Radiopharmaceutical Quality Control

4

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4.1 Introduction

Like all other drugs intended for human administration, radiopharmaceuticals should undergo strict and routine quality testing procedures in addition to their own specific tests for radionuclidic and radiochemical purity. Radiopharmaceutical quality control tests can be simply classified as *Physiochemical* and *Biological* tests [1–3].

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4.2 Physiochemical Tests

4.2.1 Physical Tests

Physical characteristics should be observed for any radiopharmaceutical for the first and frequent use. Color alterations should be identified for both true solution and colloidal preparations. True solutions should also be checked for turbidity and presence of any particulate matter; in colloidal preparations, determination of particle size is of most interest.

4.2.2 pH and Ionic Strength

The administered radiopharmaceutical should have a proper pH (hydrogen ion concentration) with an ideal value of 7.4 (pH of blood), but it can vary between 2 and 9 because of the high buffer capacity of the blood.The pH of any radiopharmaceutical preparation can be measured by a pH meter [4].

Ionic strength, isotonicity, and osmolality should be observed properly in any radiopharmaceutical preparation so it is suitable for human administration.

Note: Since pH and ionic strength are important factors for the stability of a radiopharmaceutical, the preparation diluent is preferred to be the same solvent used in the original preparation.

4.2.3 Radionuclidic Purity

The term *radionuclidic* purity refers to the presence of radionuclides other than the one of interest and is defined as the proportion of the total radioactivity

M.M. Khalil (ed.), *Basic Sciences of Nuclear Medicine*, DOI: 10.1007/978-3-540-85962-8_4,

present as the stated radionuclide. Measurement of radionuclide purity requires determination of the identity and amounts of all radionuclides that are present. The radionuclide impurities which, vary according to the method of radionuclide production, can affect dramatically the obtained image quality in addition to the overall patient radiation dose.

Radionuclidic impurities may belong to the same element of the desired radionuclide or to a different element. Examples of radionuclidic impurities are ^{99}Mo in ^{99m}Tc-labeled preparations and iodide isotopes in ^{131}I-labeled preparations [5]. Radionuclidic purity is determined by measuring the half-lives and characteristic radiations emitted by each radionuclide. The γ-emitters can be differentiated by identification of their energies on the spectra obtained by an NaI(Tl) crystal or a lithium-drifted germanium [Ge(Li)] detector coupled to a multichannel analyzer (MCA). The β-emitters can be tested by a β-spectrometer or a liquid scintillation counter. Since a given radiation energy may belong to a number of radionuclides, half-life must be established to identify each radionuclide.

4.2.4 Radiochemical Purity

Radiochemical purity is defined as the proportion of the stated radionuclide that is present in the stated chemical form. Image quality (as a function of the radiopharmaceutical biological distribution) and the radiation absorbed dose are directly related to the radiochemical purity. Radiochemical impurities are produced from decomposition due to the *action of solvent*, *change in temperature or pH*, *light*, *presence of oxidation or reducing agents, and radiolysis*.

Free and hydrolyzed ^{99m}Tc forms in many ^{99m}Tc-labeled preparations; secondary hexamethylpropylene amine oxime (HMPAO) complex in ^{99m}Tc-HMPAO preparations and free ^{131}I-iodide in ^{131}I-labeled proteins are good examples of the radiochemical impurities [6].

The stability of a compound is time dependent on exposure to light, change in temperature, and radiolysis, and the longer the time of exposure is, the higher the probability of decomposition will be. Sodium ascorbate, ascorbic acid, and sodium sulfite are normally used for maintaining the stability of a radiopharmaceutical. Several analytical methods are used to determine the radiochemical purity of a given radiopharmaceutical, and these methods are discussed next.

4.2.4.1 Thin-Layer Chromatography

Thin-layer chromatography (TLC), which was developed by Hoye in 1967 is considered the most commonly used method for determination of radiochemical purity in nuclear medicine. The main principle of a TLC chromatography system is that a mobile phase (solvent) migrates along a stationary phase (adsorbent) by the action of the capillary forces [7]. Depending on the distribution of components between the stationary and the mobile phases, a radiopharmaceutical sample spotted onto an adsorbent will migrate with different velocities, and thus impurities are separated.

