of most radiopharmaceuticals that contain free pertechnetate and/or colloidal Tc. Acetone has been replaced by MEK because artificially high values of pertechnetate have been obtained, caused by its higher water content (Carpenter 1986).

More recently, developed ^{99m}Tc complexes (e.g. mercaptoacetyltriglycine [MAG_3], monodentate methoxyisobutyl isocyanide [MIBI], hexamethylpropylene amine oxime [HMPAO], etc.) require more sophisticated analytical methods to detect additional impurities in the injection solution. This is inherent in the chemical properties of certain ^{99m}Tc pharmaceuticals that need more complex kit formulations and contain several labeled impurities. In addition, special composed solvent systems have to be used.

9.1.1.4 Spotting of the Sample

The sample size has a considerable effect on the separation characteristics of a certain system. Therefore, the sample diameter on the plate should be kept as small as possible (< 3 mm). Inefficient separations and artificial results are caused by too-large spots. The standard TLC technique for applying a sample onto the plate is to use micropipets or glass capillaries for single use. The volume is typically 5 µl. A certain drawback of this technique is the fact that the sample is withdrawn aseptically with a syringe from the vial and needs to be transferred for spotting.

Therefore, to reduce handling, the sample is withdrawn using a 1-ml syringe with a fine needle (> 25 gauge) and is spotted directly onto the plate using a single drop from the needle. The volume corresponding to a drop from a vertically held needle (25 gauge) is approximately 6 µl. If the syringe is held in the horizontal position, the volume might double (Robbins 1983). With experience, reproducibly small spots are applied to the TLC plate, suitable for analysis. The technique also avoids contamination and minimizes sample exposure to air, which may affect certain ^{99m}Tc radiopharmaceuticals.

9.1.1.5 Development of the Chromatogram

For the development of chromatograms, a small beaker might be used, which is closed with a glass plate or covered with a foil to maintain a saturated atmosphere inside the vessel. Small tanks for chromatography are recommended, especially when organic solvents are used. If an open beaker is used, evaporation of the solvent may affect the separation properties of a system (Levit 1980; Manger 1986).

The solvent should cover the bottom not more than 5 mm high; the solvent level has to be below the start line on the plate/strip.

After applying the sample, the plate must be placed into the tank and developed immediately, without drying the spot. Dried samples may lead to artificial results due to oxidation of ^{99m}Tc complexes and formation of free pertechnetate (Mallol 1990; Manger 1986).

An exception to this rule is made for mobile phases that do not dissolve the aqueous sample (e.g., ethylacetate, chloroform). In this case, a wet sample will produce a poor resolution and high background activity along the track. The sample spot should be dried in a gentle stream of nitrogen, and heating the plate must be avoided.

The plate/sheet is placed vertically into the chamber, carefully avoiding any damage of the surface. Materials with limited mechanical resistance like certain chromatography paper should be supported (e.g., clipped to the lid of the chamber), otherwise they will slip into the solution or touch the chamber wall during development.

The usual developing time is between 2 and 15 min, depending on the stationary phase and (to a lesser extent) on the solvent. The SF is not always visible, especially when using ITLC materials. Therefore, SF (end of chromatogram) is marked with a color pen (water soluble for aqueous solutions; water resistant for organic solvents) (Fig. 9.1.1.2). When the solvent gets in contact with the marker, the color migrates with the solvent, indicating the end of the development. The strip should be marked in such a way that the color does not interfere with the sample track to avoid artificial results (Levitt 1980). When the solvent has reached SF, the strip is removed from the chamber and then dried.

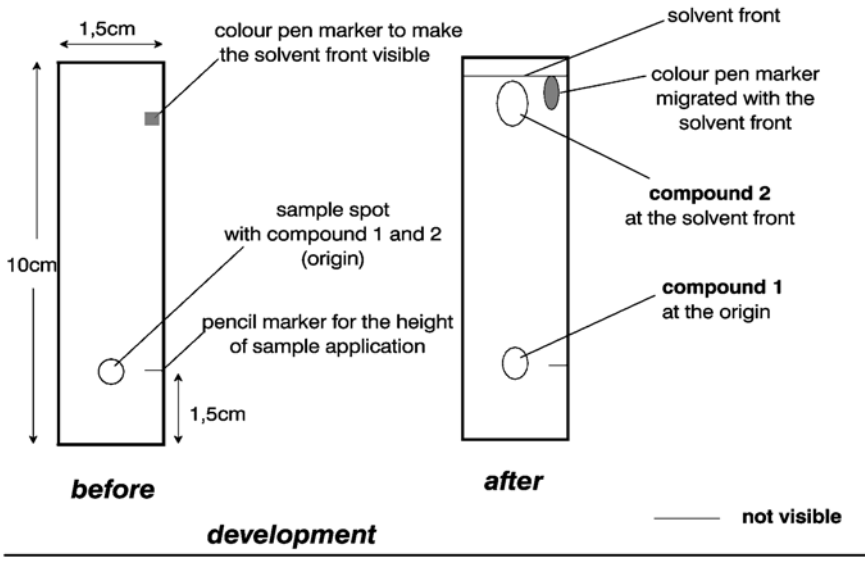

Fig. 9.1.1.2. ITLC plates before and after development (typical dimensions). The application point is marked with a line; a color pen marker may be used to mark the solvent front. When the solvent reaches the marker, the color migrates with the solvent. Markers are positioned outside the track to avoid interference with the sample

Procedure for the determination of radiochemical purity by ITLC or paper chromatography:

- Fill a 100-ml beaker with the solvent (about 10 ml, solvent 3–5 mm high); close the beaker with a tight lid or parafilm.
- Prepare the strip: Mark the solvent front with a color pen and the start with a pencil.
- Take a small sample of the preparation ready for injection (< 100 µl).
- Apply one small drop of the sample with a thin needle onto the strip; the drop must not dry.
- Immediately put the strip into the beaker, the spot must remain above the solvent level.
- When the solvent has reached the front, take the strip out and let it dry.
- Quantify the regional distribution of radioactivity on the strip.

9.1.1.6 Measurement of Radioactivity

There are several methods for the quantification of radiochromatograms. Depending on the available instrumentation, the resolution of analysis will vary, and the amount of radioactivity used for analysis will differ considerably. In any case, the results of measurements are used to calculate the radiochemical purity of a radiopharmaceutical (percentage) as the ratio between the radioactivity corresponding to the main component, divided by the total recovered radioactivity of the chromatogram:

$$\text{Radiochemical purity (\%)} = \frac{\text{Radiopharmaceutical (dpm)}}{\text{Total recovered activity (dpm)}} \times 100$$

The relative merits of quantification methods are:
- Resolution
- Sensitivity
- Linearity
- Time needed to perform
- Practicability
- Costs

Autoradiography. Autoradiography is one of the oldest methods used for semiquantitative measurement of radioactivity. The chromatogram is placed on an x-ray film and exposed in the dark for usually less than 1 h (a detailed procedure is given elsewhere) (Theobald 1994). This method is no longer in use since it is time-consuming and inaccurate. Visualization of the radioactivity distribution on a film may be useful for documentation purposes.

Gamma Camera. Measurement of a radiochromatogram with a gamma scintillation camera might be first choice when no specific instrumentation is available. The dried strip is placed at close distance to the head of the gamma camera, and images are acquired. Using the regions of interest (ROI) technique (drawing regions over distinct areas of activity), the radiochemical purity is expressed as a fraction of the total recovered activity. A certain advantage is the use of undiluted sample for chromatography (measurement of high count rates). The main disadvantage of this technique is that it is a rather time-consuming procedure (measurement and analysis) at a time when the camera is needed for patients.

In cases when no other device is available, visualization of the radioactivity distribution by an activity profile might be a good indicator of the amount of impurities present in the injection solution.

Ionization Chamber. This method is frequently used and is recommended in many official procedures (Robbins 1983). It might be used for simple separation techniques (compounds with an R_f of 0 or 1). The strip is cut into two segments (one corresponding to the main compound and the other to the impurity) and measured in the ionization chamber. This facilitates quick analysis before the radiopharmaceutical is injected.

