TECHNICAL REPORTS SERIES NO. 466

Technetium-99m Radiopharmaceuticals: Manufacture of Kits

IAEA
International Atomic Energy Agency

TECHNETIUM-99m RADIOPHARMACEUTICALS: MANUFACTURE OF KITS

The following States are Members of the International Atomic Energy Agency:

AFGHANISTAN
ALBANIA
ALGERIA
ANGOLA
ARGENTINA
ARMENIA
AUSTRALIA
AUSTRIA
AZERBAIJAN
BANGLADESH
BELARUS
BELGIUM
BELIZE
BENIN
BOLIVIA
BOSNIA AND HERZEGOVINA
BOTSWANA
BRAZIL
BULGARIA
BURKINA FASO
CAMEROON
CANADA
CENTRAL AFRICAN REPUBLIC
CHAD
CHILE
CHINA
COLOMBIA
COSTA RICA
CÔTE D'IVOIRE
CROATIA
CUBA
CYPRUS
CZECH REPUBLIC
DEMOCRATIC REPUBLIC OF THE CONGO
DENMARK
DOMINICAN REPUBLIC
ECUADOR
EGYPT
EL SALVADOR
ERITREA
ESTONIA
ETHIOPIA
FINLAND
FRANCE
GABON
GEORGIA
GERMANY
GHANA
GREECE
GUATEMALA
HAITI
HOLY SEE
HONDURAS
HUNGARY
ICELAND
INDIA
INDONESIA
IRAN, ISLAMIC REPUBLIC OF
IRAQ
IRELAND
ISRAEL
ITALY
JAMAICA
JAPAN
JORDAN
KAZAKHSTAN
KENYA
KOREA, REPUBLIC OF
KUWAIT
KYRGYZSTAN
LATVIA
LEBANON
LIBERIA
LIBYAN ARAB JAMAHIRIYA
LIECHTENSTEIN
LITHUANIA
LUXEMBOURG
MADAGASCAR
MALAWI
MALAYSIA
MALI
MALTA
MARSHALL ISLANDS
MAURITANIA
MAURITIUS
MEXICO
MONACO
MONGOLIA
MONTENEGRO
MOROCCO
MOZAMBIQUE
MYANMAR
NAMIBIA
NETHERLANDS
NEW ZEALAND
NICARAGUA
NIGER
NIGERIA
NORWAY
PAKISTAN
PALAU
PANAMA
PARAGUAY
PERU
PHILIPPINES
POLAND
PORTUGAL
QATAR
REPUBLIC OF MOLDOVA
ROMANIA
RUSSIAN FEDERATION
SAUDI ARABIA
SENEGAL
SERBIA
SEYCHELLES
SIERRA LEONE
SINGAPORE
SLOVAKIA
SLOVENIA
SOUTH AFRICA
SPAIN
SRI LANKA
SUDAN
SWEDEN
SWITZERLAND
SYRIAN ARAB REPUBLIC
TAJIKISTAN
THAILAND
THE FORMER YUGOSLAV REPUBLIC OF MACEDONIA
TUNISIA
TURKEY
UGANDA
UKRAINE
UNITED ARAB EMIRATES
UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND
UNITED REPUBLIC OF TANZANIA
UNITED STATES OF AMERICA
URUGUAY
UZBEKISTAN
VENEZUELA
VIETNAM
YEMEN
ZAMBIA
ZIMBABWE

The Agency's Statute was approved on 23 October 1956 by the Conference on the Statute of the IAEA held at United Nations Headquarters, New York; it entered into force on 29 July 1957. The Headquarters of the Agency are situated in Vienna. Its principal objective is "to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world".

TECHNICAL REPORTS SERIES No. 466

TECHNETIUM-99m RADIOPHARMACEUTICALS: MANUFACTURE OF KITS

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 2008

COPYRIGHT NOTICE

Sales and Promotion, Publishing Section
International Atomic Energy Agency
Wagramer Strasse 5
P.O. Box 100
1400 Vienna, Austria
fax: +43 1 2600 29302
tel.: +43 1 2600 22417
email: sales.publications@iaea.org
http://www.iaea.org/books

Printed by the IAEA in Austria
August 2008
STI/DOC/010/466

IAEA Library Cataloguing in Publication Data

Technetium-99m radiopharmaceuticals : manufacture of kits. — Vienna : International Atomic Energy Agency, 2008.
p. ; 24 cm. — (Technical reports series, ISSN 0074–1914 ; no. 466)
STI/DOC/010/466
ISBN 978–92–0–100408–6
Includes bibliographical references.

1. Technetium — Therapeutic use. 2. Radiopharmaceuticals — Quality control. 3. Radiopharmaceutical industry. — I. International Atomic Energy Agency. II. Series: Technical reports series (International Atomic Energy Agency); 466.

IAEAL 08–00527

FOREWORD

Technetium-99m radiopharmaceuticals are in widespread use owing to the availability and affordability of $^{99}Mo/^{99m}Tc$ generators and the variety of kits for formulating the desired products. Together, they provide an array of specific tools for diagnosing a large number of diseases affecting the bones and major organs of the body such as the heart, brain, liver, kidney and thyroid. Nuclear medicine requires high quality radiopharmaceuticals and kits that are safe for administration and efficacious for a given application. The IAEA supports Member States in building capacity in the area of ^{99m}Tc generators and 'cold kits' through coordinated research projects (CRPs), technical cooperation activities and the publication of relevant reports that help disseminate useful information. These efforts have contributed greatly to reducing costs and increasing the efficiency of resource utilization, thereby helping to make nuclear medicine cost effective in Member States.

This publication presents the theoretical basis of and describes the procedures for preparing 23 selected kits. Details of the preparation of ten of the active ingredients are also included. The procedures described here can be used to develop manuals, monographs and standard operating procedures. This report is expected to serve as a guide to radiopharmaceutical manufacturing centres and centralized pharmacies involved in the production of such kits. It will be a useful resource for the many hospital radiopharmacies that routinely use the kits to compound ^{99m}Tc radiopharmaceuticals, and a useful source of information for regulators of radiopharmaceuticals.

The IAEA thanks J. Környei and K. Ozker for compiling and reviewing the manuscript, the experts who provided input for the report, and the reviewers for their valuable suggestions and comments. The IAEA officer responsible for this publication was M.R.A. Pillai of the Division of Physical and Chemical Sciences.

EDITORIAL NOTE

Although great care has been taken to maintain the accuracy of information contained in this publication, neither the IAEA nor its Member States assume any responsibility for consequences which may arise from its use.

The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

The mention of names of specific companies or products (whether or not indicated as registered) does not imply any intention to infringe proprietary rights, nor should it be construed as an endorsement or recommendation on the part of the IAEA.

CONTENTS

1. INTRODUCTION

1.1. BACKGROUND

1.1.1. Technetium-99m radiopharmaceuticals

The growth of nuclear medicine has been due mainly to the availability of ^{99m}Tc radiopharmaceuticals; this single isotope is used in over 80% of all diagnostic procedures. Each year, roughly 25 million procedures are carried out with ^{99m}Tc radiopharmaceuticals, and this figure is projected to grow at a rate of about 15% per annum. The availability of short lived ^{99m}Tc (half-life: 6 h) from the ^{99}Mo/^{99m}Tc generator, as the daughter product of long lived ^{99}Mo (half-life: 67 h), is a major factor behind the universal use of this radioisotope. The parent radionuclide, ^{99}Mo, is prepared in abundant quantities by the fission of ^{235}U in a nuclear reactor, with a fission yield of 6%. There are several major producers that collectively have the capacity to produce enough ^{99}Mo to meet current global demand. It is also possible to enhance the capacity of ^{99}Mo production to meet the expected increased demand.

Technetium-99m radiopharmaceuticals are used in several diagnostic procedures, from the use of pertechnetate for thyroid uptake to the use of ^{99m}Tc-octreotide derivatives for imaging neuroendocrine tumours. Owing to its multiple oxidation states, ^{99m}Tc has a versatile chemistry, making it possible to produce a variety of complexes with specific desired characteristics, which is a major advantage of ^{99m}Tc for radiopharmaceutical development. There are hundreds of ^{99m}Tc complexes useful for diagnostic procedures, of which over thirty are used in clinical studies.

Research on ^{99m}Tc radiopharmaceuticals began after the development of the ^{99}Mo/^{99m}Tc generator at the Brookhaven National Laboratory, USA, in the early 1960s [1.1]. The search for new and more efficacious ^{99m}Tc radiopharmaceuticals has been a continuous process for nearly half a century, as can be seen from the increasing number of scientific papers published on this topic every year.[1]

Technetium-99m radiopharmaceuticals can be categorized as first, second or third generation products, depending on their level of complexity.

[1] A search of the Pubmed web site (www.pubmed.gov) shows over 40 000 publications on the development and/or use of ^{99m}Tc radiopharmaceuticals.

1.1.1.1. First generation ^{99m}Tc radiopharmaceuticals

The first generation of technetium radiopharmaceuticals was developed mainly by taking advantage of the simple absorption, distribution, metabolism and excretion properties of the common complexes of ^{99m}Tc. These studies led to ^{99m}Tc radiopharmaceuticals for the thyroid ($^{99m}TcO_4^-$), liver (^{99m}Tc-colloids), bone (^{99m}Tc-phosphonates) and kidney (^{99m}Tc-DTPA) [1.2, 1.3]. The majority of the procedures carried out in a typical nuclear medicine department make use of these radiopharmaceuticals.

1.1.1.2. Second generation ^{99m}Tc radiopharmaceuticals

The ability to determine the exact molecular structure of the coordination compounds using powerful modern analytical tools such as nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and X ray diffraction helped researchers to understand the structure–activity relationships underlying the biological behaviour of the ^{99m}Tc agents. As a consequence, careful design of new ligands and their ^{99m}Tc complexes led to the discovery of imaging agents for perfusion in the myocardium and brain. The widely used cardiac imaging agents ^{99m}Tc-MIBI (sestamibi, Cardiolite®) and ^{99m}Tc-tetrofosmin (Myoview®), and the brain imaging agents ^{99m}Tc-HMPAO (exametazime, Ceretec®) and ^{99m}Tc-ECD (bicisate, Neurolite®) are the result of the above strategy in the development of ^{99m}Tc complexes. The in vivo behaviour of these radiopharmaceuticals is driven by their molecular properties, such as size, charge and lipophilicity. These products, including the novel renal agent ^{99m}Tc-MAG_3 (Mertiatide) and hepatobiliary agents such as ^{99m}Tc-mebrofenin, are generally referred to as second generation ^{99m}Tc radiopharmaceuticals [1.4–1.6].

1.1.1.3. Third generation ^{99m}Tc radiopharmaceuticals

Current designs of imaging agents are based on the careful selection of suitable biomolecules to function as effective vectors for in vivo delivery of radioactivity to more specific biological targets such as receptors and transporters. This strategy implies that the labelling approach employed for introducing a radionuclide into a biomolecule should not lead to any distortion of that part of the molecule responsible for its biological activity. Thus, these agents have required the development of sophisticated labelling approaches that go beyond the technologies previously used. The introduction of the bifunctional chelating agent (BFCA) concept and new chemistries such as the Tc-tricarbonyl, Tc-nitrido, Tc-HYNIC and mixed ligand complexes have

helped to achieve that objective. The radiopharmaceuticals ^{99m}Tc-HYNIC-EDDA-TOC, developed as an alternative to ^{111}In-octreotide, and ^{99m}Tc-TRODAT-1 are the best examples of third generation ^{99m}Tc radiopharmaceuticals [1.7–1.9]. The former is useful for imaging neuroendocrine tumours, and the latter is the first, and to date the only, ^{99m}Tc compound for receptor studies in the brain.

1.1.2. Future role of ^{99m}Tc radiopharmaceuticals

Applications of ^{99m}Tc radiopharmaceuticals for morphological and dynamic imaging of renal, liver, hepatobiliary, bone, cardiac and similar well entrenched areas are expected to increase in the future. New labelling techniques developed in the 1990s using, for example, HYNIC and carbonyl can be harnessed to develop new tracers for oncology, cardiology and neurology. Some of these radiopharmaceuticals will find broad acceptance owing to their low cost and wide availability.

Although positron emission tomography/computed tomography (PET/CT) images are inherently superior to single photon emission computed tomography (SPECT) images, this improvement comes at a significant price increase. As a result of current advances in detector technologies and reconstruction algorithms, the spatial resolution of SPECT images is rapidly approaching that of PET images, without a decrease in sensitivity. The introduction of SPECT/CT has taken this imaging modality a step closer to the detection characteristics of PET/CT. These improvements of SPECT/CT, together with improved technetium radiopharmaceuticals, will provide as high a level of functional and anatomical information as is attainable by PET/CT. All of the above developments are expected to increase the demand for and utility of ^{99m}Tc radiopharmaceuticals and their kits.

1.1.3. Kits for formulation of ^{99m}Tc radiopharmaceuticals

Technetium-99m radiopharmaceuticals are generally formulated from kits prepared in authorized manufacturing facilities. A cold kit contains the ligand to which ^{99m}Tc is to be complexed, an adequate quantity of reducing agent, buffer to adjust the pH to suit the labelling conditions, stabilizing agents and excipients. The kits are prepared in a freeze-dried form and have a long shelf life, ranging from several months to years. The kits can be transported at room temperature; however, storage in a refrigerator at 2–8°C is advantageous and ensures a long shelf life in most cases.

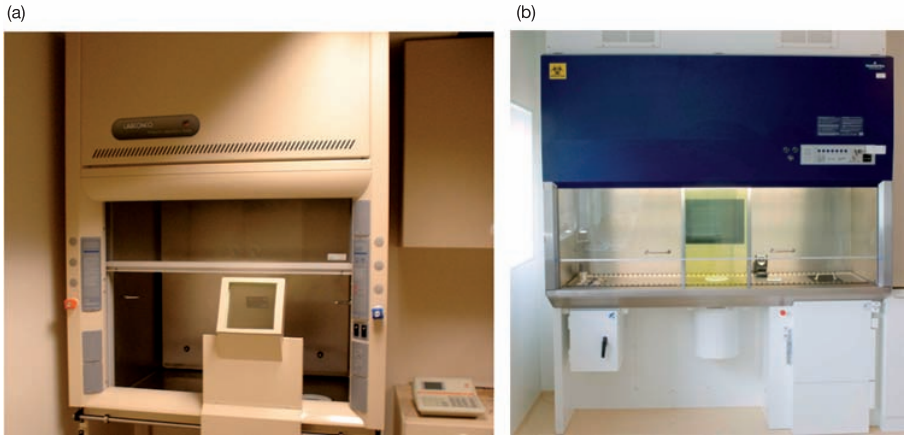


FIG. 1.1. Laminar flow benches with sliding lead shields for compounding sterile radiopharmaceuticals (source: Graymont Inc. and Veenstra Instruments).

1.1.4. Compounding of radiopharmaceuticals in hospital radiopharmacies

The compounding of ^{99m}Tc radiopharmaceuticals using kits is fairly easy, as it involves the addition of $^{99m}TcO_4^-$ eluted from a generator, generally at room temperature but at times with heating. The use of chromatographic techniques such as paper chromatography, instant thin layer chromatography (ITLC) or high performance liquid chromatography (HPLC) is recommended for estimation of the radiochemical purity of the final product, prior to administration to patients [1.10]. Guidelines for aseptic compounding and dispensing of radiopharmaceuticals are available in chapters 707 and 1075 of the United States Pharmacopeia (USP) [1.11]. According to these guidelines, radiopharmaceuticals are considered to be compounded sterile products, and it is recommended that compounding of ^{99m}Tc radiopharmaceuticals be carried out in an ISO 5 (class 100, grade A) laminar flow bench located in a clean room (with a buffer zone). This guideline is in force as of 2008. Two types of laminar flow bench with sliding lead shields used in radiopharmacy for compounding sterile radiopharmaceuticals are shown in Fig. 1.1.

1.1.5. Good manufacturing practices in kit production

The manufacture of radiopharmaceuticals requires that current good manufacturing practices (GMPs) be employed. Ensuring that current GMPs are used in the manufacture of radiopharmaceuticals requires that several aspects of the production process be considered, addressed and verified prior

to, during and after production. These include the training and qualification of personnel, use of controlled materials and procedures, availability of qualified equipment, production of products in designated clean areas, application of validated processes and analytical methods, full documentation of the process, registration of the products and release of the final product by a certified person.

1.2. OBJECTIVE

In 1992, the IAEA published a report on the preparation of kits for ^{99m}Tc radiopharmaceuticals [1.12] that gave technical inputs for the preparation of 13 of the 'first generation' ^{99m}Tc radiopharmaceuticals. Since that time, several ^{99m}Tc radiopharmaceuticals not described in the report have come into routine clinical use, some of which are already described in the USP and the European Pharmacopoeia (EP). On the basis of a review of all currently used ^{99m}Tc radiopharmaceuticals, it was decided to expand the report to include the production methods of the most widely used kits. The requirements for manufacturing the radiopharmaceuticals have also undergone significant changes, with regulations becoming more stringent and compliance with them becoming mandatory. The current report provides details of 23 kits and 10 active ingredients. The GMPs described here are those followed by the contributors to this report and served as the basis for obtaining licences from the regulatory authorities.

1.3. SCOPE

This report provides information on the various aspects of the production of kits for formulating ^{99m}Tc radiopharmaceuticals. It draws on the collective experience of experts in the installation and operation of kit production facilities and is expected to serve as guidance for kit manufacturers and as a reference for kit users.

Issues such as principles of kit manufacture, GMP in kit production, general procedures for production and quality control of kits, and quality specifications of generally used equipment and materials are detailed in the initial sections. Product specific information on selected kits is provided in Section 7 of this book. Information is given on the production of kits, starting with the active ingredients, and details are provided concerning the reagents required, the chemical composition of the kits, manufacturing formulas, manufacturing details, freeze-drying conditions, storage and stability of the products, methods

for radiolabelling, features of the labelled products, quality requirements and recommended quality control tests of the labelled products. Whereas the majority of the active ingredients used for the production of the kits are available from commercial manufacturers, the active ingredients of the new products are not widely available. Details on the synthesis of active ingredients are included in Section 8. The 11 annexes to the report provide useful information on general procedures for the manufacture and quality control of kits.

REFERENCES TO SECTION 1

[1.1] RICHARDS, P., "A survey of the production at Brookhaven National Lab of the isotopes for medical research", Trans. 5th Nucl. Cong., 7th Int. Electr. Nucl. Symp., Rome (1960).

[1.2] SRIVASTAVA, S.C., RICHARDS, P., "Technetium-99m labeled compounds", Radiotracers for Medical Applications (RAYUDU, G.V.S., Ed.), CRC Press Inc., Boca Raton, FL (1983) 107–185.

[1.3] SCHWOCHAU, K., Technetium radiopharmaceuticals — Fundamentals, synthesis, structure, and development, Angew. Chem. Int. Ed. Engl. **33** (1994) 2258–2267.

[1.4] DEUTSCH, E.A., LIBSON, K., JURISSON, S., "Technetium chemistry and technetium radiopharmaceuticals", Progress in Inorganic Chemistry (LIPPARD, S.J., Ed.), John Wiley & Sons, New York (1983) 75–139.

[1.5] NUNN, A.D., LOBERG, M.D., CONLEY, R.A., A structure-distribution relationship approach leading to the development of ^{99m}Tc-mebrofenin: An improved chole-scintigraphic agent, J. Nucl. Med. **24** (1983) 423–430.

[1.6] BANERJEE, S., PILLAI, M.R.A., RAMAMOORTHY, N., Evolution of Tc-99m in diagnostic radiopharmaceuticals, Semin. Nucl. Med. **31** (2001) 260–277.

[1.7] RICCABONA, G., DECRISTOFORO, C., Peptide targeted imaging of cancer, Cancer Biother. Radiopharm. **18** (2003) 675–687.

[1.8] GABRIEL, M., et al., An intrapatient comparison of ^{99m}Tc-EDDA/HYNIC-TOC with ^{111}In-DTPA-octreotide for diagnosis of somatostatin receptor-expressing tumors, J. Nucl. Med. **44** (2003) 708–716.

[1.9] KUNG, H.F., Development of Tc-99m labeled tropanes: TRODAT-1 as a dopamine transporter imaging agent, Nucl. Med. Biol. **28** (2001) 505–508.

[1.10] ZOLLE, I. (Ed.), Technetium-99m Pharmaceuticals: Preparation and Quality Control in Nuclear Medicine, Springer, Berlin (2007).

[1.11] UNITED STATES PHARMACOPEIAL CONVENTION, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006).

[1.12] INTERNATIONAL ATOMIC ENERGY AGENCY, Preparation of Kits for ^{99m}Tc Radiopharmaceuticals, IAEA-TECDOC-649, IAEA, Vienna (1992).

2. TECHNETIUM CHEMISTRY: THE STATE OF THE ART

2.1. BASIC CHEMICAL PROPERTIES OF TECHNETIUM

Technetium, the 43rd element in the periodic table, belongs to the group of transient metals. Owing to its electron configuration of $4d^5\ 5s^2$, technetium provides several opportunities for complex formation with a large number of different ligands, and its oxidation state (OS) can change from +1 up to +7. The OS is considered to be a main parameter determining the chemical nature of the complexes. Technetium can form chemical bonds consisting of both sigma and pi electrons, and the sigma bonds can be of colligative and coordinative types when spin compensation and electron pair donation occur, respectively.

The structure of technetium complexes can also be characterized by the coordination number (N), which can vary from 4 to 7, allowing tetrahedral (N = 4), tetragonal pyramidal (N = 5), octahedral (N = 6), capped octahedral (N = 7) or pentagonal bipyramidal (N = 7) geometry. The third parameter for characterization of technetium complexes is the electric charge (Z) of the whole molecule, which may provide an anionic (Z = –1), neutral (Z = 0) or cationic (Z = +1) character [2.1]. A summary of the different kinds of complexing centre and the parameters OS, N and Z is presented in Table 2.1.

The high variability of the complexing centres results in different stabilities for the various complexes. Pertechnetate (N = 4, OS = +7, Z = –1) is the most stable form of technetium in aqueous media. The presence of free pertechnetate in the solution of a technetium compound is possible, especially after long periods of post-labelling storage. At lower oxidation states, the Tc-S, $Tc\text{-}P^{(III)}$ and $Tc\text{-}C^{(II)}$ chemical bonds are quite stable in the appropriate geometry. At the same time, phosphonates, in which six oxygen atoms are bound to technetium, are of a lower stability and are liable to form oligomers, that is, polynuclear complexes [2.2]. The hexacoordinated N_2O_4 and N_3O_3 complexes such as DTPA, EDTA or HIDA derivatives are also of a relatively low stability and partially transform to heptacoordinated pentagonal bipyramidal geometry, which might be an alternative structure provided by these complexing centres and an additional oxo-oxygen.

2.2. FIRST GENERATION TECHNETIUM RADIOPHARMACEUTICALS

When the first generation technetium radiopharmaceuticals were produced, it was obvious that the reduction of technetium needed to be

TABLE 2.1. CHARACTERIZATION OF VARIOUS TECHNETIUM COMPLEXES

N	OS	Z	Complexing centres of Tc by:		Compound	Organ/tissue specificity
			Sigma bond	Pi bond		
4	+7	–1	—	$(=O)_4$	Pertechnetate	Thyroid
5	+5	–1	O_4	= O	Gluconate	Red blood cell labelling
			N_3S	= O	MAG_3	Kidney
			N_2S_2	= O	EC	Kidney
			S_4	= O	DMSA(V)	Soft tissue tumours
5	+5	0	N_4	= O	HMPAO	Brain, white blood cells
			N_2S_2	= O	ECD	Brain
			S_4	≡ N	NOET	Myocardium
6	+1	+1	C_6	—	MIBI	Myocardium
	+1	+1	C_3N_3, C_3N_2O	—	'Tricarbonyl'	Various
	+3	+1	$N_2O_2P_2$	—	Q 12	Myocardium
	+5	+1	P_4	$(=O)_2$	Tetrofosmin	Myocardium
6	+4		N_3O_3	—	DTPA	Kidney
	+3	–1	S_3O_3	—	DMSA(III)	Kidney
		–1	N_2O_4	—	HIDA derivatives	Hepatobiliary system
	+4	–1	O_6	—	Phosphonates	Bone
7	+3	0	N_6Cl	—	Teboroxime	Myocardial flow
7	+5	–1	N_2O_4	= O	EDTA, DTPA	Kidney
					HIDA derivatives	Hepatobiliary system

Note: N = coordination number; OS = oxidation state; Z = electric charge.

ensured. As the short physical half-life of technetium required 'on the spot' labelling, kit formulation had to be provided by freeze-drying the appropriate ligand with Sn(II)-chloride as a reducing agent. This resulted in a 'one vial' labelling system, and the kit was reconstituted simply by injecting the pertechnetate (the generator eluate) into the freeze-dried product under aseptic conditions. The most important first generation radiopharmaceuticals are summarized below.

Among the earliest technetium radiopharmaceuticals were ^{99m}Tc-gluconate and ^{99m}Tc-glucoheptonate; however, no experimental evidence of the pentacoordinated geometry has been found. Although these complexes are of a hydrophilic character, they show high plasma protein binding (50–70%). Therefore, both glomerular filtration and tubular excretion by the kidneys occur with slow pharmacokinetics [2.3], and only

70% of the injected activity is washed out after one day. This is the reason why ^{99m}Tc-gluconate and ^{99m}Tc-glucoheptonate are no longer used for renal studies. Owing to their low stability, they are used (i) for red blood cell labelling [2.4] and (ii) as a ligand exchange partner for labelling other molecules.

Another well known radiopharmaceutical with a hydrophilic character is ^{99m}Tc-DTPA. However, only up to 10% of the ^{99m}Tc-DTPA is bound to plasma proteins, and almost complete glomerular excretion occurs via the kidneys [2.5]. The pharmacokinetics of ^{99m}Tc-DTPA is much faster than that of ^{99m}Tc-gluconate, and more than 90% of the injected activity is washed out within 2 h for ^{99m}Tc-DTPA, compared with 12 h for ^{99m}Tc-gluconate. Other applications of ^{99m}Tc-DTPA include blood flow studies (brain, heart, extremities), cerebrospinal fluid circulation studies, studies of transport in the gastrointestinal tract with labelled drinks and foods, and inhalation studies using ^{99m}Tc-DTPA aerosol.

In an alkaline medium (≥pH8), all of the free –SH groups of dimercaptosuccinic acid (DMSA) are ready to react with technetium, forming a pentacoordinated biscomplex, ^{99m}Tc-DMSA(V). This biscomplex accumulates both in the kidneys and in soft tissue tumours such as medullar carcinomas [2.6]. If the pH does not exceed 9, the compound is of an appropriate stability and is injectable. When labelling of DMSA is performed in acidic media, a hexacoordinated asymmetric biscomplex is formed in which one molecule is bound to technetium via two -S- bridges and one -O- bridge, while the other is bound via one -S- bridge and two -O- bridges, and one –SH remains free. This kind of structure of ^{99m}Tc-DMSA(III) is complete if the pH is around 3. The asymmetric complex, when injected, is taken up by the kidneys [2.7] and remains bound owing to a ligand exchange reaction occurring between the DMSA ligand and the protein located in the proximal tubules of the kidney. Complete uptake of ^{99m}Tc-DMSA(III) is observed 2–4 h post-injection. Renal scintigraphy using ^{99m}Tc-DMSA(III) provides quantitative information about the functional mass of each individual kidney, since the renal uptake is proportional to the functional mass.

All ^{99m}Tc-phosphonates with ligands such as MDP, HMDP and HEDP can be characterized by the general formula $H_2O_3P\text{-}X\text{-}PO_3H_2$, where X = $-CH_2$, $-CH(OH)-$ and $-C(OH)(CH_3)-$ for MDP, HMDP and HEDP, respectively. Independent of the substituents, phosphonates show a tendency to form oligomers, which can be avoided by adding antioxidants such as ascorbic acid prior to freeze-drying the product. Their main field of application is bone scintigraphy [2.8, 2.9], because technetium phosphonates are taken up by normal bone and bone lesions by chemisorption, followed by exchange on the hydroxyapatite, the inorganic matrix of the bone. Higher uptake is observed

when the regional blood flow is higher owing to hydroxyapatite formation accompanied by increased osteoblastic activity. Thus 2–8 times the activity of technetium phosphonates can accumulate in bone lesions with increased osteoblastic activity (rupture, tumour metastases, etc.) compared with normal bone. The different substituents in the phosphonates affect the pharmacokinetics (^{99m}Tc-HMDP is faster than the others) and the lesion to normal bone activity ratio (^{99m}Tc-HEDP gives the highest contrast). In general, it is recommended that imaging be performed 2–4 h post-injection.

Pyrophosphate is used for in vivo labelling of red blood cells. The cold lyophilizate, Sn(II)-pyrophosphate, can be injected after reconstitution in saline; this non-radioactive compound accumulates in the red blood cells. Free pertechnetate is injected into the patients 20 min post-injection of the Sn(II)-pyrophosphate, resulting in red blood cell labelling under in vivo conditions (this was the first example of the 'pretargeting' technique) [2.10]. Technetium-99m-pyrophosphate can also be used to perform red blood cell labelling by in vitro methods. Another application of pyrophosphate is as a ligand exchange partner for labelling sensitive molecules such as HMPAO.

Derivatives of (N-phenyl-carbamoylmethyl)-imino diacetic acid have been developed as bilirubin analogues for investigating the functioning of the liver and the hepatobiliary system [2.11]. These compounds are octahedral or heptacoordinated pentagonal bipyramidic biscomplexes possessing a negative electric charge. After injection, they are excreted by the hepatocytes in the liver, similar to the bilirubin, followed by elimination via the bile routes, gall-bladder and duodenum within 45–60 min. By varying the substituents on the aromatic ring, the lipophilic character of the molecule can be increased and various HIDA derivatives can be obtained that are mostly excreted by the liver. These more lipophilic complexes of ligands, such as diisopropyl-IDA [2.12] and trimethyl-bromo-IDA [2.13], can be used for hepatobiliary studies even in cases of imperfect liver function.

When injected, ^{99m}Tc colloids with a particle size of 0.1–2 μm are accumulated in the liver by phagocytosis in the Kupffer cells [2.14]. The larger particles appear in the spleen. The first species of this kind of radiopharmaceutical were the ^{99m}Tc-sulphur colloids [2.15] prepared either from sodium thiosulphate ($Na_2S_2O_3$) in an acidic medium (4.6M HCl) or from sodium dithionite ($Na_2S_2O_4$) in a neutral medium by reducing the technetium at an elevated temperature (88–100°C). The ^{99m}Tc-tin colloid [2.16] may contain more fine particles when prepared from Sn(II)-fluoride reacting with pertechnetate. In this compound, the reduced hydrolysed technetium is bound to the tin oxide/hydroxide colloidal particles. Technetium-99m-rhenium-sulphide colloid particles for liver scintigraphy can be prepared from sodium thiosulphate in the presence of hydrochloric acid and potassium perrhenate at an

elevated temperature. Recently, the ^{99m}Tc-rhenium-sulphide colloid has been used in sentinel lymph node detection [2.17]. In addition to colloids prepared in vitro, the ^{99m}Tc-phytate colloid can be formed in situ in the blood by interacting the phytate ligand with calcium content in serum [2.18].

Technetium-99m-rhenium-sulphide colloids with a particle size of 10–80 nm can also be used for bone marrow scintigraphy, lymphoscintigraphy and detection of inflammation [2.19]. In this two-step procedure, a technetium pyrophosphate complex is prepared, followed by reaction with freeze-dried rhenium sulphide at 100°C for 15–30 min. A ^{99m}Tc nanocolloid of similar particle size can prepared from human serum albumin (HSA) as well.

Technetium-99m macroaggregates (particle size: 10–45 μm) can be obtained from HSA when the heat treated alkaline HSA solution is neutralized at pH5.2, and ^{99m}Tc microspheres (particle size: 5–75 μm) can be attained when the heat treated alkaline HSA solution is completely denatured [2.20]. Technetium-99m macroaggregates are used for perfusion lung scintigraphy, since the accumulation in the lung is proportional to the blood flow (i.e. embolisms are cold areas, having no blood supply).

2.3. SECOND GENERATION TECHNETIUM RADIOPHARMACEUTICALS

After the success of the first generation of technetium radiopharmaceuticals was established, there was demand for radiopharmaceuticals in which the conventional radionuclides, such as ^{51}Cr, ^{131}I, ^{197}Hg, were replaced with technetium. Although the task was difficult, a wide range of small organic molecules giving neutral, lipophilic or positively charged complexes were synthesized and labelled with technetium. These efforts resulted in the technetium radiopharmaceuticals currently used for brain and myocardial perfusion studies as well as those for the investigation and quantification of renal tubular function. Thus, as cost effective alternatives to non-technetium radiopharmaceuticals, the second generation products are of high clinical importance.

Technetium-99m-MAG_3 (mercaptoacetyltriglycine) was developed as an alternative to o-radioiodo-hippurate [2.21]. To evaluate complete renal function, radiopharmaceuticals with high tubular secretion are needed. Tubular secretion of a compound is facilitated enzymatically by making use of a special geometry consisting of three of the molecule's oxygen atoms. The highest tubular secretion was observed in the case of para-amino-hippuric acid (90%), which loses its biological activity when any kind of non-physiological radionuclide (i.e. other than carbon, oxygen or nitrogen isotopes) is introduced into

the molecule. If the amino group in the para position is replaced with a hydrogen atom, and at the same time radioiodine is introduced into the ortho position, the resulting o-radioiodo-hippurate molecule shows renal tubular excretion of about 85%. At the same time, routine clinical use of radioiodohippuran is limited, since ^{125}I and ^{131}I are not suitable for gamma camera renography and ^{123}I is too expensive in most countries. On the other hand, it was recognized that an approximately ideal geometry of the above mentioned oxygen trio would be provided by the –COOH terminal and the adjacent carbonyl group of a simple tripeptide like triglycine, while the three nitrogen atoms could take part in complexation with technetium. To ensure a stable complex with coordination number 5, a mercaptoacetyl group was attached to the nitrogen terminal of the triglycine, resulting in an N_3S complex (MAG_3).

The formulation of the MAG_3 kit consists mainly of S-benzoyl-mercaptoacetyl-triglycine, Sn(II)-chloride and tartrate as a ligand exchange partner. The S-benzoyl group protects the active substance from oxidation (i.e. avoiding the formation of intermolecular -S-S- bridges from the free –SH groups). During labelling, the S-benzoyl group is eliminated by applying an elevated temperature. Up to 90% of the ^{99m}Tc-MAG_3 binds to plasma proteins but, owing to the enzymatically facilitated tubular excretion, 50% of the activity is found in the kidneys at 5 min post-injection. After 3 h, more than 90% of the injected dose is washed out via urine. This pharmacokinetics allows the evaluation of the effective renal plasma flow (ERPF) as well.

Technetium-99m-HMPAO (hexamethylpropylene amine oxime) was the first neutral lipophilic technetium compound able to pass through the blood–brain barrier [2.22]. Due to the asymmetric carbon atoms in the ligand, different stereoisomers of the active substance and the labelled compound are known [2.23]. The brain uptake of the *d* and *l* (trans) isomers is high (up to 4% of the injected dose), while the *meso* (syn) isomers do not show acceptable accumulation in the brain tissues. The concentration of the appropriate trans isomers in the brain is constant from 2 to 4 min post-injection, since the trans isomers are intracellularly transformed into hydrophilic compounds and captured in the brain cells. The brain uptake of ^{99m}Tc-HMPAO is proportional to the perfusion in the tissues.

Labelling of HMPAO requires great care, since a very small amount — only a few micrograms — of Sn(II) is used in the kit for ligand exchange labelling via pyrophosphate. If the eluate from a generator that is not regularly eluted is used for radiolabelling of HMPAO, the amount of Sn(II) will not be sufficient to reduce the total amount of technetium (^{99m}Tc + ^{99g}Tc) and a greater amount of free pertechnetate will remain in the solution. This is the reason why the use of fresh eluate from a continuously used generator is recommended for labelling HMPAO. Technetium-99m-HMPAO is of poor

stability, and hence the complex must be used within 30–45 min of preparation [2.24]. Technetium-99m-HMPAO also penetrates the white blood cells (leucocytes, lymphocytes), platelets and even macrophages in vitro, and hence is used for cell labelling studies for imaging infection and inflammation.

Technetium-99m-MIBI (methoxyisobutylisonitrile), or ^{99m}Tc-sestamibi, is a lipophilic complex with a positive charge that is in frequent clinical use [2.25]. To ensure the positive charge of the complex, a technetium atom in a low oxidation state (+1) is reacted with the monodentate isonitrile ligand to obtain $[Tc(-C{=}NR)_6]^+$ with a hexacoordinated (octahedral) structure. Each carbon atom bound to the technetium possesses a non-paired electron (overlapping with the lone pair of the adjacent nitrogen), thus the technetium-sestamibi molecules are paramagnetic. Since isonitriles are volatile, not very stable compounds, MIBI is available in stabilized form as copper tetrafluoroborate adduct, $[Cu(MIBI)_4]BF_4$, which should be decomposed during labelling, with the procedure carried out at an elevated temperature by immersing the vial in boiling water for 10 min [2.26].

Technetium-99m-MIBI is taken up by the cells of the myocardium in passive diffusion [2.27] and then appears in the cytosol and is localized in the mitrochondria. The uptake is proportional to the myocardial perfusion, and the washout is rather slow (excluding considerable redistribution). At stress, more than 3% of the injected dose is accumulated in the myocardium, while the non-bound part is eliminated via the hepatobiliary route. Technetium-99m-MIBI is also taken up in tumours and metastases, expanding its clinical application.

Cationic technetium complexes can also be obtained if technetium is in an oxidation state of +3, such as with compound Q12, or +5, as with tetrofosmin. Development of compound Q12 was stopped after patient studies, while tetrofosmin [2.28, 2.29] is currently available for clinical use. In the latter biscomplex, four trivalent phosphorus atoms and two oxo-oxygen atoms are coordinated to technetium (+5). Tetrofosmin can be labelled at ambient temperature, and its uptake in the myocardium and in tumours and metastases is similar to that of ^{99m}Tc-MIBI (passive diffusion, no redistribution).

Technetium-99m-ECD (ethylene-L,L-dicysteine diethylester) is a neutral, lipophilic technetium complex with high stability, owing to the N_2S_2 core, that can be used even several hours after preparation. It can easily pass through the blood–brain barrier and is captured in the brain cells owing to enzymatic hydrolysis, resulting in EC-monoester-monoacid and EC-diacid in the first and second steps, respectively. Compared with ^{99m}Tc-HMPAO, ^{99m}Tc-ECD exhibits different pharmacokinetics in humans, thus a direct comparison of these two tracers cannot be made: the use of one or the other tracer may be preferable, depending on the clinical case [2.30].

Technetium-99m-ECD is far superior to ^{99m}Tc-HMPAO regarding stability; however, it is not suitable for white cell labelling.

Ethylene-L,L-dicysteine (EC) is the free acid derivative of ECD [2.31]. Owing to the two free –COOH groups, intramolecular interaction takes place between the molecule's two protons and two nitrogen atoms, resulting in two intramolecular rings in the EC. For this reason, only the –SH groups of EC are able to react with technetium, unless the intramolecular rings have been broken by ensuring a strong alkaline medium (≥pH12). In this medium, both the nitrogen atoms and the sulphur atoms are able to bind to technetium and a N_2S_2 monocomplex can be obtained. In this complex, the oxo-oxygen atom of the technetium and two oxygen atoms of a –COOH group form a geometry (i.e. a triangle consisting of the oxygen trio) similar to those of iodo-hippuran and ^{99m}Tc-MAG_3. Thus ^{99m}Tc-EC is also a tubular renal agent showing rapid renal uptake and washout [2.32–2.34], and providing high image quality for gamma camera renography.

Labelling of EC can be accomplished using a three-vial kit formulation, with the EC in a strong alkaline buffer (in which the stannous ions are not stable enough) in the first vial, the Sn(II)-tartrate as a reducing agent and ligand exchange partner in the second vial, and an acidic buffer to neutralize the strong alkaline solution just after labelling in the third vial. The procedure can be carried out at ambient temperature, with the ^{99m}Tc-EC remaining stable for several hours [2.32].