In TLC, each component in a given sample is identified by an R_f value, which is defined as "the ratio of the distance traveled by the sample component to the distance the solvent front has advanced from the original point of starting the chromatography test in the stationary phase."

$$R_f = \text{Distance traveled by the component/distance of the solvent front}$$

The R_f values range from 0 to1. If a component migrates with the solvent front, the R_f is [1], while the R_f for the component remaining at the origin is [0]. R_f values are established with known components and may vary under different experimental conditions. The main principles of separation are adsorption (electrostatic forces), partition (solubility), and ion exchange (charge), and the movement of the mobile phase may take either ascending or descending modes. When the solvent front moves to the desired distance, the strip is removed from the testing container, dried, and measured in an appropriate radiation detection system; histograms are obtained for the activity of all sample components [8].

Stationary Phases

Standard TLC materials: Standard TLC plates are available as glass plates, as plastic or aluminum foils

covered with the stationary phase. A wide range of stationary phases are commercially available, including silica gel, reversed-phase silica, aluminum oxide, synthetic resins, and cellulose. The main advantage of standard TLC materials is that they have the ability to provide relatively high- resolution tests, while the relatively long developing time of the mobile phase (mainly >30 min) through the high-particle-size adsorbent material (20 μm) is considered its main disadvantage [9].

High-performance TLC (*HPTLC*): HPTLC provides a good solution to the long chromatographic developing time by use of materials with a smaller particle size.

Instant TLC (*ITLC*): ITLC plates are made of fiberglass sheets integrated with an adsorbent, usually silica gel, and can be cut to any size, developing an economic chromatographic solution. Due to the fine mesh material, the migration properties are increased manyfold compared to the standard TLC materials, reducing the chromatographic time to less than 5 min without affecting the separation of radiochemical impurities. Because of the advantages mentioned, ITLC materials are the most frequently used for the stationary phase in nuclear medicine since they fulfill the need for a rapid and accurate method for testing the radiochemical purity of a radiopharmaceutical sample. Silica stationary phases have been produced for ITLC as silica gel (ITLC-SG) and silicic acid (ITLC-SA). ITLC-SG is the most widely used adsorbent for routine radiochemical purity determination [10].

Paper: Papers (e.g., Whatman no. 1 and Whatman 3MM) were commonly used in the early days of chromatography, although they are still used and recommended for many chromatographic procedures.

The main disadvantage of paper chromatography is the poor resolution it provides for radiochemical purity tests; however, Whatman 3MM is the material of choice for partition chromatography procedures.

Aluminum oxide: Aluminum-coated plates are commonly used for separation of some radiopharmaceuticals (e.g., Sesta-MIBI[methoxyisobutyl isonitrile]) depending on the aluminum oxide (Al_2O_3) polar properties.

Cellulose: Cellulose can interact with water and serve as a stationary phase for separation of polar substances by paper chromatography; also, it can be used in the powder form as an adsorbent for TLC.

Mobile Phases

Saline, *Water*, *Acetone*, *Methyl Ethyl Ketone* (*MEK*), *Ethanol*, *Acetic Acid*, *Chloroform,* and *Acetonitrile* represent the most common group of mobile phases used as the mobile partner in most TLC chromatographic procedures.

4.2.4.2 TLC Chromatography Procedure

To determine the radiochemical purity by ITLC or paper chromatography for a radiopharmaceutical sample, the following steps should be applied [11]:

- Fill a small beaker with about 10 ml (3–5 mm high) with the proper solvent and then cover the beaker with a glass plate or with a foil sheet.
- Prepare the chromatography strip (5–10 cm long) and mark the solvent front ($R_f = 0$) with a colored pen and the start point ($R_f = 1$) with a pencil.
- Using a 1-ml syringe with a fine needle (25 gauge), put one small drop of the sample onto the starting point on the strip.
- Immediately, insert the strip into the beaker so the spot sample does not dry,observing that initially the solvent level is below the starting point.
- When the solvent has reached the front, the strip should be removed and dried.
- Quantify the regional distribution of radioactivity on the strip using any of the radiation detection methods discussed next.

Figure 4.1 shows a sample procedure for determination of radiochemical purity of a radiopharmaceutical by ITLC using two different solvents.