Limitations are low resolution of measurements, an overestimation of the amount of impurities, and if not cut properly, results may be wrong. This problem can be avoided by cutting the strip into more segments, which are measured separately (Bish et al. 1980).

Second, the sensitivity is limited by the sensitivity of the ionization chamber. In practicee, 3 MBq or more should be applied on the plate, which would correspond to an injection solution of 600 MBq/ml if 5 µl are applied (Robbins 1983). Results with lower concentrations of radioactivity should be judged with caution.

Cut and Count. Chromatographic plates may be analyzed using a NaI(Tl) scintillation counter. The plates are cut in segments (up to ten) and transferred into tubes to be counted. The radioactivity on the plate can be plotted as a histogram. The sensitivity is much higher compared with the ionization chamber (even dilution may be necessary), and the resolution is dependent on the number of segments. It is important to keep the

same counting geometry for all segments at the bottom of the counting tube. Cutting the plate in many segments permits assessment of the quality of separation. The background radioactivity is subtracted from each count leading to a more accurate analysis. The main limitations of this method are that the high activities of the main component may paralyze the counter (Robbins 1983), and the procedure is time-consuming (cutting samples and filling into test tubes, time of measurements, and calculation of the relative percentages).

Chromatogram Scanning. The advantage of this method is the fact that a slit-collimated detector is moved along the thin-layer plate, and the radioactivity distributed between the start and SF of a chromatogram is recorded. The detector is coupled to a scaler ratemeter, with the ratemeter output signal passed on to a chart recorder producing a radioactivity profile (Janshold et al. 1980). A useful addition is a chromatography integrator to measure the area of radioactive peaks, which is proportional to the detected radioactivity. Quantification of single peaks as a percentage of the total measured radioactivity will produce a purity report for each radiopharmaceutical.

For measuring ^{99m}Tc and other gamma emitters, a NaI(Tl) scintillation detector is used. The resolution of the scanner is dependent on the width of the slit-collimator, the distance between chromatogram and detector, and the window settings on the scaler. Artificial results may be obtained if the peaks are not symmetrical and comparable.

Linear Analyzer. The linear analyzer was developed for the measurement of beta-emitting radionuclides and was introduced in 1980 by Berthold Analytical Instruments (Filthuth 1986). It operates as a position-sensing proportional counter, measuring a fixed number of channels along the length of the chromatographic plate. The system is equipped with analytical software for quantification.

Further developments have increased the potential of proportional counting by placing a grid of anode wires between two large plates, 1.5 mm above and below, serving as cathodes. Ions formed in the gas-filled volume drift toward the nearest anode wire where they undergo multiplication; each event is automatically localized. Thus, two-dimensional localization may be accomplished by these position-sensing detectors.

The *digital autoradiograph* is a multiwire proportional counter; each electrode is composed of 100 wires (gold-plated tungsten) arranged at a 2-mm distance. The gas-filled proportional chamber has a window of 24×24 cm for the measurement of chromatograms at a fixed distance (0.5 mm). Each channel is connected with a time analyzer for registration of position specific pulses (nanoseconds). Finally, the analog signals are digitalized for computer analysis and quantification.

Gas flow proportional counters exhibit higher sensitivity for beta-emitters, but also pure gamma-emitters like ^{99m}Tc are measured with high resolution. When placing a chromatogram into the counting chamber the developed strip must be completely dry.

The main advantages over a conventional TLC scanner are:

- Increased sensitivity because of the high specific ionization produced in the gas-filled proportional chamber using argon/methane (89:10)
- Two-dimensional positioning of radioactivity
- A "photographic" image of the radioactivity distribution is generated
- Several chromatograms (strips) are analyzed during one exposure
- The linear analyzer and the digital autoradiograph may be used with any radionuclide
- Quantification of chromatograms, storage, and display of data
- Resolution may decrease with high activities.

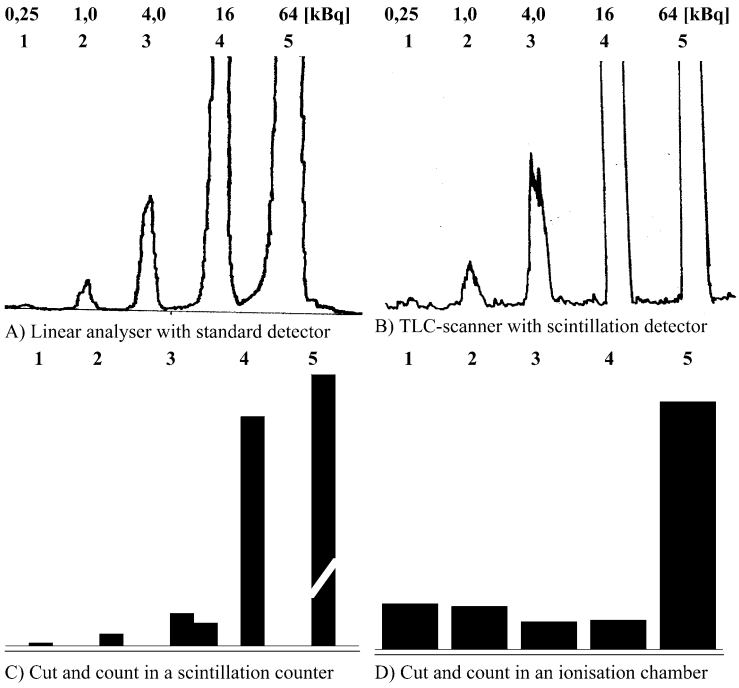

Fig. 9.1.1.3. "Phantom" chromatographic plate analyzed by four different methods of measurement. The highest sensitivity is achieved with A and C, the best resolution with B and C. Using the ionization chamber (D), activities less than 20 kBq (peak 1, 2, 3, 4) are not resolved accurately.

A comparison of four different methods of quantification is shown in Fig. 9.1.1.3. A "phantom" chromatographic plate with increasing amounts of radioactivity (0.25, 1.0, 4.0, 16.0, and 64.0 kBq) spotted at exact intervals was measured by: A. linear analyzer, B. conventional scanner, C. cut/measure, and D. ionization chamber (Capintec). The highest sensitivity was achieved with A. and C., the best resolution with B. and C. Using the ionization chamber indicated low detection efficiency of radioactivity below 20 kBq (peaks 1, 2, 3, and 4).

Linear analyzers and conventional TLC scanners are standard equipment in nuclear medicine. These instruments offer high detection efficiency and resolution characteristics combined with speed of analysis, which is required for the analysis of short-lived radiopharmaceuticals.

Electronic Autoradiography. New methods have been developed with the aim of replacing the time-consuming film autoradiography. Two different systems, the so-called phosphor imagers and the microchannel plate analyzers, are currently available for quantifying TLC plates, ITLC, or paper strips. While phosphor imagers require two steps for quantification of an autoradiogram (exposure and "development"), microchannel plate analyzers provide a direct measurement of radioactivity. The main advantages are a short analysis time, high sensitivity, a broad dynamic range, system stability at high radioactive concentrations, online two-dimensional imaging, direct quantification of radioactivity, and detectability of all radionuclides used in nuclear medicine. A de-

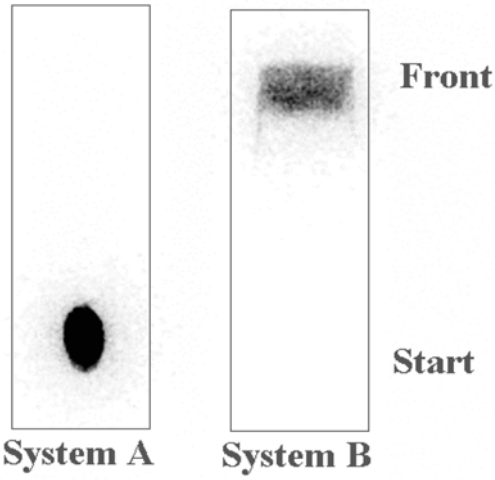

Fig. 9.1.1.4. Electronic autoradiogram of two ITLC-SG strips showing the radiochemical purity of ^{99m}Tc-diethylene triamine pentaacetate (DTPA) in two solvent systems: *A* MEK: ^{99m}Tc-DTPA remains at the start, *B* MeOH (80%): ^{99m}Tc-DTPA moves with SF. Strip dimensions: 1.5×10 cm. (Instant Imager, Canberra Packard, acquisition time: 60 s)

tailed description of such a system (Instant Imager, Canberra Packard) for radiopharmaceutical applications is described in (Decristoforo 1977). Figure 9.1.1.4 shows an electronic autoradiogram of the radiochemical purity of ^{99m}Tc-diethylene triamine pentaacetate (DTPA). These instruments offer a wide range of radioanalytical applications besides radiochemical purity determinations, yet the high cost may limit their use in nuclear medicine.