The chemical formulas of selected first and second generation technetium radiopharmaceuticals are shown in Fig. 2.1.

2.4. NOVEL TECHNETIUM CHEMISTRY

Novel technetium chemistry involves labelling of tissue specific molecules such as receptor ligands, metabolic agents, peptides, proteins, antibodies and antibiotics with technetium such that these biomolecules retain their biochemical and physiological activities [2.35, 2.36]. To achieve this, the complexing core must be as far as possible from the biospecific part of the molecules. At the same time, the labelled species should possess high specific activity, since their binding sites in living tissue are limited. The following three methods can be considered the most important in which monodentate ligands play a role.

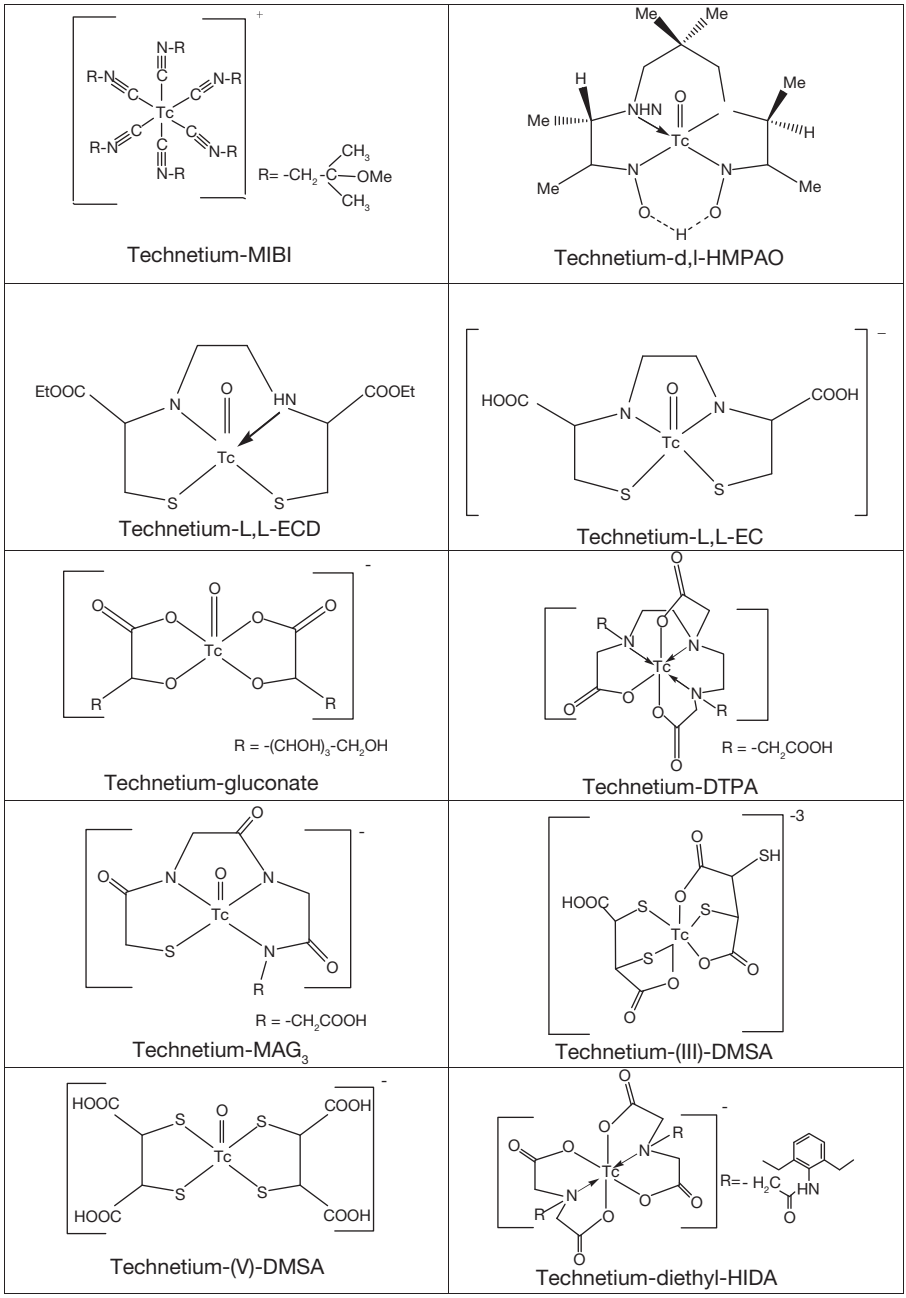
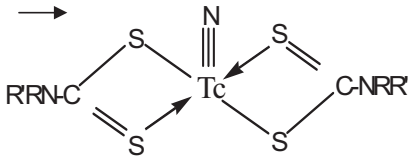


FIG. 2.1. Chemical formulas of selected technetium radiopharmaceuticals.

$[TcO_4]^{-1} + SnCl_2 + H_2N\text{-}NH\text{-}CO\text{-}(CH_2)_2\text{-}CO\text{-}NH\text{-}NH_2 \longrightarrow [Tc{\equiv}N]$ synthon



$[Tc{\equiv}N]$ synthon + 2 $RR'N\text{-}CS_2Na$

FIG. 2.2. Preparation of Tc-nitrido complex using the [Tc≡N] synthon.

2.4.1. Nitrido labelling

As was indicated by chemical evidence, replacing the oxo-oxygen atom in the technetium complexes with a nitrido group containing a triple bond between the technetium and the nitrogen atom results in a more compact complexing core and much higher stability [2.37]. The first of the nitrido technetium compounds was NOET (technetium-nitrido-N-ethyl-N ethoxy-dithiocarbamate), which was designed as a neutral lipophilic complex [2.38]. The Tc≡N structure can easily be prepared if hydrazino derivatives and tin chloride are reacted with pertechnetate at ambient or an elevated temperature. This technetium-nitrido intermediate is then transformed by the appropriate ligand to the desired nitrido complex. Dithiocarbamate derivatives are shown in Fig. 2.2 as an example of this reaction route.

When R = -Et and R′ = -OEt, the resulting neutral lipophilic NOET molecule shows myocardial uptake by being intercalated in the phospholipid layer of the myocytes [2.39]. A redistribution effect is observed owing to a dynamic equilibrium involving washout and uptake, which is unique among the potential technetium myocardial agents [2.40]. The registration procedure of NOET was not completed. By varying R and R′ groups, a series of neutral compounds of lipophilic character can be obtained, which could serve as, for example, potential agents for brain studies [2.41].

Symmetric nitrido complexes can be prepared, not only with a S_4 core such as NOET, but also with an $S_2P_2^{(III)}$ core when the reaction partner is $RR'P\text{-}CH_2\text{-}CH_2\text{-}SH$ (Fig. 2.3(a)). At the same time, asymmetric nitrido complexes can be prepared by using bidentate phosphino ligands with a bidentate coligand (Fig. 2.3(b)).

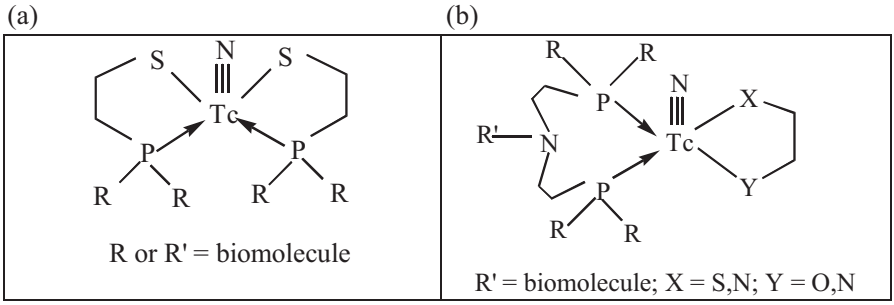


FIG. 2.3. Various nitrido complexes of technetium: (a) symmetric labelling and (b) asymmetric labelling.

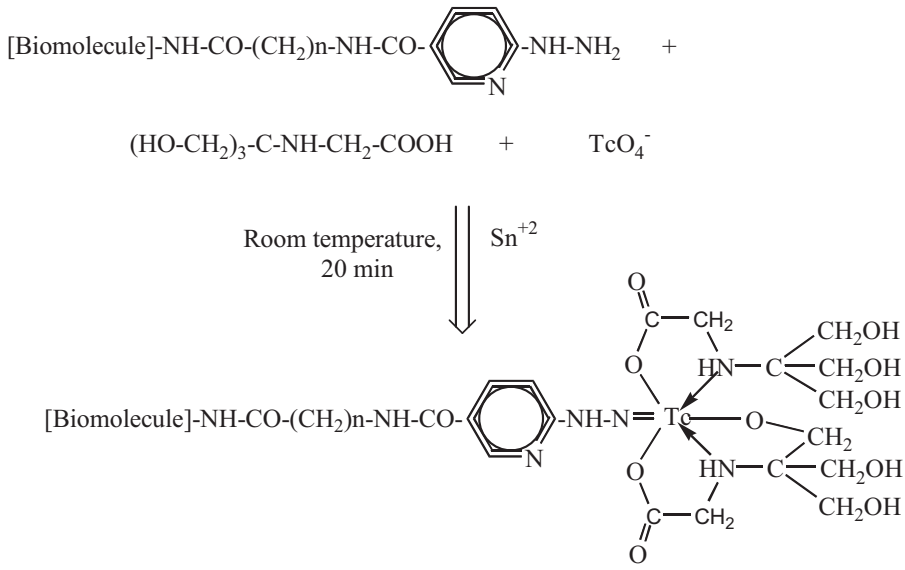


FIG. 2.4. Technetium-99m labelling of a biomolecule through the HYNIC substrate.

2.4.2. HYNIC labelling

HYNIC, the hydrazinonicotinamide introduced by Abrams et al. [2.42], functions as a BFCA, forming a bridge between the biomolecule and the technetium [2.43]. The HYNIC conjugated molecules react as monodentate ligands, while the coligands may be tricine or EDDA (ethylene-diamine-diacetic acid). A typical HYNIC-tricine labelling is shown in Fig. 2.4.

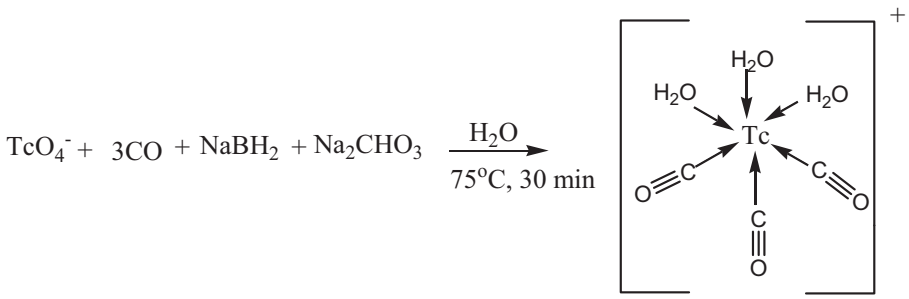


FIG. 2.5. Synthesis of a Tc-tricarbonyl synthon.

HYNIC labelling ensures mild conditions, avoiding the reduction of the -S-S- cystine bridges of the biomolecules; ^{99m}Tc-somatostatin derivatives can also be prepared in this manner. The best version of these derivatives is ^{99m}Tc-EDDA-HYNIC-TOC, in which the modified octreotide (containing tyrosine instead of phenylalanine in the amino acid chain (TOC)) and EDDA (as the co-ligand) are present in the molecule [2.44, 2.45]. Technetium-99m-EDDA-HYNIC-TOC is a useful diagnostic tool for imaging tumours and metastases expressing somatostatin receptors on their surfaces.

HYNIC provides a successful labelling methodology in the case of the phosphatidylserine specific protein Annexin V for the imaging of programmed cell death (apoptosis). HYNIC also allows the labelling of various agents for imaging infection and inflammation, such as the antimicrobial peptide ubiquicidine (UBI 29-41) [2.46], interleukin-8 [2.47], leukotriene B4 (LTB-4) receptor antagonists [2.48], the RP-463 chemotactic peptide-containing sequence of formyl-methionyl-leucyl-phenylalanyl-lysine-HYNIC [2.49] and liposomes [2.50]. HYNIC coupled lysine is a simple amino acid to which technetium may be bound [2.51], and HYNIC coupled antisense DNA [2.52] can be considered a useful tool for the application of technetium in biomedical research.

2.4.3. Tricarbonyl labelling

The use of organometallic carbonyl compounds in technetium chemistry was first proposed in 1993, and such compounds were first fully synthesized in an aqueous medium in 1998 [2.53]. It was observed that, when carbon monoxide is flushed in an alkaline medium in the presence of sodium carbonate and sodium borohydride, pertechnetate can be reduced at elevated temperatures by forming a transient cationic complex (Fig. 2.5).

In the presence of a desired biomolecule containing an imidazol ring at its terminal (due to the attached histamine or histidine) as a chelating agent, the

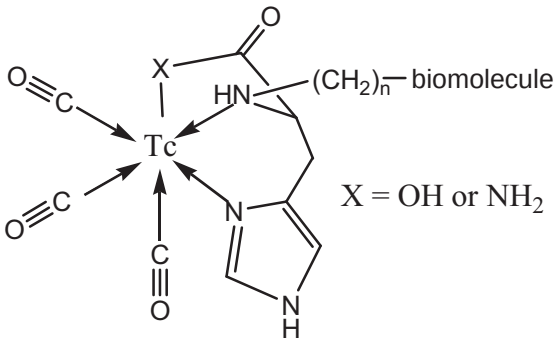


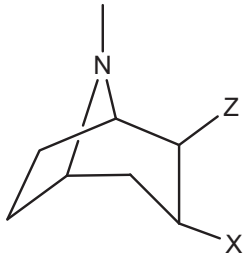
FIG. 2.6. Technetium labelling of a biomolecule through the Tc-tricarbonyl synthon.

transient cationic complex may be transformed into a highly stable cationic technetium complex (see Fig. 2.6).

Initially, the labelling technique required that the reaction mixture be flushed with carbon monoxide. A freeze-dried kit containing sodium tartrate, sodium tetraborate, sodium carbonate and sodium boranocarbonate is now available for easy synthesis of Tc-carbonyl complexes. The transient (intermediary) complex is prepared by adding pertechnetate to the freeze-dried kit and immersing it in boiling water for 20 min. In the second step, the reaction of the intermediary complex with the biomolecules containing a histamine or histidine terminal can take place at ambient temperature, providing mild conditions for labelling. Tricarbonyl labelling has been carried out with somatostatin derivatives [2.54], bombesin derivatives [2.55], neurotensin pseudopeptides [2.56], surface protein-B, isonitriles [2.57] and even glucose [2.58].

2.5. DESIGNED MOLECULES AND BIOCONJUGATES

High biological specificity is one of the most important requirements of the novel radiopharmaceuticals, and knowledge of the localization mechanism can help in developing optimal molecules. Systematic studies of receptor–ligand or antigen–antibody interactions can provide sufficient information for computer aided molecular modelling of potential bioconjugates. The computing systems not only may provide the ideal means of storing, visualizing and manipulating molecular structures, but may also facilitate quantitative analysis of the structural data, based on quantum chemistry. Thus, the bioactive part of the molecular structure can be identified, giving an opportunity to



Cocaine	TRODAT
Z = -$COOCH_3$	Z = -CH_2-[N_2S_2(Tc=O)]
X = -O-CO-Ph	X = -C_6H_4Cl

FIG. 2.7. Structures of cocaine and technetium–TRODAT.

design, synthesize and use small molecules appropriate for specific biological interactions.

For example, molecular design played an important role when ^{99m}Tc-TRODAT was developed for imaging Parkinson's disease. In this case, the cocaine molecule was taken as a model. The structural similarity of cocaine and TRODAT is presented in Fig. 2.7.

As the figure shows**,** there are some differences between substituents Z and X. Concerning TRODAT, Z was designed as the complexing core for technetium, but the difference in X is to ensure a lipophilic character similar to that of cocaine, which would not be the case with substituent Z. With these modifications, ^{99m}Tc-TRODAT has become a successful agent in neuro-psychiatry [2.59].

Technetium-99m labelled bioconjugates can be prepared by the preformed chelate approach or by post-conjugation techniques. The first preformed chelate approach was developed by Fritzberg et al. [2.60] when a carboxyethyl derivative of an N_2S_2 core was transformed into an active ester with 2,3,5,6-tetrafluorophenol, followed by the reaction with the –NH_2 terminal of the biomolecule (Fig. 2.8) [2.60].

The post-conjugation labelling concept involves two steps: first, the BFCAs are coupled efficiently with the bioactive compounds, and then either pertechnetate and a reducing agent or a freshly prepared weak complex of technetium such as gluconate or glucoheptonate is added. Triamidemono-thiolates (MAG_3-like compounds) [2.61], diaminodithiolates (BAT- or EC-like compounds) [2.62] and propyleneaminoxime derivatives (HMPAO-like compounds) [2.63] can be used as BFCAs. Peptides, monoclonal antibody fragments and other compounds such as spiperone derivatives or some steroids can be successfully labelled with technetium using the post-conjugation approach.

$$[(Tc{=}O)N_2S_2]\text{-}CH_2CH_2COOH + C_6HF_4OH \xrightarrow{\text{carbodiimide}} [(Tc{=}O)N_2S_2]\text{-}CH_2CH_2COOC_6HF_4$$

$$+ [\text{biomolecule}]\text{-}NH_2 \longrightarrow [(Tc{=}O)N_2S_2]\text{-}CH_2CH_2CO\text{-}NH\text{-}[\text{biomolecule}]$$

where $[(Tc{=}O)N_2S_2]$- is

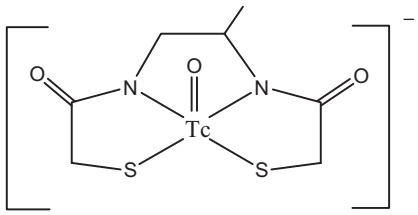


FIG. 2.8. Synthesis of a ^{99m}Tc labelled peptide by the post-conjugation technique.

2.6. PRECURSORS AND CHELATING AGENTS NEEDED FOR THE LABELLING OF BIOMOLECULES

Increasingly, laboratories are seeking to perform procedures with various technetium compounds prepared using novel labelling approaches. For such procedures to be successful, some practical issues must be addressed. For example, preformed chelators, including the technetium labelled active esters from such precursors as tetrafluorophenol or tetrafluoro-thiophenol, are not stable and hence need to be freshly prepared. Another issue concerning tricarbonyl labelling is the technique of obtaining carbon monoxide for preparation of the ^{99m}Tc-tricarbonyl synthon. Flushing carbon monoxide gas into the solution is a simple but not very convenient method that can be avoided by using kit formulated, commercially available boranocarbonate (ISOLINK).

Labelling of biomolecules is often performed in the ligand exchange reaction. The reaction partners can be divided into the following two groups:

— Conventional ligand exchange partners, which originally were clinical radiopharmaceuticals (e.g. kits of gluconate, glucoheptonate or pyrophosphate);
— Weak chelators such as tartrate, citrate and EDTA.

Tartrate was first used in the MAG_3 kit as an ingredient for labelling by ligand exchange reaction. Tartaric acid neutralized with sodium hydroxide, Sn(II)-chloride and ascorbic acid (as a stabilizer) can be freeze-dried together, resulting in a kit formulated agent for ligand exchange. This composition is

available in the three-vial EC kit as well. EDTA is used as a ligand exchange partner in the ECD and MIBI kit, but it can be formulated separately as well.

In nitrido, HYNIC and tricarbonyl labelling, in addition to the biomolecule, co-ligands should be incorporated into the ^{99m}Tc complexes to attain the optimal structure and biological activity. In HYNIC labelling, first tricine and later EDDA were used in this way. Separate formulation of such co-ligands could be useful in the future.

REFERENCES TO SECTION 2

[2.1] MELNIK, M., VAN LIER, J.E., Analyses of structural data of technetium compounds, Coord. Chem. Rev. **77** (1987) 275–324.

[2.2] LIBSON, K., DEUTSCH, E., BARNETT, B.L., Structural characterization of a ^{99}Tc-diphopsonate complex: Implications for the chemistry of ^{99m}Tc skeletal imaging agents, J. Am. Chem. Soc. **102** (1980) 2478–2480.

[2.3] BOYD, R.E., et al., ^{99m}Tc gluconate complexes for renal scintigraphy, Br. J. Radiol. **46** (1973) 604–612.

[2.4] GUTKOWSKY, R.F., DWORKIN, H.J., Kit-produced ^{99m}Tc-labelled red cells for spleen imaging, J. Nucl. Med. **15** (1974) 1187–1191.

[2.5] ARNOLD, R.W., et al., Comparison of ^{99m}Tc complexes for renal imaging, J. Nucl. Med. **16** (1975) 357–367.

[2.6] OHTA, H., et al., Clinical evaluation of tumour imaging using ^{99m}Tc(V) dimercaptosuccinic acid, a new tumour-seeking agent, Nucl. Med. Commun. **9** (1988) 105–116.

[2.7] IKEDA, I., INOUE, O., KURATA, K., Preparation of various ^{99m}Tc dimercaptosuccinate complexes and their evaluation as radiotracers, J. Nucl. Med. **18** (1977) 1222–1229.

[2.8] SUBRAMANIAN, G., McAFEE, J.J., BLAIR, R., ^{99m}Tc methylenediphosphonate: A superior agent for skeletal imaging — Comparison with other Tc complexes, J. Nucl. Med. **16** (1977) 744–754.

[2.9] BERGQVIST, L., et al., Clinical comparison of bone scintigraphy with ^{99m}Tc-DPD, ^{99m}Tc-HDP, ^{99m}Tc-DPD, Acta Radiol. Diagn. **25** (1974) 217–223.

[2.10] SMITH, R.K., ARTERBURN, G., Detection and localization of gastrointestinal bleeding using ^{99m}Tc pyrophosphate in vivo labelled red blood cells, Clin. Nucl. Med. **5** (1980) 55–60.

[2.11] HARVEY, E., LOBERG, M.D., COOPER, M., A new radiopharmaceutical for hepatobiliary imaging, J. Nucl. Med. **16** (1975) 533–540.

[2.12] ASCHER, S.A., SARKAR, S.D., SPIVAK, W.B., Hepatic uptake of ^{99m}Tc diisopropyl iminodiacetic (DISIDA) is not impaired by very high serum bilirubin levels, Clin. Nucl. Med. **13** (1988) 1–3.

[2.13] NUNN, A.D., LOBERG, M.D., CONLEY, R.A., A structure-distribution-relationship approach leading to the development of ^{99m}Tc mebrofenin: An improved cholescintigraphic agent, J. Nucl. Med. **24** (1983) 423–430.
[2.14] DRUM, D.E., Current status of radiocolloid hepatic scintiphotography for space-occupying disease, Sem. Nucl. Med. **12** (1982) 64–74.
[2.15] FRIER, M., GRIFFITHS, P., RAMSEY, A., The physical and chemical characteristics of sulphur colloids, Eur. J. Nucl. Med. **6** (1981) 255–260.
[2.16] WHATELEY, T.L., STEELE, G., Particle size and surface charge studies of a tin colloid radiopharmaceutical for liver scintigraphy, Eur. J. Nucl. Med. **10** (1985) 353–357.
[2.17] WATANABE, T., et al., Sentinel node biopsy with ^{99m}Tc colloidal rhenium sulphide in patients with breast cancer, Br. J. Surg. **88** (2001) 704–707.
[2.18] ARZOUMANIAN, A., ROSETHALL, L., SETO, H., Clinical comparison of ^{99m}Tc-labelled preformed phytate colloid and sulfur colloid: Concise communication, J. Nucl. Med. **18** (1977) 118–120.
[2.19] TSOPELAS, C., Particle size analysis of ^{99m}Tc-labelled and unlabelled antimony trisulphide and rhenium sulphide colloids intended for lymphoscintigraphic application, J. Nucl. Med. **42** (2001) 460–466.
[2.20] AL-JANABI, M.A., YOUSIF, Z.M., KADIM, A.H., AL-SALEM, A.M., A new technique for the preparation of ready-to-use macroaggregated albumin (MAA) kits to be labelled with ^{99m}Tc for lung scanning, Int. J. Appl. Radiat. Isot. **34** (1983) 1473–1478.
[2.21] BORMANS, G., et al., Investigation of the labelling characteristics of ^{99m}Tc-mercaptoacetyltriglicine, Nucl. Med. Biol. **22** (1995) 339–349.
[2.22] NEIRINCKX, R.D., Evaluation of regional cerebral blood flow with ^{99m}Tc-d,l-HMPAO and SPECT, Neurosurg. Rev. **10** (1987) 181–184.
[2.23] BANERJEE, S., SAMUEL, G., KOTHARI, K., SARMA, H.D., PILLAI, M.R.A., On the synthesis, isolation and radiochemical studies for the preparation of in-house kit for ^{99m}Tc-meso- and d,l-HMPAO: A few additional observations, Nucl. Med. Biol. **26** (1999) 327–338.
[2.24] TUBERGEN, K., CORLIJA, M., VOLKERT, W.A., HOLMES, R.A., Sensitivity of ^{99m}Tc-d,l-HMPAO to radiolysis in aqueous solution, J. Nucl. Med. **32** (1991) 111–115.
[2.25] WACKERS, F.J., et al., Technetium-99m hexakis 2-methoxyisobutyl isonitrile: Human biodistribution, dosimetry, safety and preliminary comparison to thallium-201 for myocardial perfusion imaging, J. Nucl. Med. **30** (1989) 301–311.
[2.26] PROULX, A., BALLINGER, J.R., GULENCHYN, K.Y., Routine determination of radiochemical purity of ^{99m}Tc-MIBI, Int. J. Rad. Appl. Instrum. A **40** (1989) 95–97.
[2.27] SAVI, A., et al., Biodistribution of ^{99m}Tc methoxy-isobutyl-isonitrile (MIBI) in humans, Eur. J. Nucl. Med. **15** (1989) 597–600.
[2.28] KELLY, J.D., et al., Technetium-99m-tetrofosmin as a new radiopharmaceutical for myocardial perfusion imaging, J. Nucl. Med. **34** (1993) 222–227.

[2.29] NAKAJIMA, K., et al., Myocardial perfusion imaging and dynamic analysis with ^{99m}Tc tetrofosmin, J. Nucl. Med. **34** (1993) 1478–1484.
[2.30] ASENBAUM, S., BRUCKE, T., PIRKER, W., PIETRZYK, U., PODREGA, I., Imaging of cerebral blood flow with technetium-99m HMPAO and technetium-99m-ECD: A comparison, J. Nucl. Med. **39** (1998) 613–618.
[2.31] KABASAKAL, L., Technetium-99m ethylene dicysteine: A new renal tubular function agent, Eur. J. Nucl. Med. **27** (2000) 351–357.
[2.32] VAN NEROM, C.G., BORMANS, G.M., DE ROO, M.J., VERBRUGGEN, A.M., First experience in healthy volunteers with technetium-99m L,L-ethylene-dicysteine, a new renal imaging agent, Eur. J. Nucl. Med. **20** (1993) 738–746.
[2.33] KABASAKAL, L., et al., Clinical comparison of technetium-99m-EC, technetium-99m-MAG3 and iodine-131-OIH in renal disorders, J. Nucl. Med. **36** (1995) 224–228.
[2.34] NIELSEN, S.S., et al., Clearance and renal extraction of technetium-99m-L,L-ethylenedicysteine, I-131-ortho-iodohippurate and I-125-iothalamate in pigs, Clin. Physiol. **19** (1999) 338–343.
[2.35] SUNBERG, M.W., MEARES, C.F., GOODWIN, D.A., DIAMANTI, C.I., Chelating agents for the binding of metal ions to macromolecules, Nature **250** (1974) 587–588.
[2.36] DELMON-MOINGEON, L.I., MAHMOOD, A., DAVISON, A., JONES, A.G., Strategies for labelling monoclonal antibodies and antibody-like molecules with technetium-99m, J. Nucl. Biol. Med. **35** (1991) 47–59.
[2.37] BALDAS, J., BONNYMAN, J., Effect of the Tc-nitrido group on the behaviour of ^{99m}Tc-radiopharmaceuticals, Int. J. App. Radiat. Isot. **36** (1985) 919–923.
[2.38] PASQUALINI, R., et al., Bis(sithiocarbamato) nitrido technetium-99m radiopharmaceuticals: Neutral myocardial imaging agents, J. Nucl. Med. **35** (1994) 334–341.
[2.39] GHEZZI, C., et al., Myocardial kinetics of TcN-NOET: A neutral lipophilic complex tracer for regional myocardial blood flow, J. Nucl. Med. **36** (1995) 1069–1077.
[2.40] JOHNSON, G., NGUYEN, K.N., PASQUALINI, R., OKADA, R.D., Interaction of technetium-99m NOET with blood elements: Potential mechanism of myocardial redistribution, J. Nucl. Med. **38** (1997) 138–143.
[2.41] BELLANDE, E., et al., Synthesis and biodistribution of nitrido technetium-99m radiopharmaceuticals with dithiophosphinate ligands: A class of brain imaging agents, Nucl. Med. Biol. **22** (1995) 315–320.
[2.42] CALLAHAN, R.J., BARROW, S.A., ABRAMS, M.J., RUBIN, R.H., FISHMAN, A.J., Biodistribution and dosimetry of technetium-99m hydrazino nicotinamid IgG: Comparison with indium-111-DTPA-IgG, J. Nucl. Med. **37** (1996) 843–846.
[2.43] RENNEN, H.J., BOERMAN, O.C., KOENDERS, E.B., OYEN, V.J., CORSTENS, F.H., Labelling proteins with ^{99m}Tc via hydrazinonicotinamide (HYNIC): Optimization of the conjugation reaction, Nucl. Med. Biol. **27** (2000) 599–604.

[2.44] DECRISTOFORO, C., et al., ^{99m}Tc-EDDA/HYNIC-TOC: A new ^{99m}Tc-labelled radiopharmaceutical for imaging somatostatin receptor-positive tumours: First clinical results and intra-patient comparison with ^{111}In-labelled octreotide derivatives, Eur. J. Nucl. Med. **27** (2000) 1318–1325.

[2.45] PLACHCINSKA, A., et al., Clinical usefulness of ^{99m}Tc-EDDA/HYNIC-TOC scintigraphy in oncological diagnostics: A preliminary communication, Eur. J. Nucl. Med. Mol. Imaging **30** (2003) 1402–1406.

[2.46] WELLING, M.M., et al., Radiochemical and biological characteristics of ^{99m}Tc-UBI 29-41 for imaging bacterial infections, Nucl. Med. Biol. **29** (2002) 413–422.

[2.47] RENNEN, H.J., BOERMAN, O.C., OYEN, W.J., CORSTENS, F.H., Kinetics of ^{99m}Tc-labelled interleukin-8 in experimental inflammation infection, J. Nucl. Med. **44** (2003) 1502–1509.

[2.48] LIU, S., HARRIS, A.R., WILLIAMS, N.E., EDWARDS, D.S., ^{99m}Tc-labelling of hydrazinonicotinamide-conjugated LTB(4) receptor antagonist useful imaging infection and inflammation, Bioconj. Chem. **13** (2002) 881–886.

[2.49] EDWARDS, D.S., et al., RP463: A stabilized ^{99m}Tc complex of a hydrazino nicotinamide derivatized chemotactic peptide for infection imaging, Bioconj. Chem. **10** (1999) 884–891.

[2.50] SZEBENI, J., et al., Role of complement activation in hypersensitivity reactions to doxil and hynic PEG liposomes: Experimental and clinical studies, J. Liposome Res. **12** (2002) 165–172.

[2.51] GREENLAND, W.E., HOWLAND, K., HARDY, J., FOGELMAN, I., BLOWER, P.J., Solid-phase synthesis of peptide radiopharmaceuticals using Fmoc-N-epsilon-(hynic-Boc)-lysine, a technetium-binding amino acid: Application to ^{99m}Tc-labelled salmon calcitonin, J. Med. Chem. **46** (2003) 1951–1757.

[2.52] ZHANG, Y.M., et al., Electrostatic binding with tat and other cationic peptides increases cell accumulation of ^{99m}Tc-antisense DNAs without entrapment, Mol. Imaging Biol. **5** (2003) 240–247.

[2.53] ALBERTO, R., et al., A novel organometallic aqua complex of technetium for the labelling of biomolecules: Synthesis of $/^{99m}Tc(OH_2)_3(CO)_3/^+$ from $/^{99m}TcO_4/^-$ in aqueous solution and its reaction with a bifunctional ligand, J. Am. Chem. Soc. **120** (1998) 7987–7988.

[2.54] MARMION, M.E., BUGAJ, J.E., SCHMIDT, M.A., SRINIVASAN, A., Preparation and biological evaluation of ^{99m}Tc-(I)-carbonyl labelled octreotate conjugates, J. Nucl. Med. **41** (2000) 40 (abstract).

[2.55] LA BELLA, R., et al., A ^{99m}Tc-(I)-postlabelled high affinity bombesin analogue as potential tumour imaging agent, Bioconj. Chem. **13** (2002) 599–604.

[2.56] GARCIA-GARAYOA, E., et al., Preclinical evaluation of a new, stabilized neurotensin(8-13) pseudopeptide radiolabelled with ^{99m}Tc, J. Nucl. Med. **43** (2002) 374–383.

[2.57] MARMION, M.E., MACDONALD, J.R., Preparation and biodistribution of ^{99m}Tc-(I)-tricarbonyl complexes containing isonitrile and phosphine ligands, J. Nucl. Med. **41** (2000) 124 (abstract).

[2.58] PETRIG, J., SCHIBLI, R., DUMAS, C., ALBERTO, R., SCHUBIGER, P.A., Derivatization of glucose and 2-deoxyglucose for transition metal complexation: Substitution reactions with organometallic ^{99m}Tc and precursors and fundamental NMR investigations, Chem. **7** (2001) 1868–1873.
[2.59] KUNG, H.F., Development of ^{99m}Tc labelled tropanes: TRODAT-1 as dopamine transporter imaging agent, Nucl. Med. Biol. **28** (2001) 505–508.
[2.60] FRITZBERG, A.R., et al., Specific and stable labelling of antibodies with technetium-99m with a diamide dithiolate chelating agent, Proc. Natl. Acad. Sci. USA **85** (1988) 4025–4029.
[2.61] RAM, S., BUCHSBAUM, D., A peptide-based bifunctional chelating agent for ^{99m}Tc- and ^{188}Re-labelling of monoclonal antibodies, Cancer Res. **3** (1994) 769–773.
[2.62] BAIDOO, K.E., LEVER, S.Z., Synthesis of diaminedithiol bifunctional chelating agent for incorporation of technetium-99m into biomolecules, Bioconjug. Chem. **1** (1990) 132–137.
[2.63] LINDER, K.E., et al., TcO/PnAO-1-(2-nitromidazole)/, A new technetium containing nitroimidazole complex for imaging hypoxia: Synthesis, characterization and xanthine oxydase-catalyzed reduction, J. Med. Chem. **37** (1994) 9–17.

3. GOOD MANUFACTURING PRACTICE

The manufacture of kits is currently considered to be conventional pharmaceutical production and hence needs to fulfil all the basic requirements of the pharmaceutical industry. Consequently, manufacturers of kits should be licensed for compliance with GMP by the regulatory authorities.

3.1. MAIN COMPONENTS OF GMP

Good manufacturing practice involves requirements in several areas, including:

— Adequate premises, space, equipment and materials;
— Appropriately qualified and trained personnel;
— Clear definition of manufacturing processes;
— Validation of critical steps in the process;
— Validation of any significant changes to the process;
— Approved instructions and procedures for production, quality control (QC), product release, etc.;

— Quality assurance (QA) and QC activities, independent of production;
— Records of manufacture and complete batch history;
— Controlled product release;
— Suitable storage and transport of finished products;
— Means to recall any batch from sale or supply;
— Examination of complaints and investigation of quality defects.

3.2. QUALITY MANAGEMENT

All activities of kit production (i.e. pharmaceutical production) are controlled by the quality management system (QMS), based on an interrelated basic concept of QA, GMP and QC. QA covers all matters that, individually or collectively, may influence the quality of the product. Thus QA is the totality of organized arrangements for ensuring the quality required for the intended use (i.e. the safety of pharmaceutical products). To implement QA, all activities must be performed according to the rules of GMP covering the areas listed above. QC activity covers all sampling, testing and monitoring, including starting materials, packaging materials, and intermediate, bulk and finished products. It also requires that trained personnel be employed and that validated test/analytical methods and approved procedures be used.

3.3. PERSONNEL

3.3.1. Key personnel

The following three persons are considered to be key personnel in a production setup: the head of production (HP), the head of QC (HQC) and the qualified person (QP).

The HP ensures that appropriate conditions for production and storage exist and approves the standard operating procedures (SOPs). The HP also ensures that the appropriate validations and evaluations of production records are made, and checks that equipment is properly maintained.

The HQC approves or rejects the starting and packaging materials, and bulk and finished products. He or she ensures that all necessary tests are carried out, evaluates the analytical records and ensures the appropriate validations of analytical procedures.

The QP, who is approved by the local pharmaceutical regulatory authorities, ensures that every batch is produced and tested in accordance with the pertinent directives and specifications, and releases all batches.

3.3.2. Training

The manufacturer must provide basic training for all personnel and special training to certain groups of personnel according to their assigned duties. The training programme has to be planned for each year and performed several times a year. Personnel deployed for kit preparation should have a university degree in chemistry, radiochemistry or pharmacy, and all support personnel should be trained in GMP.

3.3.3. Personnel hygiene

Regular medical examinations of the personnel involved in production should be performed and registered. Involvement in pharmaceutical preparation is prohibited in the case of illness (e.g. infectious diseases, open lesions, injuries). Sterile/aseptic conditions are to be maintained by appropriate clothing, hand washing and disinfection. Eating, drinking and smoking in production and storage areas are prohibited.

3.3.4. Organization and functions

The QMS requires clearly defined functions and responsibilities. For this reason, QA and production should be completely separate — that is, they have to be independent units of the manufacturer. While QC may be a sub-unit of QA, the HQC and the QP must be two different persons. Thus, product release by the QP is the crucial step in pharmaceutical production. The release decision of the QP is based on evaluation of the analytical and batch processing records as well as the environmental monitoring results. A schematic presentation of GMP organization and functions is given in Fig. 3.1. In the figure, the above mentioned functions are represented by dashed arrows.

3.4. PREMISES AND EQUIPMENT

3.4.1. Premises

Kit preparation must be performed on premises meeting defined cleanliness criteria. Production areas can be divided into four groups according to the grade of sterility/aseptic conditions. This classification is presented in Table 3.1.

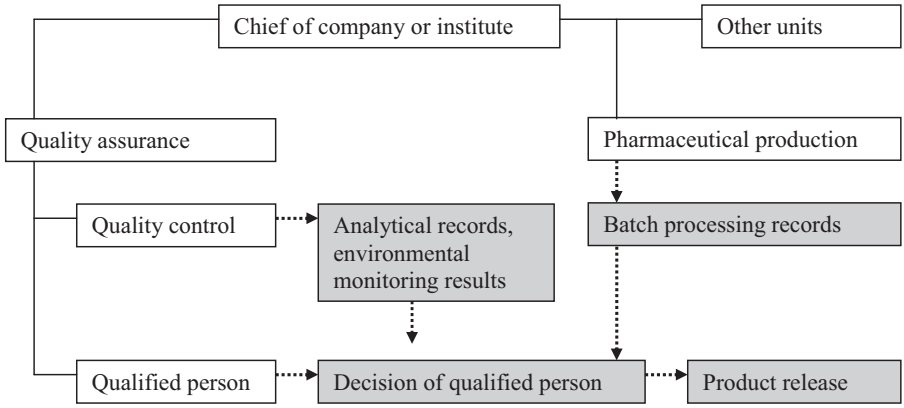


FIG. 3.1. Schematic presentation of GMP organization and functions.