4.2.4.3 Methods for Regional Radioactivity Measurement in TLC Chromatography

Many analytical methods were developed for determination of the radioactivity percentage of the different radiochemical species along the TLC chromatography strip, starting with the *autoradiography* method by which a chromatogram is placed on an x-ray film and exposed in the dark for about 1 h. However, the most popular methods in nuclear medicine were achieved using one of the following systems [9]:

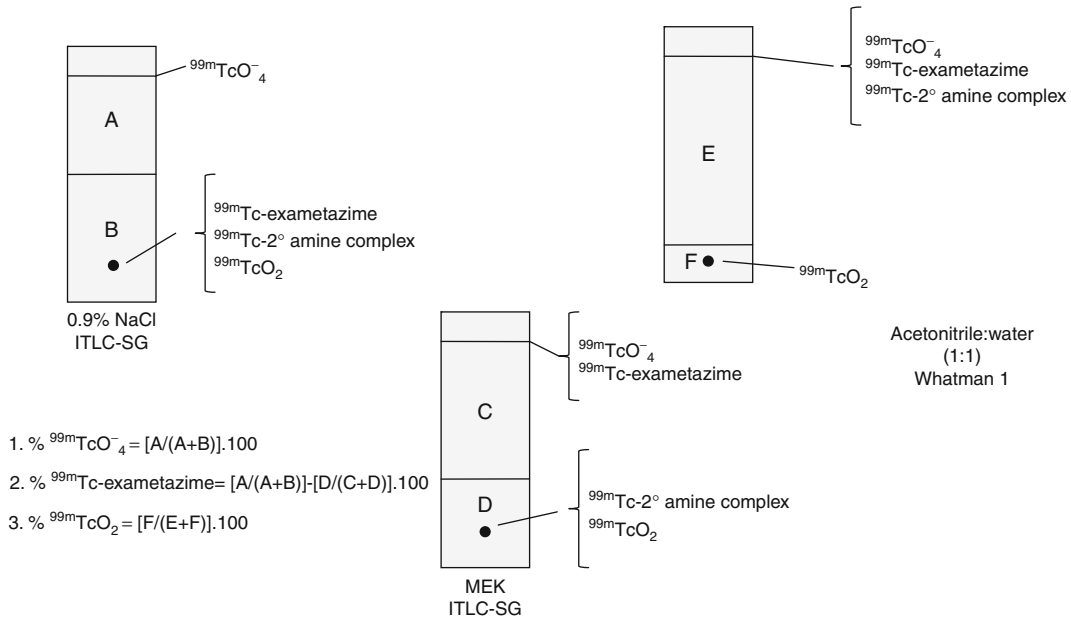

Fig. 4.1 Chromatographic system for determination of radiochemical purity of Tc-99m labeled HMPAO. (Adapted from [7])

- *Gamma camera*: A dried strip is placed close to the detector head of the camera, and an image is obtained. The region of interest (ROI) is drawn for each radioactive area. The radiochemical purity is expressed as a fraction of the total recovered activity.
- *Ionization chamber*: The dried strip is cut into two segments and measured in the chamber. This method is frequently used for simple separation techniques (compounds of $R_f = 0$ or 1) but is not applicable in multisegmental strips or samples with low radioactive concentration [12].
- *NaI(Tl) scintillation counter*: Chromatographic plates are cut in segments (up to ten) and counted in the scintillation counter. This method is preferrede to the ionization chamber because it provides more sensitive results, giving the chance to determine the radiochemical purity of a sample at different and close R_f values.
- *Chromatography scanner*: A slit-collimated radiation detector [mainly NaI(Tl) scintillation counter] is moved along the thin-layer plate, and the radioactivity distribution between the start and the solvent front points is recorded and plotted as radioactive peaks [13]. This method is considered the most accurate one because it provides values with high sensitivity and resolution .
- *Linear analyzer*: This device operates as a position-sensing proportional counter, measuring a fixed number of channels along the length of the chromatographic plate. It gives us the most sensitive results, but resolution is less than that obtained with NaI(Tl) scintillation counters [14].

A comparison of four different methods of quantification is shown in Fig. 4.2, in which a phantom chromatographic plate with increasing amount of radioactivity (0.25, 1.0, 4.0, 16.0, and 64.0 kBq) spotted at exact intervals was measured bythe following:

A:	Linear analyzer
B:	Conventional scanner
C:	Cut-and-measure scintillation counter
D:	Ionization chamber

The highest sensitivity was achieved in A and C, while the best resolution occured with B and C.

The ionization chamber indicated a low detection efficiency of radioactivity below 20 kBq (peaks 1, 2, 3, and 4).