9.1.1.7 Summary

The determination of the radiochemical purity in nuclear medicine may be performed with little expenditure of material.

Minimal equipment for routine determination of the radiochemical purity with TLC:

- Two chromatographic chambers for small volumes or laboratory beakers (about 100 ml size, 10 cm high), foil or lid for covering the beakers
- 1 ml syringes, fine needles (25 gauge), gloves
- Small flasks for solvents (MEK, saline, etc.)
- Chromatographic plates:
  - ITLC-SG plates (Gelman No. 61886, 20×20 cm, cut into strips [1.5×10 cm])
  - Paper (Whatman 3MM No. 3030861, 20×20 cm, cut into strips [1.5×10 cm])
- Pencil for marking the strips, color pens
- Scissors for cutting
- Plastic tubes for the measurement of the segments
- Ionization chamber

For handling of radioactive samples, only single-use materials should be used in order to minimize the risk of contamination of the sample and the staff. The TLC analysis

should be performed in an area of the hot lab with a low probability of contamination from other working procedures. If possible, a separate working area should be used. There should be a place with adequate ventilation (hood) for drying the strips after development in organic solvents (MEK, ethylacetate, etc.).

The determination of the radiochemical purity of radiopharmaceuticals by ITLC requires an experienced operator to produce accurate results. Quality control should be performed daily and not on demand, when abnormal distribution or poor image quality is reported.

References

Bish RE, Silverstein D, Bede JI (1980) Radiopharmaceutical-radiochromatography quality assurance with a radioisotope calibrator. J Radioanal Chem 57:565–573

Carpenter AP (1986) Radioanalytical techniques: ITLC, TLC, mini columns and electrophoresis. In: Wieland DM, Tobes MC, Mangner TJ (eds) Analytical and chromatographic techniques in radiopharmaceutical chemistry. Springer, Berlin Heidelberg New York

Council of Europe (1982) Sodium pertechnetate [^{99m}Tc] injection (fission), Monograph (124). European pharmacopeia. Strasbourg

Decristoforo C, Zaknun J, Kohler B, Oberladstaetter M, Riccabona G (1997) The use of electronic autoradiography in radiopharmacy. Nucl Med Biol 24:361–365

Dickey EE (1953) Separation of radium D, E and F by paper chromatography. J Chem Ed 30:525

Fairbrother JE (1984) Advances in TLC in two parts: Part 1 – Techniques. Pharm J 10 March:293–297; Part 2 – Applications. Pharm J 7 July:23–28

Filthuth H (1986) Detection of radiochromatograms and electropherograms with position-sensitive wire chambers. In: Wieland DM, Tobes MC, Mangner TJ (eds) Analytical and chromatographic techniques in radiopharmaceutical chemistry. Springer, Berlin Heidelberg New York

Frier M, Hesslewood S (1980) Quality assurance of radiopharmaceuticals – a guide to hospital practice. Nucl Med Comm, Special Issue 3

Hammermaier A, Reich E, Bögl W (1986) Qualitätskontrolle von in-vivo Radiopharmaka, ein Handbuch für das nuklearmedizinische Labor zur Analytik radiochemischer Verunreinigungen [in German]. ISH-Heft 94

Höye, A (1967) Determination of radiochemical purity of some radiochemicals and pharmaceuticals by paper chromatography, thin-layer chromatography and high voltage electrophoresis. J Chromatograpy 28:379

Izmailov, N.A. Shraiber MS (1938) The application of analysis by drop chromatography to pharmacy. Farmezia 3:1

Janshold, AL, Krohn KA, Vera DR, Hines HH (1980) Design and validation of a radiochromatogram scanner with analog and digital output. J Nucl Med Tech 8:222

Levit N (1980) Radiopharmacy laboratory manual for nuclear medicine technologists. University of New Mexico College of Pharmacy Albuquerque, pp 69–80

Mallol J (1990) Analytical artifacts in quality control of Tc-99m radiopharmaceuticals. Nucl Med Biol 17:609–611

Mangner TJ (1986) Potential artifacts in the chromatography of radiopharmaceuticals. In: Wieland DM, Tobes MC, Mangner TJ (eds) Analytical and chromatographic techniques in radiopharmaceutical chemistry. Springer, Berlin Heidelberg New York

Miller JM (2004) Chromatography: concepts and contrasts, 2nd edn. Wiley, New York

Robbins PJ (1983) Chromatography of technetium-99m radiopharmaceuticals – a practical guide. The Society of Nuclear Medicine, New York

Stahl E (1956) Dünnschicht-Chromatgraphie [in German]. Pharmazie 11:633

Theobald AE (1994) Quality control of radiopharmaceuticals. In: Sampson CB (ed) Textbook of radiopharmacy: theory and practice, 2nd enlarged edn. Gordon and Breach, Philadelphia

Westera G, Gadze A, Horst W (1985) A convenient method for the preparation of 99m Tc(V)dimercaptosuccinic acid (^{99m}Tc(V)-DMSA). Int J Appl Radiat Isot 36:349–355

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

9.1.2 Column Chromatography

F. Rakiás and J. Imre

Column chromatography, or liquid chromatography, is a separation method by which a ^{99m}Tc pharmaceutical is resolved into its components when passed through a chromatographic column with a mobile phase (solvent). Interaction of the sample with the solvent and the column matrix is affected by solubility, electrostatic forces, and charge, resulting in the retention of components on the chromatographic column and separation of impurities. Physical and chemical forces include van der Waal's, dipole, hydrogen bonding, dielectric, and electrostatic interactions, which cause separation of components with different physicochemical properties.

Several separation methods were derived from liquid chromatography, depending on the choice of the stationary phase and the solvent used for a particular compound, i.e., adsorption chromatography, partition chromatography (normal or reversed phase), ion-exchange chromatography, ion-pair chromatography, gel-permeation chromatography, and affinity chromatography.

For ^{99m}Tc-labeled pharmaceuticals, the most frequently used separation techniques are gel permeation chromatography at low pressure, and high-performance reversed-phase, ion-pair, ion-exchange, and size-exclusion chromatography.

9.1.2.1 Limitations of Liquid Chromatography for Quality Control of ^{99m}Tc Pharmaceuticals

In planar radiochromatography (thin-layer chromatography), the detection of the components of any ^{99m}Tc pharmaceutical is performed directly on the chromatographic plate; thus, the total applied ^{99m}Tc activity – theoretically all ^{99m}Tc constituents – can be determined, depending on sample and the system performance.

In the case of column chromatographic techniques, the separated ^{99m}Tc impurities are detected indirectly, after separation, accepting incomplete sample recovery. Some components of a ^{99m}Tc pharmaceutical remain on the column. In fact, reduced, hydrolized ^{99m}Tc activity is commonly retained on the column.

Since determination of radiochemical purity relies on the complete recovery of all constituents of a ^{99m}Tc pharmaceutical applied to the column, calculations based on the peak area of eluted components will overestimate the value of radiochemical purity.

The retention of ^{99m}Tc radiocolloid has been determined indirectly by comparing the precolumn and postcolumn ^{99m}Tc activity, using two detection loops. However, this method depends on the accurate calibration and validation of the system.