TABLE 3.1. CLEAN AIR CLASSIFICATIONS WITH REFERENCE TO ISO-14644 STANDARDS

ISO class	Grade (class)	Maximum permitted number of particles/m^3 of air			
		At rest		In operation	
		Particles ≤0.5 μm	Particles ≤5 μm	Particles ≤0.5 μm	Particles ≤5 μm
5	A (100)	3 500	<1	3 500	<1
6	B (1 000)	3 500	<1	350 000	<1
7	C (10 000)	350 000	2 000	3 500 000	20 000
8	D (100 000)	3 500 000	20 000	Not defined	Not defined

Since kits cannot be sterilized terminally (i.e. in their final containers), aseptic preparation is required. This means that:

— Handling of items after washing should be performed in a grade D area. The items will be sterilized before use.
— Measuring of chemicals for solutions to be filtered should be done in a grade C area.
— Aseptic preparation (that is, making solutions, setting the pH, sterile filtering and dispensing) is to be carried out in a grade A area. (A grade B area is not a dedicated area for any operation; it can be considered as the background area or 'buffer zone' for a grade A quality laminar flow bench.)

The controlled areas should be provided with an air supply that is filtered through a microbe retaining filter and enters through an airlock. To ensure a grade A area for aseptic preparation, a laminar flow bench is necessary. Material transport into the grade A area should be performed either via the transport window of an interlocking system (chemical and previously sterilized and hermetically packed items) or via the dual door heat sterilizer (items to be heat sterilized). The freeze-dryer should be placed outside the grade A area; only the door of the freeze-dryer and the space where the vials are put for freeze-drying may be in contact with the grade A area. After freeze-drying and closing, the vials can leave the grade A area through a different transfer window with an interlocking system for packaging. Thus the material flow can be represented as a single direction line that does not cross the other flow lines.

In Fig. 3.2, the concept of the clean room design is presented graphically. Lower grade areas surround the higher grade areas. It should not be possibile to enter the grade A area from grade C or lower areas without a proper change of laboratory gowns in changing rooms. The flow of materials and personnel should be separated. Pass boxes should be installed between rooms of different grades. Before entering the grade B area, an air shower is needed to remove particulate contamination from personnel after gowning and to maintain the high grade of air purity when entering the grade B room. Bulk solution can be prepared in the grade C area, but its sterility must be assured before it can be dispensed in the grade A area by sterile filtration.

A typical layout of a clean room facility for the production of kits is given in Fig. 3.3.

The conditions of the production area must be controlled and monitored. The pressure difference before and after the air filters must be checked, and pressure differences between the areas of different grades must be checked and registered. The temperature also needs to be controlled, and air samples from different areas must be collected and evaluated regularly.

Although the control of particulates is important for the pharmaceutical industry, avoiding contamination of products by microorganisms, bacteria or yeast is critical in terms of the premises involved in the production of injectables. Recommended limits for microbiological monitoring of clean areas during operation are given in Table 3.2.

Figures 3.4(a)–(c) are typical views of a GMP compliant kit manufacturing facility. Figure 3.4(a) shows a worker ready to enter the aseptic manufacturing zone after changing into sterile attire. Figure 3.4(b) shows the inside of the clean room and the loading chambers of the two freeze-dryers. Figure 3.4(c) is a dispensing operation under a laminar flow station using a semi-automatic

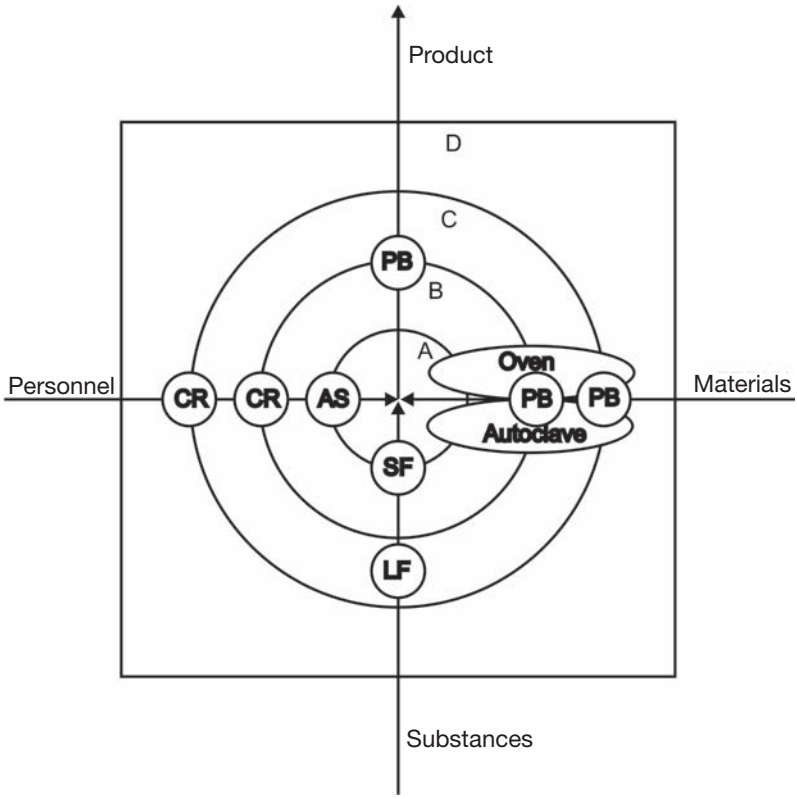


FIG. 3.2. Clean room concept; AS: air shower; CR: changing room, PB: pass box; SF: sterile filtration.

dispenser. Note that only the minimum essential equipment is provided in the laboratory.

Apart from the clean room for dispensing, separate stores should be designated for starting materials and finished products. Regarding starting materials, separate places should be defined for chemicals (active ingredients and excipients), primary packaging materials (vials and stoppers in contact with the pharmaceutical products) and secondary packaging materials (paper boxes, labels). Laminar flow should be ensured in the store for chemicals so as to avoid any contamination during their sampling. Controlled temperature and air humidity should be provided in the starting material store. Places for quarantine, approved products and rejected products should be provided separately. These places should also be distinguished from finished product storage.

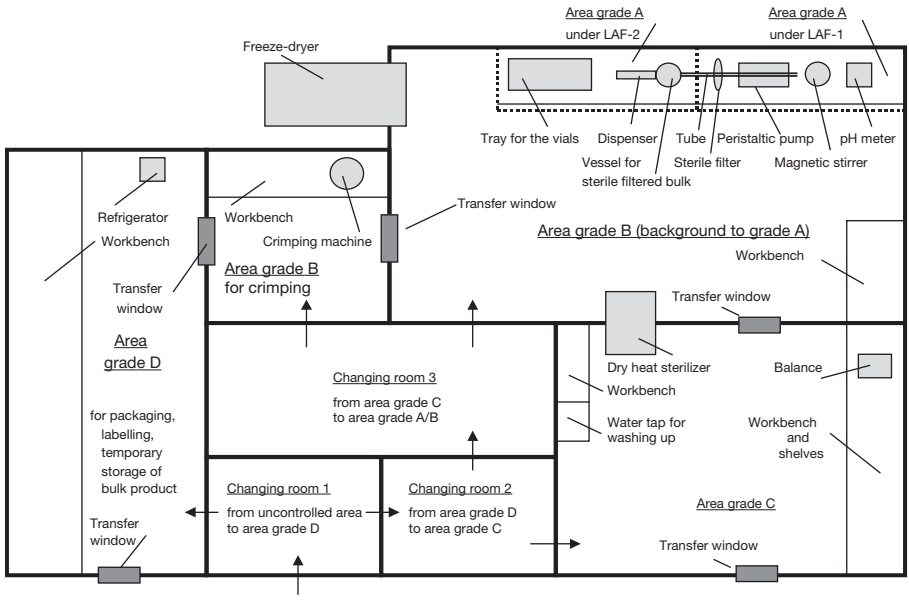


FIG. 3.3. Typical layout of a kit production laboratory.

TABLE 3.2. MICROBIOLOGICAL LIMITS DURING OPERATION OF CLEAN AREAS

Grade (class)	Recommended limits for microbial contamination			
	Air sample, CFU/m^3	Settle plates (dia.: 90 mm), CFU/4 h	Contact plates (dia.: 55 mm), CFU/plate	Glove print five fingers, CFU/glove
A (100)	<1	<1	<1	<1
B (1 000)	10	5	5	5
C (10 000)	100	50	25	—
D (100 000)	200	100	50	—

Note: CFU: Colony-forming unit.

3.4.2. Equipment and its validation

All equipment such as dry heat and steam sterilizers, pH meters, membrane filtration devices, freeze-dryers and air supply systems (including laminar flow benches) is to be qualified. Installation qualification indicates that this equipment meets the technical requirements for its intended use.

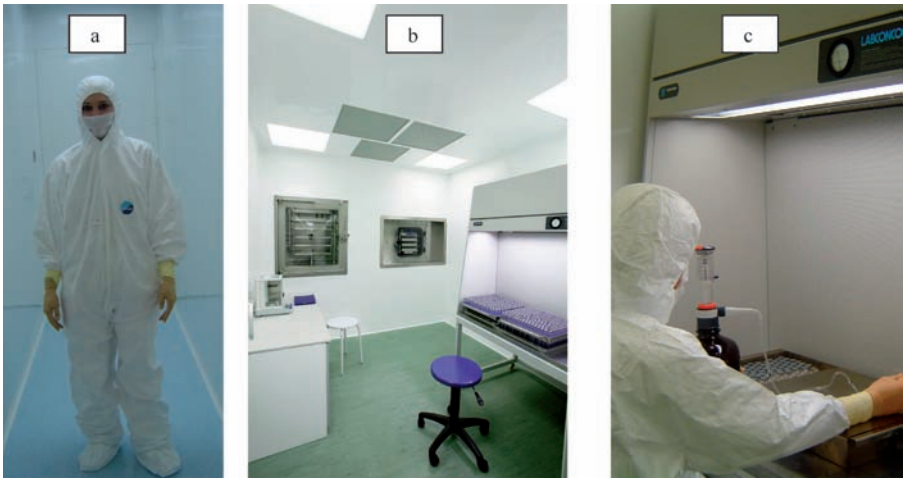


FIG. 3.4(a)–(c). Internal views of a kit production facility; see text for explanation of figures (source: Monrol A.S.).

Operation qualification shows that this equipment also meets all the requirements in model experiments. Performance qualification is considered to be successful if the equipment operates appropriately in producing trial batches of the intended products.

3.4.2.1. Sterilizers

Steam and heat sterilizers (Fig. 3.5) are used in kit production, depending on the conditions and heat tolerances of the different items. The minimum conditions for steam and dry heat sterilization are 121°C for 15 min and 160°C for 2 h, respectively. Other combinations of temperature and time may also be used if it is demonstrated that the process delivers an adequate and reproducible level of microbiological lethality in routine operation within the established tolerances. Temperature distribution within the sterilizers (i.e. the coldest point) should be determined, and the required level of microbiological lethality must be ensured at the coldest points as well.

3.4.2.2. Balances

Balances of appropiate measuring ranges must be used and calibrated regularly. After each calibration, the expiry of the calibration period is to be indicated on a green label stuck to the balance. After the expiry date of the calibration period, the balance must be recalibrated before it is used. Any

FIG. 3.5. Steam sterilizer and dry heat oven installation in a kit production facility (source: Monrol A.S.).

calibration action should be recorded in a logbook, which should be available at all times, especially for audits and inspections.

3.4.2.3. pH meters

Qualified pH meters are to be used. The pH of the standard solutions used for calibration must cover the range over which the pH setting is carried out.

3.4.2.4. Membrane filtration devices

Pre-sterilized disposable or reusable membrane filtration devices (pore size: 0.22 μm) made of plastic, glass or stainless steel can be used. The membrane filter should be checked for leakage ('bubble point' test) and should not be used more than once. For nitrogen gas purging of solutions or for sealing of vials under a nitrogen atmosphere, membrane filters suitable for gas filtration are to be used.

3.4.2.5. Freeze-dryer

Freeze-dryers that are GMP compliant are currently available, and their use is recommended for kit production. The chamber of the freeze-dryer is

autoclavable and provides for monitoring of the freeze-drying parameters. Many small scale kit manufacturers have older freeze-dryers that cannot be upgraded for self-steam sterilization; these are to be sterilized before use, followed by proper validation.

Shelf type freeze-drying units capable of accommodating two hundred or more 10 mL vials are commonly used. The unit should have mechanical facilities for closing the vials with split rubber closures under vacuum or nitrogen gas. Other operational features are: (i) a mechanical condenser capable of reaching temperatures below –40°C, (ii) an ice removing capacity of more than 2 kg in 24 h of operation, (iii) the capacity to retain a vacuum better than 50 torr without load, (iv) a facility for cooling and heating the shelf from –40 to +40°C by a circulating fluid and (v) a facility for monitoring the above parameters and the product temperature.

Upon installation of the equipment, and prior to each loading of samples, the freeze-dryer is to be operated without load and performance of the machine is to be checked with respect to the above parameters.

freeze-drying is an intricate process, and careful standardization of the various parameters is essential for obtaining a good product. To ensure aseptic conditions within the chamber of the freeze-dryer, specific and reliable procedures should be elaborated, unless the machine is capable of self-steam sterilization.

3.4.2.6. Laminar flow bench

Standard laminar flow benches (e.g. the vertical type) that meet the requirements outlined in Tables 3.1 and 3.2 can be used. Air supply to the bench should be filtered through high efficiency particulate air (HEPA) filters to achieve a minimum efficiency of 99.995%. After installation of a laminar flow bench, its conformation to the standard must be verified prior to its use in kit preparation. The bench must be cleaned and disinfected according to the standard procedures used in clean rooms. The performance of the bench is to be checked regularly by measurement of air velocity and by periodic exposure of nutrient agar culture plates in the working area to detect microbial contamination.

3.5. DOCUMENTATION

3.5.1. Specifications

Specifications must be elaborated for starting materials such as active ingredients, excipients and primary packaging materials, and for finished products. For starting materials, the following parameters are to be included: description, identification, assay, impurities, storage conditions and shelf life. Only those starting materials that completely fulfil all the requirements of the specifications can be approved and used for production. The pharmaceutical form, labelling conditions and efficiency, sterility and apyrogenicity should be added for the specification of the final product.

3.5.2. Manufacturing formulas

Manufacturing formulas are to be specified for the batch size according to the number of vials to be produced in one batch. A list of the appropriate amounts of all materials used in each batch must be included.

3.5.3. Procedures

All procedures should be detailed in written form as SOPs. The most important SOPs are as follows:

Procedure specific SOPs:

— Rules for cleaning the aseptic area;
— Rules for dressing in the aseptic area;
— Rules for preparation and sterilization of vials and stoppers for kit production;
— Rules for preparation and sterilization of other items and devices used in kit production;
— Rules for the weighing of chemicals and for making solutions in the aseptic area;
— Manner of pH setting;
— Rules for membrane filtering;
— Rules for dispensing solutions into vials;
— Manner of freeze-drying and container closure;
— Rules for the preparation of labels and labelling of vials;
— Packaging instructions.

Product specific SOPs:

— Order in which materials are to be added when making solutions;
— Individual prescriptions for pH and volumes to be dispensed;
— Freeze-drying protocol (temperature and vacuum as a function of time; these may be different functions depending on the product);
— Individual storage conditions.

3.5.4. Batch processing records

Batch processing records are to contain all the product specific step descriptions. The name of the product, date, batch number, and names of the operators and the responsible person are to be registered. Operators' signatures are required at every step. For those steps identified as 'important steps', such as weighing of components, two signatures are required: one from the operator carrying out the procedure and one from a second operator present and supervising the step. A separate table must be included in the batch processing record for notes on special problems, including technical details. If any deviation from the manufacturing formulas or from the SOPs occurs, the signed authorization of the responsible person is required.

Batch processing records contain attachments such as original records and diagrams. Label printing records are also required, containing information on how many empty labels, correctly printed labels and incorrectly printed labels (with errors) were made for each batch. Batch packaging records prove that packaging was carried out in an appropriate manner, registering how many vials were packed, how many boxes of the kit were produced and how many samples were delivered for QC.

3.6. PRODUCTION

Kit production is to be carried out according to clearly defined procedures as detailed in the SOPs and the batch processing record. Production must be performed and supervised by competent, well trained people.

All incoming materials are to be tested; during this time they must be kept in quarantine (indicated with a yellow label). After passing the test, they become 'approved' materials (indicated with a green label). Rejected materials are to be placed in a separate, closed area.

The general formulation process for kit preparation essentially consists of the following simple steps:

— Weighing and dissolution of stannous chloride in dilute hydrochloric acid;
— Weighing and dissolution of the ligand;
— Addition of the required amount of stannous chloride solution to the ligand solution with constant stirring;
— Adjustment of the pH to the required value using a pH meter;
— Sterile filtering of the above solution by membrane filtration (pore size: 0.22 μm);
— Dispensing of this solution aseptically into sterile vials;
— Freeze-drying.

The steps in the freeze-drying process are:

— Introducing the vials containing the dispensed solution into the freeze-dryer;
— Freezing the dispensed solution (alternatively, this can be done outside, either in a deep-freezer or by immersing the vials in liquid nitrogen and introducing the frozen samples into the freeze-dryer);
— Cooling the condenser to below –40°C;
— Evacuating the system to less than 13 Pa;
— Providing controlled heat input to the product during the freeze-drying cycle;
— Sealing the vials under vacuum or under nitrogen gas in the freeze-dryer after completion of the drying cycle.

The freshly made batches are placed in quarantine (indicated with a yellow label). Once the QC tests have been done and the products have met all acceptance criteria, the QP can release the batch, which is then clearly marked (with a green label) and placed in a separate, dedicated area. Rejected products are to be clearly marked (with a red label) and placed in a separate, closed area. The prescribed storage conditions are to be ensured in the pharmaceutical store.

To obtain products of reliable and reproducible quality, all process parameters must be continuously monitored, registered and properly recorded for every batch. Deviations are to be avoided.

All critical production steps must be identified and validated. For kit production, the following steps are generally considered to be critical:

— Sterile filtering of the solution containing the active ingredient and excipients;
— Dispensing of the sterile filtered solution;
— Freeze-drying.

Validation of sterile filtering

As an example, aqueous solutions of different volumes (100–1800 mL) and different pH levels (2.8–12) are filtered. The flow rate is set in the range of 35–100 mL/min. Microbiological tests of the filtered solutions are carried out. The acceptance criteria are that the chosen range of volume and the pH should not affect the microbiological purity (sterility).

Validation of dispensing

The number of samples for validation of dispensing can be determined as the square root of the batch size, which should not be fewer than ten vials for each dispensed volume. Samples containing 1 and 2 mL volumes are prepared by using a dispensing device, and the weights of these samples are measured. Precision, average, highest value, lowest value, median and deviation of the average from the median are determined. The acceptance criteria are that imprecision must be less than 1.5% and the relative standard deviation must be less than 1%.

Validation of freeze-drying

A heat distribution study must be performed on the empty chamber of the freeze-dryer. The temperature is measured on the trays at a minimum of five different points at three different temperatures (–40, –10 and +40°C). The acceptance criteria are that the average difference in the measured temperature between two measuring points must be less than ±2°C and the deviation must be less than ±4°C.

Vacuum leak testing should be done at –40°C. After switching off the vacuum, the pressure in the chamber is measured for up to 30 min. The acceptance criterion is that the rise of the pressure in the chamber must be not more than 500 μbar during a 30 min period.

3.7. QUALITY CONTROL

The QC unit is independent of production and carries out sampling and subsequently performs all tests included in the quality specifications of starting materials, bulk products (if any) and finished products. These quality specifications are component specific declarations about the description, manner of identification, content determination, storage conditions and expiry date of starting materials. Regarding final products, the quality specifications also

define the pharmaceutical form, labelling conditions and efficiency, sterility and apyrogenicity. Only those starting materials that have passed the tests described in the quality specifications are to be approved for production. Similar criteria are valid for finished products.

The starting material chemicals, stock solutions and ligands for use in kit preparation should be given an in-house batch number after acceptance by due analysis, and be labelled with the date of analysis and the date beyond which their use is not recommended. A complete record of their procurement, use, analysis and disposal is to be maintained in a logbook. In addition to the original container labels giving the manufacturer's batch number, the in-house identification numbers for use in kit preparation — such as the batch number, date of analysis and name of the manufacturing institution — should be affixed on the container to clearly indicate that the reagent is for use in radiopharmaceutical production. Since the quantities of reagents and ligands used in kit preparation are small, it may be advantageous to redistribute them in smaller vials, close them with an airtight seal and store them safely in order to avoid contaminating the bulk reagents and ligands by frequent handling. Once they have been identified for use in kit preparation, it is preferable to store them in safe custody for exclusive use in kits to avoid mixing them up with other chemicals.

QC is to be carried out according to procedure or test method specific SOPs. All analytical methods are to be validated, and all instruments used for the measurements are to be calibrated. The data of the different tests must be registered in analytical records, which should also include all original supplementary information and diagrams. Based on these data, the QC unit will issue an analytical certificate for each production batch; these certificates are clear and brief summaries of the analytical records indicating both the acceptance limits and values actually obtained.

Carrying out environmental monitoring of the production areas is also the responsibility of the QC unit. Some pharmacopoeias also require biodistribution and pharmacokinetic studies for the batches produced; carrying out or managing these investigations is the responsibility of the QC unit.

3.8. STABILITY TESTING

The stability of the kit depends on its contents, such as the active ingredient, reducing agent and excipients used in the formulation of the kit, as well as on the freeze-drying process used. Many of the above factors are optimized during the development of the kit in order to obtain highly stable products. A well formulated and well freeze-dried kit can be stable for up to a

year. The main factor affecting the stability of the product from batch to batch is the freeze-drying process. The main problem is the loss of Sn(II) content in the kit, leading to incomplete reduction of the pertechnetate. Degradation of the active ingredient is also seen in many products during storage. The residual moisture content plays a large role in determining the stability of the freeze-dried kits, and hence the moisture content is estimated immediately after completion of the production batch. A moisture content of less than 3% is not expected to contribute to product instability. Every batch of kits must be tested periodically for stability. To test the stability of the kits, they are reconstituted with the maximum recommended amount of pertechnetate solution, and the radiochemical purity is estimated by the recommended methods. The radiochemical purity of the formulated compound is monitored until the end of the recommended shelf life of the reconstituted product. This process is continued until the end of the shelf life of the kit itself. In the case of a loss of product integrity, a decision is taken to stop the supply of the kits and to recall the unused kits.

When stability studies are planned, the guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) can be taken into consideration. According to the ICH, three types of stability study for different sample storage conditions can be performed. These are illustrated in Table 3.3.

It is up to the manufacturer to decide whether long term stability studies are performed at 25 ± 2°C or 30 ± 2°C. If the latter condition is chosen, the intermediate study can be omitted.

In the case of products normally stored in the refrigerator, the long term stability study should be performed at 2–8°C for 12 months without controlling the relative humidity. At the same time, an accelerated study should also be performed with those products at 25 ± 5°C and 60 ± 5% relative humidity for 6 months.

To ensure the appropriate temperature and relative humidity, Climacell equipment should be used, with automatic control of the conditions.

TABLE 3.3. GENERAL TYPES OF STABILITY STUDY ACCORDING TO ICH GUIDELINES

Type of study	Storage conditions	Minimum time period (months)
Long term	25 ± 2°C, 60 ± 5% relative humidity	12
Intermediate	30 ± 2°C, 65 ± 5% relative humidity	6
Accelerated	40 ± 2°C, 75 ± 5% relative humidity	6

When the manufacturer recieves marketing authorization for the product, so-called post-marketing stability studies need to be performed periodically (e.g. every 2–5 a). Samples of these studies are to be stored under normal product storage conditions.

3.9. CONTRACT MANUFACTURE AND ANALYSIS

It is possible that certain production steps or analytical tests are not carried out in the manufacturer's facility. In these cases, a written contract is required between the manufacturer and the partner clearly defining the product quality specifications, production or analytical procedures, and any other conditions and validations. This contract will detail the responsibilities of the partner concerning the purchase of materials, production, performance of QC tests and release of products.

3.10. COMPLAINTS AND PRODUCT RECALLS

Written procedures are to be included in the manufacturer's QA system describing all the actions to be taken in the case of complaints. In this case, the QP plays the main role. In the case of complaints, detailed investigations are to be performed, followed by a written report. All customers have to be informed about the results of such investigation.

If the safety of patients might be endangered due to any uncertainties or failures concerning a pharmaceutical product, the batch must be promptly recalled. Thus, recall operations and actions should be available to be performed immediately at any time. All competent authorities of all countries involved (i.e. those to which the product may have been distributed) should be informed promptly.

3.11. SELF-INSPECTIONS

Self-inspections are to be carried out to monitor the implementation of and compliance with GMP. They should be performed by independent and competent persons employed by the manufacturer (such inspections are generally initiated by the QP and performed by the QA). All data concerning self-inspections are to be recorded and archived. The self-inspection records are evaluated by the regulatory authorities during GMP licensing as well as at licence renewal.

3.12. RELATIONSHIP BETWEEN GMP AND ISO REGULATION

On the one hand, GMP applies to pharmaceutical manufacture to ensure the most important issue, i.e. the safety of the patient. On the other hand, ISO standards may regulate all activities of a manufacturer, including R&D, commercial and logistical activities, and the 'company philosophy'. Thus GMP, GLP (good laboratory practice for preclinical studies) and even GCP (good clinical practice in human trials) can easily be inserted into the ISO standards, ensuring a complete system of total QA.

There are several useful publications on GMP applicable to medicinal products and kits that may be used as guidelines.

BIBLIOGRAPHY

EUROPEAN ASSOCIATION OF NUCLEAR MEDICINE, Guidelines on Current Good Radiopharmacy Practice (cGRP) in the Preparation of Radiopharmaceuticals, EANM, Vienna (2007), http://www.eanm.org/scientific_info/guidelines/gl_radioph_cgrpp.pdf

EUROPEAN COMMISSION, EU Guidelines to Good Manufacturing Practice, EUDRALEX, Vol. 4, Medicinal Products for Human and Veterinary Use: Good Manufacturing Practice, EU, Luxembourg (2003), http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/homev4.htm

HEALTH CANADA, Draft Guidance for Industry: Preparation of the Quality Information for Radiopharmaceuticals (Schedule C Drugs), Ottawa (2001), http://www.hc-sc.gc.ca/dhp-mps/brgtherap/applic-demande/guides/radiopharm/qisr-sdqr_guide_e.html

INTERNATIONAL ATOMIC ENERGY AGENCY, "Guidelines for good manufacturing practices of radiopharmaceuticals", Quality Assurance and Good Manufacturing Practices for Radiopharmaceuticals (East Asia Pacific Reg. Tech. Co-op. Proj. RAS 2/009), IAEA, Vienna (2001).

WORLD HEALTH ORGANIZATION, A WHO Guide to Good Manufacturing Practice (GMP) Requirements, WHO, Geneva (1997), http://www.who.int/vaccines-documents/DocsPDF/www9666.pdf

WORLD HEALTH ORGANIZATION, Supplementary Guidelines on Good Manufacturing Practices for Heating, Ventilation and Air Conditioning (HVAC) Systems for Non-Sterile Dosage Forms, WHO, Geneva (2005), http://www.who.int/medicines/services/expertcommittees/pharmprep/qas048rev2hvac_withoutfigs.pdf

4. GENERAL PROCEDURES FOR PRODUCTION OF KITS

The production of kits for the formulation of ^{99m}Tc radiopharmaceuticals is considered to be the manufacture of sterile medicinal products, according to current GMP. Therefore, it is subject to special requirements to minimize the risk of particulate, microbiological and pyrogen contamination. Minimization of this risk depends on the skill, training and attitudes of the personnel involved. Quality assurance is particularly important, and this type of manufacture must strictly follow the carefully established and validated preparation methods and procedures.

The following are general requirements for the aseptic preparation of kits:

— Components should be handled in at least a grade D environment after washing.
— Solutions are to be sterile filtered and should be prepared in a grade C environment.
— Handling and filling of aseptically prepared products should be done in a grade A environment with a grade B background.
— Following dispensing, the semi-sealed vials should be transferred to the freeze-dryer either in a grade A environment with a grade B background or in sealed transfer trays in a grade B environment.

Personnel involved in the aseptic process of production of kits for ^{99m}Tc labelling should strictly follow the hygiene standards established in the general GMP introduction. Only authorized personnel are allowed to enter the clean rooms where the aseptic process is carried out.

The process should be documented in the batch processing record. It is important that this record be filled in while the process is taking place, not after it has been completed. All records should be written clearly and confirmed by the signature and identification of the person who wrote them. When necessary, another staff member or a supervisor should check the records. All deviations from the routine should also be recorded, and their potential impact on the quality of the product should be evaluated.

The completed batch processing records should be stored for at least three years after expiry of the product to allow tracing of the history of the product and of the process and starting materials used for its manufacture.

4.1. BATCH PLANNING

In the production of kits, batch planning takes into consideration the capacity of the freeze-dryer, and the stability and shelf life of and expected demand for the kits. The size of the batch should be defined prior to the production process, and appropriate manufacturing formulas containing all the weights, concentrations, quantities and volumes of the starting materials needed for the specific batch size should be applied. Only one kit composition should be processed at a time. The starting materials — that is, the active substance, excipients and packaging materials — should be prepared in sufficient quantities. Each processed batch should be given a batch number, which will later help to identify the product. A sample batch record is given in Annex I.

4.2. WASHING AND STERILIZATION OF GLASSWARE AND STOPPERS

A grade D environment is required for washing of glassware and stoppers. After final sterilization, the glassware should be placed in a grade C environment for use in the preparation of bulk solutions. Sterilized glass vials and stoppers should be placed in a grade A environment with a grade B background shortly before filling.

Glass vials and glassware used for bulk solution preparation should be washed using hot purified water. Final washing should be done using water for injection. The vials and glassware are to be dried and then heated in an oven at 250°C for 3 h for depyrogenation. Rubber stoppers are washed in the same way as glass vials and then steam sterilized in an autoclave at 121°C for 30 min.

Elements of the dosing pump, glassware, tanks and tubing used for each batch of kits are treated in the same manner. To reduce the risk of improper handling, materials should be processed (with a small excess amount) for a single batch at a time. After sterilization, the materials should be used for production on the same day and not be stored for later use. The intervals between the washing, drying and sterilization of components, containers and equipment, as well as between their sterilization and use, should be minimized. If the materials are not used but are returned to storage, they must be washed and sterilized again before being used for another batch preparation.

Protective garments for use only in the aseptic area preferably should be washed in a separate washing machine and then steam sterilized and wrapped in paper packets. Sterilization records should be filed for each sterilization run and should be approved as a part of the batch release procedure.

A typical standard for steam sterilization is achieved at 121°C after 20–30 min at 106 kPa. It is important to refer to the manufacturer's instructions for operation, since exposure times can vary according to the design of the particular sterilizer.

4.3. STARTING MATERIALS

Only those materials released by the QC unit can be used for production, and they must be used within their prescribed shelf life. The required quantities should be calculated according to the planned batch size.

Weighing of starting materials

Starting materials should be weighed in clean and sterilized vials in a grade C environment. The weights of the materials should be recorded in the batch record, and the supervisor should check the records. The vials should be labelled with the name of the starting material, the name of the product and its batch number. The balance used for weighing should be regularly calibrated using standard weights.

4.4. PREPARATION OF BULK SOLUTIONS

Stock solutions for the manufacture of kits should be prepared in a grade C environment in a laminar flow bench. Generally, the following processes are involved in stock solution preparation:

— Weighing and dissolution of stannous chloride in dilute hydrochloric acid;
— Weighing and dissolution of the ligand (active substance);
— Weighing and dissolution of excipients;
— Mixing, heating and stirring of the dissolved ingredients;
— Adjustment of the pH to the required value.

The diluted solutions of NaOH or HCl used for pH adjustment should be freshly prepared in sterilized vials using water for injection. Only qualified pH meters should be used; these should be calibrated using the standard buffer solutions before batch processing. The results of readings obtained with the standard buffers (pH4, 7 and 11) should be recorded. Only the authorized person is allowed to adjust the settings of the pH meter.

The stock solution should be bubbled with sterile nitrogen gas to reduce the concentration of dissolved oxygen and prevent the oxidation of stannous tin. The nitrogen should be passed through a sterile filter.

Stirring of the stock solution is necessary to obtain a uniform concentration of components. The pH is usually a critical parameter for kits and should be checked only when the stirring has been stopped. Use of pH papers (colour indicators) is not recommended in the preparation of bulk solution.

The time between the start of the preparation of a solution and its sterilization by filtration should be minimized. A sample of stock solution should be taken for QC in order to determine the bioload of the solution before sterilization by filtration and to predict the efficiency of this sterilization using a microorganism retaining filter.

4.5. STERILE FILTRATION

Sterilization by filtration through 0.22 μm filters is performed using filter holders. Preferably, filter integrity will be checked before and after filtration to make sure that the filter has not been damaged during the process. The product should be filtered into a previously sterilized container located close to the filling point.

The same filter should not be used for more than one batch filtration (within one working shift).

4.6. DISPENSING

Sterilized stock solution should be dispensed into the sterile glass vials in a grade A environment with a grade B background. Usually the dispensed volume is 1 mL of stock solution. The dose of the dispensing unit (pipette, peristaltic pump, etc.) should be set to the required volume, and the precision of the dose should be checked by weight prior to dispensing as well as during (in the middle of the batch) and after filling. Directly after dispensing the solution into the vials, sterile stoppers should be placed on the vials (with the necks open for freeze-drying) and the vials should be placed on the precooled shelves of the freeze-dryer. If the freeze-dryer is not equipped with the desired precooling option, the kits should first be frozen and then placed in the freeze-dryer immediately before it is switched on. The freeze-drying cycle is started and carried out in the time required to complete the process. The parameters of freeze-drying are individually adjusted depending on the product. Freeze-dryer parameters such as pressure and temperature should be monitored during the

process. When the process is finished, the freeze-dryer chamber is filled with sterile nitrogen (or another inert gas). Closing of the stoppers is done automatically by shifting the shelves and pressing the stoppers in.

4.7. CRIMPING

The crimping device should be located in a grade B environment. The sealed vials containing the product are transferred from the freeze-dryer to the crimping device for capping. Sterilized aluminium caps are placed on the rubber stoppers and crimped. The quality of crimping should be checked. Usually the crimping process is accompanied by visual inspection of the vials containing the product.

4.8. SUMMARY OF IN-PROCESS CONTROLS

The environment in which aseptic operations are performed should be monitored frequently using methods such as volumetric air and surface sampling (e.g. swabs and contact plates) and settle plates. Sampling methods used during operation should not interfere with zone protection. Results from monitoring should be taken into consideration when reviewing batch documentation for finished product release. Surfaces and personnel should be monitored after critical operations. (See Table 3.2 for recommended limits for microbiological monitoring of clean areas during operation.)

The pH of the stock solution is a critical parameter influencing product performance; therefore, it should be strictly monitored during stock solution preparation.

As mentioned in Section 4.5, filter integrity is to be tested to confirm that the entire surface of the filter remains intact throughout the process. The methods used for this purpose are the bubble point, diffusive flow or pressure hold test. A filter integrity test should be performed on each processed batch.

The volume of dispensed solution is also critical for uniform distribution of kit components in the vials.

Inspection of freeze-dried product

Visual inspection: The crimped vials should be inspected individually for extraneous contamination or other defects. Visual inspection should be done under suitable and controlled lighting and background conditions. The results of the inspection should be recorded.

Rejected vials: Vials that have not been properly sealed and/or crimped, and those with visible cracks, are rejected and collected in a separate container. After the production process is finished, the rejected vials are destroyed. The batch yield is calculated and the results are recorded.

4.9. QUARANTINE

Properly crimped vials are placed in the containers and transferred to a quarantine area. The containers should be labelled with the product name, code, batch number and production date. The finished product should be stored in the quarantine area at the prescribed temperature (2–8°C). A number of samples sufficient for performing all required QC tests should be taken. The product containers should be moved to the finished product store when the QC is completed and the product is released for sale. The labels on the containers should indicate that the product has been released.

4.10. PACKAGING

Printed labels, carton boxes and package inserts should be taken from the starting material store in the quantity required for batch packaging. Information on printed material release (the QC release certificate number) should be available. Production personnel usually print the information regarding the product batch number and expiry date during packaging; it is necessary that the production supervisor verify this information.

The packaging process is recorded in the batch packaging record. Printed materials damaged during packaging are to be destroyed. Undamaged excess materials can be returned to the store of starting materials. The account of printed materials used for one batch must be balanced.

4.11. LEAVING THE PRODUCTION PREMISES

After completing the production process and before leaving each production zone, the personnel must clean up workbenches, and wash and disinfect surfaces such as the ceiling, walls and floor. Waste materials are to be removed from the clean area. Exchangeable elements of production equipment are to be removed from grade A, B and C areas to the grade D environment, where they are washed and dried.

BIBLIOGRAPHY

EUROPEAN ASSOCIATION OF NUCLEAR MEDICINE, Guidelines on Current Good Radiopharmacy Practice (cGRP) in the Preparation of Radiopharmaceuticals, EANM, Vienna (2007),
http://www.eanm.org/scientific_info/guidelines/gl_radioph_cgrpp.pdf

EUROPEAN COMMISSION, EU Guidelines to Good Manufacturing Practice, EUDRALEX, Vol. 4, Medicinal Products for Human and Veterinary Use: Good Manufacturing Practice, Luxembourg (2003),
http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/homev4.htm

WORLD HEALTH ORGANIZATION, A WHO Guide to Good Manufacturing Practice (GMP) Requirements, WHO, Geneva (1997),
http://www.who.int/vaccines-documents/DocsPDF/www9666.pdf

5. GENERAL PROCEDURES IN QUALITY CONTROL

QC measures are necessary to ensure that a product complies with all the requirements and specifications laid out for it. The QC unit should have well documented procedures for QC, which are to be undertaken for each starting material used for production as well as for finished products. It is suggested that the manufacturers refer to national pharmacopoeias, the USP, the EP or any other international pharmacopoeia when designing appropriate QC specifications and methods.

5.1. QUALITY CONTROL OF STARTING MATERIALS

All starting materials, including active pharmaceutical ingredients (active substances), excipients and primary packaging materials used for kit production, need to be approved before use. Generally, the starting materials — such as buffer salts and reducing agents — are used in many types of kit and are to be analysed when a new bottle is opened. The specifications for such substances are described in various pharmacopoeias. However, it should be borne in mind that ^{99m}Tc radiopharmaceuticals are a special class of products in which 'no carrier added' grade ^{99m}Tc is used to form a complex with ligands, most often in the presence of a reducing agent such as Sn^{+2} salts. The presence

of even small quantities of competing metal ions or oxidants could cause problems in the formation of the desired radiopharmaceutical. Thus it is difficult to provide complete specifications for all the starting materials with respect to the components that should not be present. Often, the use of high quality materials from reputed manufacturers is adequate to ensure good quality products. QA for the material that forms the radiopharmaceutical (along with the ligand and other materials, which are pretested) is advisable. A QC certificate from the manufacturer should be procured. Although the compliance certificate from the manufacturer may appear to be adequate, compliance with the rules laid out by the local regulatory authorities is desirable. Throughout the world, the laws governing the manufacture and sale of medical and pharmaceutical products are modified from time to time, becoming progressively more stringent and specific. In most countries, when a new product is manufactured for use in humans, all the starting materials are to be tested for their quality. This can be done by having the starting materials analysed at an approved laboratory; alternatively, the QC analysis can be done in the manufacturer's own laboratory. The quality of all the materials should comply with the specifications in the pharmacopoeias or recommended by the regulatory body of the country. The vials and rubber closures should be purchased from approved manufacturers, and a certificate of quality compliance should be obtained and archived (see Section 6).

5.2. QUALITY CONTROL OF ACTIVE SUBSTANCES USED IN THE KIT

Many of the active substances used for kit manufacture can be purchased from commercial sources. However, certain active ingredients are synthesized in the manufacturing facility. Regardless of the source, they must undergo QC analysis before being used to produce kits. Chemical identity, purity and amount (or assay) of the ligand are the three most important criteria, and these need to be ascertained by identification of the ligand, estimation of the purity of the preparation and assay of the amount of the ligand in the starting material.