For ^{99m}Tc, the main impurities are the *free pertechnetate* (TcO_4) and the *reduced, hydrolyzed technetium* (TcO_2) (in another words, colloidal ^{99m}Tc).

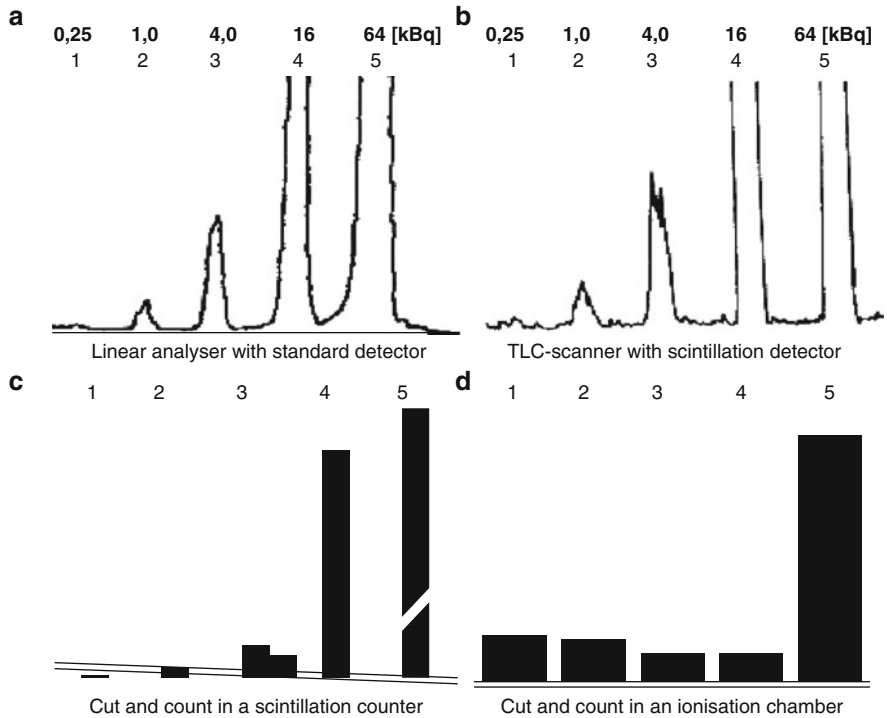

Fig. 4.2 Phantom chromatography plate analyzed by four different methods of measurement. The highest sensitivity was achieved with a and c, the best resolution with b and c. Using the ionization chamber (d), activities less than 20 kBq (peaks 1, 2, 3, 4) were not resolved accurately. (From [9] with permission from Springer)

Free pertechnetate migration properties and hence its R_f value are governed by the type of mobile and stationary phases used for the TLC test, while reduced, hydrolyzed technetium is present mainly at the origin ($R_f = 0$) due to its insolubility properties (like all other colloidal components), explaining why reduced, hydrolyzed technetium species cannot be recognized in colloidal and particulate preparations (e.g., macroaggregated albumin [MAA] preparations).

Tables 4.1 and 4.2 summarize the TLC chromatographic data for Tc-99m and non-Tc-99m radiopharmaceuticals, respectively.

4.2.5 Precipitation

Precipitation is the method of separating a radiochemical form from another with an appropriate chemical reagent. The precipitate is separated by centrifugation. $^{51}Cr^{3+}$ in ^{51}Cr-sodium chromate can be estimated by precipitating chromates as lead chromate and determining the radioactivity of the supernatant.

4.2.6 Distillation

Distillation is a method applicable to compounds with different vapor pressures. The two compounds can be separated by simple distillation at a specific temperature. The compound with higher vapor pressure is distilled off first, leaving the other compound in the distilling container. Iodide impurities can be separated from any iodine-labeled compound by distillation.

4.2.7 Ion Exchange

Ion exchange is performed simply by passing a radiopharmaceutical sample through a column of ionic resin and eluting the column with a suitable solvent. Separation of different chemical forms is dependent on the ability and affinity of exchange of ions from a solution onto the resin. Polymerized and high molecular weight resins are of two kinds: cation exchange and anion exchange resins.