Trapping of reducing tin(II) salts (in colloidal form) in the pores of the column material has also been observed to affect radiochemical purity, because the pertechnetate content in the subsequent samples will be reduced, providing false values.

These problems exclude conventional column chromatography from routine use for quality control; however, application of minicolumns has offered an alternative to thin-layer chromatography in the cases of ^{99m}Tc-hexamethylpropylene amine oxime (HMPAO) and others, discussed in the respective monographs (Part 2).

Because of its high resolving capacity, conventional liquid chromatography is an efficient tool in research, when analysis of chemically similar byproducts (secondary, tertiary, etc., complexes), metabolites or products resulting from radiolysis, is required.

9.1.2.2 Gel Permeation or Size-Exclusion Chromatography

This method is based on the accessibility of the pores in the stationary phase for ^{99m}Tc-labeled molecules of different molecular sizes. The sample is eluted from a vertical column packed with porous beads of the gel by gravity or low pressure. Smaller ^{99m}Tc species penetrate the pores and are retained on the column, while larger molecules are excluded and are therefore rapidly eluted from the column. This separation technique has particular application for macromolecules, proteins (serum albumin, immunoglobulins [e.g., monoclonal antibodies and their fragments]), but has also been used for separation of small-molecular-weight ^{99m}Tc-diphosphonate complexes.

Quantification was performed by external scanning of the column and analysis of the activity profile of the retained ^{99m}Tc compounds (mercapto complexes, diphosphonates, etc.), using a modified thin-layer scanner.

Several types of gel column filling material are available (e.g., Sephadex, Sepharose, etc.).

9.1.2.3 High-Performance Liquid Chromatography

J. Imre

High-performance liquid chromatography (HPLC) provides faster separation and higher resolution of the eluted components of a ^{99m}Tc pharmaceutical than does the conventional column chromatography. While in column chromatography the mobile phase passes through the stationary phase by gravity or low pressure, in HPLC the mobile phase is pumped through the column by high pressure up to 6000 psi (~ 400 bar).

HPLC Instrumentation. The schematic diagram of a radio-HPLC system can be seen in Fig. 9.1.2.1.

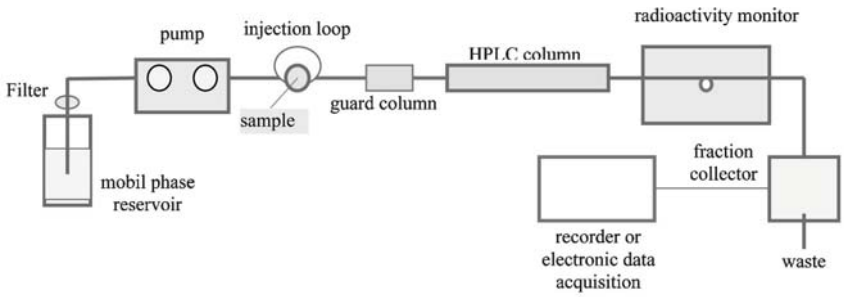

Fig. 9.1.2.1. Components of a radio-high-performance liquid chromatography (HPLC) system

Table 9.1.2.1. Modified silica packing materials with chemically bonded phases

Type of modification	Application
C_2 Dimethyl – RP-2	Reversed-phase and ion-pairing chromatography Shorter retention time than with other RP
C4 Butyl – RP-4	Reversed-phase and ion-pairing chromatography Separation of peptides and proteins
C_8 Octyl – RP-8	Reversed-phase and ion-pairing chromatography Moderately to highly polar (water-soluble compounds)
C_{18} Octadecyl – RP-18	Reversed-phase and ion-pairing chromatography Nonpolar or moderately polar compounds
C_6H_5 Phenyl	Reversed-phase and ion-pairing chromatography Moderately polar compounds, polar aromatics etc.
CN, cyano	Straight- or reversed-phase chromatography
NO_2, nitro	Separation of compounds with double bonds
OH, alkanol – DIOL	Straight- or reversed-phase chromatography
NH_2 amino	Straight-phase, weak anion-exchange, and reversed-phase chromatography
$N(CH_3)_2$ dimethylamino	Weakly basic anion exchanger
Quaternary ammonium	Strongly basic anion exchanger
Sulfonic acid	Strongly acidic cation exchanger

The Eluent Delivery Pump. HPLC pumps deliver the mobile phase from the reservoir to the column. Mainly double piston-actuated pumps accurately deliver solvent over a broad range of flow rates (0.1 µl/min–20 ml/min). Gradient elution (flow rate or composition) can be used in HPLC separations where the use of only one mobile phase (isocratic separation) would not have achieved adequate resolution.

The Sample Application Unit. In the case of radio-HPLC, the more useful system is the injector valve with a sample loop of desired volume, allowing safe filling of the ^{99m}Tc sample.

HPLC Column. In HPLC, a number of chromatographic techniques are readily available. Highly efficient HPLC columns contain small particles of less than 10 µm in diameter (greater than 20 µm for preparative HPLC), tightly packed into 100 to 300 mm length and of small internal diameter (2–5 mm) glass or steel tubes, and the result is good sample resolution and narrow peaks that elute from the column. Columns with high resolving capacity are now commercially available (Table 9.1.2.1) and can be used for several times.

Packing Materials. Packing materials include modified silica with chemically bonded phases, e.g., nonpolar groups – dimethyl, butyl, octyl, octadecyl, and phenyl; and polar groups – amino (NH_2), cyano (CN), nitro (NO_2) dimethylamino [$N(CH_3)_2$], and alkanol (OH). Quaternary ammonium facilitates basic anion exchange, and sulfonic acid the acidic cation exchange, offering separation conditions for a vast variety of chemically different compounds. The use of prefilters and guard columns may considerably improve the life span of a column.

Radioactivity Monitor. The homemade detector systems – where the detector loop is placed into a well-type scintillation detector – are frequently used (De Groot et al.

1986a; Nunn and Fritzberg 1986). Most commercially available radioactivity monitors use flow cells positioned between two photomultipliers with high counting efficiencies. The signal pulses from the photomultipliers are measured in coincidence to suppress noise. The signals are amplified and are then usually subjected to various data processing, integration, etc.

HPLC Applications for ^{99m}Tc Pharmaceuticals. Examples of typical applications of HPLC for separation of ^{99m}Tc-labelled species are shown in Table 9.1.2.2. The most frequently used HPLC techniques (Hnatowich 1986; Millar 1989) are reversed-phase partition chromatography for nonpolar or weakly polar components of ^{99m}Tc pharmaceuticals (HMPAO [Hung et al. 1988; Neirinckx et al. 1987; Weisner et al. 1993], monodentate methoxyisobutyl isocyanide [MIBI; Carvalho et al. 1992; Hung et al. 1991], mercaptoacetyltriglycine [MAG_3; Brandau et al. 1990; Coveney and Robbins 1987; Millar et al. 1990; Shattuck et al. 1994], tetrofosmin [Cagnolini et al. 1998; Graham and Millar 1999; Kelly et al. 1993], iminodiacetic acid [IDAs; Fritzberg and Lewis 1980; Nunn 1983; Nunn et al. 1983], peptides [Vallabhajosula 1986; Zinn et al. 2000], etc.), ion-pair (both ionized and less polar constituents: pertechnetate, diphosphonates; De Groot et al. 1986b; Hoch and Pinkerton 1986; Huigen et al. 1988; Nieuwland et al. 1989; Tanabe et al. 1983, etc.), ion-exchange (ionized forms: pertechnetate, diphosphonates, etc.), and size exclusion chromatography (separation based on molecular size: diphosphonates, human serum albumin [HSA; Vallabhajosula et al. 1982], and antibodies and their fragments [Hnatowich 1986], etc.).

Since HPLC is nondestructive, it can also be used as a preparative technique. The fraction containing the ^{99m}Tc pharmaceutical is formulated and applied in the clinic.