5.2.1. Identification

To identify the ligand in the starting material, one or a combination of several analytical methods — such as melting point determination, elemental analysis, IR spectroscopy, UV/VIS spectroscopy, NMR spectroscopy, mass spectroscopy and HPLC analysis — should be used. If the ligand is synthesized

in-house, the progress and completion of the reaction need to be monitored, which again is done with the aid of the above techniques. Often, a few independent analytical techniques need to be used to prove the identity of the product unequivocally. It is desirable to keep a set of typical analysis results in the monograph file for each product. The specific absorption pattern (IR, UV/VIS or NMR spectroscopy) of each molecule could act as a 'fingerprint' for identifying the molecule and ensuring the absence of impurities in the preparation. A brief description of the information available from different analytical methods is given below:

- Melting point determination is a simple method often used to confirm the identity of the substance, although not for establishing it unequivocally.
- Elemental analysis is a tool for ascertaining the identity of the product. Although the values for elemental composition vary to some extent, it is a useful technique for proving the identity when other techniques fail to provide sufficient information.
- IR spectroscopy is based on the selective absorption of energy in the IR region by asymmetric bonds such as C=O (vibrational transitions). Thus, the presence of such asymmetric IR active groups is identified and quantified using IR spectroscopy. IR spectroscopy is also an important technique for monitoring the progress of synthesis and ensuring the absence of impurities, when such groups are involved.
- UV/VIS spectroscopy is based on the absorption of light in the UV/VIS region by the molecule as a result of electronic transitions. In the presence of conjugated double bonds, metal complexes where d-d transitions occur in the UV/VIS region are often identified and quantified by the UV/VIS spectrum.
- NMR spectroscopy is based on the absorption of energy by the nucleus of atoms that have net nuclear magnetic moment. Some of the most widely used NMR active atoms are ^{1}H, ^{13}C and ^{31}P. The pattern of the energy absorption (chemical shift) is indicative of the chemical environment of the atom, and hence of the groups present. NMR patterns are very useful in determining structures, confirming reactions and determining the purity of the preparation. Proton NMR is the most widely used, as nearly all the molecules contain a large proportion of ^{1}H, and a quick NMR can indicate the distribution of various kinds of hydrogen atoms in the molecule. On the other hand, ^{13}C and ^{31}P NMR give information on the number and distribution of carbon and phosphorus atoms, respectively. Most of the time, a 'fingerprint' ^{1}H NMR spectrum is adequate to prove the identity and purity of a ligand. However, the presence of very similar compounds or precursors cannot be completely ruled out by perusing the

^{1}H NMR alone. Also useful for establishing the identity of the compound/ligand are ^{13}C NMR and ^{31}P NMR (when phosphorus is present in the ligand, which is not always the case).

— Mass spectrometry is a well established technique based on the principle that when a charged particle passes through a magnetic field, it is deflected along a circular path of a radius that is proportional to the mass to charge ratio. Mass spectrometry is a powerful tool that can distinguish a difference of just one mass number in the molecules. Typically, a small amount of the substance is injected into the sample port, where it is vaporized and ionized. The deflection/path of the ions is used to calculate the mass. Since under ionization conditions many cleavages take place, mass spectra are invariably a set of lines corresponding to a series of ions, the highest giving a clue as to the mass of the molecule and the others corresponding to the various ions formed by cleavages of labile groups in succession. Thus, it is possible to logically prove the identity of the compound. Although mass spectroscopy is a useful tool for identifying and testing the purity of a compound, difficulties associated with purchasing and maintaining such an expensive instrument limit its use. More often, laboratories have the samples analysed at an institute with a mass spectrometer.

5.2.2. Purity

The ligand can be contaminated with trace amounts of unreacted starting materials used for synthesis and with degradation products formed during storage. Thus the estimation of impurities in the substance is important. The tests used to prove the identity of the substance can also give valuable information about the purity of the product.

HPLC is the most commonly used technique for estimating such impurities. It is a very sensitive tool for separation based on different physico-chemical characteristics (e.g. residual charge on the various groups in the molecule, lipophilicity/hydrophilicity, molecular weight). The compounds are separated from the chromatographic columns by elution at different retention times. The separation is facilitated by the use of a variety of columns such as C-18, PRP and silica gel, as well as by the choice of an appropriate solvent system. The use of a combination of solvents of different polarities and of gradient elution is often required to obtain complete separation of the ligand from closely related substances.

5.2.3. Assay

Assaying the exact amount of the ligand is vital for preparation of the kit vials. A quantitative estimate of the ligand can be done by simple gravimetry, if the ligand is of high purity. In the preparation of kits for technetium radiopharmaceuticals, the ligands are used in the range of milligrams per vial, which translates into handling gram quantities per batch. With the availability of weighing balances that can weigh a tenth of a milligram with high accuracy, gravimetry is the simplest, most ideal mode of quantification. However, if the ligand is present with salts, it must be assayed. Ideally, any of the methods used for testing the purity can also be used for the assay. Standards of known concentration/amount are used for calibration/comparison. Spectrophotometry (UV/VIS or IR) is an easy method frequently used for assaying the ligands.

5.3. QUALITY CONTROL OF FINISHED KITS

QC analysis of the finished kit starts with sampling from the lot, followed by different types of analysis including visual inspection of the kit vials and analytical tests of kit content dissolved in saline or water for injection and of the ^{99m}Tc labelled product.

5.3.1. Sampling

Sampling of the finished product for QC is an important step to ensure that the samples collected are representative of the batch. QC personnel should randomly pick 2% of the total number of vials or a minimum of 20 vials, whichever is greater, for testing. Pharmacopoeias give guidelines for the number of samples needed for sterility testing. USP 30 requires 10 vials for sterility testing when the batch size is 100–500, and 2% or 20 vials for larger batches, whichever is less.

5.3.2. Control of pharmaceutical form

The physical appearance of each of the reagents, the solubility of the ligand and other reagents in the defined medium, and the pH of the dissolved product are tested to ascertain the product's compliance with requirements. At this stage, a failure of any of the parameters to comply with requirements, such as non-dissolution of the contents or presence of suspended particles in the vial, indicates that the ligand has undergone some degradation either during storage

or during the freeze-drying cycle. Similarly, a pH value far outside the expected range indicates erroneous reagent preparation at the production end. Whenever the values are not within the expected range, the tests are to be repeated and the quality of the reagents used for testing, as well as the glassware, needs to be checked. Whenever multiple components from different vials are used for kit formulation, the test should be done in each component vial and then in the final product.

5.3.3. Sn(II) content assay

Almost all kits for technetium radiopharmaceuticals employ stannous ions to reduce the technetium from +7 to the desired oxidation state. In most cases, the amount of Sn(II) is not critical. However, in certain cases it is crucial to maintain a minimum Sn(II) level, as very low amounts of Sn(II) will result in inadequate oxidation of technetium, while high amounts will damage the compound formed. One such example is the kit for ^{99m}Tc-HMPAO.

It is necessary to measure the Sn(II) content in the kit vial. Estimation of the Sn(II) content could be carried out by simple methods such as titration with iodine or N-bromosuccinimide. However, interference owing to the presence of other reducing agents is possible, and it is necessary to ensure that such interference does not occur. Alternatively, polarography could be used. Iodometry is very sensitive (up to 1.5 ppm levels) and is often used for Sn(II) estimation. Certain laboratories employ spot test methods (such as the one based on ammonium phosphomolybdate colour correlated to stannous content) for semi-quantitative estimation of Sn(II) in order to ensure that the levels are within prescribed limits. Methods of assaying Sn(II) content are discussed in Annex V. A limit for Sn(II) contamination is often fixed by the manufacturer, for example, not less than 50% of the quoted value at the expiry of the kit.

5.3.4. pH of reconstituted kit

Since radiolabelling depends on the pH level, and since the quality of the product is affected by the quality of the reagents used in its preparation, checking the pH level of the reconstituted kit gives an idea of whether or not the reagents used were of the right quality.

5.3.5. Microbiological safety

Tests for sterility and the absence of pyrogens are used to ensure the microbiological safety of a product. However, since ^{99m}Tc radiopharmaceuticals are constituted using the reagents provided in the kit, any breach of

sterility or apyrogenicity can in turn be due to the presence of microbial organisms in one or more of the reagents. Hence, in the case of kits, each reagent needs to be certified for microbiological safety. The microbiological safety of the product is established by conducting sterility tests and tests for bacterial endotoxins. These tests are conducted on each component of the kit when a fresh batch is made.

Sterility testing

Conventional tests for sterility are well established. The tests can be carried out by membrane filtration of the product or by direct inoculation of the culture medium with the product to be examined. Direct inoculation involves aseptic transfer of the contents of the vial into two kinds of growth medium — soybean casein digest medium (SCDM) and fluid thioglycollate medium (FTM) — to determine if the product is free of viable bacterial and fungal contamination. These broths are incubated for 14 d, as required by the USP (or for the period required by the regulatory requirements of the country), and inspected for evidence of bacterial and fungal growth. Briefly, the contents of the vial to be tested are reconstituted with tested sterile solvent (water or saline, as necessary), and an aliquot (typically 100 µL) is taken with a syringe and inoculated into the media, taking care to use sterile glassware and carrying out the work in a clean work area, such as a laminar flow bench/hood. The agar plates and the tubes are covered, placed in an incubator at 37ºC and monitored daily for any growth for 14 d. The absence of growth indicates adequate sterility of the product. The membrane filtration technique is recommended for filterable aqueous preparation, as is the case with kits.

Bacterial endotoxin test

Conventionally, the absence of pyrogens was tested by injecting an aliquot of the preparation into rabbits and watching for any increase in their body temperature. If gram-negative bacteria are present in a product, they are destroyed during sterilization, and the endotoxins are released from their cell walls. A bacterial endotoxin test (BET) using Limulus amebocyte lysate (LAL) is now used to determine the presence of endotoxins much faster and with far greater sensitivity than the rabbit based pyrogen test. Briefly, the BET is based on the principle that the bacterial endotoxins react with LAL and form a gel-like precipitate. The pyrogenicity is expressed in BET units; the limits for BET are well established for various products and depend on the volumes generally injected into patients. For example, vehicles such as saline and water for injection have a very low limit of 0.25 BET units, whereas a finished radiopharmaceutical

product that is expected to be injected in small volumes has a limit of 25 BET units. This test needs to be carried out in a clean environment in a laminar flow hood. Appropriate amounts of the product to be tested (depending on the limits set) are allowed to react with the LAL reagents, along with positive and negative controls, and are monitored for the formation of gel at the end of the incubation period (typically 30 min). The absence of gel formation indicates the absence of bacterial endotoxins.

Annex VII discusses sterility testing; environmental monitoring is discussed in Annex VIII; and Annex IX discusses the bacterial endotoxin test.

5.3.6. Radiochemical purity

The radiochemical purity of the labelled product is generally estimated with a chromatographic technique such as paper chromatography, thin layer chromatography, HPLC or column chromatography. Other techniques such as paper electrophoresis are also used to estimate the radiochemical purity of ^{99m}Tc radiopharmaceuticals. (See Section 7 for specific QC tests for estimating the radiochemical purity of individual radiolabelled kits.)

5.3.7. Biodistribution

The biodistribution of the radiopharmaceutical is an important criterion for product utility. It is ascertained by biological QC, where the radiolabelled product is administered to an appropriate small animal (rat or mouse) and the biodistribution is examined at the end of a prescribed time interval. Such biological control is essential when a product is first introduced on the market until consistent results are obtained for several consecutive batches. After approval, the tests may be performed at a frequency required by the regulatory authority. However, in a few cases, such as with mebrofenin or MDP, biodistribution control studies are carried out on every batch. Details on conducting biodistribution studies are given in Annex X.

BIBLIOGRAPHY

BRAITHWAITE, A., SMITH, F.J. (Eds), Chromatographic Methods, Chapman & Hall, London (1995).

EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, European Pharmacopoeia, 5th Edn, EDQM, Council of Europe, Strasbourg, France (2005).

GORDON. M.H., MacRAE, R. (Eds), Instrumental Analysis in the Biological Sciences, Springer, New York (1989).

HARRIS, D.C. (Ed.), Quantitative Chemical Analysis, 7th Edn, W.H. Freeman, New York (2007).

ROBBINS, P.J., Chromatography of Technetium-99m Radiopharmaceuticals: A Practical Guide, Society of Nuclear Medicine, New York (1984).

SAHA, G.B. (Ed.), Fundamentals of Nuclear Pharmacy, 4th Edn, Springer, New York (2004).

SKOOG, D.A., HOLLER, F.J., NIEMAN, T.A. (Eds), Principles of Instrumental Analysis, 5th Edn, Saunders College Publishing, New York (1998).

THEOBALD, A.E., "Quality control of radiopharmaceuticals", Textbook of Radiopharmacy: Theory and Practice, 2nd Edn (SAMPSON, C.B., Ed.), Gordon and Breach, Amsterdam (1999).

UNITED STATES PHARMACOPEIAL CONVENTION, United States Pharmacopeia (USP) 25–29, USP Convention, Rockville, MD (2002–2006).

ZOLLE, I. (Ed.), Technetium-99m Pharmaceuticals: Preparation and Quality Control in Nuclear Medicine, Springer, Berlin and Heidelberg (2007).

6. QUALITY SPECIFICATIONS

Quality specifications for the premises, equipment, supplies and processes are important elements of a kit production facility design that conforms with GMP. Kit production must employ all aspects of aseptic processes, because the final product will not be terminally sterilized. In the manufacturing process, a presterilized product is combined with a presterilized container that is then closed with a presterilized closure inside a grade A clean room. Major components of the facilities needed for GMP manufacturing, such as clean rooms and equipment, are discussed in detail in Sections 3 and 4. The operational parameters of a GMP controlled area are discussed here.

6.1. CLEAN ROOMS

Clean rooms are controlled environments where parameters such as airflow, microbiological and particulate quality of air, equipment, surfaces and garments (dress, shoes, gloves, hat and mask) are controlled. All the above need to be monitored and kept within the specified limits (see Tables 3.1 and 3.2). The flow of personnel and material, and the operation within the premises, should be controlled to minimize the risk of contamination. Operations in a clean room are to be well defined, with strict adherence to SOPs. A system for cleaning and disinfecting the room and equipment should be enforced. Microbiological evaluation should be carried out periodically to control the aseptic process in a clean room, and this process should be validated.

6.2. FREEZE-DRYING

Freeze-drying is the method of choice for preserving a wide variety of healthcare products, including kits. Freeze-drying ensures the overall stability of the product, inhibits bacterial growth and facilitates transport. Also important, it helps to ensure the stability of the stannous ion in the kit.

In the freeze-drying process, the contents are first frozen, the surrounding pressure is reduced and enough heat is added to allow the frozen water in the material to sublimate directly from the solid to the gas phase. There are three stages in the freeze-drying process: freezing, primary drying and secondary drying. Freezing is preferably done using a freeze-dryer with a built-in cooling facility, although occasionally material is frozen outside the freeze-dryer. In this step, it is important to freeze the material at a temperature below its eutectic point (i.e. the temperature at which all the three phases — solid, liquid and gas — coexist) to ensure that sublimation rather than melting occurs in subsequent steps.

During the primary drying phase, a vacuum is applied to ensure that the water in the substance sublimates. In this initial drying phase, which is slow, about 98% of the water in the material is sublimated, and pressure and temperature are carefully controlled. A condenser chamber is built into the freeze-drying unit to allow the removed water vapour to resolidify. This condenser prevents water vapour from reaching the vacuum pump, thus ensuring the pump's performance. Condenser temperatures are typically kept below –50°C.

The secondary drying phase aims to sublimate the water molecules that are adsorbed during the freezing process (the mobile water molecules are sublimated in the primary drying phase). In this phase, the temperature is

raised even higher than in the primary drying phase to break any physico-chemical interactions that may have formed between the water molecules and the frozen material. Usually, the pressure is also lowered in this stage to encourage sublimation. After the freeze-drying process is complete, the chamber of the freeze-dryer is filled with an inert gas such as nitrogen before the vials are sealed.

The general procedure for validation of the freeze-dryer is specified in Section 3.4.2.

6.3. PACKING MATERIALS: VIALS, STOPPERS AND CAPS

Kit components are dispensed in type I neutral borosilicate glass vials (clear or amber) with a capacity of 5–10 mL. Specially designed rubber stoppers with split ends must be used for freeze-drying. This kind of closure functions as a vent, allowing water vapour to escape during freeze-drying. It also allows easy penetration with a syringe needle for reconstitution of the kits, for labelling with ^{99m}Tc-pertechnetate solution and for subsequent use. Grey butyl rubber is the starting material recommended for stopper manufacture.

The vials and stoppers should be certified under USP or EP specifications, or should meet the legal standards of the country where the kits are produced. The containers may be washed using a sequence of deionized water and steam, followed by final rinsing with water for injection. The washing equipment may be batch type, where containers are washed in a rack, or continuous, where containers are washed individually in a continuous washer. The glassware is then sterilized and depyrogenated using dry heat in an oven at 170–300ºC. Rubber stoppers must first be washed to remove particulates and any residues left over from the stopper manufacturing operation. Washing is often a batch operation in which the stoppers are held in a basket that allows water to flow around them. Finally, the stoppers must be rinsed with water for injection. After cleaning, they are siliconized and steam sterilized in an autoclave; alternatively, they can be dried in an oven at 80–90ºC and sterilized by gamma irradiation. A cleaning and sterilizing protocol should be established. Aluminium seals with standard openings are used to ensure a perfect fit of the closures.

6.4. WATER FOR INJECTION

Water is one of the critical elements of kit preparation. It is used not only for formulating products, but also for cleaning, rinsing and sterilizing materials.

Therefore, regulatory standards set by the USP or EP strictly regulate the quality of water for pharmaceutical use, water for injection (Ref. [6.1], p. 3473; Ref. [6.2], p. 2692) and purified water (Ref. [6.1], p. 3474; Ref. [6.2], p. 2697). The producer must be committed to validating its water system according to these quality standards, because sterile water for injection is a starting material for kit manufacturing throughout the world.

The USP allows water for injection to be made from tap water by distillation or a purification system that is equivalent or superior to distillation in the removal of chemicals and microorganisms. Water for injection to be used for manufacturing freeze-dried kits must be a clear, colourless and odourless liquid that is sterile, deionized, free of particulate matter and free of endotoxins. USP 29, and later USP 30, proposed new specifications for water for injection relating to quantitative conductivity and total organic carbon (TOC). The impetus behind these changes was to create a means of analysis that is less laborious and more reflective of the water quality. The USP and EP recommended specifications of water for injection are shown in Table 6.1.

6.5. NITROGEN GAS

Freeze-drying equipment for kit production must employ the procedures for closing the vials under an inert atmosphere at the end of the process in order to stabilize the stannous ion and ensure the shelf life of the kit. Nitrogen

TABLE 6.1. CHARACTERIZATION OF WATER FOR INJECTION

Parameter	USP 30	EP Addendum 2001
Origin	Potable water/CEE or EP regulations	Potable water/CEE or EP regulations
Preparation method	Distillation or equivalent method	Distillation
Conductivity	≤1.3 μS/cm at 25°C	≤1.1 μS/cm at 20°C
TOC	≤500 ppb	≤500 ppb
NO_3/NO	—	≤0.2 ppm
Heavy metals	—	≤0.1 ppm
Aluminium	—	≤10 μg/L
Endotoxins	≤0.25 EU/mL	≤0.25 EU/mL
Microbial contamination	≤10 CFU/100 mL	≤10 CFU/100 mL

Note: CFU: colony-forming unit; EU: endotoxin unit.

gas is usually used as an inert gas for this purpose. It is also used to prepare oxygen free water for injection by bubbling the water before and during the bulk solution preparation to eliminate possible oxidation of the stannous ion (Ref. [6.1], p. 1167). This is especially important when the formulation of the kit contains only a few micrograms of tin. It is recommended that the gas be filtered through a 'gas drying unit' and a sterile hydrophobic filter in line with a sterile 0.22 μm pore size filter to remove microbial contamination and to eliminate humidity.

6.6. COMMONLY USED CHEMICALS

The active ingredient of the kit and the freeze-drying process used influence the product formulation design. Freeze-dried preparations have some adjunct materials that are generally used for the kit formulations. Some of these materials are classified as excipients, for example:

— Stannous chloride dihydrate, used as a reducing agent; use ACS reagent grade (Ref. [6.1], p. 795).
— Ascorbic acid, used for stabilizing the reducing agent; use ACS reagent grade (Ref. [6.1], p. 1441).
— Sodium chloride, used as an excipient for isotonicity; use ACS reagent grade (Ref. [6.1], pp. 792 and 3189).

Other substances are also used during the preparation of the kit, for example:

— Hydrochloric acid, used for adjusting the pH; use ACS reagent grade (Ref. [6.1], pp. 773 and 1132).
— Sodium hydroxide, inorganic base used for adjusting the pH; use ACS reagent grade (Ref. [6.1], pp. 793 and 1211).

All the above chemicals are commercially available and must be obtained from recognized sources having certified conformity to pharmacopoeia reagent specification and official monographs.

6.7. OTHER EXCIPIENTS

There are a number of agents for controlling the pH level, beyond inorganic acids and bases such as hydrochloric acid and sodium hydroxide.

Organic acids such as the amino acids glycine and arginine may be used. The formulation can also utilize a buffering system such as phosphate (monobasic and dibasic sodium phosphate) and acetate buffer (sodium acetate and acetic acid). Each buffering system has its own advantages and influences freeze-dried preparations. Bulking agents such as mannitol (Ref. [6.1], p. 2544) are used as excipients in some kit formulations to provide a sufficient mass of substances to achieve a well-shaped pellet of the freeze-dried product.

REFERENCES TO SECTION 6

[6.1] UNITED STATES PHARMACOPEIAL CONVENTION, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006).

[6.2] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINE, European Pharmacopoeia, 5th Edn, EDQM, Council of Europe, Strasbourg (2006).

BIBLIOGRAPHY

EUROPEAN ASSOCIATION OF NUCLEAR MEDICINE, Guidelines on Current Good Radiopharmacy Practice (cGRP) in the Preparation of Radiopharmaceuticals, EANM, Vienna (2007),
http://www.eanm.org/scientific_info/guidelines/gl_radioph_cgrpp.pdf

EUROPEAN COMMISSION, EU Guidelines to Good Manufacturing Practice, EUDRALEX, Vol. 4, Medicinal Products for Human and Veterinary Use: Good Manufacturing Practice, Luxembourg (2003),
http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/homev4.htm

HEALTH CANADA, Draft Guidance for Industry: Preparation of the Quality Information for Radiopharmaceuticals (Schedule C Drugs), Ottawa, Ontario (2001),
http://www.hc-sc.gc.ca/dhp-mps/brgtherap/applic-demande/guides/radiopharm/qisr-sdqr_guide_e.html

WORLD HEALTH ORGANIZATION, A WHO Guide to Good Manufacturing Practice (GMP) Requirements, WHO, Geneva (1997),
http://www.who.int/vaccines-documents/DocsPDF/www9666.pdf

7. PRODUCTION METHODS AND SPECIFICATIONS FOR KITS

Table 7.1 lists the products detailed in this publication. Specific protocols for preparation of the kits are elaborated in the same order in this section.

GENERAL COMMENTS

- It is expected that the products described in this publication will be produced in manufacturing facilities following GMP. Production of the kits in clinical radiopharmacies is not recommended.
- Matters such as national regulations and intellectual property rights should be considered before the products described in this publication are commercialized.
- Multiple formulations are available for the production of different kits. The most commonly used formulations having a record of satisfactory performance in manufacturing facilities are included here.
- The kits are inactive products; however, QC will involve the preparation of radiolabelled products, which involves the handling of radioactivity. All necessary precautions, such as the availability of an appropriate laboratory, shielded hoods, workbenches and radiation protection monitoring, need to be taken care of in accordance with the Basic Safety Standards[2], the IAEA radiation safety standards in nuclear medicine[3], and national regulations.
- Many of the products described in this publication are mentioned in the EP or USP. The QC procedures discussed here are harmonized with the

[2] FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, INTERNATIONAL ATOMIC ENERGY AGENCY, INTERNATIONAL LABOUR ORGANISATION, OECD NUCLEAR ENERGY AGENCY, PAN AMERICAN HEALTH ORGANIZATION, WORLD HEALTH ORGANIZATION, International Basic Safety Standards for Protection against Ionizing Radiation and for the Safety of Radiation Sources, Safety Series No. 115, IAEA, Vienna (1996).

[3] INTERNATIONAL ATOMIC ENERGY AGENCY, INTERNATIONAL LABOUR OFFICE, INTERNATIONAL ORGANIZATION FOR MEDICAL PHYSICS, PAN AMERICAN HEALTH ORGANIZATION, WORLD FEDERATION OF NUCLEAR MEDICINE AND BIOLOGY, WORLD HEALTH ORGANIZATION, Applying Radiation Safety Standards in Nuclear Medicine, Safety Reports Series No. 40, IAEA, Vienna (2005).

descriptions in the pharmacopoeias to the greatest extent possible. For products not described in the pharmacopoeias, information from the contributors to this report based on their experience is given.

— Many regulators will require an assay of the main ingredients of the kit, such as the active ingredient and Sn(II) content.
— Most manufacturers quote 50% as the lower limit for Sn(II) content. However, this is indicative, and for products with very low Sn(II) content, such as HMPAO, problems may arise if the Sn(II) content is 50%.
— The general recommendation is for storage at 2–8ºC; however, the stability of each kit should be established at the manufacturing centre.
— Estimation of the eutectic temperature is important for developing a freeze-drying cycle to obtain a good quality product. The eutectic point given for different products is that measured by the contributors to this report. It is advisable that the eutectic point of each product be measured at the user's laboratory and that the freeze-drying cycle be optimized.
— Residual humidity in freeze-dried kits is a major reason for poor kit stability. The requirement set out by most manufacturers is 3%, which can serve as a guidance figure. The residual humidity can be estimated by using an appropriate test.
— The values for maximum radioactivity mentioned for reconstitution of the kit are indicative and need to be checked by assessing the quality of the labelled product.
— The stability of the labelled product given here is indicative and needs to be assessed.
— While carrying out the biodistribution studies, national regulations on animal handling and ethics should be followed.
— Adequate caution is required when biological products of human or animal origin are used in the kit. A pyrogen test in rabbits is mandatory when using human or animal products.
— For several of the products, biodistribution is not mentioned in the USP or EP. When guidance is not available in these pharmacopoeias, indicative values based on the experience of the contributors to this report are provided.
— GMP rules are changing and need to be updated to reflect the current regulations. The user must follow national requirements.
— Most kit manufacturers use active ingredients from commercial manufacturers. Most of these products are not meant for human use. Adequate precautions should be taken when using these starting materials so that the procedure is acceptable to the regulators.
— Adequate caution is required when using commercial materials not prepared under GMP as starting materials for radiopharmaceutical

production. GMP regulations require qualification of the starting materials in such cases.

7.1. PREPARATION OF KIT FOR ^{99m}Tc-MDP

7.1.1. Reagents

— Methylene diphosphonic acid (MDP);
— Ascorbic acid;

TABLE 7.1. KITS DETAILED IN THIS PUBLICATION

Section	Kit
7.1	^{99m}Tc-MDP
7.2	^{99m}Tc-HMDP
7.3	^{99m}Tc-pyrophosphate
7.4	^{99m}Tc-DTPA
7.5	^{99m}Tc-glucoheptonate
7.6	^{99m}Tc-MAG_3
7.7	^{99m}Tc-EC
7.8	^{99m}Tc-DMSA(III)
7.9	^{99m}Tc-DMSA(V)
7.10	^{99m}Tc-mebrofenin (Bromo-HIDA)
7.11	^{99m}Tc-EHIDA
7.12	^{99m}Tc-phytate
7.13	^{99m}Tc-sulphur colloid
7.14	^{99m}Tc-tin colloid
7.15	^{99m}Tc-rhenium-sulphide colloid
7.16	^{99m}Tc–human serum albumin (HSA) nanocolloid
7.17	^{99m}Tc–human serum albumin (HSA) colloid
7.18	^{99m}Tc-microspheres
7.19	^{99m}Tc–human immunoglobulin
7.20	^{99m}Tc-ECD
7.21	^{99m}Tc-d,l–HMPAO
7.22	^{99m}Tc-MIBI
7.23	^{99m}Tc-tetrofosmin

— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.1.2. Chemical composition of kit

— MDP: 10 mg;
— Stannous chloride dihydrate: 1 mg;
— Ascorbic acid: 2 mg.

7.1.3. Manufacturing formulas

Final volume (mL)	MDP (g)	Ascorbic acid (g)	Stannous chloride dihydrate (g)
100	1.0	0.2	0.1
250	2.5	0.5	0.25
500	5.0	1.0	0.5
800	8.0	1.6	0.8
1000	10.0	2.0	1.0

7.1.4. Preparation of kit solution for a final volume of 500 mL

— Use water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 500 mg of stannous chloride dihydrate using 50 mL of 0.2N HCl (or 0.4 mL of concentrated HCl, adjusting the volume to 50 mL) just before it is added to the final solution.
— Dissolve 5 g of MDP in approximately 400 mL of water for injection.
— Add 1 g of ascorbic acid; the pH will be in the range of 3.5–4.0 after the addition.
— Slowly add solution A to the MDP solution, with continuous N_2 bubbling and stirring.
— Adjust the pH to between 4 and 5 using 1N NaOH or 1N HCl.
— Adjust the final pH to 5.8–6.0 using a pH meter.
— Adjust the final volume to 500 mL.
— Filter the solution through a sterile 0.22 μm filter.
— Dispense 1 mL per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Dried temperature	Time
–30ºC	24ºC	24–48 h

7.1.5. Storage

Store refrigerated at 2–8ºC.

7.1.6. Radiolabelling

— Reconstitute the freeze-dried kit using 4 mL of freshly eluted $^{99m}TcO_4^-$ solution containing a maximum of 500 mCi (18.5 GBq) of activity.
— Stir for 1 min and use after 5 min.
— The ^{99m}Tc-MDP labelled in this manner should be stable for over 6 h after labelling.

7.1.7. Labelling features

— MDP: 2.5 mg/mL;
— Stannous chloride dihydrate: 0.25 mg/mL;
— pH: 5–7;
— Radiochemical purity: >95%;
— Pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <5%.

7.1.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper	ITLC-SG
Solvent	MEK/acetone	Saline
R_f ^{99m}Tc-MDP	0.0	0.9–1.0
R_f $^{99m}TcO_4^-$	0.9–1.0	0.9–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Note: MEK: methyl ethyl ketone.

Main ingredients content: Determination of the content of MDP may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value.

A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-MDP in mice at 2 h post-injection is as follows:

Organ	%i.d./organ	%i.d./g
Bone (femur)	≥60	≥2
Liver	≤3	≤1
Kidneys	≤5	≤1

Note: i.d.: percentage of injected dose.
The USP and EP do not require a biodistribution test for ^{99m}Tc-MDP. The values given here are for guidance only.

[7.1.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) medronate injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 859.

[7.1.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) medronate injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3281–3282.

7.2. PREPARATION OF KIT FOR ^{99m}Tc-HMDP

7.2.1. Reagents

— Hydroxymethylenediphosphonic acid (HMDP, HDP);
— Ascorbic acid;
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.2.2. Chemical composition of kit

— HMDP: 2 mg;
— Stannous chloride dihydrate: 0.5 mg;
— Ascorbic acid: 2 mg.

7.2.3. Manufacturing formulas

Final volume (mL)	HMDP (g)	Ascorbic acid (g)	Stannous chloride dihydrate (mg)
100	0.2	0.2	50
250	0.5	0.5	125
500	1.0	1.0	250

7.2.4. Preparation of kit solution for a final volume of 250 mL

— Use water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 125 mg of stannous chloride dihydrate using 50 mL of 0.2N HCl (or 0.4 mL of concentrated HCl, adjusting the volume to 50 mL) just before it is added to the final solution.
— Dissolve 0.5 g of HMDP in approximately 150 mL of water for injection.
— Add 0.5 g of ascorbic acid.
— Slowly add solution A to the HMDP solution, with continuous N_2 bubbling and stirring.
— Adjust the pH to between 4 and 5 using 1N NaOH or 1N HCl.
— Adjust the final pH to 5.8–6.0 using a pH meter.
— Adjust the final volume to 250 mL.
— Filter the solution through a sterile 0.22 μm filter.
— Dispense 1 mL per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Primary drying temperature	Dried temperature	Time
–30ºC	–3ºC	24ºC	24–48 h

7.2.5. Storage

Store refrigerated at 2–8ºC.

7.2.6. Radiolabelling

Reconstitute the freeze-dried kit using 4 mL of freshly eluted $^{99m}TcO_4^-$ solution containing a maximum of 300 mCi (11.1 GBq) of activity.

— Stir for 1 min and use after 5 min.
— The ^{99m}Tc-HMDP labelled in this manner should be stable for over 6 h after labelling.

7.2.7. Labelling features

— HMDP: 0.5 mg/mL;
— Stannous chloride dihydrate: 0.125 mg/mL;
— pH: 5–7;
— Radiochemical purity: >95%;
— Pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <5%.

7.2.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper	ITLC-SG
Solvent	MEK/acetone	Saline
R_f ^{99m}Tc-HMDP	0.0	0.9–1.0
R_f $^{99m}TcO_4^-$	0.9–1.0	0.9–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Main ingredients content: Determination of the content of HMDP may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-HMDP in mice at 1 h post-injection is as follows:

Organ	%i.d./organ	%i.d./g
Bone	≥50	≥2 (femur)
Liver	≤3	≤0.5
Kidneys	≤3	≤1.0

Note: Biodistribution tests of ^{99m}Tc-HMDP should be the same as those for ^{99m}Tc-pyrophosphate injection in accordance with the USP. The values given here are for guidance only.

[7.2.1] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) oxidronate injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3283.

7.3. PREPARATION OF KIT FOR ^{99m}Tc-PYROPHOSPHATE

7.3.1. Reagents

— Sodium pyrophosphate: $Na_4P_2O_7.10H_2O$;
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 1N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.3.2. Chemical composition of kit

— Sodium pyrophosphate: 8 mg;
— Stannous chloride dihydrate: 0.8 mg.

7.3.3. Manufacturing formulas

Final volume (mL)	Sodium pyrophosphate (mg)	Stannous chloride dihydrate (mg)
100	800	80
150	1200	120

7.3.4. Preparation of kit solution for a final volume of 100 mL

— Use cold water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 80 mg of stannous chloride dihydrate using 0.4 mL of concentrated HCl and adjust the volume to 10 mL just before it is added to the final solution.
— Dissolve 800 mg of sodium pyrophosphate in approximately 80 mL of water for injection and stir well while bubbling the solution with nitrogen.
— Slowly add solution A, with continuous N_2 bubbling and stirring.
— Adjust the final pH to between 6.3 and 6.5 with 1N NaOH or 1N HCl using a pH meter.
— Adjust the final volume to 100 mL.
— Filter the solution through a 0.22 μm sterile filter.

— Precool the vial inside the freeze-dryer or using liquid nitrogen.
— Dispense 0.5 mL of the solution per vial, keeping the vials as cool as possible.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–45ºC	–1ºC	24ºC	24–48 h

7.3.5. Storage

Store refrigerated at 2–8ºC.

7.3.6. Radiolabelling

— Reconstitute the freeze-dried kit using 5 mL of freshly eluted $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 GBq) of activity.
— Stir well until completely dissolved (about 2 min).
— The ^{99m}Tc-pyrophosphate labelled in this manner should be stable for over 4 h after labelling.

7.3.7. Labelling features

— Sodium pyrophosphate: 1.6 mg/mL;
— Stannous chloride dihydrate: 0.16 mg/mL;
— pH: 6.0–7.0;
— Radiochemical purity: >90%;
— Pertechnetate (TcO_4^-): <5%;
— ^{99m}Tc reduced/hydrolysed: <5%.

7.3.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper	ITLC-SG
Solvent	MEK or acetone	Saline or 136 g/L sodium acetate
R_f ^{99m}Tc-pyrophosphate	0.0	0.9–1.0
R_f $^{99m}TcO_4^-$	0.9–1.0	0.9–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Main ingredients content: Determination of the content of sodium pyrophosphate may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: There is no requirement for biological distribution in the EP. The USP limits are: not less than 1% of the injected activity in the femur and not more than 5% in the liver or kidneys at 1 h post-injection.

[7.3.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) tin pyrophosphate injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 865.

[7.3.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) pyrophosphate injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3285.

7.4. PREPARATION OF KIT FOR ^{99m}Tc-DTPA

7.4.1. Reagents

— Ethylenediamine pentaacetic acid (DTPA, Na_5DTPA or Na_3CaDTPA);[4]
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N);
— Sodium hydroxide: NaOH (3N, 1N);
— Water for injection;
— Nitrogen gas.

7.4.2. Chemical composition of kit

— Na_5DTPA: 10 mg;
— Stannous chloride dihydrate: 0.8 mg.

[4] The weight is to be adjusted on the basis of the salt used in order to give the desired concentration of DTPA. The weight given here is for Na_5DTPA.

7.4.3. Manufacturing formulas

Final volume (mL)	Na_5DTPA (g)	Stannous chloride dihydrate (mg)
100	1.0	80
250	2.5	200
500	5.0	400

7.4.4. Preparation of kit solution for a final volume of 250 mL

— Use cold water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 200 mg of stannous chloride dihydrate using 25 mL of 0.2N HCl (or 0.4 mL of concentrated HCl, adjusting the volume to 25 mL) just before it is added to the final solution.
— Dissolve 2.5 g of DTPA in approximately 10 mL of 3N NaOH and add 150 mL of water for injection.
— Slowly add solution A, with continuous N_2 bubbling and stirring.
— Control the pH at between 4 and 5 using 1N NaOH or 1N HCl.
— Adjust the final pH to 5.0–5.5 using a pH meter.
— Adjust the final volume to 250 mL.
— Filter the solution through a 0.22 μm sterile filter.
— Precool the vial inside the freeze-dryer or by using liquid nitrogen.
— Dispense 1 mL per vial, keeping the vials as cool as possible.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–1ºC	24ºC	24–48 h

7.4.5. Storage

Store refrigerated at 2–8ºC.

7.4.6. Radiolabelling

— Reconstitute the freeze-dried kit using 5 mL of freshly eluted $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 GBq) of activity.
— Stir until completely dissolved (about 2 min).
— The ^{99m}Tc-DTPA labelled in this manner should be stable for over 4 h after labelling.

7.4.7. Labelling features

— Na_5DTPA: 2 mg/mL;
— Stannous chloride dihydrate: 0.16 mg/mL;
— pH: 5.0–7.5;
— Radiochemical purity: >95%;
— Pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <5%.

7.4.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper	ITLC-SG
Solvent	MEK or acetone	Saline
R_f ^{99m}Tc-DTPA	0.0	0.9–1.0
Rf $^{99m}TcO_4^-$	0.9–1.0	0.9–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Main ingredients content: Determination of the content of DTPA may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: There is no requirement for biodistribution tests in the EP. The USP requires that not more than 5% of the injected activity be retained in the whole body 24 h post-injection.

[7.4.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) pentetate injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 862.
[7.4.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) pentetate injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3283.

7.5. PREPARATION OF KIT FOR ^{99m}Tc-GLUCOHEPTONATE

7.5.1. Reagents

— Glucoheptonic acid calcium salt (GH);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.5.2. Chemical composition of kit

— Glucoheptonic acid calcium salt (GH): 100 mg;
— Stannous chloride dihydrate: 0.5 mg.

7.5.3. Manufacturing formulas

Final volume (mL)	GH (g)	Stannous chloride dihydrate (mg)
100	10.0	50
250	25.0	125
500	50.0	250

7.5.4. Preparation of kit solution for a final volume of 250 mL

— Use water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 125 mg of stannous chloride dihydrate using 25 mL of 0.2N HCl (or 0.4 mL of concentrated HCl, adjusting the volume to 25 mL) just before it is added to the final solution.
— Dissolve 25 g of calcium glucoheptonate in approximately 200 mL of water for injection.
— Slowly add solution A to the GH solution, with continuous N_2 bubbling and stirring.
— Adjust the pH to between 4 and 5 using 1N NaOH or 1N HCl.
— Adjust the final pH to 5.0–5.5 using a pH meter.
— Adjust the final volume to 250 mL.
— Filter the solution through a sterile 0.22 μm filter.

— Dispense 1 mL per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–1ºC	24ºC	24–48 h

7.5.5. Storage

Store refrigerated at 2–8ºC.

7.5.6. Radiolabelling

— Reconstitute the freeze-dried kit using 4 mL of freshly eluted $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 GBq) of activity.
— Stir for 1 min and use after 5 min.
— The ^{99m}Tc-GH labelled in this manner should be stable for over 6 h after labelling.