An example of that method is the determination of the presence of $^{99m}TcO_4^-$ in ^{99m}Tc-labeled albumins,

Table 4.1 Chromatographic data for ^{99m}Tc-radiopharmaceuticals

^{99m}Tc-Radiopharmaceutical	Stationary Phase	Solvent	R_f		
			$^{99m}TcO_4$	^{99m}Tc-Complex	Hydrolyzed ^{99m}Tc
Bone radiopharmaceuticals					
^{99m}Tc-PYP	ITLC-SG	Acetone	1.0	0.0	0.0
	ITLC-SG	Saline	1.0	1.0	0.0
^{99m}Tc-MDP	ITLC-SG	Acetone	1.0	0.0	0.0
	ITLC-SG	Saline	1.0	1.0	0.0
^{99m}Tc-HDP (hydroxymethylene diphosphonate)	ITLC-SG	Acetone	1.0	0.0	0.0
	ITLC-SG	Saline	1.0	1.0	0.0
Renal radiopharmaceuticals					
^{99m}Tc-DTPA	ITLC-SG	Acetone	1.0	0.0	0.0
	ITLC-SG	Saline	1.0	1.0	0.0
^{99m}Tc-MAG_3	Whatman 3MM	Acetone	1.0	0.0	0.0
	Whatman 3MM	Water	1.0	1.0	0.0
^{99m}Tc-gluceptate	ITLC-SG	Acetone	1.0	0.0	0.0
	ITLC-SG	Saline	1.0	1.0	0.0
^{99m}Tc(III)-DMSA (dimercaptosuccinic acid)	Whatman 3MM	Acetone	1.0	0.0	0.0
	ITLC-SA	Butanol	0.9	0.5	0.0
^{99m}Tc(V)-DMSA	ITLC-SG	MEK (methyl ethyl ketone)	1.0	0.0	0.0
	ITLC-SG	Saline	1.0	1.0	0.0
^{99m}Tc-IDAs (iminodiacetic acids)	Whatman 3MM	MEK	0.9	0.0	0.0
	ITLC-SG	Water	1.0	1.0	0.0
Cardiac radiopharmaceuticals					
^{99m}Tc-sestamibi	Whatman 3MM	Ethyl acetate	0.0–0.1	0.5–0.8	0.0–0.1
^{99m}Tc-tetrofosmin	ITLC-SG	Acetone-dichloromethane (35:65)	0.9–1.0	0.4–0.7	0.0–0.1
Brain radiopharmaceuticals					
^{99m}Tc-HMPAO	ITLC-SG	MEK	1.0	1.0 (primary)	0.0
	ITLC-SG	Saline	1.0	0.0	0.0
	Whatman 1	50% acetonitrile	1.0	1.0	0.0
^{99m}Tc-bicisate	Whatman 3MM	Ethyl acetate	0.0	1.0	0.0

(*continued*)

Table 4.1 (continued)

^{99m}Tc-Radiopharmaceutical	Stationary Phase	Solvent	R_f		
			$^{99m}TcO_4$	^{99m}Tc-Complex	Hydrolyzed ^{99m}Tc
Miscellaneous					
^{99m}Tc-MAA	ITLC-SG	Acetone	1.0	0.0	0.0
^{99m}Tc-HSA (human serum albumin)	ITLC-SG	Ethanol:NH_4OH:H_2O (2:1:5)	1.0	1.0	0.0
^{99m}Tc-SC (sulfur colloid)	ITLC-SG	Acetone	1.0	0.0	0.0
^{99m}Tc-nanocolloid	Whatman ET-31	Saline	0.8	0.0	0.0
^{99m}Tc-architumomab	ITLC-SG	Acetone	1.0	0.0	0.0
^{99m}Tc-nofetumomab	ITLC-SG	12% TCA in H_2O	1.0	0.0	0.0

Data adapted from [9, 21] and UKRG Handbook (Bev Ellis, United Kingdom Radiopharmaceutical Group, London, 2002)
Adapted from [2].
ITLC-SA instant thin-layer chromatography silicic acid, *ITLC-SG* instant thin-layer chromatography silica gel

Table 4.2 Chromatographic data of radiopharmaceuticals other than 99mTc-complexes