Reversed-Phase Chromatography. At acidic pH, octadecylsilane (ODS or C-18)-coated silica particles are frequently used as a stationary phase, although polymer-based columns (PRP-18) are also popular because of their wider operating pH range (1.0–13.0). The used mobile phases are polar solvents such as water, which is mixed with varying concentrations of miscible organics (e.g., methanol, acetonitrile). Solvent strength can be varied by changing the composition ratios of the mobile phase.

Ion-Pair Chromatography. A number of ^{99m}Tc complexes are ionized by deprotonation at higher pH. It is possible to separate both ionized and nonionized ^{99m}Tc species using reversed-phase chromatography. Ionic charges can be suppressed by manipulation of pH or by the use of an ion-pair reagent. The preferred ion-pairing agents are quaternary ammonium compounds (tetrabutyl ammonium, dodecyltrimethyl ammonium, etc.) for the analysis of anionic ^{99m}Tc complexes, and *N*-alkyl sulfonates for HPLC of cationic ^{99m}Tc compounds dissolved in a solvent similar to that used in reversed phase method.

Ion-Exchange Chromatography. In the case of ^{99m}Tc pharmaceuticals typically weak anion (e.g., amino-modified silica) or cation exchangers are used. The resolution can be affected by the ionic strength and pH of the buffered mobile phase, and gradient elution might be required to hasten elution.

Gel Permeation Chromatography. ^{99m}Tc-labeled proteins on polymer-based column packing can be purified from precursor reagents and unbound radiolabeling. The first eluted macromolecules are separated according to their size from the smaller molecules.

Table 9.1.2.2. High-performance liquid chromatography (HPLC) separation methods applied to ^{99m}Tc pharmaceuticals

Radiopharmaceutical	Column	Isocratic/ Gradient	Solvent(s)	Reference
^{99m}Tc-HMPAO (exametazime)	C-18	Isocratic	For "A" impurity determination: A: acetonitrile B: 0.1 *M* phosphate buffer, pH 3.0 A:B 33:67	Council of Europe 2005
	PRP-1	Gradient	A: 20 m*M* phosphate buffer, pH 7.4 B: tetrahydrofuran 0–25% B over 6 min	Neirinckx 1987
	PRP-1	Gradient	A: 10 m*M* potassium phosphate, pH 7.0 or water containing 1% methanol B: acetonitrile 0–50% B over 5 min	Hung 1988
	PRP-1	Gradient	A: 50 m*M* sodium acetate, pH 5.6 B: tetrahydrofuran 0–100% B over 17 min	Weisner 1993
^{99m}Tc-MIBI (sestamibi)	C-18	Isocratic	For "C" impurity determination: A: acetonitrile B: 50 m*M* ammonium sulfate C: methanol A:B:C, 20:35:45	Ccouncil of Europe 2005
	C-8	Gradient	A: 50 m*M* ammonium sulfate B: methanol 0–95% B over 5 min	Carvalho 1992
	C-18	Isocratic	A: methanol B: 50 m*M* ammonium sulfate C: acetonitrile A:B:C, 45:35:20	Hung 1991
^{99m}Tc-MAG$_3$ (mertiatide)	C-18	Isocratic with wash	A: ethanol B: 10 m*M* phosphate buffer, pH 6.0 A:B 7:93 After 20 min, wash with methanol:water, 90:10	Council of Europe 2005
	C-18	Isocratic with wash	A: ethanol B: 10 m*M* phosphate buffer, pH 6.5 A:B 5:95 After peak, wash with methanol–water, 90:10	Millar 1990
	C-18	Gradient	A: 10 m*M* potassium phosphate with 1% triethylamine, pH 5.0 B: tetrahydrofuran 0–8% B over 30 min	Shattuck 1994
^{99m}Tc-tetrofosmin	PRP-1	Gradient	A: 10 m*M* phosphate buffer, pH 7.5 B: tetrahydrofuran 0–100% B over 17 min	Kelly 1993
	PRP-1	Isocratic	A: acetonitrile B: 10 m*M* ammonium carbonate A:B, 70:30	Graham 1999
	PRP-1	Isocratic	A: 5 m*M* monopotassium phosphate B: acetonitrile A:B, 50:50	Cagnolini 1998

Table 9.1.2.2 (continued)

Radiopharmaceutical	Column	Isocratic/ Gradient	Solvent(s)	Reference
^{99m}Tc-MDP	Aminex	Isocratic	850 m*M* sodium acetate	Tanabe 1983
	C-18	Isocratic	10 m*M* sodium acetate, 2 m*M* tetrabutylammonium hydroxide, 3% ethyl acetate, pH 6.0	Hoch 1986
^{99m}Tc-EHDP	Aminex	Isocratic	850 m*M* sodium acetate	Huigen 1988
	C-18	Isocratic	50 m*M* EHDP, 10 m*M* sodium acetate, 3 m*M* tetrabutylammonium hydroxide	Nieuwland 1989
^{99m}Tc-IDA	C-18	Isocratic	25 m*M* phosphate buffer pH 6.0	Nunn 1983
	Ultrasphere ODS	Isocratic	A: 10 m*M* phosphate buffer, pH 6.8 B: methanol A:B 50:50	Fritzberg 1980
^{99m}Tc-HSA	Silica gel	Isocratic	100 m*M* phosphate buffer, 100 m*M* sodium chloride, 8 m*M* sodium azide	Council of Europe 2005
	Spherogel-TSK SW	Isocratic	100 *M* phosphate buffer	Vallabhajosula 1982
^{99m}Tc-depreotide	C-18	Gradient	A: 0.1% TFA in water B: 0.1% TFA/90% acetonitrile/H_2O 20% B over 30 min	Vallabhajosula 1996; Zinn 2000

HMPAO hexamethylpropylene amine oxime, *MIBI* monodentate methoxyisobutyl isocyanide, *MAG$_3$* mercaptoacetyltriglycine, *MDP* methylenediphosphonate, *EHDP* etidronate, *IDA* iminodiacetic acid, *HSA* human serum albumin, *TFA* trifluroroacetic acid

The use of a dual detector system, which is a radioactive monitor with a UV detector working at 280 nm, is practical. The mobile phase is usually buffered water.

9.1.2.4 Minicolumns for Routine Quality Control

There is a small-scale version of column chromatography that can compete with the planar chromatographic techniques for routine analysis of certain ^{99m}Tc pharmaceuticals. A small-sized tube or syringe is packed with appropriate stationary phase, forming a short-bed column. Several types of filling material can be used: reversed-phase packing (e.g., C18-modified silica for separation of ^{99m}Tc complexes of hepatoiminodiacetic acids ([HIDAs], MIBI, HMPAO, MAG3, etc.), preswollen gel (e.g., Sephadex for gel filtration of ^{99m}Tc-labeled HSA, immunoglobulin [HIG, monoclonal antibodies]) or aluminium oxide (for adsorption of anionic pertechnetate from inert ^{99m}Tc complexes). The ^{99m}Tc-sample is applied onto the top of the short bed and eluted with a small amount of solvent. The elution may be performed even in a plastic syringe. The separation is fast and simple, and the activity retained on the column or of the collected fractions is measured in an ionization chamber; thus, higher amounts of radioactivity of ^{99m}Tc label may be used than in the case of the planar method. The results depend on the column performance, affected by the tightness of packing and the flow rate; therefore application of standardized, prepacked columns is suggested (e.g., Chromabond, SEP-PAK) at optimal elution speed.