7.5.7. Labelling features

— GH: 25 mg/mL;
— Stannous chloride dihydrate: 0.125 mg/mL;
— pH: 5.0–5.5;
— Radiochemical purity: >95%;
— Pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <5%.

7.5.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper	ITLC-SG
Solvent	MEK or acetone	Saline
R_f ^{99m}Tc-GH	0.0	0.9–1.0
R_f $^{99m}TcO_4^-$	0.9–1.0	0.9–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Main ingredients content: Determination of the content of glucoheptonate may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-GH in rats at 1 h post-injection is as follows:

Organ	%i.d./organ
Kidneys, bladder and urine	≥70
Blood	<3
Liver	<5
Gastrointestinal tract	<15

The USP requires that not less than 15% of the injected activity be in the kidneys, and not more than 5, 15 and 5% be in the blood, gastrointestinal tract and liver, respectively, at 1 h post-injection.

[7.5.1] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) glucoheptate injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3278–3279.

7.6. PREPARATION OF KIT FOR ^{99m}Tc-MAG_3

7.6.1. Reagents

— S-benzoylmercaptoacetyl-tryglicine (MAG_3);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Disodium glucoheptonate;
— Disodium tartrate dihydrate;
— Lactose;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N, 0.001N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.6.2. Chemical composition of kit

— MAG_3: 1 mg;
— Disodium glucoheptonate: 20 mg;
— Disodium tartrate dihydrate: 40 mg;
— Lactose: 20 mg;
— Stannous chloride dihydrate: 0.1 mg.

7.6.3. Manufacturing formulas

Final volume (mL)	MAG_3 (mg)	Disodium glucoheptonate (g)	Disodium tartrate dihydrate (g)	Lactose (g)	Stannous chloride dihydrate (mg)
100	100	2.0	4.0	2.0	10.0
150	150	3.0	6.0	3.0	15.0

7.6.4. Preparation of kit solution for a final volume of 100 mL

— Use cold water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 100 mg of stannous chloride dihydrate using 10 mL of 0.2N HCl (or 0.5 mL of concentrated HCl, adjusting the volume to 10 mL) just before it is added to the final solution.
— Dissolve 100 mg of MAG_3 in approximately 80 mL of water for injection.
— Add 2.0 g of disodium glucoheptonate and 4.0 g of disodium tartrate dihydrate and allow to dissolve.
— Slowly add 1 mL of solution A, with continuous N_2 bubbling and stirring.
— Control the pH at between 4 and 5, using 1N NaOH or 1N HCl.
— Adjust the final pH to 5.0–5.5 using a pH meter.
— Add 2.0 g of lactose and allow to dissolve.
— Adjust the final volume to 100 mL.
— Filter the solution through a 0.22 μm sterile filter.
— Precool the vial inside the freeze-dryer or using liquid nitrogen.
— Dispense 1 mL per vial, keeping the vials as cool as possible.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–1ºC	24ºC	24–48 h

7.6.5. Storage

Store refrigerated at 2–8ºC.

7.6.6. Radiolabelling

— Reconstitute the freeze-dried kit using 3 mL of freshly eluted $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 GBq) of activity.
— Stir for 1 min and allow to stand for 5 min.
— Heat the vial in a boiling water bath for 15 min and allow to cool to room temperature.
— The ^{99m}Tc-MAG_3 labelled in this manner should be stable for over 6 h after labelling.

7.6.7. Labelling features

— MAG_3: 0.33 mg;
— Disodium glucoheptonate: 6.66 mg;
— Disodium tartrate dihydrate: 13.33 mg;
— Lactose: 6.66 mg;
— Stannous chloride dihydrate: 0.033mg/mL;
— pH: 5.0–5.5;
— Radiochemical purity: >95%;
— Pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <5%.

7.6.8. Quality control analyses

— Activate a Sep-Pak C-18 column with 5–10 mL of ethanol.
— Wash with 5–10 mL of 0.001N HCl.
— Add 0.1 mL of ^{99m}Tc-MAG_3 and elute the column as follows, counting each fraction:
  • A : Eluent contains $^{99m}TcO_4^-$, ^{99m}Tc-reduced/hydrolysed, etc.
  • B: Elute with 10 mL of ethanol:saline (1:1, vol./vol.); eluent contains ^{99m}Tc-MAG_3
  • C: Activity in column
  • Radiochemical purity = $\frac{B \times 100}{A + B + C}$
— Elute with 10 mL of 0.001N HCl.

Radiochemical purity: Ascending chromatography

Support	ITLC-SG	ITLC-SG or Whatman No. 1 paper
Solvent	Octanol	Saline
R_f ^{99m}Tc-MAG_3	0.0	0.9–1.0
R_f $^{99m}TcO_4^-$	0.9–1.0	0.9–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

The use of HPLC is recommended by the USP and EP. Whatman paper chromatography in acetonitrile:water (6:4, vol./vol.) for ^{99m}Tc reduced/hydrolysed is recommended by the USP and EP.

Main ingredients content: Determination of the content of MAG_3 may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-MAG_3 in rats at 30 min post-injection is as follows:

Organ	%i.d./organ
Kidneys	≤2
Bladder and urine	≥80
Liver	<2

Note: A biodistribution test of ^{99m}Tc-MAG_3 is not required by the USP and EP. The values given here are for guidance only.

[7.6.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) mertiatide injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 860.

[7.6.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) mertiatide injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3281–3282.

7.7. PREPARATION OF KIT FOR ^{99m}Tc-EC

7.7.1. Reagents

— N,N-Ethylene-L,L-dicysteine (EC);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Calcium/sodium glucoheptonate;
— Sodium dihydrogen phosphate dihydride;
— Disodium hydrogen phosphate dihydride;
— Hydrochloric acid (concentrated, 1N);
— Sodium hydroxide: NaOH (0.1N);
— Water for injection;
— Nitrogen gas.

7.7.2. Chemical composition of kit

The kit comprises three different components necessary for preparation of the radiopharmaceutical:

— Component A: 40 mg of calcium/sodium glucoheptonate and 0.1 mg of stannous chloride dihydrate, freeze-dried;
— Component B: 1 mg of EC, freeze-dried;
— Component C: 1 mL of 0.5M Na_2HPO_4 solution at pH4–5.

7.7.3. Manufacturing formulas

Component A:

Final volume (mL)	Ca/Na glucoheptonate (g)	Stannous chloride dihydrate (mg)
100	4.0	10.0
250	10.0	25.0

Component B:

Final volume (mL)	EC (mg)
100	100
250	250

Component C:

Final volume (mL)	$Na_2HPO_4.2H_2O$ (g)
100	15.6
250	37.5

7.7.4. Preparation of kit solution for a final volume of 100 mL

Component A:

- Solution A: Dissolve 50 mg of stannous chloride dihydrate in 0.1 mL of concentrated HCl and adjust the volume to 5 mL just before it is added to the final solution.
- Weigh 4.0 g of calcium/sodium salt of glucoheptonate and dissolve in 90 mL of water for injection in a sterile measuring flask. Adjust the pH of the solution to ~7 by dropwise addition of 1N HCl.
- Add 1 mL of solution A to the glucoheptonate solution and adjust the pH of the solution to 6.5 by dropwise addition of 0.1N NaOH.
- Adjust the total volume to 100 mL with water for injection after thorough stirring.
- Filter the solution through a sterile 0.22 μm filter.
- Dispense 1 mL aliquots per vial.
- Precool the vials using liquid nitrogen or cool them inside the freeze-dryer.
- Freeze-dry using the following conditions:

Freeze temperature	Primary drying	Secondary drying	Drying	Time
–44ºC	5°C every 4 h until –5° C	10ºC every 2 h until 35ºC	1–2 h at 35ºC	40–44 h

Component B:

- Prepare 0.1M phosphate buffer at pH12–13 by dissolving 3.5 g of disodium hydrogen phosphate dihydrate and 800 mg of sodium hydroxide in 200 mL of water for injection.
- Weigh 100 mg of EC and dissolve in 100 mL of 0.1M phosphate buffer at pH12–13.
- Filter the solution through a sterile 0.22 μm filter.

— Dispense 1 mL aliquots per vial.
— Freeze-dry using the conditions described above.

Component C:

— Weigh 7.8 g of sodium dihydrogen phosphate dihydride and dissolve in water for injection in a sterile volumetric flask of 100 mL capacity.
— Mix well and adjust the volume to 100 mL.
— Filter the solution through a sterile 0.22 μm filter.
— Dispense 1 mL aliquots per vial into sterile 10 mL vials and autoclave.

7.7.5. Storage

Store components A and B refrigerated at 2–8ºC.

7.7.6. Radiolabelling

— Reconstitute the freeze-dried kit by adding 3 mL of freshly eluted $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 GBq) of activity to component A with a syringe and withdrawing an equal amount of air; shake the contents of the vial.
— Add 1 mL of water for injection to component B, mix well and transfer the contents to the reaction vial using a syringe.
— Mix well by shaking the vial and heat in a boiling water bath for 10 min.
— Remove the vial from the water bath and allow it to stand at ambient temperature for 5 min.
— Withdraw 0.5 mL of component C in a syringe and add it to the reaction vial.
— The preparation is now ready for use.
— Store the product at room temperature; use within 4 h of preparation.

7.7.7. Labelling features

— EC: 1 mg/4.5 mL;
— pH: 5–8;
— Radiochemical purity: >90%;
— Pertechnetate (TcO_4^-): <5%;
— ^{99m}Tc reduced/hydrolysed: <5%.

7.7.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	Whatman No. 1 paper	
Solvent	Acetone	Saline
R_f ^{99m}Tc-EC	0.0–02	0.8–1.0
R_f $^{99m}TcO_4^-$	0.8–1.0	0.8–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Main ingredients content: Determination of the content of EC may be required by local regulations. The average amount of stannous chloride must be at least 50% of the quoted value.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-EC in mice at 1 h post-injection is as follows:

Organ	%i.d./organ
Bladder and urine	≥80
Kidneys	<5
Total carcass (except bladder and kidneys)	<20

Note: EC is not described in the EP or USP.

7.8. PREPARATION OF KIT FOR ^{99m}Tc-DMSA(III)

7.8.1. Reagents

— Dimercaptosuccinic acid (DMSA);
— Ascorbic acid;
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Sodium bicarbonate;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N);
— Sodium hydroxide: NaOH (3 and 1N);
— Water for injection;
— Nitrogen gas.

7.8.2. Chemical composition of kit

— DMSA: 3 mg;
— Stannous chloride dihydrate: 1 mg;
— Ascorbic acid: 3 mg.

7.8.3. Manufacturing formulas

Final volume (mL)	DMSA (g)	Ascorbic acid (g)	Stannous chloride dihydrate (mg)
100	0.3	0.3	100
250	0.75	0.75	250
500	1.5	1.5	500

7.8.4. Preparation of kit solution for a final volume of 250 mL

— Use water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 250 mg of stannous chloride dihydrate using 50 mL of 0.2N HCl (or 0.4 mL of concentrated HCl, adjusting the volume to 50 mL) just before it is added to the final solution.
— Dissolve 750 mg of DMSA in approximately 180 mL of water for injection at pH4.0–4.5.
— Add 750 mg of ascorbic acid.
— Slowly add solution A to the DMSA solution, with continuous N_2 bubbling and stirring.
— Adjust the pH to 3–4 using 1N NaOH or 1N HCl.
— Adjust the final pH to 2.8–3.0 using a pH meter.
— Adjust the final volume to 250 mL.
— Filter the solution through a sterile 0.22 μm filter.
— Dispense 1 mL per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–3ºC	24ºC	48 h

7.8.5. Storage

Store refrigerated at 2–8ºC.

7.8.6. Radiolabelling

— Reconstitute the freeze-dried kit using 3 mL of freshly eluted $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 GBq) of activity.
— Stir for 1 min and use after 20 min.
— The ^{99m}Tc-DMSA(III) labelled in this manner should be stable for over 4 h after labelling.

7.8.7. Labelling features

— DMSA: 1.0 mg/mL;
— Stannous chloride dihydrate: 0.33 mg/mL;
— pH: 2.3–3.5;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-): <5%.

7.8.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper
Solvent	MEK or acetone
R_f ^{99m}Tc-DMSA(III)	0.0
R_f $^{99m}TcO_4^-$	0.9–1.0

Main ingredients content: Determination of the content of DMSA may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-DMSA(III) in rats at 1 h post-injection is as follows:

Organ	% i.d./organ	% i.d./g
Kidneys	≥40	
Femur		<1
Liver	<5	

The USP requires that the uptake in kidneys be not less than 40% of the injected activity and that the kidneys/(liver + spleen) ratio be not less than 6:1 at 1 h post-injection.

[7.8.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) succimer injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 865.
[7.8.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) succimer injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3270–3271.

7.9. PREPARATION OF KIT FOR ^{99m}Tc-DMSA(V)

Technetium-99m–DMSA(V) is prepared using the same DMSA kit that is used for ^{99m}Tc-DMSA(III), except that it requires the addition of a second component (component B) for adjusting the pH to 8–9 prior to labelling. See Sections 7.8.1–7.8.5 for information on the preparation of component A.

7.9.1. Manufacturing formulas for 100 kits

Component B:

Bicarbonate buffer, 4.4%, pH9.0

Final volume (mL)	$NaHCO_3$ (g)
100	4.4
250	11.0

7.9.2. Preparation of kit solution for a final volume of 250 mL

— Use water for injection bubbled with nitrogen gas.
— Dissolve 11 g of $NaHCO_3$ in 80 mL of water for injection.
— Adjust the pH to 9.0.
— Adjust the final volume to 250 mL.
— Filter the solution through a sterile 0.22 μm filter.
— Dispense 2 mL per vial.

7.9.3. Radiolabelling

— Reconstitute the freeze-dried kit using 1.0 mL of bicarbonate buffer at pH9.0, mix well.
— Add 2 mL of $^{99m}TcO_4^-$ solution containing a maximum of 30 mCi (1.11 MBq) of activity.
— Stir for 1 min and allow to stand for 20 min.
— The ^{99m}Tc-DMSA(V) labelled in this manner should be stable for over 4 h after labelling.

7.9.4. Labelling features

— DMSA: 1.0 mg/mL;
— Stannous chloride dihydrate: 0.33 mg/mL;
— pH: 8–9;
— Radiochemical purity: >85%;
— Free pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <15%.

7.9.5. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper	Whatman 3MM paper
Solvent	MEK or acetone	ButOH:HAc:H_2O (3:2:3, vol./vol./vol.)
R_f ^{99m}Tc-DMSA(V)	0.0	0.7–0.8
R_f $^{99m}TcO_4^-$	0.9–1.0	0.9
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Note: The values reported are based on the data provided by one of the contributors to this report. The USP and EP do not provide guidance for ^{99m}Tc-DMSA(V).

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-DMSA(V) in rats at 1 h post-injection is as follows:

Organ	%i.d./organ	%i.d./g
Kidneys	<10	
Femur		>1.5
Liver	< 2	

7.10. PREPARATION OF KIT FOR ^{99m}Tc-MEBROFENIN (BROMO-HIDA)

7.10.1. Reagents

— Mebrofenin (N-(2,4,6-trimethyl-3-bromacetanilid)-iminodiacetic acid (bromo-HIDA));
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 0.2N, 1N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.10.2. Chemical composition of kit

— Bromo-HIDA: 20 mg;
— Stannous chloride dihydrate: 0.24 mg;
— NaCl: 0.7 mg.

7.10.3. Manufacturing formulas

Final volume (mL)	Bromo-HIDA (g)	Stannous chloride dihydrate (mg)	NaCl (mg)
100	2.0	24	70
250	5.0	60	175

7.10.4. Preparation of kit solution for a final volume of 100 mL

— Use cold water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 24 mg of stannous chloride dihydrate using 10 mL of 0.2N HCl (or 0.5 mL of concentrated HCl, adjusting the volume to 10 mL) just before it is added to the final solution.
— Dissolve 2 g of bromo-HIDA in approximately 4 mL of 1N NaOH and add 50 mL of water for injection.
— Adjust the pH to 6.5 by dropwise addition of 1N NaOH.
— Slowly add solution A, with continuous N_2 bubbling and stirring.

— Control the pH at between 6 and 6.5, using 1N NaOH or 1N HCl, and adjust the final pH to 6.0–6.5 using a pH meter.
— Adjust the final volume to 100 mL.
— Filter the solution through a 0.22 μm sterile filter.
— Precool the vial using liquid nitrogen.
— Dispense 1 mL per vial, keeping the vials as cool as possible.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–35ºC	–1ºC	24ºC	24–48 h

7.10.5. Storage

Store refrigerated at 2–8ºC.

7.10.6. Radiolabelling

— Reconstitute the freeze-dried kit using 5 mL of $^{99m}TcO_4^-$ solution containing a maximum of 50 mCi (1.85 MBq) of activity.
— Stir for 1 min to dissolve and use after 30 min.
— The ^{99m}Tc-bromo-HIDA labelled in this manner should be stable for over 4 h after labelling.

7.10.7. Labelling features

— Bromo-HIDA: 4 mg/mL;
— Stannous chloride dihydrate: 0.05 mg/mL;
— pH: 5.5–7.5;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <5%.

7.10.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SA	Whatman No. 1 paper
Solvent	Saturated NaCl	Ethylene glycol:water (1:1)
R_f ^{99m}Tc-bromo-HIDA	0.0	0.8–1.0
R_f $^{99m}TcO_4^-$	0.9–1.0	0.8–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Main ingredients content: Determination of the content of bromo-HIDA may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-bromo-HIDA in mice at 30 min post-injection is as follows:

Organ	%i.d./organ
Gall-bladder, small and large intestine	≥80
Liver	<3
Kidneys	<2

The USP requires that not less than 75% of the injected activity be in the gastrointestinal tract, and not more than 10 and 5% be in the liver and kidneys, respectively, at 30 min post-injection.

[7.10.1] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) mebrofenin injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3280–3281.

7.11. PREPARATION OF KIT FOR ^{99m}Tc-EHIDA

7.11.1. Reagents

— N-(2,6-diethylacetanilido) iminodiacetic acid (EHIDA);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N);

— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.11.2. Chemical composition of kit

— EHIDA: 10 mg;
— Stannous chloride dihydrate: 0.5 mg.

7.11.3. Manufacturing formulas

Final volume (mL)	EHIDA (g)	Stannous chloride dihydrate (mg)
100	1.0	50
250	2.5	125

7.11.4. Preparation of kit solution for a final volume of 100 mL

— Use water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 50 mg of stannous chloride dihydrate using 25 mL of 0.2N HCl (or 0.4 mL of concentrated HCl, adjusting the volume to 25 mL) just before it is added to the final solution.
— Dissolve 1 g of EHIDA in approximately 50 mL of water for injection.
— Slowly add solution A to the EHIDA solution, with continuous N_2 bubbling and stirring.
— Control the pH at between 4 and 5 using 1N NaOH or 1N HCl.
— Adjust the final pH to 5.8–6.0 using a pH meter.
— Adjust the final volume to 100 mL.
— Filter the solution through a sterile 0.22 μm filter.
— Dispense 1 mL per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–1ºC	24ºC	24 h

7.11.5. Storage

Store refrigerated at 2–8ºC.

7.11.6. Radiolabelling

— Reconstitute the freeze-dried kit using 5 mL $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 MBq) of activity.
— Stir for 1 min and use after 5 min.
— The ^{99m}Tc-EHIDA labelled in this manner should be stable for over 4 h after labelling.

7.11.7. Labelling features

— EHIDA: 2.5 mg/mL;
— Stannous chloride dihydrate: 0.125 mg /mL;
— pH: 5–7;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <5%.

7.11.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SA	ITLC-SG (Whatman No. 1 paper)
Solvent	Saturated NaCl	Methanol (ethylene glycol: water, 1:9, vol./vol.)
R_f ^{99m}Tc-EHIDA	0.0	0.9–1.0
R_f $^{99m}TcO_4^-$	0.9–1.0	0.9–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0

Main ingredients content: Determination of the content of EHIDA may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-EHIDA in rats at 1 h post-injection is as follows:

Organ	% i.d./organ
Gall-bladder and intestine	>70
Kidneys	<10
Liver	<10
Stomach	<3
Blood	<3

7.12. PREPARATION OF KIT FOR ^{99m}Tc-PHYTATE

7.12.1. Reagents

— Sodium phytate: Na_{12} phytate;
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 1N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas;
— Methanol.

7.12.2. Chemical composition of kit

— Sodium phytate: 10 mg;
— Stannous chloride dihydrate: 1.0 mg.

7.12.3. Manufacturing formulas

Final volume (mL)	Sodium phytate (g)	Stannous chloride dihydrate (mg)
100	1.0	100
200	2.0	200
500	5.0	500

7.12.4. Preparation of kit solution for a final volume of 100 mL

— Use cold water for injection bubbled with N_2 gas during solution preparation.
— Solution A: Dissolve 300 mg of stannous chloride dihydrate in 0.3 mL of concentrated HCl with warming in a hot water bath and adjust the volume to 4.5 mL with water for injection.
— Solution B: Weigh 1 g of sodium phytate accurately and dissolve in 80 mL of water for injection.
— Mix thoroughly and adjust the pH of the solution to between 7 and 8 by dropwise addition of 1N HCl. To this, add 1.5 mL of freshly prepared solution A, mix and, using a pH meter, adjust the pH to 6.5 by dropwise addition of 1N NaOH.
— Adjust the total volume to 100 mL with water for injection after thorough stirring.
— Filter the solution through a 0.22 μm sterile filter fitted in a sterile filter assembly.
— Dispense 1 mL aliquots per vial into sterile precooled 10 mL vials.
— Precool the vials using liquid nitrogen or cool them inside the freeze-dryer.
— Freeze-dry using the following conditions:

Freeze temperature	Primary drying	Secondary drying	Drying	Time
–44ºC	5°C every 4 h until –5°C	10ºC every 2 h until 35ºC	1–2 h at 35ºC	40–44 h

7.12.5. Storage

Store refrigerated at 2–8ºC.

7.12.6. Radiolabelling

— Reconstitute the freeze-dried kit using 3 mL of $^{99m}TcO_4^-$ solution containing a maximum of 50 mCi (1.85 MBq) of activity.
— Mix well and use after 10 min.
— The ^{99m}Tc-phytate prepared in this manner should be stable for over 4 h after labelling.

7.12.7. Labelling features

— Sodium phytate: 3.3 mg/mL;
— pH: 5–8;
— Radiochemical purity: >90%;
— Free pertechnetate (TcO_4^-) + ^{99m}Tc reduced/hydrolysed: <10%.

7.12.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper
Solvent	85% methanol
R_f ^{99m}Tc-phytate	0.0–0.1
R_f $^{99m}TcO_4^-$	0.55–0.7

Main ingredients content: Determination of the content of phytate may be required by local regulations. The average amount of stannous chloride must be at least 50% of the quoted value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-phytate in mice at 30 min post-injection is as follows:

Organ	%i.d./organ
Liver	>80
Kidneys	≤5
Lungs	≤5

7.13. PREPARATION OF KIT FOR ^{99m}Tc-SULPHUR COLLOID

7.13.1. Reagents

— Sodium thiosulphate pentahydrate;
— Sodium dihydrogen phosphate dihydride: $NaH_2PO_4.2H_2O$;
— Disodium hydrogen phosphate dihydride: $Na_2HPO_4.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 0.3N);
— Concentrated nitric acid: HNO_3;

— Sodium hydroxide solution (1N);
— 3.5% gelatin solution;
— Water for injection;
— Nitrogen gas.

7.13.2. Chemical composition of kit

The kit comprises three different components necessary for preparation of the radiopharmaceutical:

— Component A: 0.5 mL of 0.3N HCl;
— Component B: 1 mL of solution containing 10% sodium thiosulphate and 3.5% gelatin;
— Component C: 1 mL of 0.08M phosphate buffer at pH7.4 containing 136 mg of Na_2HPO_4 and 12 mg of NaH_2PO_4.

7.13.3. Manufacturing formulas

Component A:

Final volume (mL)	Concentrated HCl (mL)
100	1.5
250	3.75

Component B:

Final volume (mL)	3.5% gelatin solution (mL)	10% sodium thiosulphate solution (mL)
100	85	10
250	212.5	25

Component C:

Final volume (mL)	Disodium dihydrogen phosphate dihydrate (g)	Sodium dihydrogen phosphate dihydrate (g)
100	13.6	1.2
250	34.0	3.0

7.13.4. Preparation of kit solution for a final volume of 100 mL

Use cold water for injection bubbled with N_2 gas, and bubble N_2 gas while preparing the solutions.

Component A:

- To 1.5 mL of concentrated HCl, add 53.5 mL of water for injection with stirring.
- Mix thoroughly and filter through a 0.22 μm membrane filter.
- Dispense 0.5 mL aliquots per vial into sterile 10 mL vials.

Component B:

- Prepare ~100 mL of 3.5% gelatin solution and sterilize in an autoclave.
- Weigh 1.5 g of sodium thiosulphate pentahydrate and dissolve in 10 mL of water for injection.
- Mix well and adjust the volume to 15 mL with water for injection to obtain 10% thiosulphate solution.
- Add 10 mL of the 10% sodium thiosulphate solution to 85 mL of 3.5% gelatin solution.
- Mix well and dispense 1 mL aliquots into 10 mL clean sterile vials under aseptic conditions.
- Autoclave the vials.

Component C:

- Weigh 1.2 g of sodium dihydrogen phosphate dihydrate and 13.6 g of disodium dihydrogen phosphate dihydrate.
- Dissolve in 80 mL of water for injection.
- Mix well and adjust the volume to 100 mL with water for injection.
- Filter the solution through a sterile 0.22 μm filter.
- Dispense 1 mL aliquots into sterile 10 mL vials.

7.13.5. Storage

Store components A and C at room temperature, and component B refrigerated at 2–8ºC.

7.13.6. Radiolabelling

— Add 3 mL of $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 MBq) of activity to component A.
— Transfer 0.5 mL of component B to the reaction vial containing component A.
— Mix well and place the vial in a boiling water bath for 3–5 min.
— Allow the vial to cool to room temperature (5 min) and then transfer 0.5 mL of component C into the reaction vial and mix; use after 5 min.

7.13.7. Labelling features

— ^{99m}Tc-sulphur colloid: colloidal suspension;
— pH: 4–7;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-): <5%.

7.13.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper
Solvent	Acetone or saline
R_f ^{99m}Tc-sulphur colloid	0.0–0.1
R_f $^{99m}TcO_4^-$	0.9–1.0

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-sulphur colloid in mice at 30 min post-injection is as follows:

Organ	%i.d./organ
Liver and spleen	>80
Lungs	≤5

[7.13.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) colloidal sulphur injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 852.
[7.13.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) sulphur colloid injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3288.

7.14. PREPARATION OF KIT FOR ^{99m}Tc-TIN COLLOID

7.14.1. Reagents

— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Sodium fluoride;
— Sodium chloride;
— Hydrochloric acid: HCl (1N, 0.02N);
— Povidone;
— Water for injection;
— Nitrogen gas.

7.14.2. Chemical composition of kit

— Stannous chloride dihydrate: 0.3 mg;
— Sodium fluoride: 1.0 mg;
— Sodium chloride: 3.6 mg;
— Povidone: 0.5 mg.

7.14.3. Manufacturing formulas

Final volume (mL)	Stannous chloride dihydrate (mg)	Sodium fluoride (mg)	Sodium chloride (mg)	Povidone (mg)
100	30	100	360	50
200	60	200	720	100
500	150	500	1800	250

7.14.4. Preparation of kit solution for a final volume of 200 mL

— Use cold water for injection bubbled with nitrogen gas.
— Dissolve 200 mg of sodium fluoride in 80 mL of saline solution.
— Add 100 mg of povidone and stir until completely dissolved.
— Dissolve 60 mg of stannous chloride dihydrate in 2 mL of 0.02N HCl and add to the final solution, with continuous N_2 bubbling and stirring.
— Adjust the pH to between 5.5 and 5.7 using 1N HCl.
— Adjust the final volume to 200 mL.
— Filter the solution through a sterile 0.22 μm filter.
— Precool the vial inside the freeze-dryer or by using liquid nitrogen.
— Dispense 0.5 mL per vial, keeping the vials as cool as possible.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–1ºC	24ºC	24–48 h

7.14.5. Storage

Store refrigerated at 2–8ºC.

7.14.6. Radiolabelling

— Reconstitute the freeze-dried kit using 3 mL of $^{99m}TcO_4^-$ solution containing a maximum 100 mCi (3.7 MBq) of activity.
— Stir for 1 min and use after 20 min.
— The ^{99m}Tc-tin colloid labelled in this manner should be stable for over 4 h after labelling.

7.14.7. Labelling features

— Stannous chloride dihydrate: 0.1 mg/mL;
— Sodium fluoride: 0.3 mg/mL;
— Sodium chloride: 1.2 mg/mL;
— pH: 4–7;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-): <5%.

7.14.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG
Solvent	Saline
R_f ^{99m}Tc-tin colloid	0
R_f $^{99m}TcO_4^-$	0.9–1.0

Main ingredients content: The determination of the content of tin chloride may be required by local regulations. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-tin colloid in mice at 20 min post-injection is as follows:

Organ	%i.d./organ
Liver and spleen	≥80
Lungs	≤5

[7.13.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) colloidal tin injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 853.

7.15. PREPARATION OF KIT FOR ^{99m}Tc-RHENIUM-SULPHIDE COLLOID

7.15.1. Reagents

— Rhenium metal;
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Gelatin;
— Ascorbic acid;
— H_2O_2;
— Sodium pyrophosphate: $Na_4P_2O_7.10H_2O$;

— Hydrochloric acid: HCl (4.5N, 1N, 0.5N);
— Sodium hydroxide: NaOH (5N);
— DOWEX 1X8;
— Water for injection;
— H_2S gas;
— Nitrogen gas.

7.15.2. Chemical composition of kit

Component A:

— Rhenium (as sulphide): 0.15 mg;
— Gelatin: 9.6 mg;
— Ascorbic acid: 7 mg;
— Water for injection: 1 mL.

Component B:

— Sodium pyrophosphate: 3 mg;
— Stannous chloride dihydrate: 0.1 mg.

7.15.3. Manufacturing formulas

Component A:

Final volume (mL)	Rhenium metal (mg)	Gelatin (g)	Ascorbic acid (g)
100	15	0.96	0.7
150	22.5	1.8	1.05

Component B:

Final volume (mL)	Sodium pyrophosphate (g)	Stannous chloride dihydrate (mg)
100	0.3	10
150	0.45	15

7.15.4. Preparation of kit solution for a final volume of 100 mL

Component A:

— Dissolve 15 mg of rhenium metal in 5 mL of H_2O_2, evaporate to dryness in a water bath, take up the residue with water for injection and evaporate to dryness; repeat the operation two times using 5–10 mL of water for injection.
— Dissolve the residue in 10 mL of 4.5N HCl.
— Prepare gelatin by dissolving 0.96 g of gelatin in 20 mL of 4.5N HCl; filter the solution through a 0.45 μm filter to obtain solution 1.
— Prepare the diluted gelatin solution (1:10) by adding 90 mL of water for injection to the 10 mL of gelatin solution to obtain solution 2.
— To the 10 mL of perrhenate in 4.5N HCl, add 10 mL of gelatin solution 1.
— Mix the solution by bubbling with H_2S gas for 20 min.
— Remove the excess H_2S gas by bubbling the solution with nitrogen.
— Add 60 mL of gelatin solution 2 and adjust the pH to ~5 using 5N NaOH; fill to 100 mL with water for injection.
— Activate 9 g of DOWEX 1X8 with 1N HCl and wash with water for injection to neutral pH; add the DOWEX to the rhenium-sulphide colloid solution and mix for 15 min.
— Filter the suspension on the glass frit; to the filtrate, add 700 mg of ascorbic acid and mix well until it is completely dissolved.
— Adjust the pH to 5; filter the solution through a 0.45 μm filter and dispense 1 mL per vial under nitrogen.

Component B:

— Use cold water for injection bubbled with nitrogen gas.
— Dissolve 300 mg of sodium pyrophosphate in 66 mL of water for injection.
— Add 10 mg of stannous chloride dihydrate dissolved in 33 mL of 0.5N HCl.
— Mix and adjust the pH to 6.0–6.1 using 5N NaOH.
— Filter the solution through a 0.22 μm sterile filter.
— Dispense 1 mL per vial, keeping the vials as cool as possible.
— Freeze-dry components A and B using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–1ºC	24ºC	24–48 h

7.15.5. Storage

Store refrigerated at 2–8ºC.

7.15.6. Radiolabelling

— Reconstitute the freeze-dried content of component B in 4 mL of water for injection and stir well.
— Transfer 2 mL of the component B solution to component A using a syringe.
— Add 3 mL of $^{99m}TcO_4^-$ containing a maximum 100 mCi (37 MBq) of activity to the above vial.
— Heat the vial in a boiling water bath for 15 min and leave to cool at room temperature.
— The ^{99m}Tc-rhenium-sulphide colloid labelled in this manner should be stable for over 4 h after labelling.

7.15.7. Labelling features

— Rhenium (as sulphide): 0.05 mg/mL;
— Gelatin: 3.2 mg/mL;
— Ascorbic acid: 1.2 mg/mL;
— Sodium pyrophosphate: 0.5 mg/mL;
— Stannous chloride dihydrate: 0.16 mg/mL;
— pH: 5.0–6.0;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-): <5%.

7.15.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	Whatman No. 1 paper
Solvent	Saline
R_f ^{99m}Tc-Re-sulphide colloid	0.0
R_f $^{99m}TcO_4^-$	0.6
Other ^{99m}Tc impurities	0.8–0.9

Main ingredients content: Determination of the content of rhenium-sulphide may be required by local regulations.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-Re-sulphide colloid in mice at 20 min post-injection is as follows:

Organ	%i.d./organ
Liver and spleen	≥80
Lungs	≤5

[7.15.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) colloidal rhenium sulphide injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 851.

7.16. PREPARATION OF KIT FOR ^{99m}Tc–HUMAN SERUM ALBUMIN (HSA) NANOCOLLOID

7.16.1. Reagents

— Human serum albumin (HSA 25%);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Poloxamer 210 (F88);
— Anhydrous disodium hydrogen phosphate: Na_2HPO_4;
— Ascorbic acid;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.16.2. Chemical composition of kit

— HSA: 1.0 mg;
— Poloxamer 210: 2.0 mg;
— Anhydrous disodium hydrogen phosphate: 2.2 mg;
— Stannous chloride dihydrate: 0.5 mg;
— Ascorbic acid: 1.0 mg.

7.16.3. Manufacturing formulas

Final volume (mL)	HSA (g)	Poloxamer 210 (mg)	Ascorbic acid (mg)	Stannous chloride dihydrate (mg)
100	0.1	200	100	50
200	1.0	400	200	100

7.16.4. Preparation of kit solution for a final volume of 100 mL

— Use water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 50 mg of stannous chloride dihydrate using 10 mL of 0.2N HCl (or 0.2 mL of concentrated HCl, adjusting the volume to 10 mL) just before it is added to the final solution.
— Solution B: Anhydrous disodium hydrogen phosphate, 40 mg/mL.
— Solution C: Dissolve 200 mg of Poloxamer 210 in approximately 80 mL of water for injection, and add 0.4 mL of HSA (25%) and 100 mg of ascorbic acid.
— Adjust the pH to 5.5–6.0 using a pH meter.
— Slowly add solution A to solution C, with continuous N_2 bubbling and stirring.
— Add 5 mL of solution B.
— Control the pH at between 6.0 and 6.5 using 1N NaOH or 1N HCl.
— Filter the solution through a sterile 0.22 μm filter to a sterile stoppered crimped vial containing a magnetic stirring bar.
— Heat the solution to 85ºC in a circulating water bath on top of a magnetic stirrer for 30 min; the mixing should be rapid and steady without bumping of the stirring bar.
— Cool the solution for 30 min by stirring at room temperature.
— Add 0.4 mL of HSA (25%) and adjust the final volume to 100 mL.
— Filter the solution through a 0.22 μm sterile filter.
— Dispense 1 mL per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–3ºC	24ºC	24–48 h

7.16.5. Storage

Store refrigerated at 2–8ºC.

7.16.6. Radiolabelling

— Reconstitute the freeze-dried kit using 2 mL of $^{99m}TcO_4^-$ solution containing a maximum of 50 mCi (1.85 MBq) of activity.
— Stir for 1 min and use after 5 min.
— The ^{99m}Tc-HSA nanocolloid labelled in this manner should be stable for over 4 h after labelling.

7.16.7. Labelling features

— HSA: 1.25 mg/mL;
— Stannous chloride dihydrate: 0.125 mg/mL;
— pH: 5.5–6.0;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-): <5%;
— Particle size: 0.020–0.1 μm.[5]

7.16.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG
Solvent	Saline or MEK
R_f ^{99m}Tc-HSA nanocolloid	0.0
R_f $^{99m}TcO_4^-$	0.9–1.0

Main ingredients content: Determination of the content of HSA may be required by local regulations. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

[5] Particle size measurement may not be performed as a routine QC method.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-HSA nanocolloid in rats at 2 h post-injection is as follows:

Organ	%i.d./organ
Blood	≤3
Liver/bone marrow	≥80
Lungs	≤3

7.17. PREPARATION OF KIT FOR ^{99m}Tc-HUMAN SERUM ALBUMIN (HSA) COLLOID

7.17.1. Reagents

— Human serum albumin (HSA 25%);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Poloxamer 210 (F88);
— Anhydrous sodium hydrogen phosphate: Na_2HPO_4;
— Ascorbic acid;
— Hydrochloric acid: HCl (concentrated, 1N, 0.2N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.17.2. Chemical composition of kit

— HSA: 5.0 mg;
— Poloxamer 210: 2.0 mg;
— Na_2HPO_4: 2.0 mg;
— Stannous chloride dihydrate: 0.5 mg;
— Ascorbic acid: 1.0 mg.

7.17.3. Manufacturing formulas

Final volume (mL)	HSA (g)	Poloxamer 210 (mg)	Ascorbic acid (mg)	Na_2HPO_4 (mg)	Stannous chloride dihydrate (mg)
100	0.5	200	100	200	50
200	1.0	400	200	400	100

7.17.4. Preparation of kit solution for a final volume of 100 mL

— Use water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 50 mg of stannous chloride dihydrate using 10 mL of 0.2N HCl (or 0.2 mL of concentrated HCl, adjusting the volume to 10 mL) just before it is added to the final solution.
— Solution B: Dissolve 400 mg of anhydrous Na_2HPO_4 in 10 mL of water for injection to obtain a concentration of 40 mg/mL.
— Solution C: Dissolve 200 mg of Poloxamer 210 in approximately 80 mL of water for injection, add 0.4 mL of HSA (25%) and 100 mg of ascorbic acid.
— Adjust the pH to 4.5.
— Slowly add solution A to solution C with continuous N_2 bubbling and stirring.
— Add 5 mL of solution B.
— Control the pH at between 5.0 and 5.5 using 1N NaOH or 1N HCl.
— Filter the solution through a sterile 0.22 μm filter to a sterile stoppered crimped vial containing a magnetic stirring bar.
— Heat the solution at 65ºC in a circulating water bath on a magnetic stirrer for 30 min; the mixing should be rapid and steady.
— Cool the solution for 30 min by stirring at room temperature.
— Add 1.6 mL of HSA (25%) and adjust the final volume to 100 mL.
— Filter the solution through a sterile 0.22 μm filter.
— Dispense 1 mL per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–3ºC	24ºC	48 h

7.17.5. Storage

Store refrigerated at 2–8ºC.

7.17.6. Radiolabelling

— Reconstitute the freeze-dried kit using 2 mL of saline; add 2 mL of $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 MBq) of activity.
— Stir for 1 min and use after 5 min.
— The ^{99m}Tc-HSA colloid labelled in this manner should be stable for over 4 h after labelling.