Radiopharmaceutical	Stationary phase	Solvent	R_f values	
			Labeled product	Impurities
125I-RISA (radioiodinated serum albumin)	ITLC-SG	85% methanol	0.0	1.0(I^-)
131I-hippuran	ITLC-SG	$CHCl_3$:acetic acid (9:1)	1.0	1.0(I^-)
131I-NP-59	ITLC-SG	Chloroform	1.0	1.0(I^-)
131I-MIBG	Silica gel plated plastic	Ethyl acetate:ethanol (1:1)	0.0	0.6(I^-)
131I-NaI	ITLC-SG	85% methanol	1.0	0.2(IO^{-3})
51Cr-sodium chromate	ITLC-SG	*n*-Butanol saturated with 1N HCl	0.9	0.2(Cr3+)
67Ga-citrate	ITLC-SG	$CHCl_3$:acetic acid(9:1)	0.1	1.0
111In-DTPA (diethylenetriaminepentaacetate)	ITLC-SG	10% ammonium acetate: methanol (1:1)	1.0	0.1(In3+)
111In-capromab pendetide	ITLC-SG	Saline	0.0	1.0(In3+)
18F-FDGh	ITLC-SG	CH_3CN/H_2O (95:5)	0.37	0.0
90Y-,111In-ibritumomab tiuxetan		0.9%NaC1 solution	0.0	1.0

Adapted from [21]
ITLC-SG instant thin-layer chromatography silica gel

by which the $^{99m}TcO_4^-$ is adsorbed to the Dowex-1 resin, leaving the ^{99m}Tc-labeled albumins and the hydrolyzed ^{99m}Tc to go with the elute when using 0.9% NaCl as a solvent to wash the resin column.

4.2.8 Solvent Extraction

When two immiscible solvents are shaken together, any solute present will distribute between the two

phases according to its solubility in each phase. Equilibrium is reached and governed by the partition coefficient D:

$$D = \text{Concentration in organic phase/Concentration in aqueous phase}$$

Lipid-soluble molecules may have $D \geq 100$, so it becomes highly concentrated in the organic phase. If the solvent is volatile (e.g., ether or chloroform), the solute can be recovered by distillation or evaporation. An example of this method is the separation of ^{99m}Tc-pertechnetate by dissolving ^{99}Mo in a strongly alkaline solution (e.g., potassium hydroxide) and then extracting with MEK. ^{99m}Tc-pertechnetate dissolved in the organic phase can be recovered by evaporation to dryness; this method has been used initially to prepare ^{99m}Tc-pertechnetate for ^{99m}Tc-labeled radiopharmaceuticals [15].

4.2.9 Electrophoresis

The basic idea of electrophoresis is the property of charged molecules (atoms) to migrate in an electric field. The migration rate is dependent on the charge and size of the molecule. The apparatus used in electrophoresis consists simply of a direct current power supply to provide a potential difference of 400 V or greater, connected through an electrolyte buffer solution (barbitone/barbituric acid) to either end of a strip of support medium, which may be cellulose acetate or filter paper. Since ^{99m}Tc-pertechnetate is considered a small ion, it migrates rapidly and is readily separated from larger negatively charged complexes, such as ^{99m}Tc-DTPA (diethylenetriaminepentaacetate) or insoluble $^{99m}TcO_2$, which remain at the origin [16].

4.2.10 Gel Filtration

Gel filtration chromatography is a process obtained when a mixture of solutes is passed down a column of suitable medium, such as *cross-linked dextran Sephadex,* and then eluted with a proper solvent, allowing the large molecules to be released first while the smaller ones are selectively retarded due to penetrating the pores of the gel polymeric structure. Sequential fractions of the eluate are collected by an automated fraction collector, and the radioactivity is measured for each fraction.

Gel chromatography is the method of choice for separating proteins of different molecular weights. In addition, this method is equally important in detecting impurities in ^{99m}Tc-labeled radiopharmaceuticals. To obtain the percentages of free, bound, and unbound hydrolyzed ^{99m}Tc species, a Sephadex gel-saline filtration system is widely used. In this case, ^{99m}Tc-chelate is eluted first, followed by the free $^{99m}TcO_4^-$, while the hydrolyzed ^{99m}Tc is retained by the column.

It has been observed that some ^{99m}Tc chelates may bind to or dissociate on the column, producing inaccurate results.

4.3 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is a recent modification of the gel chromatography method; the liquid phase is forced at a high pressure through an adsorbent, densely packed column. When the different species of the sample are eluted from the column, they pass through a radiation detector or any other detector, which records its presence, normally in a graph form. The stationary phase most frequently consists of beads of silica bounding an organic pad containing long-chain (C_{18}) carbon groups. A wide range of stationary and mobile phases has been used; by suitable choice, this technique is extremely valuable in the development and evaluation of new radiopharmaceuticals. In addition, HPLC is considered a suitable technique for estimation of short-lived radiopharmaceuticals because of its rapidity [17].