References

Brandau W, Bubeck B, Schober O, Weber E, Taylor DM (1990) ^{99m}Tc-MAG$_3$: chemistry and biokinetics of by-products. In: Blaufox MD, Hollenberg NK, Raynaud C (eds) Radionuclides in nephrourology. Karger, Basel pp 11–16

Cagnolini A, Whitener D, Jurisson S (1998) Comparison of the kit performance of three ^{99m}Tc myocardial perfusion agents. Nucl Med Biol 25:435–439

Carvalho PA, Chiu ML, Kronauge JF, Kawamura M, Jones AG, Holman BL, Piwnica-Worms D (1992) Subcellular distribution and analysis of technetium-99m-MIBI in isolated perfused rat hearts. J Nucl Med 33:1516–1522

Council of Europe (2005) European pharmacopeia. Council of Europe, Maisonneuve, Sainte-Ruffine

Coveney JR, Robbins MS (1987) Comparison of technetium-99m MAG3 kit with HPLC-purified technetium-99m MAG3 and OIH in rats. J Nucl Med 28:1881–1887

De Groot GJ, Das HA, deLigny CL (1986a) A system for high performance liquid chromatography of Tc-99m complexes with on-line radiometric detection and data processing. Int J Appl Radiat Isot 37:349–355

De Groot GJ, Das HA, deLigny CL (1986b) Separation of Tc-99m(Sn)HEDP complexes by HPLC and GPC. Int J Appl Radiat Isot 37:23–27

Fritzberg AR, Lewis D (1980) HPLC analysis of Tc-99m iminodiacetate hepatobiliary agents and a question of multiple peaks: concise communication. J Nucl Med 21:1180–1184

Graham D, Millar AM (1999) Artefacts in the thin-layer chromatographic analysis of ^{99}Tc^{m}-tetrofosmin injections. Nucl Med Commun 20:439–444

Hnatowich DJ (1986*)* HPLC of radiolabelled antibodies. In: Wieland DM, Tobes MC, Manger TJ (eds) Analytical and chromatographic techniques in radiopharmaceutical chemistry. Springer, Berlin Heidelberg New York

Hoch DJ, Pinkerton TC (1986) Reversed-phase HPLC of ^{99m}Tc methylene diphosphonate bone imaging kits with quantification of pertechnetate. Int J Rad Appl Instrum 37:593–598

Huigen YM, Gelsema WJ, de Ligny C (1988) Effect of the composition of the eluent in the chromatography of ^{99m}Tc-diphosphonate complexes. Int J Rad Appl Instrum 39:381–384

Hung JC, Corlija M, Volkert WA, Holmes RA (1988) Kinetic analysis of technetium-99m D,L-HM-PAO decomposition in aqueous media. J Nucl Med 29:1568–1576

Hung JC, Wilson ME, Brown ML, Gibbons RJ (1991) Rapid preparation and quality control method for technetium-99m-2-methoxy isobutyl isonitrile (technetium-99m-sestamibi). J Nucl Med 32:2162–2168

Kelly JD, Forster AM, Higley B, Archer CM, Booker FS, Canning LR, Chiu KW, Edwards B, Gill HK, McPartlin M (1993) Technetium-99m-tetrofosmin as a new radiopharmaceutical for myocardial perfusion imaging. J Nucl Med 34:222–227

Millar AM (1989) Quality assurance of radiopharmaceuticals. In: Theobald AE (1989) Radiopharmaceuticals and radiotracers in pharmacy. Ellis Horwood, Chichester, UK

Millar AM, Wilkinson AG, McAteer E, Best JJK (1990) ^{99m}Tc-MAG$_3$: in vitro stability and in vivo behaviour at different times after preparation. Nucl Med Commun 11:405–412

Neirinckx RD, Canning LR, Piper IM, Nowotnik DP, Picket RD, Holmes RA, Volkert WA, Foster AM, Weisner PS, Marriot JA, Chaplin SB (1987) Tchnetium-99m D,L-HM-PAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood flow. J Nucl Med 28:191–202

Nieuwland RJ, Das HA, de Ligny CL (1989) Improvement of the reproducibility of ion-pair HPLC of ^{99m}Tc(Sn)EHDP complexes and the influence of the Sn(II) concentration on the composition of the reaction mixture. Int J Rad Appl Instrum 40:153–157

Nunn AD (1983) Structure-distribution relationships of radiopharmaceuticals. Correlation between the reversed-phase capacity factors for Tc-99m phenylcarbamoyl-methyliminodiacetic acids and their renal elimination. J Chromatogr 255:91–100

Nunn AD, Fritzberg AR (1986) Components for the design of a radio-HPLC system. In: Wieland DM, Tobes MC, Manger TJ (eds) Analytical and chromatographic techniques in radiopharmaceutical chemistry. Springer, Berlin Heidelberg New York

Nunn AD, Loberg MD, Conley RA (1983) A structure–distribution–relationship approach leading to the development of Tc-99m-mebrofenin: an improved cholescintigraphic agent. J Nucl Med 24:423–430

Shattuck LA, Eshima D, Taylor AT, Anderson TL, Graham DL, Latino FA, Payne SE (1994) Evaluation of the hepatobiliary excretion of technetium-99m-MAG$_3$ and reconstitution factors affecting radiochemical purity. J Nucl Med 35:349–355

Tanabe S, Zodda JP, Deutsch E, Heineman WR (1983) Effect of pH on the formation of Tc($NaBH_4$)-MDP radiopharmaceutical analogues. Int J Appl Radiat Isot 34:1577–1584
Vallabhajosula S, Goldsmith SJ, Pollina R, Lipszyc H (1982) Radiochemical analysis of Tc-99m human serum albumin with high pressure liquid chromatography. J Nucl Med 23:326–329
Vallabhajosula S, Moyer BR, Lister-James J, McBride BJ, Lipszyc H, Lee H, Bastidas D, Dean RT (1996) Preclinical evaluation of technetium-99m-labelled somatostatin receptor-binding peptides. JNM 37:1016–1022
Weisner PS, Bower GR, Dollimore LA, Forster AM, Higley B, Storey AE (1993) A method for stabilising technetium-99m exametazime prepared from a commercial kit. Eur J Nucl Med 20:661–666
Zinn KR, Buchsbaum DJ, Chaudhuri TR, Mountz JM, Grizzle WE, Rogers BE (2000) Noninvasive monitoring of gene transfer using a reporter receptor imaged with a high-affinity peptide radiolabeled with ^{99m}Tc or ^{188}Re. J Nucl Med 41:887–895

9.1.3 Electrophoresis

Gy. Jánoki

Electrophoresis is widely used in the separation of inorganic ions and different classes of molecules. The physical principle underlying electrophoresis can be stated as: Particles carrying an electric charge are accelerated when placed in an electric field; this driving force is very rapidly balanced by the friction forces arising in the medium; from that moment, the particles move at a constant speed proportional to their charge. Electric charge carried by molecules originates either from dissociation or from selective adsorption. For polyelectrolytes such as proteins, dissociation of the acidic groups COOH or NH_3^+ constitutes the principal source.

The electromigration rate is directly related to the charge and inversely related to the ionic radius of the complex being separated. It means that the direction of migration of cationic species is toward the cathode while the migration of the anionic species moves toward the anode.

Electrophoresis has been used for quality control of certain radiopharmaceuticals (Belkas and Archimandritis 1979; Pauwels and Feitsma 1979). Paper electrophoresis has been applied successfully for the determination of the relative charge on some ^{99m}Tc complexes.

HPLC, TLC, and ITLC analysis methods available today are more convenient and reproducible than electrophoresis. Despite this, electrophoresis continues to be used as a research tool in radiopharmaceutical studies. The reason for this is that electrophoresis gives information on the charge of ^{99m}Tc complexes that cannot be easily obtained by other techniques. From the standpoint of determining whether a specific radiolabeled complex is anionic, cationic or neutral, electrophoresis is the best technique currently available.

In addition, polyacrylamide gels (PAGE) are used as supports for protein and macromolecular electrophoresis including radiolabeled antibodies and other proteins (Wieme 1965).

References

Belkas EP, Archimandritis S (1979) Quality control of colloid and particulate ^{99m}Tc-labelled radiopharmaceuticals. Eur J Nucl Med 4:375
Pauwels EKJ, Feitsma RIJ (1977) Radiochemical quality control of ^{99m}Tc-labelled radiopharmaceuticals. Eur J Nucl Med 2:97
Wieme RJ (1965) Agar gel electrophoresis. Elsevier, Amsterdam

9.2 Determination of Tin(II)

F. Rakiás

Radiopharmaceutical kits for labeling with ^{99m}Tc eluate contain tin(II)-ion for reduction of sodium pertechnetate to lower valency states, which are chemically reactive. Tin(II) salts are easily oxidized, even by the oxygen in air. Certain chemicals are also assumed to enhance the oxidation of tin(II) to tin(IV). Therefore, determination of tin(II) in radiopharmaceutical kits is an important aspect of quality control.