7.17.7. Labelling features

— HSA: 1.25 mg/mL;
— Stannous chloride dihydrate: 0.125mg/mL;
— pH: 5.5–6.0;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-): <5%;
— Particle size: 0.1–0.22 μm.[6]

7.17.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper
Solvent	Saline or MEK
R_f ^{99m}Tc-HSA colloid	0.0
R_f $^{99m}TcO_4^-$	0.9–1.0

Main ingredients content: Determination of the content of HSA may be required by local regulations.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-HSA colloid in rats at 2 h post-injection is as follows:

Organ	%i.d./organ
Blood	≤3
Liver	≥80
Lungs	≤3

7.18. PREPARATION OF KIT FOR ^{99m}Tc-MICROSPHERES

Technetium-99m-microspheres ranging in size between 5 and 90 μm in diameter are used to study lung perfusion. The microspheres are made of HSA,

[6] Particle size measurement may not be performed as a routine QC method.

intended for human use. Two methods for preparation of such microspheres are described.

7.18.1. Preparation of human serum albumin microspheres

HSA microspheres are white/pale yellow particles between 5 and 90 μm in size, and are insoluble in water. They are a denatured preparation of HSA.

Method 1:

An aqueous solution of HSA is allowed to drop from a reservoir at a constant pressure onto a spinning disc that disperses the droplets. These droplets are allowed to fall over a distance of about 1 m into a dry chamber, during which time they dry and form spheres. The size of these spheres is controlled by the drop size, drop rate and spinning rate of the disc. Typically, for HSA microspheres, 10% HSA solution is dropped at a rate of 0.5 mL/min. The dried microspheres are collected and heated in an oven at 120–130°C for 2–3 h, sealed in a sterile vial and stored in a refrigerator at 2–10°C.

Method 2:

Typically, 4–5 mL of 20% HSA solution is added through a 23 gauge needle and syringe to 500–600 mL of clear vegetable oil in a beaker placed on a heating mantle, with vigorous and continuous stirring. After slowly heating the emulsion to 140–160°C, the suspension is rapidly cooled and 500 mL of n-heptane is added to disperse the spheres. This preparation is then filtered through a Whatman filter paper by applying suction. The microspheres retained on the filter are washed repeatedly with n-heptane to remove traces of oil and then air-dried. The size of the spheres depends on the emulsion consistency, which in turn depends on the stirring speed, rate of introduction of the albumin solution, etc. The dried microspheres are sieved to exclude large particles (>75 μm) and stored in sealed vials in a refrigerator at 2–10°C.

7.18.2. Reagents

— HSA microspheres (90% of spheres between 5 and 90 μm in diameter);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 1N);
— Polysorbate 80 (Tween 80);
— Sodium pyrophosphate decahydrate;
— Saline solution;

— Sodium chloride;
— Water for injection.

7.18.3. Chemical composition of kit

— HSA microspheres: 10 mg;
— Sodium pyrophosphate decahydrate: 12 mg;
— Stannous chloride dihydrate: 1.0 mg.

7.18.4. Manufacturing formulas

Final volume (mL)	HSA microspheres (g)	Sodium pyrophosphate decahydrate (g)	Polysorbate 80 (for rinsing) (mL)	Stannous chloride dihydrate (mg)
100	1.0	1.2	0.4	100
200	2.0	2.4	0.8	200
500	5.0	6.0	2.0	500

7.18.5. Preparation of kit solution for a final volume of 100 mL

— Use cold water for injection bubbled with N_2 gas during solution preparation.
— Dissolve 200 mg of stannous chloride in 0.3 mL of concentrated HCl, with warming if necessary. Adjust the volume to 2 mL with water for injection.
— Dilute 0.4 mL of polysorbate 80 to 100 mL with saline and autoclave the solution.
— Dissolve 1.2 g of sodium pyrophosphate decahydrate in 10 mL of water for injection and add 1 mL of the stannous chloride solution dropwise with stirring. Adjust the pH to ~2 with 1N HCl. Filter the solution by passing through a sterile 0.22 μm filter fitted into a sterile filter assembly.
— Weigh 1 g of denatured HSA microspheres and suspend in the above solution under aseptic conditions.
— Sonicate the solution in an ultrasound bath for 10 min and heat in a boiling water bath for 15 min.
— Cool and pass the suspension through a sterile 0.45 μm filter.
— Rinse with the polysorbate solution.

— Suspend the microspheres retained on the filter in 100 mL saline; dispense 1 mL aliquots per vial into sterile precooled 10 mL vials.
— Freeze-dry using the following conditions:

Freeze temperature	Primary drying	Secondary drying	Drying	Time
–44ºC	5°C every 4 h until –5°C	10ºC every 2 h until 35ºC	1–2 h at 35ºC	40–44 h

7.18.6. Storage

Store refrigerated at 2–8ºC.

7.18.7. Radiolabelling

— Reconstitute the freeze-dried kit with 2 mL of $^{99m}TcO_4^-$ solution containing a maximum of 50 mCi (1.85 MBq) of activity.
— Mix well and use after 10 min.
— The ^{99m}Tc microspheres labelled in this manner should be stable for over 4 h after labelling.

7.18.8. Labelling features

— HSA microspheres: 5 mg/mL;
— pH: 5–8;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-): <5%.

7.18.9. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG or Whatman No. 1 paper
Solvent	Methanol 85%
R_f ^{99m}Tc microspheres	0.0
R_f $^{99m}TcO_4^-$	0.55–0.7

The radioactivity associated with the microspheres is estimated by filtering the radiolabelled product through a 0.22 or 0.45 μm membrane filter,

washing it twice with 3 mL of saline each time and counting the activity associated with the supernatant and the washings. The activity in the washings and supernatant should not be more than 10% of the total activity.

Particle size: The microsphere preparation is reconstituted in saline and a small aliquot is taken for viewing under a microscope. The size of at least 100 particles is measured using the microspheres suspended in mineral oil under a light microscope. At least 90% of the particles should be between 5 and 90 μm in size, and no particle should be larger than 100 μm.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc microspheres in rats at 10 min post-injection is as follows:

Organ	%i.d./organ
Lungs	>80
Liver and spleen	≤5

[7.18.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) microspheres injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 861.

7.19. PREPARATION OF ^{99m}Tc–HUMAN IMMUNOGLOBULIN

7.19.1. Reagents

— Human immunoglobulin (HIG);
— 2-Mercaptoethanol (2ME);
— Sephadex G-75;
— Sodium dihydrogen phosphate: $NaH_2PO_4.2H_2O$;
— Disodium hydrogen phosphate: $Na_2HPO_4.2H_2O$;
— Sodium hydroxide;
— Sodium chloride;
— Phosphate buffered saline, 0.04M, pH7.4 (PBS);
— Water for injection;
— Nitrogen gas.

7.19.2. Chemical composition of kit

Component A:

— Reduced HIG: 0.5 mg.

Component B:

— Glucoheptonate: 100 mg;[7]
— Stannous chloride: 0.5 mg.

7.19.3. Manufacturing formulas

Final volume (mL)	Reduced HIG (mg)
100	60
250	150
500	300

7.19.4. Preparation of kit solution for a final volume of 100 mL of component A

— Prepare 500 mL of 0.04M phosphate buffer at pH7.4 using the sodium dihydrogen phosphate and disodium hydrogen phosphate salts and water for injection.
— Add 4.5 g of sodium chloride to the buffer and mix well; filter the mixture through a sterile 0.22 μm membrane filter assembly.
— Weigh an amount of the HIG reagent equivalent to 60 mg of HIG (often, only part of the weight of the HIG preparation is HIG, and hence a proportionally higher amount should be taken to obtain 60 mg of HIG, e.g. 60 mg of HIG is present in 156 mg of sandoglobulin).
— Dissolve the HIG in 1 mL of sterile saline solution and add 60 μL of 2ME to it.
— Load the above mixture over a 14 cm × 1 cm column of Sephadex G-75 gel that has been prepared and equilibrated with 0.04M PBS at pH7.4.
— Elute the column with PBS at a rate of 1 mL/min and collect fractions of 0.5 mL each in clean sterile tubes containing 0.5 mL of PBS.
— Measure the absorbance (OD) of the fractions at 280 nm in a UV-VIS spectrophotometer and identify the protein peak; the immunoglobulins will be eluted in the void volume, which will generally be within 8–12 fractions.
— Pool the 4–5 protein fractions forming the peak of HIG.

[7] See Section 7.5 for a description of the preparation of the kit for ^{99m}Tc-glucoheptonate.

— Measure the absorbance of the pooled solution and estimate the HIG content; assign the value of HIG concentration to this pooled solution.
— Dilute the solution with sterile PBS to obtain a HIG concentration of 1 mg/mL; filter the solution through a sterile 0.22 μm filter.
— Dispense 0.5 mL aliquots per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Primary drying	Secondary drying	Drying	Time
–44ºC	5°C per 4 h until –5°C	10ºC every 2h until 35ºC	1–2 h at 35ºC	40–44 h

7.19.5. Storage

Store refrigerated at 2–8ºC.

7.19.6. Radiolabelling

— Add 0.5 mL of water for injection to component A and mix gently by hand; this is the reaction vial.
— Reconstitute component B with 5 mL of normal saline and add 100 μL aliquot to the reaction vial.
— Add 2 mL of $^{99m}TcO_4^-$ solution containing a maximum of 20 mCi (0.74 MBq) activity to the reaction vial.
— Mix gently and use after 30 min.
— The ^{99m}Tc-HIG labelled in this manner should be stable for 1 h after labelling.

7.19.7. Labelling features

— HIG: 0.5 mg/2.6 mL;
— pH: 6.5–8;
— Radiochemical purity: >95%;
— Free pertechnetate (TcO_4^-) + other impurities: <5%.

7.19.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG	ITLC-SG
Solvent	Acetone	Saline
R_f ^{99m}Tc-HIG	0.0–0.1	0.0–0.1
R_f $^{99m}TcO_4^-$	1.0	0.9–1.0
R_f ^{99m}Tc-glucoheptonate	0.0	0.9–1.0

Biodistribution: This product is not described in the EP or USP.

7.20. PREPARATION OF KIT FOR ^{99m}Tc-ECD

7.20.1. Reagents

— L,L-ethyl cysteinate dimer (ECD);
— Stannous chloride dihydrate;
— Disodium salt of ethylene diamine tetra-acetic acid (disodium EDTA);
— Mannitol;
— Sodium dihydrogen phosphate: $NaH_2PO_4.2H_2O$;
— Disodium hydrogen phosphate: $Na_2HPO_4.2H_2O$;
— Hydrochloric acid: HCl (concentrated and 1N);
— Sodium hydroxide (1N);
— Saline;
— Water for injection;
— Nitrogen gas.

7.20.2. Chemical composition of kit

The kit is composed of two different components for preparation of the radiopharmaceutical:

— Component A: 1 mg of ECD, 80 μg of stannous chloride dihydrate, 0.3 mg of disodium EDTA and 20 mg of mannitol, freeze-dried;

— Component B: 1 mL of 0.02M phosphate buffer at pH7.5–8.

7.20.3. Manufacturing formulas

Component A:

Final volume (mL)	ECD (mg)	Disodium EDTA (mg)	Stannous chloride dihydrate (mg)	Mannitol (g)
100	100	30	8	2
250	250	75	20	5

Component B:

Final volume (mL)	Disodium hydrogen phosphate (mg)	Sodium dihydrogen phosphate (mg)
100	310	50
250	775	125

7.20.4. Preparation of kit solution for a final volume of 100 mL

Use cold water for injection bubbled with N_2 gas, and bubble N_2 gas while preparing the solutions.

Component A:

- Weigh 50 mg of stannous chloride dihydrate and dissolve in 0.1 mL of concentrated HCl, with slight warming. Dilute the solution to 5 mL with water for injection in a measuring flask of 50 mL capacity. Just prior to final formulation, dilute this solution to 50 mL with water for injection and use immediately.
- Weigh 100 mg of ECD and dissolve in 57 mL of saline.
- Weigh 30 mg of disodium EDTA and dissolve in 10 mL of water for injection.
- Weigh 2 g of mannitol and dissolve in 25 mL of water for injection.
- To the 57 mL of ECD solution, add 8 mL of freshly prepared stannous chloride solution, 10 mL of EDTA solution and 25 mL of mannitol solution; check the pH with pH paper and confirm that it is between 2.5

and 3; mix well and filter the solution through a 0.22 μm sterile filter; dispense 1mL aliquots per vial.
— Freeze-dry using the following conditions:

Freeze temperature	Primary drying	Secondary drying	Drying	Time
–44ºC	5°C every 4 h until –5°C	10ºC every 2 h until 35ºC	1–2 h at 35ºC	40–44 h

Component B:

— Weigh 50 mg of sodium dihydrogen phosphate dihydride and 310 mg of disodium hydrogen phosphate dihydride and dissolve them together in a sterile beaker in water for injection to a final volume of 100 mL.
— Mix well and confirm that the pH of the solution is between 7.5 and 8.0.
— Filter the solution through a sterile 0.22 μm filter; dispense 1 mL aliquots per vial.

7.20.5. Storage

Store component A refrigerated at 2–8ºC; component B can be stored at room temperature.

7.20.6. Radiolabelling

— Add 3 mL of $^{99m}TcO_4^-$ solution containing a maximum of 100 mCi (3.7 MBq) of activity to component B with a syringe. Shake the contents of the vial.
— Add 1 mL of saline to the component A vial, mix well. Transfer the contents of the component A vial to the component B vial using a syringe.
— Mix well by shaking the vial and use after 30 min.
— The ^{99m}Tc-ECD labelled in this manner should be stable for 4 h after labelling.

7.20.7. Labelling features

— ECD: 1 mg/2–3 mL;
— pH: 6–8;
— Radiochemical purity: >90%;

— Free pertechnetate (TcO_4^-): <5%;
— ^{99m}Tc reduced/hydrolysed: <5%.

7.20.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	Whatman No. 1 paper	ITLC SG[a]	Whatman No. 1 paper
Solvent	Methanol	Ethyl acetate	$CHCl_3$:methanol (9:1, vol./vol.)
R_f ^{99m}Tc-ECD	0.5–1.0	0.4–1.0	0.8–1.0
R_f $^{99m}TcO_4^-$	0.2–0.4	0.0–0.2	0.0–0.4
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0	0.0

[a] In this system, all the impurities remain below $R_f = 0.2$ (EP).

Main ingredients content: Determination of the content of ECD may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The EP and USP do not require tests of biodistribution. Unlike other radiopharmaceuticals, ^{99m}Tc-ECD does not have similar bioactivity in rats, mice or other small animals; the expected behaviour of brain perfusion is seen only in primates.

[7.20.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) bicisate injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 4181.
[7.20.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) bicisate injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3274–3275.

7.21. PREPARATION OF KIT FOR ^{99m}Tc-d,l-HMPAO

7.21.1. Reagents

— d,l-Hexamethylpropylene amine oxime (d,l-HMPAO);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Hydrochloric acid: HCl (concentrated, 0.2N);

— Sodium hydroxide (1 and 3N);
— Sodium chloride: NaCl;
— Water for injection;
— Nitrogen gas.

7.21.2. Chemical composition of kit

— d,l-HMPAO: 1 mg;
— Stannous chloride dihydrate: 0.08 mg;
— NaCl: 4.0 mg.

7.21.3. Manufacturing formulas

Final volume (mL)	d,l-HMPAO (mg)	Sodium chloride (mg)	Stannous chloride dihydrate (mg)
50	50	200	0.4
100	100	400	0.8

7.21.4. Preparation of kit solution for a final volume of 100 mL

— Use cold water for injection bubbled with nitrogen gas.
— Solution A: Dissolve 80 mg of stannous chloride dihydrate using 10 mL of 0.2N HCl (or 0.2 mL of concentrated HCl, adjusting the volume to 10 mL) just before it is added to the final solution.
— Dissolve 100 mg of d,l-HMPAO in approximately 40 mL of water for injection adjusted to pH1.5–2.0 with concentrated HCl.
— Add 400 mg of sodium chloride and adjust the pH to 3.5–4.0.
— Slowly add 100 µL of solution A to the HMPAO solution, with continuous N_2 bubbling and stirring.
— Adjust the final pH to 9.0–10.0 using a pH meter.
— Adjust the final volume to 100 mL.
— Filter the solution through a 0.22 µm sterile filter.
— Precool the vials inside the freeze-dryer or using liquid nitrogen.
— Dispense 1 mL per vial, keeping the vials as cool as possible.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–30ºC	–3ºC	24ºC	24 h

7.21.5. Storage

Store refrigerated at 2–8ºC.

7.21.6. Radiolabelling

— Reconstitute the freeze-dried kit using 4 mL of $^{99m}TcO_4^-$ solution containing a maximum of 70 mCi (2.59 MBq) of activity. The $^{99m}TcO_4^-$ eluate should not be more than 2 h old and should have been obtained from a generator eluted within 24 h prior to elution of the $^{99m}TcO_4^-$ used for labelling the ^{99m}Tc-d,l-HMPAO.
— Stir for 1 min. The ^{99m}Tc-d,l-HMPAO labelled in this manner will be stable only for 30 min after labelling.

7.21.7. Labelling features

— HMPAO: 0.25 mg/1mL;
— pH: 8.5–9;
— Radiochemical purity: >90%;
— Free pertechnetate ($^{99m}TcO_4^-$): <5%;
— ^{99m}Tc-reduced/hydrolysed: <5%.

7.21.8. Quality control analyses

Radiochemical purity: Ascending chromatography

	System 1	System 2	System 3
Support	ITLC-SG	ITLC-SG	Whatman ET 31
Solvent	MEK	Saline	CH_3CN:saline (1:1)
R_f ^{99m}Tc-HMPAO lipophilic	0.8–1.0	0.0	0.9–1.0
R_f ^{99m}Tc-HMPAO secondary[a]	0.0	0.8–1.0	0.9–1.0
R_f $^{99m}TcO_4^-$	0.8–1.0	0.8–1.0	0.8–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0	0.0	0.0

[a] HPLC is a better method for estimation of secondary complex estimation:
^{99m}Tc-HMPAO (lipophilic) = 100 – (A + B + C)
where A is ^{99m}Tc-HMPAO (secondary) (i.e. system 1 origin – system 2 origin);
A + B = free $^{99m}TcO_4^-$ + ^{99m}Tc-HMPAO (secondary) (i.e. system 2, solvent front);
C = ^{99m}Tc reduced/hydrolysed (i.e. system 3, origin).

Main ingredients content: Determination of the content of HMPAO may be required by local regulations. The tin concentration in HMPAO is critical, and a significant reduction in it can adversely affect the labelling. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: Biodistribution studies of ^{99m}Tc-HMPAO are not carried out by most manufacturers. The data on the molecular identity of HMPAO are taken as an indication of its biological behaviour.

[7.21.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) exametazime injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 854.
[7.21.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) exametazime injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3277.

7.22. PREPARATION OF KIT FOR ^{99m}Tc-MIBI

7.22.1. Reagents

— Tetrakis(2-methoxy-2-methylpropyl-1-isocyanide)copper (1+)]tetrafluoroborate (MIBI);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— L-cysteine hydrochloride monohydrate;
— Sodium citrate dihydrate;
— D-mannitol;
— Hydrochloric acid (concentrated, 1N, 0.2N);
— Sodium hydroxide: NaOH (1N);
— Water for injection;
— Nitrogen gas.

7.22.2. Chemical composition of kit

— MIBI: 1.0 mg;
— Stannous chloride dihydrate: 0.075 mg;
— L-cysteine hydrochloride monohydrate: 1.0 mg;
— Sodium citrate dihydrate: 2.6 mg;
— D-mannitol: 20 mg.

7.22.3. Manufacturing formulas

Final volume (mL)	MIBI (mg)	L-cysteine hydrochloride monohydrate (mg)	Sodium citrate dihydrate (mg)	D-mannitol (g)	Stannous chloride dihydrate (mg)
100	100	100	26	2.0	7.5
500	500	500	1300	10.0	37.5
1000	1000	1000	2600	20.0	75

7.22.4. Preparation of kit solution for a final volume of 100 mL

— Use cold water for injection bubbled with nitrogen gas.
— Solution A : Dissolve 75 mg of stannous chloride dihydrate using 10 mL of 0.2N HCl (or 0.5 mL of concentrated HCl, adjusting the volume to 10 mL) just before it will be added to the final solution.
— Dissolve 100 mg of MIBI in approximately 80 mL of water for injection.
— Add 2.0 g of D-mannitol and mix for approximately 10 min until dissolved.
— Slowly add 1 mL of solution A, with continuous N_2 bubbling and stirring.
— Add 100 mg of L-cysteine hydrochloride monohydrate and 26 mg of sodium citrate dihydrate.
— Control pH at between 5 and 5.5, using 1N NaOH or 1N HCl.
— Adjust the final pH to 5.4–5.6 using a pH meter.
— Adjust the final volume to 100 mL.
— Filter the solution through a 0.22 μm sterile filter.
— Precool the vial inside the freeze-dryer or using liquid nitrogen.
— Dispense 1 mL per vial, keeping the vials as cool as possible.
— Freeze-dry using the following conditions:

Freeze temperature	Eutectic temperature	Dried temperature	Time
–40ºC	–1ºC	24ºC	24–48 h

7.22.5. Storage

Store refrigerated at 2–8ºC.

7.22.6. Radiolabelling

— Reconstitute the freeze-dried kit using 5 mL of $^{99m}TcO_4^-$ solution containing a maximum of 400 mCi (14.8 MBq) of activity.
— Stir for 1 min; heat the vial in a boiling water bath for 12 min and leave to cool at room temperature.
— The ^{99m}Tc-MIBI labelled in this manner should be stable for over 6 h after labelling.

7.22.7. Labelling features

— MIBI: 0.2 mg/mL;
— L-cysteine hydrochloride: 0.2 mg;
— Sodium citrate: 0.52 mg/mL;
— D-mannitol: 4.0 mg/mL;
— pH: 5.0–6.0;
— Radiochemical purity: >90%;
— Free pertechnetate (TcO_4^-): <5%;
— ^{99m}Tc reduced/hydrolysed: <5%.

7.22.8. Quality control analyses

Radiochemical purity[a]: Ascending chromatography

Support	TLC-ODS[b]	TLC-Al_2O_3
Solvent	Mobile phase[c]	Ethanol
R_f ^{99m}Tc-MIBI	0.3–0.6	0.8–1.0
R_f $^{99m}TcO_4^-$	0.9–1.0	0.6–0.7
R_f ^{99m}Tc-reduced/hydrolysed	0.0–0.1	0.0

[a] The EP and USP recommend the use of HPLC.
[b] TLC: thin layer chromatography.
[c] Mobile phase: tetrahydrofuran:ammonium acetate (38.5 g/L):methanol:acetonitrile (10:20:30:40).

Main ingredients content: Determination of the content of MIBI may be required by local regulations. The average amount of stannous chloride must be at least 50% of the expected value. A non-radioactive formulation should dissolve easily in saline, giving a clear and colourless solution.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-MIBI in rats at 180 min post-injection is as follows:

Organ	% i.d./organ
Heart	>1.0
Gastrointestinal tract	≥80
Liver	<4

No requirements for tests of biodistribution are required by the USP and EP.

[7.22.1] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Technetium (^{99m}Tc) sestamibi injection, European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 863.
[7.22.2] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium Tc-99m sestamibi injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3286–3287.

7.23. PREPARATION OF KIT FOR ^{99m}Tc-TETROFOSMIN

7.23.1. Reagents

— 2-Ethoxy ethyl-3,12-dioxa-6,9-diphosphate tetradecane (tetrofosmin);
— Stannous chloride dihydrate: $SnCl_2.2H_2O$;
— Disodium sulphosalicylate;
— Sodium D-gluconate;
— Sodium hydrogen carbonate;
— Hydrochloric acid: HCl (concentrated, 1N);
— Sodium hydroxide: NaOH (1N);
— Saline;
— Water for injection;
— Nitrogen gas.

7.23.2. Chemical composition of kit

— Tetrofosmin: 0.25 mg;
— Stannous chloride dihydrate: 50 μg;
— Disodium sulphosalicylate: 0.35 mg;

— Sodium D-gluconate: 1 mg;
— Sodium hydrogen carbonate: 1.8 mg.

7.23.3. Manufacturing formulas

Final volume (mL)	Tetrofosmin (mg)	Disodium sulphosalicylate (mg)	Sodium D-gluconate (mg)	Sodium hydrogen carbonate (mg)	Stannous chloride dihydrate (mg)
100	25	35	100	180	5.0
250	62.5	87.5	250	450	12.5

7.23.4. Preparation of kit solution for a final volume of 100 mL

— Use cold water for injection bubbled with N_2 gas during solution preparation.
— Prepare 0.05M sodium bicarbonate buffer by weighing 210 mg of sodium bicarbonate and dissolving in water for injection to obtain a final volume of 50 mL; the pH of this buffer will be about 8.5.
— Weigh 50 mg of stannous chloride dihydrate and dissolve in 0.1 mL of concentrated HCl, with slight warming; dilute the solution with water for injection to 5 mL in a measuring flask of 50 mL capacity; just prior to final formulation, dilute this solution to 50 mL with water for injection and use immediately.
— Weigh 25 mg of tetrofosmin and dissolve in 43 mL of 0.05M bicarbonate buffer (pH8.5).
— Weigh 35 mg of disodium sulfosalicylate and dissolve in 10 mL of water for injection.
— Weigh 100 mg of sodium D-gluconate and dissolve in 10 mL of water for injection.
— To the 43 mL solution of tetrofosmin, add 5 mL of freshly prepared stannous chloride solution, 10 mL of disodium sulfosalicylate solution and 10 mL of sodium D-gluconate solution; adjust the pH to ~8 by dropwise addition of 1N NaOH; adjust the final volume to 100 mL; mix well and filter the solution through a 0.22 μm sterile filter.
— Dispense 1 mL aliquots per vial.

— Precool the vials using liquid nitrogen, or cool them inside the freeze-dryer.
— Freeze-dry using the following conditions:

Freeze temperature	Primary drying	Secondary drying	Drying	Time
–44ºC	5°C every 4 h until –5°C	10ºC every 2 h until 35ºC	1–2 h at 35ºC	40–44 h

7.23.5. Storage

Store refrigerated at 2–8ºC.

7.23.6. Radiolabelling

— Add 5 mL of $^{99m}TcO_4^-$ solution containing a maximum of 200 mCi (7.4 MBq) of activity.
— Mix well and use after 15 min.
— The ^{99m}Tc-tetrofosmin prepared in this manner is stable up to 12 h.

7.23.7. Labelling features

— Tetrofosmin: 50 µg/mL;
— pH: 7.5–9.0;
— Radiochemical purity: >90%;
— Free pertechnetate + ^{99m}Tc reduced/hydrolysed: <10%.

7.23.8. Quality control analyses

Radiochemical purity: Ascending chromatography

Support	ITLC-SG
Solvent	Acetone:CH_2Cl_2 (35:65, vol./vol.)
R_f ^{99m}Tc-tetrofosmin	0.3–0.7
R_f $^{99m}TcO_4^-$	0.8–1.0
R_f ^{99m}Tc reduced/hydrolysed	0.0–0.1

Main ingredients content: Determination of the content of tetrofosmin may be required by local regulations. The average amount of stannous chloride must be at least 50% of the quoted value.

Biodistribution: The typical biodistribution pattern of ^{99m}Tc-tetrofosmin in rats at 60 min post-injection is as follows:

Organ	% i.d./organ
Heart	≥1.5
Blood	≤1
Lungs	≤1
Stomach	≤5

Note: The USP does not recommend a biodistribution test.

[7.23.1] UNITED STATES PHARMACOPEIAL CONVENTION, Technetium (Tc-99m) tetrofosmin injection, United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 3288–3289.

8. SYNTHESIS OF ACTIVE INGREDIENTS

Details of the synthesis of starting materials not commercially available are provided in this section. Table 8.1 lists the ligands for which synthesis details are included.

8.1. SYNTHESIS OF HMDP

The synthetic scheme for hydroxymethylene diphosphonic acid (HMDP) disodium salt is given in Fig. 8.1.

8.1.1. Step 1: Tetrasodium carbonyl diphosphonate

Dichloromethylene diphosphonic acid (12.5 g) is dissolved in 75 mL of water, followed by the addition of 32 g of NaOH. The solution is heated under reflux for 1 h and then cooled to room temperature. Methanol (500 mL) is added to precipitate the crude product. The precipitate is redissolved in a

TABLE 8.1. ACTIVE INGREDIENTS DETAILED IN THIS PUBLICATION

Section	Ligand
8.1	Hydroxymethylene diphosphonic acid (HMDP)
8.2	Mebrofenin (bromo-HIDA)
8.3	EHIDA
8.4	Benzoyl-mercaptoacetyl-triglicine (Bz-MAG_3)
8.5	Ethylene dicysteine (L,L-EC)
8.6	Ethylene dicysteine diethylester (L,L-ECD)
8.7	d,l-HMPAO
8.8, 8.9	Methoxy-isobutylisonitrile (MIBI)
8.10	Tetrofosmin

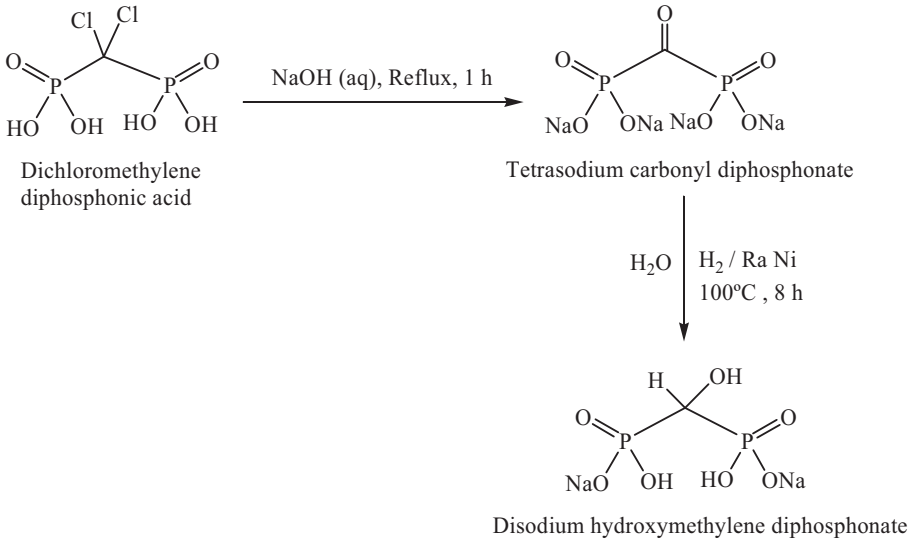


FIG. 8.1. Synthetic scheme for HMDP.

minimum amount of water, and the pH is adjusted to 10.5 with 6.0N HCl. The resulting yellow crystals are collected and washed, first with acetone and then with ethyl ether. The product has a melting point of ~300ºC. The typical yield is 91%.

8.1.2. Step 2: Disodium hydroxymethylene diphosphonate

Tetrasodium carbonyl diphosphonate (4.8 g), prepared as described above, is dissolved in 100 mL of water and subjected to hydrogen under pressure for 8 h at 100ºC in the presence of Raney nickel catalyst. The reaction mixture is cooled to room temperature and titrated to pH5.0 with 2N HCl. A small amount of ethylenediaminetetraacetic acid is added to complex the Ni^{2+} in solution, followed by the addition of methanol. The product is collected, crystallized and recrystallized from methanol:water (6:1). The typical yield is 75%.

8.1.3. Characterization

^{1}H-NMR (D_2O, δ ppm) 15.0 [d, C<u>H</u> J=15 Hz]

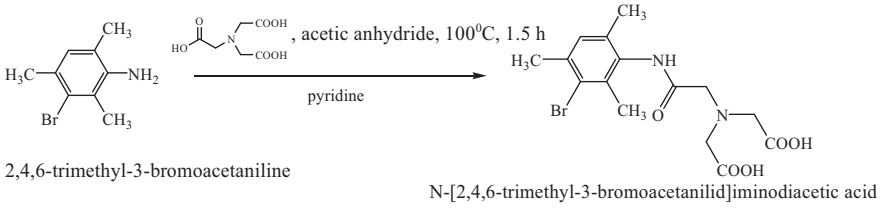
Melting point: 297–300ºC.

8.2. SYNTHESIS OF BROMO-HIDA

The synthetic scheme for N-[2,4,6-trimethyl-3-bromoacetanilid]iminodiacetic acid (bromo-HIDA, or Br-HIDA) is given in Fig. 8.2.

8.2.1. Synthesis

Nitriletriacetic acid (9.5 g, 0.05M) in liquid pyridine (100 mL) is homogenized with acetic anhydride (5.1 g, 0.05M) and 2,4,6-trimethyl-3-bromoaniline (10.45 g, 0.05M). The reaction mixture is boiled at 100ºC for about 1.5 h, cooled and vacuum evaporated. The crude product is dissolved in 10% NaOH and extracted two times with methylchloride. The solid N-[2,4,6-trimethyl-3-bromoacetanilid]iminodiacetic acid is precipitated at pH3 by



2,4,6-trimethyl-3-bromoacetaniline

N-[2,4,6-trimethyl-3-bromoacetanilid]iminodiacetic acid

FIG. 8.2. Synthetic scheme for bromo-HIDA.

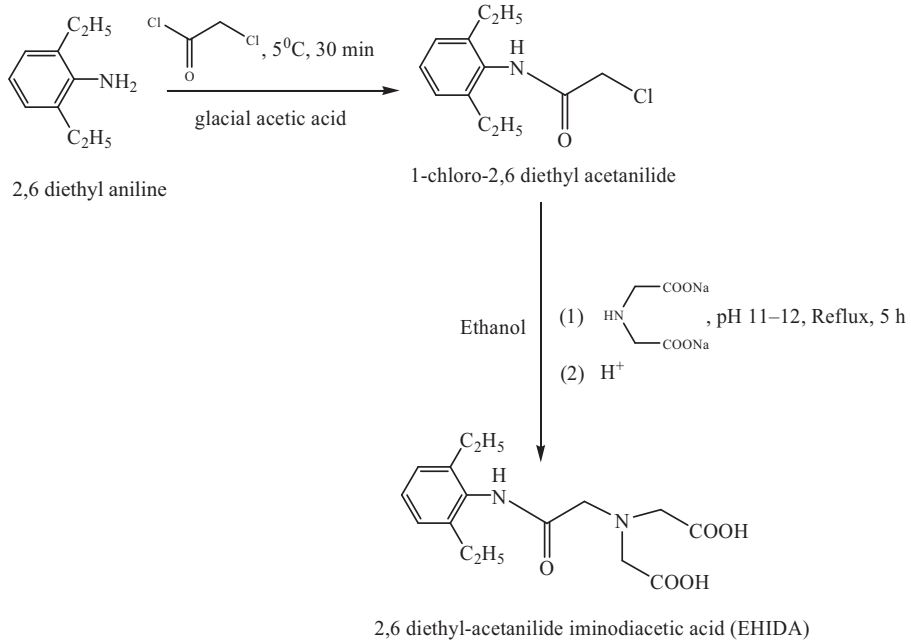


FIG. 8.3. Synthetic scheme for EHIDA.

addition of concentrated hydrochloric acid. The solid product is purified by dissolving it in 60% ethanol and boiling with charcoal. The solution is filtered and allowed to crystallize. The solid powder is filtered and washed three times with 60% ethanol and dried at 40°C. The typical yield is 70%.

8.2.2. Characterization

^{1}H-NMR (D_2O, δ ppm) 2.5 (9H, s, CH_3), 3.6 (2H, CH_2), 3.7 (4H, s, $C\underline{H}_2COOH$), 7.8 (1H, s, aromatic)

Melting point: 198–200°C.

8.3. SYNTHESIS OF EHIDA

The synthetic scheme for 2,6 diethyl-acetanilide iminodiacetic acid (EHIDA) is given in Fig. 8.3.

8.3.1. Step 1: 1-Chloro-2,6 diethyl acetanilide

2,6-Diethyl aniline (38 g) is dissolved in glacial acetic acid (120 g) and cooled to 5ºC in an ice bath. To this, 24 g of chloracetyl chloride in 120 mL of glacial acetic acid is added in a dropwise manner with continuous stirring, using a funnel with a stopcock. The temperature of the reaction mixture is maintained at less than 10ºC during the addition of the chloracetyl chloride. To this is added 63 g of sodium acetate dissolved in 267 mL of water. The reaction mixture is stirred for an additional 30 min. The product is filtered, washed with water and dried.

8.3.2. Step 2: 2,6-Diethyl-acetanilide iminodiacetic acid

1-Chloro-2,6-diethyl acetanilide (39 g), prepared as described above, is dissolved in 600 mL of 95% ethanol. To this, 84 g of iminodiacetic acid, disodium salt, dissolved in 200 mL of water, is added using a funnel. The pH of the reaction mixture is adjusted to 11–12 using 2N NaOH solution. The reaction mixture is heated and stirred until total dissolution occurs. Refluxing is continued for 4–5 h, and the mixture is then stirred overnight at room temperature. The ethanol is evaporated at 79–80ºC. The pH of the solution is adjusted to 2.8 using 5N HCl; the solution is kept refrigerated overnight. The resulting white precipitate is filtered and washed with 0.01N HCl.

8.3.3. Characterization

FT-IR (KBr, ν cm^{-1}) 1545 (-CO-); 1675 cm^{-1} (-C=O); 3020 cm^{-1} (-CH-aromatic.); 3320 cm^{-1} (-NH-)

Melting point: 177–179ºC.

8.4. SYNTHESIS OF Bz-MAG_3

The synthetic scheme for benzoyl-mercaptoacetyl-triglicine (Bz-MAG_3) is given in Fig. 8.4.

8.4.1. Step 1: S-Benzoylthioglycolic acid

Sodium hydroxide (8.8 g, 0.22 mol) and thioglycolic acid (9.1 g, 0.1 mol) are dissolved in a mixture of 75 mL toluene and 75 mL of water and cooled in an ice bath to about 10ºC. Benzoyl chloride (14.05 g, 0.1 mol) is added over the

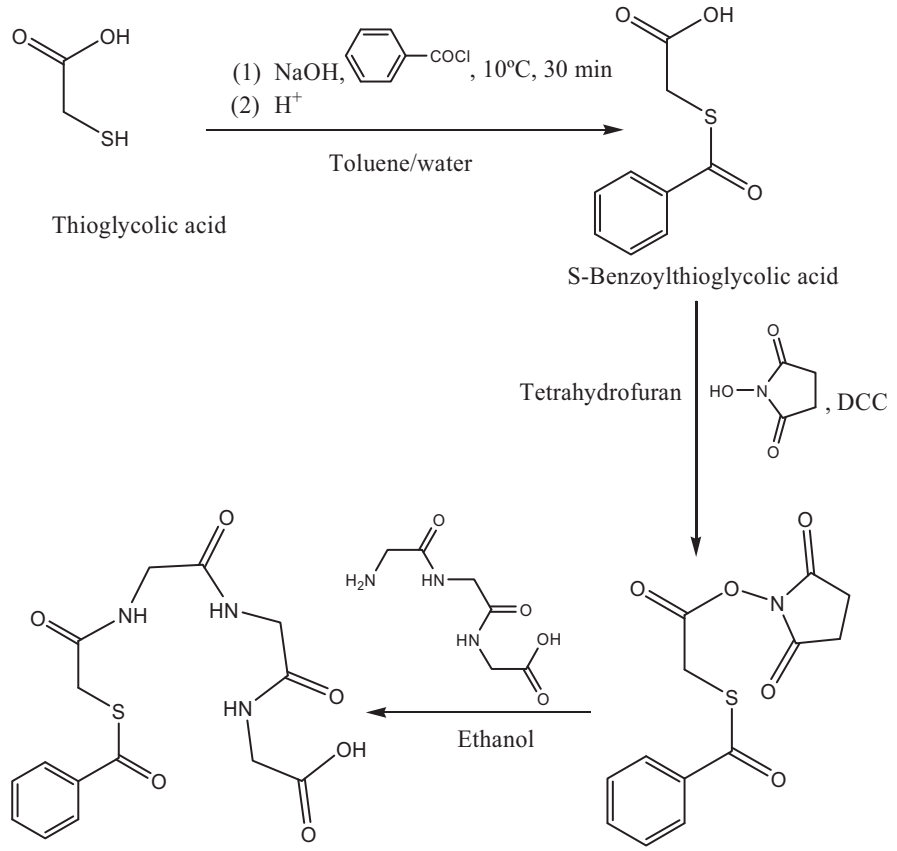


FIG. 8.4. Synthetic scheme for Bz-MAG$_3$.

course of 30 min. Stirring is continued for 30 min at 10ºC and for an additional 30 min at room temperature. The organic layer is separated and washed four times with water, and the combined aqueous phase is acidified to pH1.5 with concentrated HCl. The precipitated product is filtered and dried. Recrystallization from ethyl acetate and drying in a desiccator give ~12 g (66%) of the product as colourless crystals with a melting point of 105–108ºC.