If the components are irreversibly adsorbed on the stationary phase of the HPLC system (e.g., hydrolyzed ^{99m}Tc species), the system required is expensive; therefore, the use of HPLC is not routine in hospital practice. As an example, HPLC has been used for separation of ^{99m}Tc-MDP (methylene diphosphonate) prepared by borohydride reduction into several different components. A schematic diagram of the general components of the radio-HPLC system can be seen in Fig. 4.3.

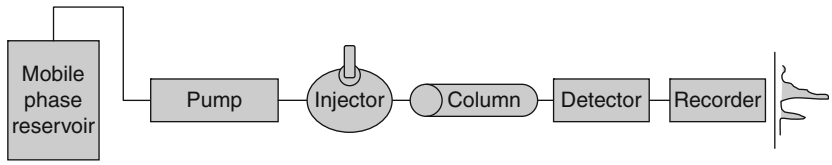

Fig. 4.3 Components of a radio-high-performance liquid chromatographic (HPLC) system

4.3.1 Sep-Pak Analysis

Sep-Pak cartridges (Millipore Water) have been developed for isolation and cleanup of sample components as a modification of the HPLC technology. This method is applicable for radiochemical analysis of ^{99m}Tc-MAG$_3$ (mercaptoacetyltriglycine), ^{99m}Tc-sestaMIBI, and radiolabeled MIBG.

4.4 Biological Tests

The presence of microorganisms (bacteria, fungi, etc.) should be examined for all pharmaceuticals intended for human administration and is defined by the sterility test.

Living organisms also can produce metabolic by-products (endotoxins) that can undesirably affect the radiopharmaceutical preparation, so special testing procedures should be applied (pyrogenicity and toxicity tests).

4.4.1 Sterility

The objective of the sterility test is to ensure that the sterilization processes, mainly by autoclaving for long-lived radiopharmaceuticals and membrane filtration for short-lived ones, are conducted properly [18]. A proper sterility test involves the incubation of the radiopharmaceutical sample for 14 days in

- *Fluid thioglycollate medium* for growth of aerobic and anaerobic bacteria
- *Soybean-casein digest medium* for growth of fungi and molds

The sterility test requires 14 days, so ^{99m}Tc-labeled compounds and other short-lived radiopharmaceuticals could be released prior to the completion of the test [19].

4.4.2 Pyrogenicity

Pyrogens are either *polysaccharides* or *proteins* produced by the metabolism of microorganisms. They are mainly soluble and heat stable, represented primarily as bacterial endotoxins. Pyrogens produce symptoms of fever, chills, malaise, joint pain, sweating, headache, and dilation of the pupils within 30 min to 2 h after administration.

Pyrogenicity testing was developed from the rabbit test (monitoring of the temperature of three healthy rabbits for 3 h after injection of the test sample) to a more sophisticated and rapid method called the *limulus amebocyte lysate* (LAL) method. LAL, which is isolated from the horseshoe crab (*limulus*), reacts with gram-negative bacterial endotoxins in nanogram or greater concentrations, forming an opaque gel. The thicker the gel, the greater the concentration of pyrogens in the sample. Gram-negative endotoxins are known as the most important source of pyrogen contamination, so the LAL test is a rapid and sensitive pyrogenicity test [20].

Generally, manufacturers are required to perform the sterility and pyrogenicity tests prior to release of their products to the end users. However, the short half-lives of ^{99m}Tc radiopharmaceuticals prohibit their testing for sterility and pyrogenicity, emphasizing aseptic labeling techniques by using laminar flow enclosures containing high-efficiency particle air (HEPA) filters improves the environment for radiopharmaceutical formulation.

4.4.3 Toxicity

Toxicity tests should be applied for all radiopharmaceuticals approved for human use. A quantity called $LD_{50/60}$ describes the toxic effect of a radiopharmaceutical by determination of the dose required to produce mortality of 50% of a species in 60 days after administration of a radiopharmaceutical dose [21].

The test should be done for at least two different species of animals. Because of the strict regulations on the use of animals for research and due to the expected differences on reactions from animal species to humans, cell culturing and computer modeling have been used to achieve toxicity tests.

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