Classic methods for the determination of tin(II) include the titration with an iodine standard solution, or absorption measurements by spectrophotometric analysis. Both methods have considerable disadvantages for the determination of tin(II) in radiopharmaceutical kits (Rakiás and Zolle 1997).

A new spectrophotometric analysis of tin(II) and tin(IV) in the same matrix was developed by Rakiás et al. (unpublished results); however, the disadvantages of sample preparation and the slow formation of a colored complex for absorption measurements led to the investigation of other methods.

Pulse polarography has been investigated because of its speed and high selectivity. Thus, square wave voltammetry has been extended to the measurement of microgram amounts of tin(II) in radiopharmaceutical kits.

Pulse polarography has offered considerable advantages for the determination of the tin(II) content in a number of radiopharmaceutical kits (Rakias et al. 1988). Square wave voltammetry shows a considerable increase in sensitivity, suitable for the measurement of very small (microgram) amounts of tin(II), as used for the reduction of ^{99m}Tc-Na-pertechnetate when labeling radiopharmaceuticals.

Two kits manufactured by NCPH–"Fredric Joliot Curie" National Research Institute for Radiobiology and Radiohygiene were examined. TromboScint and LeucoScint differ simply by the fact that LeucoScint contains twice the amount of active ingredients, i.e., 0.18 mg HMPAO and 2.28 μg tin(II). LeucoScint is used for labeling leucocytes, and TromboScint is suitable for labeling platelets.

For the determination of tin(II) in kits an EG & G polarographic analyzer (model 384) with a static mercury drop electrode (model 303) and a cabinet reference electrode was used. Model 384 is a microprocessor-based polarographic analyzer with built-in floppy disk memory to store and recall analytical curves. By controlling each step of the analysis, the microprocessor automates polarographic and voltammetric measurements. All experimental parameters may be chosen by the operator. Concentrations are computed automatically and recorded in the range from 0.001 ppb to 9999 ppm.

Square Wave Voltammetry of Tin(II) and Tin(IV). Deoxygenated 1 *N* hydrochloric acid plus 4 *N* ammonium chloride buffer (1:1, v/v) was used as an electrolyte. Sample solutions were prepared by dissolving one kit in the same buffer and adding to the electrolyte solution; recording was started from 0.0–0.6 V.

The tin(IV) maximum appeared at –0.25 V, and the tin(II) maximum at –0.45 V, as the second peak. If only 1 *N* sulfuric acid is used as an electrolyte, tin(II) may be detected selectively at –0.45 V.

The method was validated, and the following parameters were examined.

Linearity of the Instrument. To identify the highest amount of tin(II) that can be measured with a linear response, increasing concentrations of tin(II) up to 5 mg/ml were analyzed.

Linear regression analysis of the data (Booster 1982) showed a correlation coefficient > 0.999, indicating linearity of measurements over a wide range of concentration.

Precision of the Method. Measurements were performed with ten samples of each kit. In the case of TromboScint, the average value of Sn(II) was determined as 1.138 μg/kit (theoretically, 1.14 μg Sn(II)/vial) with a standard deviation of 0.050 μg and a coefficient of variation of 4.39%.

In the case of LeucoScint, the average value of Sn(II) was calculated as 2.263 μg/kit (theoretically, 2.28 μg Sn(II)/vial), with a standard deviation of 0.089 μg and a coefficient of variation of 3.93%.

Effect of Sample Volume. Since the vials were filled with 1.0 ml of the dissolved kit content in the production department, there might have been a variation in the amount of tin(II) actually added in this volume. Therefore, the precision of the method by analyzing ten matrix samples to which a homogenous solution of tin(II) chloride containing 1.14 and 2.28 μg of tin(II), respectively, were also investigated. When these known concentrations of tin(II) were analyzed together with the matrix samples, the results were identical excluding a volume effect. A recovery between 92.10 and 106.14% was observed. The average value of Sn(II) in the case of TromboScint was 1.139 μg/kit, with a standard deviation of 0.048 and a coefficient of variation of 4.21%. In the case of LeucoScint, the average value of Sn(II) was 2.266 μg/kit, with a standard deviation of 0.062 and a coefficient of variation of 2.73%.

Selectivity. Although it is stated in the literature that Sn(IV) does not interfere with the measurement of Sn(II), experimental proof adding 2 μg of tin(IV) to the test solution was obtained. When no tin(IV) was added to TromboScint, the average value of Sn(II) was determined as 1.141 μg/kit, with a standard deviation of 0.068 μg and a coefficient of variation of 5.96%. With tin(IV) added to TromboScint, the average value of Sn(II) was determined as 1.126 μg/kit, with a standard deviation of 0.055 μg and a coefficient of variation of 4.88%. Values demonstrating no interference by Sn(IV) were also shown for LeucoScint.

Based on the above-cited results, square wave voltammetry is suitable for the measurement of microgram amounts of tin(II) in radiopharmaceutical kits, with high accuracy (> 99%). Impurities causing oxidation to tin(IV) have been shown to have no effect on the recovery. Based on the obtained data, square wave voltammetry has been shown as a reliable and highly sensitive method for the determination of tin(II) in radiopharmaceutical kits.

References

Booster P (1982) Statistische Methoden im Laboratorium [in German]. Elsevier, Amsterdam

Eckelman W, Meinken G, Richards P (1971) Chemical state of Tc-99m in biomedical products. J Nucl Med 12:596–600

Rakiás F, Zolle I (1997) Stannous ion determination: importance and relevance for radiopharmaceutical kits. In: Bergmann H, Kroiss A, Sinzinger H (eds) Radioactive isotope in clinical medicine and research, vol. 22. Birkhauser, Basel, pp 401–409

Rakias F, Kelemen-Küttel I, Tömpe P, Nemeth P, PaaI TL (1988) Correlation between the Sn(II)-content of phosphonate, phytate, and gluconate kits and the quality of the obtained images. In: Höfer R, Bergmann H (eds) Radioactive isotope in clinic und research, vol. 18. Schattauer, Stuttgart, pp 417–419

9.3 Sterility Testing of Radiopharmaceuticals

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It is an integral feature of good manufacturing practice that radiopharmaceuticals for injection that are required to be sterile are prepared under conditions that exclude microbial contamination of the product. There is a higher degree of assurance of sterility of products that are terminally sterilized in their final container than for those that are prepared by aseptic technique. The majority of radiopharmaceuticals in current clinical practice fall into the latter category, and a sterility test is the only analytical method available to demonstrate the absence of microbial contamination. However, the use of commercially available sterile products and starting materials (e.g., kits) with marketing authorization is an important feature in avoiding problems with lack of sterility.

A test for sterility is laid down in the *European Pharmacopeia* for all parenteral products, and two techniques for testing are described, either membrane filtration of the product with subsequent incubation of the filter in suitable culture media (which is the preferred technique), or direct inoculation of the product into the culture medium, followed by incubation at the appropriate temperature for the specified time. Suitable media are described in the *European Pharmacopeia*; although, it is also recognized that other media may be used. In every case, however, it is necessary to demonstrate that the medium is capable of supporting the growth of microorganisms both in the presence and absence of the material to be tested.

9.3.1 Problems in Applying the *European Pharmacopeia* Test to Radiopharmaceuticals

There are specific difficulties in applying the test as written, particularly for radiopharmaceuticals based on technetium-99m, and these difficulties are acknowledged in the general pharmacopeial monograph on radiopharmaceuticals.

First, the batch size of products is often small, and in the case of a technetium product prepared in a hospital or clinic, may consist of only a single vial with a total volume of less than 10 ml.

This makes it impossible to follow the *European Pharmacopeia* requirements for the number of containers to be tested (10% or 4, whichever is the greater), without preparing extra vials of the product specifically for the test, which is economically unrealistic and also imposes an additional radiation burden on to the operators. There are also problems with the volume of the product available for testing. The *European Pharmacopeia* states that if the quantity in the container is between 4 and 20 ml, which is common for technetium products, 2 ml should be used for each culture medium being tested. If followed strictly, this would mean a large reduction in the volume of material available for patient use.