8.4.2. Step 2: Succinimidyl-S-benzoylthioglycolate

S-benzoylthioglicolic acid (9.86 g, 0.05 mol), prepared as described above, and N-hydroxysuccinimide (5.75 g, 0.006 mol) are dissolved in 60 mL of

absolute tetrahydrofuran and then cooled to –5ºC. Dicyclohexyl carbodiimide (12.38 g, 0.06 mol) dissolved in 20 mL of tetrahydrofuran is added over the course of 20 min. The reaction mixture is stirred for 2 h at –5ºC; the stirring is then continued at room temperature for 13 h. After the addition of 0.2 mL of glacial acetic acid and stirring for an additional hour, the product is filtered and the residue is extracted three times with 100 mL of boiling tetrahydrofuran. The combined filtrates are evaporated to dryness, and the colourless residue is recrystallized from ethyl acetate to obtain about 7.8 g (52%) of the product as colourless needles with a melting point of 137–140ºC.

8.4.3. Step 3: Benzoyl-mercaptoacetyl-glycyl-glycyl-glycine (Bz-MAG_3)

Glycyl-glycyl-glycine (2.83 g, 15 mmol) is dissolved in 13 mL of water by the dropwise addition of 1N NaOH. This solution is added to a warm (55ºC) solution of 5.86 g (20 mmol) of succinimidyl-S-benzoylthioglycolate in 120 mL of ethanol. The mixture is refluxed for 2.5 h and then stirred at room temperature for 12 h. After evaporation of the solvent, the colourless residue is extracted twice with boiling acetonitrile and recrystallized twice from isopropanol. The typical yield is ~5.8 g (79%) of Bz-MAG_3.

8.4.4. Characterization

HPLC system: The purity of the compound can be checked by HPLC using a C-18 ODS SP 10 μm column with a UV 254 nm detector, with THF:water (60:40) or methanol:water (75:25) as the mobile phase at a flow rate of 1 mL/min. In this system, Bz-MAG_3 can be separated from its precursor, if any is present.

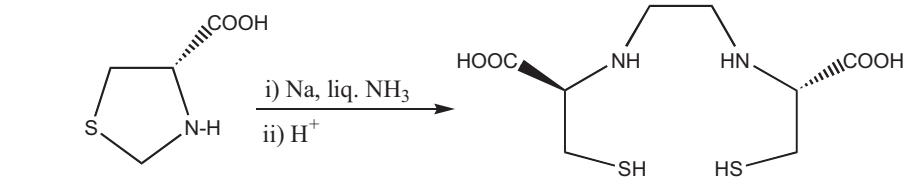
FT-IR (KBr, ν cm^{-1}) 3300 (-NH); 2850 (-N-CH_2); 1550 (-NH); 3100 (-OH); 1700 (-C=O)

1H-NMR (δ ppm) 3.74–3.79 (d, 4H, CH_2), 3.90 (s, 2H, CH_2-N), 7.55–7.96 (m, 5H, aromatic), 8.15–8.25 (t, 2H, CH_2-S), 8.5 (t, 1H, COOH)

Melting point: 196–200°C.

8.5. SYNTHESIS OF L,L-EC

The synthetic scheme for the ethylene cysteine dimer (L,L-EC) is given in Fig. 8.5.



L-thiazolidine-4-carboxylic acid

Ethylene cysteine dimer (L,L-EC)

FIG. 8.5. Synthetic scheme for L,L-EC.

8.5.1. Synthesis of EC

L-thiazolidine-4-carboxylic acid (2 g, 0.015M) is added to a 250 mL, three-necked round-bottomed flask, which is cooled over a mixture of ethyl acetate and liquid nitrogen in a Dewar flask. Liquefied ammonia (15 mL) is added to the reaction vessel. Small pieces of metallic sodium are gradually introduced into the stirred solution of L-thiazolidine-4-carboxylic acid in liquor ammonia until the blue colour persists. After 15 min, the reaction is quenched by the careful addition of ammonium chloride. The solid formed is dissolved in distilled water, and the solution is filtered. The filtrate is acidified with concentrated HCl to pH2 to obtain a white precipitate. After cooling the mixture, the precipitate is filtered, washed with ice-cold water and dried to obtain the crude product (0.6 g). Purification of the crude product is carried out by reprecipitation of the hydrochloride salt from an alkaline solution (in 5N NaOH) using concentrated HCl to give 0.8 g of the product. The typical yield is 40%.

8.5.2. Characterization

IR (KBr, ν cm^{-1}) : 1590(COOH); 1550 (NH), 3100, 2200 (amine hydrochloride)

Melting point: 251–253°C.

8.6. SYNTHESIS OF L,L-ECD

The synthetic scheme for the L,L-ethyl cysteinate dimer (L,L-ECD) is given in Fig. 8.6.

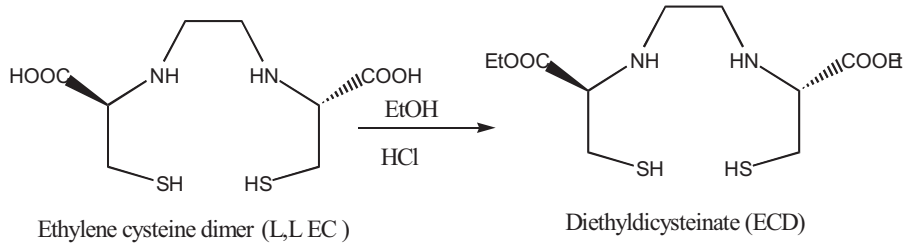


FIG. 8.6. Synthetic scheme for L,L-ECD.

8.6.1. Synthesis

A slurry of 3.5 g of EC (see Section 8.5) in anhydrous ethanol is made and saturated with dry HCl gas. The reaction mixture is heated under reflux for about 5 h. The contents are then cooled and filtered, and the precipitate obtained is washed with cold anhydrous ethanol to yield 2 g of the product. The typical product yield is ~42%.

8.6.2. Characterization

FT-IR (KBr, ν cm^{-1}) 3100, 2200 (NH_2^+), 1735–1740 (C=O)

1H-NMR ($CDCl_3$, 300 MHz, δ ppm) 1.35(t, $CH_2C\underline{H}_3$), 3.25(d:d, $C\underline{H}_2SH$), 3.65 (m, CH_2-CH_2), 4.4(q, $C\underline{H}_2CH_3$), 4.6 (t, CH)

Melting point: 196–198°C.

8.7. SYNTHESIS OF d,l-HMPAO

A two-step synthesis procedure is followed to prepare an isomeric mixture of hexamethyl-propyleneamineoxime (HMPAO) (Fig. 8.7). The isomeric mixture is repeatedly crystallized to separate the meso isomer from the d,l-HMPAO mixture.

8.7.1. Step 1: Schiff base imine

2,3-Butanedione monoxime (73 g, 0.71 mol), p-toluene sulfonic acid monohydrate (0.15 g) and benzene (725 mL) are added to a 2 L round-bottomed flask equipped with a Dean–Stark trap. The reaction mixture is

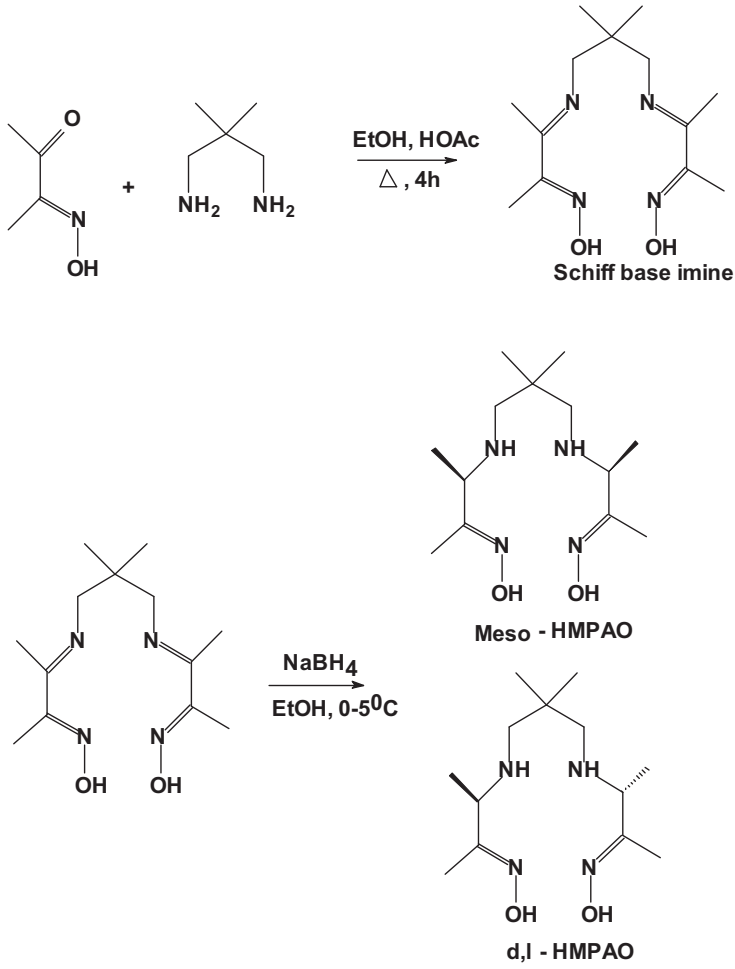


FIG. 8.7. Synthesis of 4,8-diaza-3,6,6,9-tetramethylundecane-3,6-diene-2,10-dione dioxime (mixture of HMPAO isomers).

heated and stirred for 30 min, until the solid is completely dissolved. A solution of 60 g (0.59 mol) of 2,2-dimethyl.1,3-propanediamine in 75 mL of benzene is added (3 h). The reaction mixture is refluxed until the expected quantity of water (13 mL, 0.7 mol) is collected in the trap (total reflux time ~10 h). The yellow coloured solution is then stirred for 6 h at room temperature until a white solid is obtained. The reaction mixture is kept at 4°C overnight. The solid formed is collected by Buckner filtration, washed with cold acetonitrile and dried under vacuum over P_2O_5. The yield is 87 g (90%), with a melting point of 135.6–136.4°C.

8.7.2. Step 2: HMPAO isomers

The Schiff base discussed above (52 g, 0.19 mol) and absolute ethanol (500 mL) are placed in a 1 L flask. The mixture is cooled to 0–5°C in an ice bath and stirred. Sodium borohydride (11 g, 0.24 mol) is gradually added to the mixture over a period of 1 h. The temperature of the reaction mixture is then maintained at 0–5°C, and the mixture is stirred for 2 h. Water (160 mL) is added, and stirring is continued for an additional hour at 0–5°C; the reaction mixture is then warmed to room temperature under continuous stirring. Ethanol is removed under vacuum, and the pH of the residual slurry is adjusted to 11; the slurry is kept overnight at 4°C to complete the precipitation. The white product is collected by Buckner filtration, washed with cold water and dried under vacuum over P_2O_5. The yield is 41.9 g (79%), with a melting point of 132.4–134.9°C. This is a mixture of d,l-HMPAO and meso-HMPAO.

Useful modifications: The Schiff base can also be formed by reacting 2,3-butanedione monooxime and 2,2-dimethyl-1,3-propanediamine in ethanol in the presence of p-toluenesulfonic acid and refluxing for 30 min. High purity imine is obtained by this modified procedure.

8.7.3. Separation of d,l-HMPAO and meso-HMPAO isomers

The mixture of d,l- and meso-HMPAO isomers (39.4 g) is treated with hot acetonitrile (150 mL) under magnetic stirring. The suspension is filtered under vacuum to give filtrate A (40% meso- and 60% d,l-HMPAO) and solid S (77% meso- and 23% d,l-HMPAO). The filtrate is left for 3 d at room temperature to give solid A and filtrate A*.

Solid S is treated again with 70 mL of hot acetonitrile to give filtrate B (50% d,l-HMPAO) and 17.5 g of solid S1 (85% meso-HMPAO). Filtrate B is left at room temperature for 3 d to give filtrate B* and solid S2.

Filtrates A* and B* are mixed, and the solvent is evaporated. The white residue (1.2 g, 85% d,l-HMPAO) could be further recrystallized from ethylacetate (20 mg/mL).

Solid A and solid S2 are recrystallized by dissolving in 825 mL of ethylacetate (20 mg/mL), heating and leaving at room temperature overnight. Crystals of solid S3 (86% m-HMPAO) are filtered, and the filtrate is kept at 4°C overnight for crystallization. Solid S4 is collected (3.2 g, 62% d,l-HMPAO), and the filtrate is concentrated under vacuum to give 7.2 g of solid S5 (89% d,l-HMPAO).

Solid S5 is dissolved by heating and stirring in 360 mL of ethylacetate (20 mg/mL) and kept at room temperature overnight. The solid (55 mg, 97% d,l-HMPAO) is separated by Buckner filtration, and the filtrate is concentrated

by evaporation to 300 mL and kept overnight at room temperature to give 90% d,l-HMPAO crystals. The recrystallization process is continued with ethylacetate until pure d,l-HMPAO is obtained.

8.7.4. Characterization of d,l-HMPAO

HPLC system: The purity of the compound and its precursors can be checked by HPLC using a μ-Porasil normal phase column, with a UV 210 nm detector, with methanol:0.4M NH_4OH (95:5) as the mobile phase at a flow rate of 1 mL/min. In this system, there is a difference of 1 min between the retention times of the d,l form and the meso form of HMPAO.

FT-IR (KBr, υ cm^{-1}) 3310 (-OH), 3219, 3100 (-OH, -NH)

1H-NMR (CD_3OD, 300 MHz, δ ppm) 0.891(s, 6H, gem-C$\underline{H}_3$), 1.178 (d, 6H, NH-CH-C$\underline{H}_3$), 1.778 (s, 6H, HON=C-C$\underline{H}_3$) 2,273 (4H, C$\underline{H}_2$NH), 3.235 (q, 2H, NHC$\underline{H}$-CH_3)

13C-NMR (proton decoupled) (CD_3OD, 300 MHz, δ ppm): 8.96, 19.39, 25.1, 35.26, 58.38, 58.95, 161.44

Melting point of d,l-HMPAO isomer: 132–134°C.

8.8. SYNTHESIS OF Cu(I)-MIBI TETRAFLUOROBORATE

Copper(I)tetrakis-2-methoxy-2-methyl-1-isonitrile (Cu(I)-MIBI) tetrafluoroborate is synthesized following the synthetic scheme given in Fig. 8.8.

8.8.1. Step 1: 2,2-Dimethylaziridine

Concentrated sulphuric acid (100 g, 1.12 mol) is mixed with 200 mL of water, followed by the addition of 100 g (1.12 mol) of 2-amino-2-methyl-1-propanol. The resulting warm solution is distilled at atmospheric pressure, resulting in a brown residue that is vacuum dried at 170–200°C for 6 h. The dry residue is ground and added to sodium hydroxide (100 g, 2.5 mol) in water. The suspension is heated to 110°C in a saltwater bath until dissolved, forming a black solution. The product is distilled from this solution at 70–88°C onto sodium hydroxide pellets. The remaining white precipitate is filtered through glass wool, the water layer is removed and the product is dried over sodium

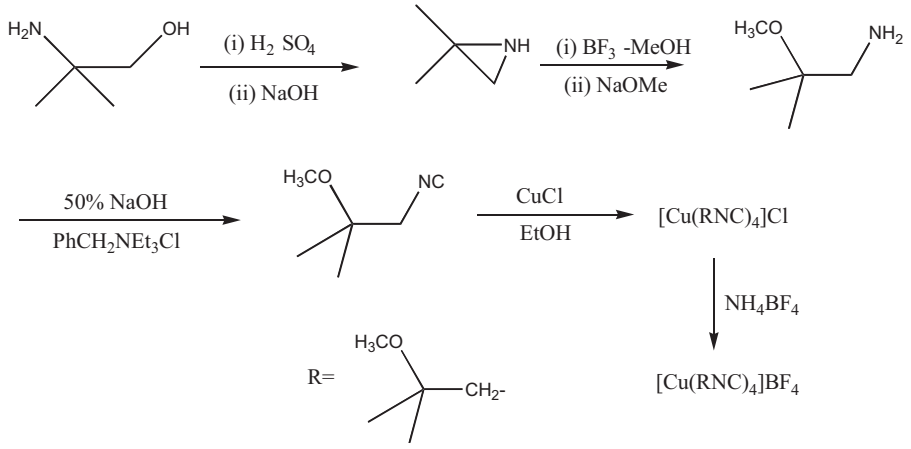


FIG. 8.8. Synthetic scheme for Cu(I)-MIBI.

hydroxide, filtered and dried over sodium metal. The product is distilled from the sodium metal at 70–73°C to yield 46.2 g of a clear, colourless product. The yield is 58%.

8.8.2. Step 2: 2-Methoxy-2-methylopropyl-1-amine

2,2-Dimethylaziridine (27.68 g, 0.39 mol) is dissolved in freshly distilled methanol and cooled to –10ºC in an ice/acetone bath. Boron trifluoride bis-methanol complex (58.32 g, 0.44 mol) is dissolved in freshly distilled methanol (50 mL) and cooled to –10ºC. Boron trifluoride bis-methanol complex is slowly added to cooled aziridine solution and allowed to warm to room temperature. The solution is stirred at room temperature for 7 d. The solution is reduced to approximately half the original volume using a rotary evaporator (25 mm Hg, 40ºC). Sodium methoxide in MeOH is added (95.04 g of 25% wt/wt solution, 0.594 mol) and a white precipitate is formed. Diethyl ether is added to the precipitate. The cloudy solution is distilled and filtered again. The distillation is continued to obtain a mixture of the two amines (14.24 g; 35%). The amines are separated by careful fractional distillation to yield pure 2-methoxy-2-methylopropyl-1-amine (boiling point: 123–124ºC).

8.8.3. Step 3: 2-Methoxy-2-methylpropyl-1-isonitrile (MIBI)

To the mixture of 2-methoxy-2-methylopropyl-1-amine (20.6 g, 0.2 mol) and chloroform (24 g, 0.2 mol), 100 mg of benzyltriethylammonium chloride and dichloromethane is added, followed by dropwise addition of 50% NaOH.

The solution is boiled for 2 h. The solution is then diluted with dichloromethane and washed twice with water. The organic layer is dried under sodium sulphate, evaporated and distilled under reduced pressure. The yield is ~15.3 g (68%) of MIBI (boiling point: 64ºC/180 mm Hg).

The water solution of MIBI absorbs light in the UV range. The maximum absorbance is obtained at 247 nm.

8.8.4. Step 4: Cu(I) tetra MIBI tetrafluoroborate ($[Cu(MIBI)_4]BF_4$)

Freshly distilled MIBI (1.23 g, 11.2 mmol) is mixed with 280 mg cuprous chloride (2.8 mmol) and stirred for 15 min in anhydrous ethanol. NH_4BF_4 (0.31 g, 0.003 mol) is added to the mixture and stirred in a water bath at 60ºC for 5 min. The solution is filtered and evaporated until a white solid appears. The complex is completely precipitated by adding ether. Purification is accomplished by dissolution in ethanol and recrystallization with the addition of ether. The yield is about 50%.

8.8.5. Characterization

NMR (D_2O, δ ppm): 1.13 (s, 6H, CH_3); 3.11 (s, 3H, OCH_3); 3.58 (m, 2H, CH_2)

FT-IR (KBr, υ cm^{-1}) 2190 (-NC), 1180, 1150, 1070 (OCH_3)

8.9. SYNTHESIS OF Cu(I)-MIBI TETRAFLUOROBORATE (ALTERNATE PROCEDURE)

Cu(I)-MIBI can also be synthesized following the synthetic scheme given in Fig. 8.9.

8.9.1. Step 1: 2-Methoxy isobutylisonitrile

To a solution of freshly fused zinc chloride (29.8 g, 0.22 mol) in anhydrous methanol, 20 mL (0.22 mol) of 2-hydroxyisobutyronitrile is added. The reaction mixture is heated overnight at 60°C. It is then poured into cold water (~0°C) and extracted with ether. The ether extract is dried over anhydrous sodium sulphate and distilled to give pure 2-methoxy isobutyronitrile. The yield is ~74%.

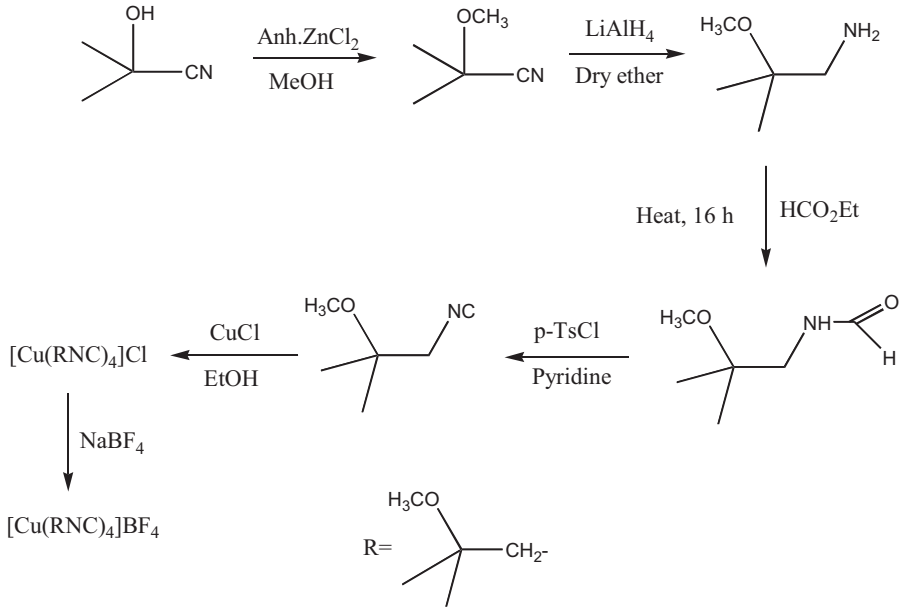


FIG. 8.9. Alternate procedure for synthesis of Cu(I)-MIBI.

^{1}H-NMR ($CDCl_3$, δ ppm) 1.31 (s, 6H, CH_3), 3.25 (s, 3H, OCH_3)

8.9.2. Step 2: 2-Methoxyisobutyl amine

To a well stirred slurry of 9 g (0.24 mol) of lithium aluminium hydride (LAH) in 500 mL of dry ether at 0°C is added 19.8 g (0.2 mol) of 2-methoxy-isobutyronitrile in 200 mL of dry ether. The reaction is continued overnight at room temperature. The excess LAH is quenched using saturated sodium sulphate solution. The mixture is filtered, and the filter cake is washed with ether. The combined ether solution is dried over anhydrous sodium sulphate, and the pure product is recovered from the ether layer on distillation to give 2-methoxyisobutylamine. The yield is 85%.

^{1}H-NMR ($CDCl_3$, δ ppm) 1.16 (s, 6H, CH_3), 1.6 (s, 2H, NH_2), 2.65 (s, 2H, CH_2), 3.26 (s, 3H, OCH_3)

8.9.3. Step 3: 2-Methoxyisobutyl N-formyl amine

Ethyl formate (12.2 mL, 0.15 mol) is added to the reaction mixture containing 15 g (0.15 mol) of 2-methoxyethylamine and a catalytic amount of

p-toluene sulphonic acid at 0°C. The reaction mixture is then brought to room temperature and refluxed overnight. The excess ethyl formate is removed by distillation, and the pure product 2-methoxyisobutyl N-formyl amine is recovered by vacuum distillation. The yield is 95% (boiling point: 75°C/ 15 mm).

^{1}H-NMR ($CDCl_3$, δ ppm) 1.13 (s, 6H, CH_3), 3.15 (s, 3H, OCH_3), 3.27 (d, 2H, CH_2), 6 (bs, 1H, NH), 8.19 (s, 1H, CHO)

8.9.4. Step 4: 2-Methoxyisobutyl isonitrile (MIBI)

2-Methoxyisobutyl N-formyl amine (15 g, 0.12 mol) and 38 g (0.2 mol) of p-toluene sulphonyl chloride are dissolved in 75 mL of pyridine. The reaction mixture is stirred at room temperature for 3 h. The final product is recovered by pouring the reaction mixture into water and extracting three times with 30 mL of ether. The combined ether extract is dried over anhydrous sodium sulphate. The ether is then removed at a low temperature (4°C/30 mm). The crude product is purified using a silica column and eluting with pentane:ether (1:1). The pure product (5.5 g, ~41%) is obtained by removing the pentane–ether mixture under vacuum at a low temperature (4°C/30 mm).

^{1}H-NMR ($CDCl_3$, δ ppm) 1.25 (s, 6H, CH_3), 3.22 (s, 3H, OCH_3), 3.38 (m, 2H, CH_2).

8.9.5. Step 5: [Cu(I)MIBI]Cl

2-Methoxyisobutyl isonitrile (MIBI) (0.5 g, 4.42 mmol), prepared as described above, is added to 0.109 g (1.1 mmol) of cuprous chloride in 1 mL of anhydrous ethanol and heated in an oil bath at 90°C for 1 h. This is then filtered through a 0.22 μm filter paper to give pure $[Cu(MIBI)_4]Cl$ in the filtrate.

8.9.6. Step 6: Cu(I)-MIBI tetrafluoroborate

To the filtrate above, 0.5 mL of a solution containing 1.1 mmol sodium tetrafluoroborate (equal in amount to the cuprous chloride added in the previous step) is added, and a white precipitate is obtained. This is filtered and dried to give 0.52 g (~78%) of $[Cu(MIBI)_4]BF_4$.

8.9.7. Characterization

1H-NMR (CD3OD, δ ppm) 1.2 (s, 6H, CH_3), 3.25 (s, 3H, OCH_3), 3.65 (m, 2H, CH_2)

8.10. SYNTHESIS OF TETROFOSMIN

The synthetic scheme for 6,9-bis-(2-ethoxy ethyl)3,12-dioxa-6,9-diphosphatetradecane (tetrofosmin) is given in Fig. 8.10.

8.10.1. Step 1: Tetraethylethylenebis(phosphonate)

Triethyl phosphite (314 g, 1.89 mol) and 1,2-dibromoethane (200 g, 1.05 mol) are placed in a two-necked, round-bottomed flask with a thermometer well. The flask is attached to a fractional column, which is connected to a distillation unit. The temperature of the reaction mixture is maintained at between 145 and 150°C for 1.5–2 h, after which ethyl bromide commences to distil. After removal of the ethyl bromide is complete, the reaction mixture containing three phosphonates is cooled to ambient temperature and subsequently fractionated under reduced pressure. The desired product, tetraethylethylenebis(phosphonate), distils at 160°C at 1 mm pressure. The typical yield is ~44%.

FT-IR (neat, ν cm^{-1}) 1150 (P=O)

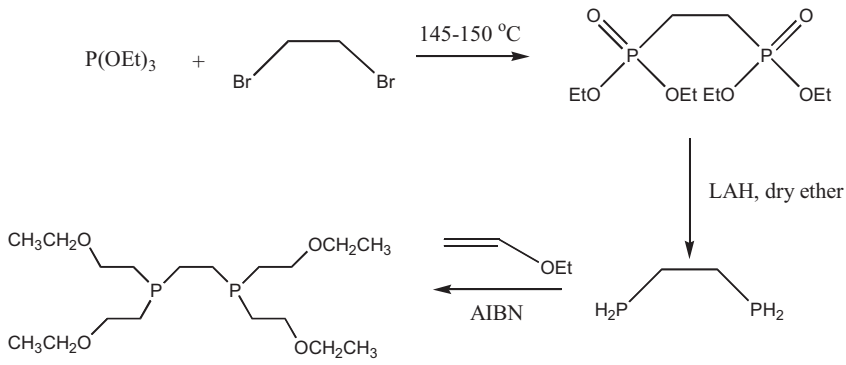


FIG. 8.10. Synthesis of tetrofosmin.

^{1}H-NMR ($CDCl_3$, δ, ppm) 1.34 (t, C-C$\underline{H}_3$), 1.98 (d, P-C$\underline{H}_2$), 4.12 (m, OC$\underline{H}_2$-C-)

8.10.2. Step 2: 1,2-bis(phosphino) ethane

A suspension of 40 g (1.05 mol) of lithium aluminium hydride in 1 L of ether is added to a three-necked flask with a nitrogen inlet, pressure equalizer and condenser connected to three traps half filled with bromine water to trap toxic vapours. The flask and its contents are cooled to 0°C, and 100 g (0.33 mol) of tetraethylethylenebis(phosphonate) in 180 mL of ether charged in a pressure equalizing funnel is added in a dropwise manner, with stirring over a period of 3 h. The reaction mixture is allowed to stand overnight at ambient temperature, after which it is hydrolysed by the slow addition of 800 mL of 6N HCl. The ether layer is separated and dried over anhydrous Na_2SO_4 for 8 h. The dried ether layer is fractionated under a slight positive pressure of nitrogen. 1,2-bis(phosphino) ethane distils at 109°C as a colourless liquid. The typical product yield is 57%.

FT-IR (neat, ν cm^{-1}) 2292 (P-H)

^{31}P-NMR ($CDCl_3$, δ, ppm) 131(s)

8.10.3. Step 3: 6,9-bis(2-ethoxyethyl)-3,12-dioxa-6,9-diphosphatetradecane (tetrofosmin)

1,2-bis(phosphino)ethane (1 mL, 10 mmol), ethyl vinyl ether (5 mL, 52.3 mmol) and azo isobutyro nitrile (AIBN) (0.1 g, 0.61 mmol) are placed in a pressure bottle. The reaction mixture is then stirred and heated at 75°C for 20 h. After cooling back to room temperature, the reaction mixture is transferred to a small round-bottomed flask, and volatile impurities are removed under vacuum. The non-distillable material is the pure compound. The typical product yield is 70%.

Note: The intermediate 1,2-bis(phosphino)ethane is extremely air sensitive and pyrophoric. Thus this reaction needs to be handled in a well ventilated fume hood with special arrangements for carrying out reactions under an inert atmosphere (under an oxygen-free atmosphere), in airtight chambers. The last step of the scheme has to be carefully carried out under pressurized conditions (pressure reaction vessels).

8.10.4. Characterization

^{31}P-NMR ($CDCl_3$, δ, ppm) -33.17

^{1}H-NMR ($CDCl_3$, δ, ppm) 1.12 (t, $OCH_2C\underline{H}_3$), 1.51 (m, PCH_2), 1.7 (t, $C\underline{H}_2CH_2OEt$), 3.4 (q, $OC\underline{H}_2CH_3$), 3.49(m, $PCH_2C\underline{H}_2OEt$)

BIBLIOGRAPHY

BANERJEE, S., et al., "On the synthesis, isolation, and radiochemical studies for the preparation of ^{99m}Tc-meso and d,l-HMPAO: A few additional observations", Nucl. Med. Biol. **26** (1999) 327–338.

BEVAN, J.A., et al., Tc-99m HMDP (hydroxymethylene diphosphonate): A radiopharmaceutical for skeletal and acute myocardial infarct imaging, I. Synthesis and distribution in animals, J. Nucl. Med. **21** (1980) 961–966.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, INTERNATIONAL ATOMIC ENERGY AGENCY, INTERNATIONAL LABOUR ORGANISATION, OECD NUCLEAR ENERGY AGENCY, PAN AMERICAN HEALTH ORGANIZATION, WORLD HEALTH ORGANIZATION, International Basic Safety Standards for Protection against Ionizing Radiation and for the Safety of Radiation Sources, Safety Series No. 115, IAEA, Vienna (1996).

FRITZBERG, A.R., et al., Synthesis and biological evaluation of technetium-MAG_3 as a hippuran replacement, J. Nucl. Med. **27** (1986) 111–116.

INTERNATIONAL ATOMIC ENERGY AGENCY, Applying Radiation Safety Standards in Nuclear Medicine, Safety Reports Series No. 40, IAEA, Vienna (2005).

INTERNATIONAL ATOMIC ENERGY AGENCY, Preparation of Kits for ^{99m}Tc Radiopharmaceuticals, IAEA-TECDOC-649, IAEA, Vienna (1992).

INTERNATIONAL ATOMIC ENERGY AGENCY, Production of ^{99m}Tc Radiopharmaceuticals for Brain, Heart and Kidney Imaging, IAEA-TECDOC-805, IAEA, Vienna (1994).

KELLY, J.D., et al., Technetium-99m-tetrofosmin as a new radiopharmaceutical for myocardial perfusion imaging, J. Nucl. Med. **34** (1993) 222–227.

NEIRINCKX, R.D., et al., Technetium-99m d,l-HM-PAO: A new radiopharmaceutical for SPECT imaging of regional blood perfusion, J. Nucl. Med. **28** (1987) 191–202.

LOBERG, M.D., et al., Development of new radiopharmaceuticals based on N-substitution of iminodiacetic acid, J. Nucl. Med. **17** (1976) 633–638.

RAMALINGHAM, K., Process for Preparing 2-Methoxy Isobutylisonitrile, US Patent 4864051 (1989).

VALLABHAJOSULA, S., et al., Technetium-99m ECD: A new brain imaging agent: In vivo kinetics and biodistribution studies in normal human subjects, J. Nucl. Med. **30** (1989) 599–604.

VAN WYK, A.J., et al., Synthesis and ^{99m}Tc labelling of MMI (MIBI) and its ethyl analog EMI, Appl. Radiat. Isot. **42** (1991) 687–689.

Annex I

SAMPLE BATCH PROCESSING RECORD

Batch Record
Kit for Preparation of ^{99m}Tc-MDP

Date of production: _____
Expiry date: _____
Batch size: 500 vials
Person in charge: _____ , Head, Production Group

1. MATERIALS

1.1. Starting materials

(i) Methylenediphosphonic acid (MDP)
(ii) Stannous chloride dihydrate
(iii) Ascorbic acid
(iv) Concentrated hydrochloric acid
(v) 1M HCl
(vi) 1M NaOH
(vii) Water for injection
(viii) Nitrogen gas

Note: Only those materials that have been released by QC can be used in the production process. The materials should be labelled with the product name, batch number and expiry date.

1.2. Packaging materials

(i) Glass vials (10 mL), penicillin type (neutral glass type I)
(ii) Rubber stoppers for lyophilizates
(iii) Aluminium caps

Note: Vials, stoppers and caps are to be washed and sterilized according to approved SOPs.

1.3. Other materials

(i) Control buffers for pH4.0 and 7.0
(ii) Elements of the dosing system that are in contact with the product
(iii) Magnetic stirrer

Note: Any equipment that comes into contact with the product must be washed and sterilized.

1.4. Glassware

(i) Glass beaker (1000 mL)
(ii) Weighing vials
(iii) Glass pipettes

Note: Glassware must be cleaned and sterilized. Only undamaged glassware is to be used. Vials are to be labelled with the product code number and batch number.

2. PRODUCTION PROCESS

Follow and complete the particle counting procedure at the manufacturing site. Record each instance where the measured values exceed limits.

2.1. Confirmation of preparatory work

(i) Washing and disinfection of premises, workbenches, chamber and trays of freeze-dryer
(ii) Washing and sterilization of vials and stoppers
(iii) Washing and sterilization of glassware and equipment that will be in contact with the product (e.g. filtration unit, dosing pump)
(iv) Sterilization of clothes, face masks, hair covering, gloves, etc.

2.2. Formulation

2.2.1. Weighing of starting materials

Weigh 0.50 g ± 0.5% of stannous chloride dihydride.

Net weight: _____ g

Operator: _____ Verified by: _____

Weigh 5.0 g ± 0.5% of MDP.

Net weight: _____ g

Operator: _____ Verified by: _____

Weigh 1.0 g ± 0.5% of ascorbic acid.

Net weight: _____ g

Operator: _____ Verified by: _____

2.2.2. Preparation of stannous chloride solution

Dissolve stannous chloride (anhydrous) in 4 mL of concentrated hydrochloric acid. Dissolve with water for injection to a volume of 250 mL just before adding to the stock solution.

Operator: _____

2.2.3. Dissolution of MDP

In the 1000 mL beaker on which the volume of 500 mL is marked, dissolve the MDP in 200 mL of water for injection. Pass this through the nitrogen and stir the solution for about 20 min.

Operator: _____

2.2.4. Preparation of stock solution

(i) Calibrate the pH meter with standard pH4.0 and 7.0 buffers. Adjust the pH meter if required.
(ii) Final reading (before stock solution measurement):

Buffer pH4.0: _____

Buffer pH7.0: _____

(iii) Add the stannous chloride solution to the nitrogen saturated MDP solution. Stir and continuously pass through the nitrogen.
(iv) Adjust the pH of the stock solution to remain between pH4 and 5. If the measured pH is lower than required, add 1M NaOH solution from the pipette in a dropwise manner.
If the measured pH is higher than required, add 1M HCl solution from the pipette in a dropwise manner. Measure the pH of the stock solution while the stirrer is off.
Record the pH of the stock solution and the volumes of 1M NaOH or 1M HCl used for adjustment:

pH of stock solution: _____

Volume of 1M NaOH solution used: _____ mL

Volume of 1M HCl solution used: _____ mL

(v) Bring the volume to 500 mL with water for injection. Continue stirring and nitrogen bubbling of stock solution for 10 min.

Operator: _____

2.2.5. Sterilizing filtration

(i) Transfer the stock solution directly from the tank to the feeding tank by sterilizing filtration using a 0.22 μm filter.
(ii) Adhere to the SOP.
(iii) Keep the temperature of the bulk solution as low as possible throughout the entire dispensing process.

2.2.6. Dispensing

Note: Distribution of the sterile products must be carried out in a class A area. During distribution, continue counting the particles in the surrounding area. Keep the sedimentation plates open.

(i) Verify that the distributor has supplied 1.0 mL ± 1% (0.99–1.01 mL).
(ii) Aliquot 1 mL ± 1% of solution into each sterile vial. Verify the distributor supply by weight during the process. Record the values in the table below. (The density of the solution will be ~1.00 g/mL. Checking the accuracy of the dispensing by weighing is a good practice.) The following table can be used for the record.

	Vial	Weight of vial (g)	Weight of vial with solution (g)	Net weight (g)	Volume (mL)
Before dispensing	1-1				
	1-2				
	1-3				
During dispensing	2-1				
	2-2				
	2-3				
After dispensing	3-1				
	3-2				
	3-3				

Operator: _____

(iii) Insert rubber stoppers into the necks of the vials. Place each filled tray in the freeze-dryer.

(iv) After distribution, verify that the filter used for the solution has not been damaged.

Record the pressure in the filter integrity test: _____

Operator: _____

(v) Control of particle counting during the process.
Note any points outside standard limits:

Time _____/_____/_____/_____

Point _____/_____/_____/_____

Source _____/_____/_____/_____

Operator: _____

2.3. Freeze-drying

(i) Follow the freeze-drying procedure using the following schedule:

Temperature:	Min: –40°C	Max: 26°C
Pressure:	Min: 0.002 mbar	Max: 0.005 mbar

Switch on the pressure and temperature recorders inside the freeze-dryer chamber.

(ii) Switch on the cooling system:

Date, hour: _____

(iii) After cooling to –40°C, switch on the vacuum pump:

Date, hour: _____

(iv) Continue freeze-drying according to the following schedule:

Hour of process	0	18	20	21	22	23	24	25	26	27	28	29	30
Actual time													
Temp. (°C)													

Hour of process	31	32	33	34	41	42
Actual time						
Temp. (°C)						

(v) In the 42nd hour of the process, switch off the pump. Fill the freeze-dryer with sterile nitrogen.

(vi) Seal the vials in the nitrogen atmosphere.

(vii) Remove the batch from the freeze-dryer; verify that the vials are properly sealed.

(viii) Attach the record of freeze-dryer parameters during the process to this batch record.

Operator: _____

2.4. Crimp sealing

(i) Crimp each vial with an aluminium cap; closure must be carried out as soon as possible after freeze-drying is completed.
(ii) Verify the yield of the process:

Number of vials taken for production: _____

Number of correctly prepared vials: _____

Number of rejected vials: _____

..... *Completion of production process* .....