Second, the radioactive nature of the product imposes handling difficulties in performing the test, since quality control departments may not have the necessary facilities for handling radioactive materials, and radiopharmacy departments may not be suitably equipped to perform sterility testing.

One method to avoid these problems would be to let the product decay for a sufficient period of time to allow the level of radiation emitted to fall to a suitably low level to facilitate handling. However, there is published evidence to suggest this technique will decrease the sensitivity of the test, since the number of any viable organisms in the preparation may decrease on storage (Brown and Baker 1986; Stathis et al. 1983; Wind 1985). It is therefore always necessary to perform a sterility test as soon as possible after preparation of the radiopharmaceutical.

Finally, it is recognized that for short-lived radiopharmaceuticals, the long incubation time of the culture media (7 days for the membrane filtration technique, 14 days for direct inoculation) means the result of the sterility test cannot be available before the product is used. In these situations, the test constitutes a control of production techniques and will give valuable information about their suitability.

9.3.2 Recommendations for Sterility Testing of Radiopharmaceuticals

In view of the difficulties in applying the *European Pharmacopeia* test, many variations have been adopted. Whatever the technique used, it is essential to perform a validation to ensure that it would be able to detect the presence of any viable microorganisms in the sample.

If following the *European Pharmacopeia* test, it is often easier to perform the direct inoculation technique. It is suggested that the remnants of a technetium vial are divided equally between two suitable culture media as soon as possible after expiration of the radiopharmaceutical. The inoculated media should then be incubated for not less than 14 days at 20–25 °C for media being used to detect fungi, or 30–35 °C for those used in the detection of bacteria. Depending on the level of radioactivity present, shielding of the culture media may be necessary during some or all of the incubation period. When macroaggregates or microspheres are being tested, it may be necessary to perform a subculture at the end of the incubation mixture, since, dependent on the volume used in the test, the radiopharmaceutical itself may produce turbidity in the culture medium that may be indistinguishable from bacterial growth.

The membrane filtration technique is technically more elaborate and requires that the radiopharmaceutical under test, after aseptic dilution, is passed through a membrane filter with a pore size of 0.45 m, which has been moistened with a sterile nutrient diluent. After filtration, the membrane is either transferred to a suitable culture medium or aseptically cut into two equal parts and one half placed in each of two suitable media. Incubation at the appropriate temperature is required for at least 7 days.

An alternative – although unofficial – technique that has been used is the addition of an equal volume of double-strength culture medium directly to the remnants of the vial of radiopharmaceutical immediately upon its expiration. The advantages and disadvantages of this technique are summarized in Table 9.3.1.

In order to make the test more meaningful, it is necessary to rotate the culture media used and ensure that each product prepared is at some stage tested with each culture me-

Table 9.3.1. Sterility testing with double-strength broth

Advantages	Disadvantages
No delay in testing	Sample size variable
Can be performed in radiopharmacy	Only one culture medium can be used
Container can be easily shielded	Not officially recognized
No dilution of culture medium below normal strength	Not applicable if preservatives need to be diluted out

dia used. After incubation for 14 days, the result is obtained by observing the culture medium for turbidity. A positive control can then be performed by inoculating one of the test organism described in the *European Pharmacopeia* directly into the vial. Again, it is necessary to ensure the test organisms are used in rotation for each product.

9.3.3 Frequency of Testing

It has already been stated that the results of a sterility test are necessarily retrospective and as such, constitute a control of production processes within the radiopharmacy department rather than forming a part of a release procedure for individual products. Sterility testing of every batch prepared, although ideal, is unrealistic in practice. The testing program should ensure that, at some stage, all different types of product prepared are tested on a regular basis. At least one batch should be tested every week. In addition, it is recommended that the remnants of the first eluate of each technetium generator and the final unused eluate should be tested.

9.4 Pyrogen Testing of Radiopharmaceuticals

The *European Pharmacopeia* requires certain radiopharmaceuticals, mainly of biological origin, to comply with a test for pyrogens, which are substances generally arising from bacteria that are capable of inducing fever. The *British Pharmacopeia* general monograph on parenteral products states that compliance is necessary for preparations where the volume in a single injection exceeds 15 ml or is less than 15 ml, but where the label says the product is apyrogenic. The test consists of measuring the rise in body temperature in rabbits, following intravenous administration of the substance and in order to pass the summed response from three rabbits must not exceed 1.15 °C. The *Pharmacopeia* also considers that for some products it may be necessary to allow decay to take place before testing in order that hyperthermia that may be due to the radioactivity of the product is avoided. In view of the sophisticated facilities required for the test and the fact that volumes of radiopharmaceuticals administered are hardly ever above 15 ml, this test is not carried out in hospitals or clinics. If necessary, samples can be submitted to specialist laboratories for testing, although it is most unlikely that this will take place routinely. For short-lived materials, the results are inevitably retrospective to the use of the product, which limits its usefulness.

For some products, a direct measurement of the level of bacterial endotoxins in the preparation is required. The test uses a lysate of amoebocytes from the horseshoe crab,

Table 9.4.1. Products in the *European Pharmacopeia* with endotoxin limits

Product	Endotoxin limit (units ml^{-1})
^{99m} Technetium human albumin	Not greater than 175/V[a]
Iodine 123 MIBG	Not greater than 175/V
Iodine 131 MIBG	Not greater than 175/V
Iodine 131 norcholesterol	Not greater than 175/V
Indium 111 pentetate	Not greater than 14/V

MIBG metaidobenzoguanidine
[a] *V* maximum recommended dose in milliliters

Limulus polyphemus, and is often referred to as *Limulus* amoebocyte lysate (LAL) test. It depends on the fact that endotoxins will produce turbidity, precipitation or, more commonly, gelation of a solution of the lysate within a period of approximately 1 h. The test is described in detail in the *European Pharmacopeia* and requires the performance of control experiments to demonstrate the sensitivity of the lysate using at least three dilutions of an endotoxin standard. It is also necessary to show that the product under test does not contain substances that interfere with the sensitivity of the test. Reagents for performing the test are commercially available and in view of the time scale required, can be used prospectively for short-lived radiopharmaceuticals, although technical expertise in performing the test is essential to avoid obtaining misleading results. Products in the *European Pharmacopeia* for which a bacterial endotoxin test is specified are listed in Table 9.4.1, together with the limits for bacterial endotoxin laid down in the monographs. This limit is the maximum allowable endotoxin concentration in units per ml. The *United States Pharmacopeia* prescribes for a wider range of radiopharmaceuticals, including those based on positron emitters. The maximum limits prescribed are the same as in the *European Pharmacopeia*.

It is known that endotoxins are approximately 1000 times more toxic following intrathecal as opposed intravenous administration and this is recognized in the pharmacopeial monographs for products used intrathecally (e.g. Indium In^{111}-pentetate) since the maximum amount of endotoxin allowed is lower than for intravenous products.

9.4.1 Recommendations for Endotoxin Determinations of Radiopharmaceuticals

The use of commercially available products with marketing authorization that have been tested by the manufacturer will remove the need for routine determination of endotoxins. However, testing should be considered if it is thought that a particular product may be giving rise to problems.

When products are prepared totally within a hospital from raw materials, the hospital assumes responsibility for their safety, quality, and efficacy. In these situations, each batch of a product, especially those intended for intrathecal administration, should be tested.

References

Brown S, Baker MH (1986) The sterility testing of dispensed radiopharmaceuticals. Nucl Med Commun 7:327–336

Stathis VJ, Miller CM, Doerr GF, Coffey JL, Hladik WB (1983) Effect of technetium Tc-99m pertechnetate on bacterial survival in solution. Am J Hosp Pharm 40:634–637

Wind K (1985) Sterility testing. In: Cox PH (ed) Radiopharmacy and radiopharmacology, yearbook 1. Gordon and Breach, London, pp 35–46