3. SAMPLING BY QUALITY CONTROL

Inform QC once the product is ready. Ask the QC Designated Person to take the vials complying with the distribution in the beginning, middle and end of the processed batch.

20 vials taken for QC
Date: _____
Operator: _____

4. QUARANTINE

(i) Place the batch in quarantine. Identify the container as follows: Quarantine, ^{99m}Tc-MDP, Batch No., Expiry date
(ii) Store refrigerated at 2–8°C.

In the quarantine area there are _____ vials of kit for preparation of ^{99m}Tc-MDP.
Batch number: _____
Date: _____
Operator: _____

5. CONTROL OF PRODUCTION FILE

Names and initials of operators:

Operator: _____
Operator: _____

Head, Production Group: _____
Date: _____

Enclosures: (i) Record of particle count in the production area
(ii) Freeze-dryer parameters record
(iii) Results of microbial monitoring

Verified
Qualified Person (QP): _____ Date: _____

6. RELEASE DECISION

Documents checked:

☐ Batch record
☐ Analytical records
☐ Environmental monitoring records
☐ QC certificate

☐ **Released** ☐ **Not released**

..... *End of document* .....

Annex II

SAMPLE QUALITY CONTROL CERTIFICATE

Manufacturer identification	Quality control certificate Kit for preparation of ^{99m}Tc-DTPA	Quality control group
		Analysis No.:
Batch No.:	Expiry date:	

Test	Specification	Method	Result
Appearance of the lyophilizate	White pellet	Visual inspection	
Assay of stannous chloride ($SnCl_2$)	≥0.33 mg/vial	Colorimetry	
Radiochemical purity	>95%		
Pertechnetate (TcO_4^-)	<5%		
Reduced/hydrolysed Tc	<5%		
pH	4.0–7.0	Potentiometry	
Biodistribution	Excretion via urine ≥90%	In rats	
Bacterial endotoxins	<175 EU/V	LAL test	
Sterility	Sterile	Membrane filtration	

Head, Quality Control:
Date:

Qualified Person:
Date:

Annex III

LABELLING OF PAPER BOXES AND VIALS

The primary container (vials) and the secondary container should be labelled. The label should contain all the relevant information. Sample labels for the MDP kit are given in Figs III–1 and III–2.

(Logo and Name of manufacturer)
Reg. No.: .....
MDP
for labelling with technetium-99m
Composition:
Each vial contains:

Methylene diphosphonate	10.0 mg
Stannum (II) chloride, dihydride	1.0 mg
Ascorbic acid	2.0 mg

Lot No.: ..... Expiry date: .....
Storage: At 2–8°C, protected from light, oxidizing agents.
Administration mode:

FIG. III–1. Label for primary container (vial) of MDP kit.

(Logo and Name of manufacturer)
Reg. No.: .....
MDP
In vivo kit for labelling with Technetium-99m
No. of vials:
Each vial contains:

Methylene diphosphonate	10.0 mg
Stannum (II) chloride, dihydride	1.0 mg
Ascorbic acid	2.0 mg

Lot No.: ..... Expiry date: .....
Storage: At 2–8°C, protected from light, oxidizing agents.
Administration mode:

FIG. III–2. Label for secondary container (box) of MDP kit.

Annex IV

SAMPLE PACKAGE INSERT

Package Insert
(Directions for Use)

1. NAME OF MEDICINAL PRODUCT

Methylene diphosphonate (MDP) in vivo kit for compounding of radiopharmaceutical product.

The pharmaceutical is to be prepared on the location of use (hospital or clinical laboratory) by mixing the contents of the product with $Na^{99m}TcO_4^-$ (pertechnetate) eluate obtained from any licensed $^{99}Mo/^{99m}Tc$ generator.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Composition of MDP in vivo kit:

MDP:	10 mg
$SnCl_2.2H_2O$:	1 mg
Ascorbic acid:	2 mg

3. PHARMACEUTICAL FORM

Pharmaceutical form of MDP in vivo kit: Powder for injection.
Pharmaceutical form of ^{99m}Tc-MDP: Radioactive, sterile injection.

4. CLINICAL PARTICULARS

4.1. Clinical indications

Isotope diagnostic bone scintigraphy:

Application is recommended for the following diseases:
— Primary bone tumour;
— Bone metastases of other tumours (e.g. prostate, breast and lung cancer);

— Osteomyelitis;
— Metabolic diseases of the bone;
— Paget's disease.

4.2. Posology and method of administration

Required activity: 370–740 MBq at the time of application.

For administration in children (see also 'contraindications' discussion below), the activity to be administered is to be determined with Webster's formula:

$$A_{\text{children}}\ (\text{MBq}) = [(N + 1) \times A_{\text{adult}}\ (\text{MBq})]/(N + 7)$$

where N is the age of the child, in years.

^{99m}Tc-MDP is administered to the patient as an intravenous injection. Imaging should begin 2–4 h post-injection.

4.3. Contraindications

Relative contraindications:

Use of the product is relatively contraindicated:
— For patients below 18 years of age;
— For pregnant or lactating women, except when the necessity and importance of acquiring the information outweigh the risk originating from the radiation exposure.

Absolute contraindications:

Use of the product is absolutely contraindicated if the patient does not agree to the administration of radioactive injection.

4.4. Special warnings and special precautions for use

The product is a pharmaceutical containing a radioisotope; thus the rules for handling, transport and storage of radioactive materials are applicable. The pharmaceutical can only be applied by properly qualified and trained personnel within designated clinical settings possessing the appropriate government authorization for the use and manipulation of radioisotopes.

4.5. Interactions with other medicinal products and other forms of interaction

No interactions have been reported.

4.6. Application during pregnancy and lactation

In general, application of the product during pregnancy and lactation is contraindicated unless the necessity and importance of acquiring the information outweigh the risk originating from the radiation exposure.

4.7. Effect of the product on ability to drive and on working in circumstances of significant accident risk

The product has no direct influence on the ability to drive or work in hazardous circumstances. In the event of unexpected side effects, the ability to drive and work in circumstances with a significant risk of accident is to be reconsidered.

4.8. Undesirable effects

Undesirable effects and symptoms are unexpected.

4.9. Overdose

No information is available about the actual occurrence of an overdose. Should such a case occur, treatment should be directed towards the support of vital functions.

5. PHARMACOLOGICAL PROPERTIES

5.1. Pharmacodynamic properties

After it is administered intravenously, ^{99m}Tc-MDP leaves the blood and is concentrated mainly in the skeleton and to a negligible extent in the soft tissues. This process is accomplished with normal bone as well, but binding is significantly more extensive where the blood supply of the bone and bone formation activity (osteoblast function) are increased.

^{99m}Tc-MDP not bound to the skeleton washes out from the body via the urine. Washout via the hepatobiliary system is negligible.

5.2. Pharmacokinetic properties

^{99m}Tc-MDP introduced intravenously leaves the bloodstream with a pharmacokinetics characterized by a three compartment model. During the initial, rapid phase, ^{99m}Tc-MDP is excreted to the extravascular space. The medium phase corresponds to bone uptake. The slow phase is the dissociation of ^{99m}Tc-MDP bound to plasma proteins of blood. Maximum bone uptake is attained 2 h post-injection.

Washout occurs via the urine. Activity observable in the liver and intestines is insignificant.

5.3. Preclinical safety data

Based on the results of intravenous acute toxicity experiments in mice, no clinical symptoms are observed up to 9 mg/kg of body weight. Consequently, application of the medicinal product can be considered safe from the point of view of toxicity.

5.4. Radiophysical properties of the radionuclide and absorbed dose values

A single dose to a patient contains 370–740 MBq of activity. For an average weight of 70 kg, 1 MBq of the injection induces the following absorbed dose in the organs listed:

Organ	Absorbed dose values (μGy/MBq)
Skeleton	12.2
Bone marrow	2.7
Kidneys	1.6
Urinary bladder	13.3
Whole body	2.7

6. PHARMACEUTICAL PARTICULARS

6.1. Excipients

Stannous (II) chloride, ascorbic acid.

6.2. Incompatibilities

No interaction with other pharmaceuticals has been reported.

6.3. Shelf life

The shelf life of the MDP kit is 12 months from the day of production.

6.4. Special precautions for storage

The MDP in vivo kit is to be stored in a refrigerator (2–8°C) in its original packaging. The ^{99m}Tc-MDP injection is to be stored at room temperature (15–25°C) in accordance with the national regulations for radioactive materials.

6.5. Nature and composition of packaging

The MDP in vivo kit contains the components as sterile, freeze-dried material. Each ampoule is labelled and closed with a rubber plug and an aluminium cap.

6.6. Instructions for handling and use

For labelling, place the glass vial containing the freeze-dried material in a small lead pot having a wall thickness of 3 mm. Under aseptic conditions, inject the required activity of sterile ^{99m}Tc-pertechnetate (up to 10 GBq) into the vial through the rubber cap with a sterile syringe. Mix the contents of the vial thoroughly and let stand for 15 min at room temperature. Thereafter, the solution (or its appropriate portion) can be administered intravenously. The quantity of the radiochemical impurities should not exceed 10%.

7. MARKETING AUTHORIZATION HOLDER: _____

8. MARKETING AUTHORIZATION NUMBER: _____

9. DATE OF FIRST AUTHORIZATION/ RENEWAL: _____

Annex V

DETERMINATION OF STANNOUS CONTENT

The cold kits used for preparation of ^{99m}Tc labelled radiopharmaceuticals contain one of the stannous salts as a reducing agent for converting technetium in the +7 state in the pertechnetate to a desired lower oxidation state, which complexes with the ligand to form the radiopharmaceutical. Stannous chloride dihydrate ($SnCl_2.2H_2O$) is the most commonly used stannous salt. Stannous ions are highly prone to oxidation, even on exposure to atmosphere or dissolved oxygen in the solution. Hence, great care must be taken to perform kit formulations using solutions bubbled with nitrogen and to carry out the dispensing procedure as quickly as possible to minimize exposure to atmosphere.

The presence of stannous ions in the kit vial is essential for preparation of the radiopharmaceutical, and in some cases the amount of stannous content present is crucial to obtaining the desired product quality. The stability of the kits depends on the continuous presence of the stannous content throughout the period, which in turn depends on, for example, the production process, freeze-drying conditions and residual humidity, as well as the storage conditions.

The following two methods are commonly used to determine the Sn(II) content of kit vials.

1. Iodometric analysis

Reagents:

— 1.5N HCl;
— 0.0015M iodine solution;
— Starch indicator.

Reconstitute the kit vial with 5 mL of saline. Add 1 mL of 1.5N HCl and two drops of starch indicator to the kit vial. Simultaneously set up a blank with 5 mL of saline. Titrate this solution against 0.0015M iodine solution until the blue colour persists. One millilitre of iodine solution corresponds to 0.564 mg of $SnCl_2.2H_2O$.

2. Spectrophotometric assay

Reagents:

— 0.1% Mo solution as sodium molybdate;
— 1.5M KSCN solution;
— Standard Sn(II) solution of 1 mg Sn(II)/mL;
— 3N HCl.

Prepare a set of vials with 5, 10, 25 and 50 µL of standard Sn(II) solution corresponding to 5, 10, 25 and 50 µg of Sn(II), respectively. Reconstitute the kit vial with 5 mL of saline and take 0.5 mL in a separate vial. Prepare a blank vial using 0.5 mL of double distilled water. To all the above vials, add 3.5 mL of 3N HCL, 0.5 mL of 0.1% Mo solution and 0.5 mL of 1.5M KSCN solution, and adjust the volume in each vial to 5.0 mL with double distilled water. Shake the vials until the solution turns orange in colour. After 15 min, measure the absorbance at 460 nm against the blank. Construct a standard curve to calculate the Sn(II) content.

BIBLIOGRAPHY

RAKIAS, F., "Determination of tin (II)", Technetium Pharmaceuticals: Preparation and Quality Control in Nuclear Medicine (ZOLLE, I., Ed.), Springer, New York (2007) 144–145.

RAKIAS, F., ZOLLE, I., "Stannous ion determination: Importance and relevance for radiopharmaceuticals kits", Radioactive Isotopes in Clinical Medicine and Research (BERGMANN, H., KROISS, A., SINZINGER, H., Eds), Vol. 22, Birkhäuser, Basel (1997) 401–409.

Annex VI

RESIDUAL MOISTURE DETERMINATION IN FREEZE-DRIED KITS

VI–1. INTRODUCTION

Determination of the residual moisture content of freeze-dried kits is important, as this moisture affects the long term stability of freeze-dried kits. The freeze-drying processes of currently available freeze-dryers can reduce the water in the bulk solution to very low levels, much less than 0.1%. In poorly freeze-dried products, the residual water damages the structure of the freeze-dried pellet in addition to significantly reducing the stability of the kits.

VI–2. METHODS FOR ESTIMATION OF RESIDUAL WATER CONTENT

There are two ways to determine the residual water content of the kits: by using a Karl Fischer apparatus, or by drying over phosphorous pentoxide (P_2O_5) and calculating the difference in weight.

VI–2.1. Karl Fischer method

The Karl Fischer method is designed to determine the water content of substances by using the quantitative reaction of water with iodine and sulphur dioxide in the presence of methanol and pyridine:

$$H_2O + I_2 + SO_2 + 3CH_5H_5N \rightarrow 2(C_5H_5N^+H)I^- + C_5H_5N.SO_3$$

$$C_5H_5N.SO_3 + CH_3OH \rightarrow (C_5H_5N^+H)O^-SO_2.OCH_3$$

Volumetric or coulometric titration is used for estimating the iodine conception and thereby the moisture content.

In the volumetric titration method, the iodine required for reaction is dissolved and its consumption is then estimated to determine the water content. In the coulometric titration method, iodine is produced by electrolysis of a reagent containing an iodide ion, and the water content in the sample is estimated by measuring the quantity of electricity needed for the electrolysis to produce the required amount of iodine.

General information on determining water content using the Karl Fischer method is available in the EP and the USP. Detailed procedures for performing the water content determination test using the Karl Fischer method are available from the commercial suppliers of the apparatus and/or reagents. Stannous ions present in the kit interfere with the reaction, hence distillation may be required prior to titration.

VI–2.2. Drying over P_2O_5 at reduced pressure

If a Karl Fischer apparatus is not available, the water content in the kits can be assessed by exposing the open freeze-dried vials to dry P_2O_5 powder. At least 5–10 vials of the kits are needed to obtain reliable results using this method. The procedure is as follows. The aluminium caps are carefully removed from the kit vials without opening the rubber stoppers. The weight of each vial and the rubber closure is determined using an analytical balance, and is recorded. The vials are placed in a desiccator in which about 200 g of fresh P_2O_5 has been placed. The vials are partially opened, until the split in the gap in the split rubber closure is visible. The desiccator is closed, and its outlet is connected to the vacuum pump and a vacuum is applied for 24 h. Once the pump is switched off, the pressure is balanced by opening the air inlet. The vials are then sealed and weighed. The water content is the difference between the weight of the sealed vials before and after drying. The percentage of water can be determined after washing and weighing the empty vials together with the rubber closures to obtain the weight of the powder.

BIBLIOGRAPHY

EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Determination of water by distillation (01/2005:20213), European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 32.

EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Water: Micro determination (01/2005:20532), European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005).

EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES, Water: Semi-micro determination (04/2007:20512), European Pharmacopoeia, 5th edn, EDQM, Council of Europe, Strasbourg (2005) 4785.

UNITED STATES PHARMACOPEIAL CONVENTION, Water determination (method 921), United States Pharmacopeia 30, USP Convention, Rockville, MD (2006) 385–387.

Annex VII

STERILITY TESTING

VII–1. INTRODUCTION

Technetium-99m radiopharmaceuticals are prepared using precautions designed to exclude bacterial contamination. It is essential that all parenteral products fulfil the pharmacopoeial requirements of being sterile and pyrogen free. Sterility tests are used to check for microbial contamination (i.e. bacteria and fungi) in these products. All components of cold kits are tested individually for sterility.

The presence of bacteria is detected by their growth during incubation of the product in fluid thioglycollate medium (FTM) at 32.5 ± 2.5°C for 14 d; the presence of fungal organisms is detected by their growth during incubation of the product in soybean casein digest medium (SCDM) at 22.5 ± 2.5°C for 14 d. The medium turns turbid if microorganisms are present, while it remains clear if the product is sterile.

Sterility tests are highly exacting and need to be performed by a well trained technician having experience in rigid aseptic techniques. The technician must be suitably attired in sterile garments (coat, gloves, mask and cap). The tests are not to be conducted under direct exposure to ultraviolet light in areas sprayed with disinfectants.

VII–2. REQUIREMENTS FOR STERILITY TESTING

VII–2.1. Reagents

— *Geobacillus stearothermophilus* (ATCC 7953);[8]
— Spore strips or discs (Raven or equivalent);
— Fumigant FTM;
— SCDM;
— Plate count agar medium;
— Double distilled water;
— Dettol;
— Teepol;

[8] Formerly, *Bacillus stearothermophilus.*

— 0.9% sodium chloride solution (sterile);
— 70% ethanol.

VII–2.2. Accessories and facilities needed for sterility testing

— Laboratory coat;
— Disposable cap;
— Disposable face mask;
— Sterile plastic plates;
— Steam sterilization indicator tape;
— Sterile, disposable syringes and needles;
— Test tube racks;
— Sterile pointed forceps;
— Scissors;
— Surgical gloves;
— Spatulas;
— Glass marking pens;
— Borosil conical flasks, 500 mL capacity;
— Borosil bottles, 250 mL capacity;
— Borosil test tubes, 20 mL capacity;
— Dispenser, 50 mL capacity;
— Measuring cylinders;
— Rubber closures;
— Non-absorbent cotton;
— Absorbent sheets;
— Aluminium foil;
— Waxed paper;
— Receptacles for discarding used test tubes;
— Plastic bags for waste disposal;
— Tissue paper rolls;
— Flammable waste discard bins;
— Non-flammable waste discard bins.

VII–2.3. Instruments

— Laminar flow bench for non-radioactive work;
— Laminar flow bench for media preparation;
— 55°C incubator;
— 37°C incubator;
— 25°C incubator;
— Autoclave;

— Hot air oven;
— Refrigerator;
— Weighing balance;
— Sealing machine;
— Fumigation equipment.

VII–3. PREPARATION OF MEDIUM FOR STERILITY TESTING

VII–3.1. Medium for detection of aerobic and anaerobic organisms

FTM, which is well known for its ability to support the growth of both aerobic and anaerobic microorganisms, is used in the detection of such organisms. The composition of FTM is given below:

— Casein enzymic hydrolysate: 15.0 g;
— Yeast extract: 5.00 g;
— Dextrose: 5.5 g;
— Sodium chloride: 2.50 g;
— L-cystine: 0.5 g;
— Sodium thioglycollate: 0.5 g;
— Sodium azide: 0.001 g;
— Agar: 0.75 g;
— Final pH (at 25°C): 7.1 ± 0.2.

Boil 29.8 g of FTM in 1 L of distilled water until it is completely dissolved. Distribute 15 mL aliquots of the solution into test tubes, plug with non-absorbent cotton and sterilize by heating in an autoclave at 1.05 kg/cm^2 (15 psi) at 121°C for 20 min. Store the sterile medium at 20–30°C. If more than 30% of the uppermost layer of the medium has changed to a pink colour, reheat in a boiling water bath to remove absorbed oxygen. Do not heat repeatedly. At the time of use, not more than the upper tenth of the medium should be pink in colour. The medium may be used for a period of one month after preparation, provided it exhibits no sign of turbidity or a change in colour.

VII–3.2. Medium for detection of fungi

SCDM is used for the detection of fungi; its composition is given below:

— Casein enzymic hydrolysate: 17.0 g;
— Papaic digest of soybean meal: 3.0 g;

— Sodium chloride: 5.0 g;
— Dibasic potassium phosphate: 2.5 g;
— Dextrose: 2.5 g;
— Final pH (at 25°C): 7.3 ± 0.2.

Boil 30 g of SCDM in 1 L of distilled water until it is completely dissolved. Distribute 15 mL aliquots of the solution into test tubes, plug the tubes with non-absorbent cotton and sterilize the medium at 121°C for 20 min. Store the sterile medium at 20–25°C. The medium may be used for a period of one month after preparation, provided it exhibits no sign of turbidity.

VII–4. SAMPLING

The number of sample vials to be provided for the test is 1% of the total number of vials or 4 vials, whichever is greater, and the volume of the solution should be not less than 1 mL.

The volume of the sample to be transferred to the medium is decided as follows:

— When the sample is in the form of a freeze-dried powder, reconstitute to 2 mL using sterile saline and transfer the entire contents to 15 mL of the sterile medium.
— When the volume of the solution is less than 2 mL, transfer the entire contents to 15 mL of the sterile medium.
— When the volume of the solution is 2–10 mL, transfer 2 mL of the sample to 15 mL of the sterile medium.
— When the volume of the solution is 10–50 mL, transfer 5 mL of the sample to 40 mL of the sterile medium.
— When the volume is greater than 50 mL, transfer 10 mL of the sample to 80 mL of the sterile medium.

VII–5. PROCEDURE FOR STERILITY TESTING

Clean the work surface of the laminar flow bench using 70% ethanol. Switch the laminar flow on at least 30 min prior to starting the test. Use sterile, disposable needles and syringes to carry out the transfer of liquid material aseptically.

Test for bacteria with FTM

- Transfer the desired volume of the sample, as described in Section VII–4 above, to the designated volume of FTM. Incubate the inoculated medium at 32.5 ± 2.5°C for observation for 14 d.
- Media control test: Expose at least one tube of FTM, in an open condition with its cotton plug removed, in the laminar flow bench for the duration of the sterility testing experiment. Plug the tube tightly at the end of the test and incubate at 32.5 ± 2.5°C for observation for 14 d.

Test for fungi with SCDM

- Transfer the desired volume of the sample as described in Section VII–4 above to the designated volume of SCDM. Incubate the inoculated medium at 22.5 ± 2.5°C for observation for 14 d, or as required by national regulatory requirements.
- Media control test: Expose at least one tube of SCDM, in an open condition with its cotton plug removed, in the laminar flow bench for the duration of the test for sterility. Plug the tube tightly at the end of the test and incubate at 20–25°C for observation for 14 d.

Interpretation

Monitor the tubes daily for growth of microorganisms and compare with the media control test container. The product is deemed to pass the test if there is no sign of turbidity before the end of 14 d.

If turbidity occurs, indicating the growth of microorganisms, take fresh containers (twice the number used in the first test) from the same batch and repeat the test. The product is deemed to have failed the sterility test if growth occurs during the second test.

Note:

- The USP recommends that, instead of transferring the product to the media, it should be filtered through a 0.22 μm filter and washed with sterile saline, and the filter should be transferred to the media.
- All material transfer for testing is carried out in a laminar flow hood. However, at present, isolators are considered a better option, as these are closed clean systems.

— On completion of the work, ensure safe disposal of used or unusable preparations of all media by autoclaving and/or by incineration to avoid contamination.
— When the test sample contains an added bactericide, the volume of the medium for the test is sufficient to make the concentration of the bactericide ineffective.

Annex VIII

ENVIRONMENTAL MONITORING

In addition to tests of product sterility (described in Annex VII), the following tests must be performed while carrying out the manufacture of parenteral preparations:

— Environmental control tests;
— Total sterility test;
— Test for sterilizing conditions.

VIII–1. ENVIRONMENTAL CONTROL TESTS

Environmental testing is conducted to establish the sterility of the atmosphere in the laminar flow benches and areas used for the production process. The testing involves exposure of plate count agar plates during production. Static exposure (to test the environment when no operation is being carried out) involves exposure for 3 h, while dynamic exposure involves exposure for the duration of the operation. After exposure, the plates are incubated for 48 h at 37°C (or for 72 h at 25°C), followed by enumeration of the microbial colonies formed.

VIII–1.1. Preparation of plate count agar

The composition of the plate count agar is as follows:

— Casein enzymic hydrolysate: 5.0 g;
— Yeast extract: 2.5 g;
— Dextrose: 1.0 g;
— Agar: 15.0 g.

Dissolve 23.5 g of the medium in 1 L of distilled or deionized water. Boil to dissolve completely. Sterilize in the autoclave for 15 min at 1.05 kg/cm^2 (15 psi) (121°C). (Final pH of 7.0 ± 0.2 at 25°C.)

Prepare plates by aseptically pouring 20 mL of medium onto sterile plastic plates in a laminar flow bench. These plates are usually prepared 4 d prior to the start of the environmental control test. Store plates at 4°C under sterile conditions and use within 3 weeks of preparation.

VIII–1.2. Method

Expose a set of plates in the working area for 3 h prior to the commencement of the production process and another set during the production operation. Incubate the plates at 30–35°C for 2 d, at the end of which the colony-forming units (CFUs) are to be counted.

VIII–1.3. Interpretation

The absence of any CFUs indicates that sterile working conditions have been maintained.

VIII–2. TOTAL STERILITY TEST

The total sterility test is carried out to verify adequate performance of all aseptic processing operations during preparation of the final product. It is done by using 'media fill' operations in those working areas where parenteral preparations are aseptically handled, filtered and distributed.

VIII–2.1. Method

During the dispensing of the injectable preparation, take 1% of the sterile vials from the same lot used for production and aseptically distribute 2 mL of sterile FTM into each of them. Incubate the vials at 37°C for 14 d.

VIII–2.2. Interpretation

Monitor the vials for growth for at least 14 d. If none of the vials show turbidity at the end of 14 d, the vials pass the total sterility test, indicating that the processing operations, the vials used and the environment are satisfactory.

VIII–3. TEST FOR STERILIZING CONDITIONS

Sterilization equipment control tests involve testing for proper performance of the autoclave and hot air oven. The testing involves exposure of spore strips containing heat resistant bacterial spores (*Geobacillus stearothermophilus* (ATCC 7953) and *Bacillus atrophaeus* (ATCC 9372 or 5230)) during the sterilization process. The methods described below can be used for this test.

VIII–3.1. Method 1

— Use spore strips/discs impregnated with *Geobacillus stearothermophilus* (ATCC 7953) spores (3.2×10^5 CFUs/strip) and a culture medium encased in a plastic ampoule with bromocresol purple functioning as a pH indicator. The acid production associated with growth causes a change in the colour of the medium from purple to yellow to facilitate the detection of growth. Each batch is supplied with a certificate of performance and the resistance characteristics of the product.
— Place one or more of the spore strips or discs in a horizontal position in the most difficult to sterilize locations of the autoclave (e.g. near the drain).
— Run a sterilization cycle (saturated steam at 121°C for 15 min at 1.05 kg/cm^2 (15 psi)).
— After the biological indicator has cooled (not longer than 15 min), seal the cap by pressing down firmly until it is flush with the tube.
— Crush the media ampoule by squeezing the sides of the plastic tube.
— Inspect the unit to make sure that the spore strip/disc has dropped into the growth medium. If the spore strip/disc has remained in the upper portion of the unit, simply hold the unit in a vertical position and tap the bottom on a hard surface until the strip/disc drops into the medium.
— Place the processed vial and one unprocessed (control) vial in a vertical position in an incubator at 55–60°C for 24 h. Begin monitoring the incubated units after 12–18 h. Record observations.

VIII–3.2. Interpretation

A failed sterilization cycle is indicated by turbidity and/or a change in colour to or towards yellow. A test ampoule that retains its purple colour indicates an adequate sterilization cycle.

Control: The control vial should exhibit a colour change and/or turbidity. If the control ampoule does not show signs of growth, the test should be considered invalid.

VIII–3.3. Method 2

— Use spore strips (2 cm × 0.5 cm) impregnated with viable spores of *Geobacillus stearothermophilus.*
— Aseptically enclose each spore strip individually, along with a drop of water for injection/saline, in sterile vials and close with a sterile rubber closure before use in the autoclave.

— Place a vial containing a spore strip in the most difficult to sterilize location of the autoclave (e.g. near the drain).
— Run a sterilization cycle (saturated steam at 121°C for 15 min at 1.05 kg/cm^2 (15 psi)). On cooling, aseptically transfer 5 mL of sterile SCD (prepared as described in Section VII–3.2 of Annex VII) to the vial using a sterile, disposable needle and syringe, without opening the vial.
— Transfer 5 mL of SCDM to a non-autoclaved spore strip to serve as a control.
— Incubate the two vials at 55–60°C for 4 d. Examine the vials daily during incubation. Record the observations.

VIII–3.4. Interpretation

A failed sterilization cycle is indicated by turbidity in the autoclaved vial. Test vials that do not show signs of growth indicate an adequate sterilization cycle.

Control: The control vial should exhibit turbidity. If the control vial does not show signs of growth, the test should be considered invalid.

VIII–4. PROCEDURE FOR FUMIGATION OF THE LABORATORY

Fumigate the sterility laboratory using a suitable fumigant (e.g. a chlorine-free fumigant containing as an active ingredient hydrogen peroxide 10% wt/vol. and diluted silver nitrate solution 0.01% wt/vol. in sterile water). The recommended dosage for aerial fumigation is 20% wt/vol. solution in demineralized water.

For a laboratory measuring 10 m × 3 m, mix 50 mL of fumigant solution with 1250 mL of demineralized water and run a fumigation machine (e.g. fog spraying equipment) for 25 min. Ensure that the laboratory is completely closed during the fumigation procedure so as to saturate the entire room with the fumigant spray. To avoid exposure of personnel, the laboratory should not be entered for at least 24 h post-fumigation. The fumigation procedure should be conducted at weekly intervals.

To assess the efficacy of the fumigation procedure, carry out environmental tests such as the colony counts test using plate count agar medium 24 h post-fumigation. Expose these plates in the fumigated area for at least 1 h. Incubate them at 37°C for at least 4 d, at the end of which the CFUs should be counted. The absence of any CFUs indicates that the fumigation procedure has been effective and that sterile working conditions have been maintained.

Precautions during the fumigation procedure: The concentrated product may cause skin irritation. In the case of accidental spillage or exposure, thoroughly wash the affected area with water. Store the fumigant in a cool, dry, dark place.

Annex IX

PYROGEN TEST AND BACTERIAL ENDOTOXIN TEST

For parenteral preparations, either the pyrogen test or the bacterial endotoxin test (BET) is required, as specified in the monograph.

IX–1. PYROGEN TEST

The pyrogen test is designed to ensure that the risk of febrile reaction in patients injected with the product concerned is limited. Pyrogen tests for kits of ^{99m}Tc radiopharmaceuticals are not recommended by the pharmacopoeias, except for products involving starting material of human or animal origin. According to the USP, injections of volumes of less than 100 mL need not be tested for pyrogens unless specified in the monograph. In most cases, the volume of technetium radiopharmaceutical injections does not exceed 10 mL.

The test involves the measurement of temperature increases in rabbits following the intravenous injection of a test solution. The USP pyrogen test procedure is simple and involves only one repeat test in the case of doubtful results. Briefly, the procedure is as follows. The cold kit vial for preparation of the ^{99m}Tc radiopharmaceutical is reconstituted with 2 mL of injectable saline. In the case of products having more than one component, the various components are reconstituted with injectable saline (2 mL per vial), and a mixture of the components is made in the same proportion as would be used in a radiopharmaceutical. The solution (1 mL/kg of body weight) is injected through the ear vein of three rabbits, and the body temperature of the animals is measured for a period of 6 h with a thermometer inserted into the anal canal. The combined rise in temperature in the three rabbits should not be more than 1.5°C, and none of the rabbits should register more than a 0.6 °C rise (British Pharmacopoeia).

IX–2. BACTERIAL ENDOTOXIN TEST

Although the terms ‘pyrogens’ and ‘endotoxins’ are often used synonymously, their meanings are not identical. Pyrogens refer to any substance that causes fever in an animal, whereas endotoxins refer to cell wall components of gram negative bacteria, which are a particular type of pyrogen. Endotoxins, which are lipopolysaccharides, are the most common cause of toxic reactions

resulting from pyrogen contamination of pharmaceutical products. Although there are a small number of pyrogens other than endotoxins, the pyrogenic effect is almost always due to the presence of endotoxins. Thus the conclusion that the absence of bacterial endotoxins in a product implies the absence of pyrogenic components is generally justified.

The kits for formulation of ^{99m}Tc radiopharmaceuticals are to be tested with the BET. The endotoxin content should not be more than 175/V endotoxin units (EU) per mL, where V is the maximum recommended total dose in mL. V is generally taken to be 7 mL (according to manufacturers of BET reagents); hence the limit for radiopharmaceuticals is 25 EU/mL. The test is to be initiated immediately after preparation of the labelled product.

The BET is a rapid and sensitive in vitro test based on the formation of a gel clot by limulus amoebocyte lysate (LAL) reagent in the presence of endotoxins. The LAL test is based on the ability of the endotoxin to produce recognizable gelation or opacity in the lysate of the amoebocytes (blood cells) of the horseshoe crab, *Limulus polyphemus* (i.e. in the LAL reagent).

IX–2.1. Method

The gel clot LAL test consists of mixing equal parts of the LAL and test specimen, and incubating the mixture for 60 min at 37°C. Formation of a firm gel clot that does not disintegrate on inversion through 180° constitutes a positive response and indicates the presence of endotoxins in excess of the LAL reagent's labelled sensitivity.

IX–2.2. Endotoxin limits for various types of product

Product	EU for a 70 kg adult	Maximum injected volume (mL)	Endotoxin limit (EU/mL)
Parenteral drugs	350	V	350/V
Intrathecal drugs	14	V	14/V
Radiotracers	175	7 (adult)	25
Large volume parenterals (e.g. saline)	—[a]	10 per kg	0.5
Water for injection	—	—	0.25

[a] Not defined.

Annex X

BIODISTRIBUTION STUDIES

X–1. INSTRUMENTS/MATERIALS NEEDED FOR ANIMAL EXPERIMENTS

— NaI(Tl) scintillation counter;
— Isotope dose calibrator;
— Balance for weighing experimental animals;
— Single pan balance for weighing organs/tissues;
— Deep-freezer (–20°C) to preserve animal carcasses;
— Refrigerator;
— Lead glass;
— Lead pots with glass windows;
— Dissection set and tray;
— Glass beakers;
— Polystyrene test tubes (5 mL);
— Glass test tubes (10 mL);
— Disposable plastic bags;
— Surgical gloves;
— Anaesthetics;
— Absolute alcohol;
— Absorbent cotton;
— Water for injection.

X–2. GENERAL PROCEDURES FOR BIODISTRIBUTION STUDIES

Rats and mice are the animals most commonly used for testing of ^{99m}Tc radiopharmaceuticals. At least three animals are studied at each time point. The animals are weighed before being injected with the radiopharmaceutical and are kept in separate numbered cages.

The ^{99m}Tc radiopharmaceutical is prepared using the cold kit vial to be tested following the instructions enclosed in the kit. Generally, 0.1–0.5 mL of the preparation is injected per animal via the tail vein. The injected activity is calculated by taking the difference between the weight of the loaded syringe and that of the syringe after injection.

At the end time point, the animals are sacrificed and a blood sample is taken by heart puncture. The organs of interest are carefully dissected, rinsed in

saline and placed in individual disposable plastic tubes or bags and accurately weighed. The tail, which is the site of injection, is removed and kept separately.

The activity in the organs, tail and carcass is measured either in an isotope dose calibrator or in a NaI(Tl) crystal scintillation counter. The total retained dose (%TRD) is calculated as follows:

$$\%\text{TRD}_{(\text{organ})} = (A/B) \times 100$$

where A is the activity or counts in the organ, and B is the activity or counts in all organs and the carcass except for the tail.

To accurately estimate the activity and to account for decay corrections in the ^{99m}Tc activity, standard solutions of the radiopharmaceuticals are prepared. A typical experiment is given below.

Preparation of standard solution

Draw 0.2 mL of the ^{99m}Tc radiopharmaceutical in a syringe and estimate its weight by weighing the empty syringe and the syringe with solution and calculating the difference. Dispense this ^{99m}Tc radiopharmaceutical solution into a clean 100 mL glass beaker and add 20 mL of distilled water. This solution is taken as the standard for estimation of the total activity that is injected into the animals.

If using an isotope dose calibrator, the activity retained in the organs is calculated as:

$$\%\text{ injected activity in the organ} = \frac{\text{Activity obtained in the organ} \times 100}{\text{Total activity injected}}$$

If using a NaI(Tl) scintillation counter, the activity retained in the organs is calculated as:

$$\%\text{ injected activity in the organ} = \frac{\text{Counts in organ} \times 100}{\text{Counts in standard} \times (W_i/W_s)}$$

where W_i is the weight of injection and W_s is the weight of the standard. All the counts are corrected for background activity.

At the end of the biodistribution studies, all the animal parts are carefully wrapped in plastic covers and stored frozen for at least one week to allow decay before disposal in an appropriate manner. National regulations for disposal of the carcasses should be followed.

CONTRIBUTORS TO DRAFTING AND REVIEW

DRAFTING

Gil Valenzuela, M.C.	C.G.M. Nuclear, Chile
Környei, J.	Institute of Isotopes Co., Ltd., Hungary
Mikolajczak, M.	Polatom, Poland
Ozker, K.	Dubai Hospital, United Arab Emirates
Pillai, M.R.A.	International Atomic Energy Agency
Venkatesh, M.	Bhabha Atomic Research Centre, India

REVIEW

Araujo, E.B.	Instituto de Pesquisas Energéticas e Nucleares, Brazil
Dondi, M.	International Atomic Energy Agency
Gomes, S.C.	Angel H. Roffo Institute of Oncology, Argentina
Koga, R.	Instituto Peruano de Energía Nuclear, Peru
Lavie, E.	Soreq Nuclear Research Center, Israel
Luo, Z.F.	China Institute of Atomic Energy (CIAE), China
Mustansar, J.	Institute of Nuclear Science and Technology, Pakistan
Narasimhan, D.V.S.	Board of Radiation and Isotope Technology, India
Paragulla, W.	Instituto Peruano de Energía Nuclear, Peru
Robles, A.	Instituto Peruano de Energía Nuclear, Peru
Verdera, S.	Centro de Investigaciones Nucleares Mataojo, Uruguay

Consultants Meetings

Vienna, Austria: 22–25 March 2004, 10–14 August 2007

COMPARATIVE EVALUATION OF THERAPEUTIC RADIOPHARMACEUTICALS
Technical Reports Series No. 458
STI/DOC/010/458 (310 pp.; 2007)
ISBN 92-0-115106-3 Price: €56.00

LABELLING OF SMALL BIOMOLECULES USING NOVEL TECHNETIUM-99m CORES
Technical Reports Series No. 459
STI/DOC/010/459 (312 pp.; 2007)
ISBN 92-0-101607-7 Price: €70.00

TRENDS IN RADIOPHARMACEUTICALS (2 VOLUMES)
STI/PUB/1294 (Vol. 1: 408 pp.; Vol. 2: 464 pp.; 2007)
ISBN 92-0-101707-3 Price: €120.00

DEVELOPMENT OF ^{99m}Tc AGENTS FOR IMAGING CENTRAL NEURAL SYSTEM RECEPTORS
Technical Reports Series No. 426
STI/DOC/010/426 (197 pp.; 2004)
ISBN 92-0-115303-1 Price: €33.00

LABELLING TECHNIQUES OF BIOMOLECULES FOR TARGETED RADIOTHERAPY
IAEA-TECDOC-1359 (198 pp.; 2003)
ISBN 92-0-107303-8 Price: €15.00

THERAPEUTIC APPLICATIONS OF RADIOPHARMACEUTICALS
IAEA-TECDOC-1228 (347 pp.; 2001) Price: €15.00

^{99m}Tc LABELLED PEPTIDES FOR IMAGING OF PERIPHERAL RECEPTORS
IAEA-TECDOC-1214 (177 pp.; 2001) Price: €15.00

This report describes the procedures for preparing kits for the formulation of 23 selected ^{99m}Tc radiopharmaceuticals. Details of the preparation of ten of the active ingredients are also included. The procedures described here can be used to develop manuals, monographs and standard operating procedures. This report is expected to serve as a guide to radiopharmaceutical manufacturing centres and centralized pharmacies involved in the production of kits. It will be a useful resource for the many hospital radiopharmacies that routinely use the kits to compound ^{99m}Tc radiopharmaceuticals, and a source of information for regulators of radiopharmaceuticals.

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA
ISBN 978–92–0–100408–6
ISSN 0074–1914