Radioactive Drugs in Drug Development Research: Quality Assurance Issues

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Abstract: Increasing number of new drugs, drug formulations and drug delivery systems is evaluated using noninvasive imaging methods. A successful use of new drugs and radiopharmaceuticals depends on their proven quality. This review provides a brief outline of the quality control procedures required for radiolabeled drugs within the context of the existing regulations.

Key Words: Radioactive drugs, radiopharmaceuticals, drug development, quality assurance, quality control.

INTRODUCTION

Noninvasive imaging technologies such as positron emission tomography (PET) or single photon emission computed tomography (SPECT) have many advantages over invasive technologies in the drug evaluation, including ethical considerations. Both techniques can identify specific drug deposition sites, biochemical and physiological processes responsible for pharmacokinetics and metabolic/catabolic fate of the drug, and thereby greatly facilitate the evaluation of new medications. The amount of radiopharmaceutical required for imaging studies is low, typically at nanomole levels for short-lived radionuclides, well below any chemical toxicity levels, allowing multiple or sequential dosing in the same subject to establish optimal administration schedules. In a well designed study, significant insights into the drug's mode of action, molecular biology and underlying pathology can also be derived from nuclear medicine images facilitating the design of new and improved drugs. The application of PET or SPECT in the drug development processes enables cost effective and rapid evaluation of new medicines often providing additional information difficult to acquire with traditional drug evaluation methods.

Radiolabeling techniques depend on the selected radionuclide, the structure of the drug to be radiolabeled, and vary accordingly in complexity; therefore the manufacturing and compounding processes are usually unique for a given radioactive drug. Successful outcomes in the use of radioactive drugs depend on their proven quality. This review provides an outline of the generally applicable quality assurance tests and procedures required for radiolabeled drugs.

REGULATORY ISSUES

Radiopharmaceutical or Radioactive Drug is defined in Section 201(g)(1) of the Federal Food, Drug and Cosmetic Act (FFDCA) as "(1) an article (A) that is intended for use in the diagnosis or monitoring of a disease or a manifestation of a disease in humans; and (B) that exhibits spontaneous disintegration of unstable nuclei with the emission of nuclear particles or photons; or (2) any nonradioactive reagent kit or nuclide generator that is intended to be used in the preparation of any such article" [1]. Radioactive drugs are regulated by the Food and Drug Administration (FDA) to the same extent that all other drugs are regulated, and with the exception of certain research uses of radioactive drugs as specified under Title 21 of the Code of Federal Regulations; 21 CFR 361.1, all radiopharmaceuticals are considered to be new drugs and subject to the applicable provisions.

In the United States certain research applications of radioactive drugs are regulated by the Code of Federal Regulations Title 21 entitled *Food and Drugs Chapter I, Subchapter D – Drugs for Human Use, Section 361.1 Radioactive drugs for certain research uses*. These particular radioactive drugs are not intended for immediate therapeutic, diagnostic, or similar purposes or to determine safety [1]. Basic research studies such as drug localization and drug kinetics can be conducted without an Investigational New Drug Application (IND) on condition that there is a review process and the approval by the Radioactive Drug Research Committee is obtained. There are several limitations set forward in the cited above section, however a detailed examination of these restrictions is beyond the scope of this review. Of importance for radioactive drugs to be administered to research subjects is the following: "361.1(c)(5)(d)(6.) Quality of radioactive drug. The radioactive drug used in the research study shall meet appropriate chemical, pharmaceutical, radiochemical, and radionuclidic standards of identity, strength, quality, and purity as needed for safety and be of such uniform and reproducible quality as to give significance to the research study conducted. The Radioactive Drug Research Committee shall determine that radioactive materials for parenteral use are prepared in sterile and pyrogen-free form".

Some radioactive drugs may have a dual purpose, i.e., the diagnostic and therapeutic application. In such instances FDA evaluates the diagnostic claims under the provisions in part 315 (for drugs) or subpart D of part 601 (for biologics), whereas the therapeutic claims are evaluated under the regu-

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lations applicable to nonradioactive drug or biologic applications and accordingly, the quality assurance specifications must be met for both objectives.

Unlike non-radioactive medications, radioactive drugs are by their very nature unstable, i.e., contain a decaying radioactive nucleus, and their clinical use, either in diagnosis or therapy, is wholly dependent on the presence of this radioactive entity. The intrinsic instability of radioactive drugs creates a need for special quality assurance requirements. Manufacturing and all quality controls are often performed on location, immediately prior to the administration into the patient. Nonetheless radioactive drugs must be manufactured and dispensed in accordance with the basic principles of good manufacturing practices (GMP). There is a General Information Chapter <1078> in USP 29 NF 24 entitled Good Manufacturing Practices that gives information on such things as process controls, testing, record keeping, training, etc. (pp. 2906-2915). The issues covered in this review should therefore be considered only as supplementary to the general requirements for GMP. Additional consideration should also be given as to whether these drugs are prepared in a manufacturing or a compounding process. If manufactured, GMP or IND protocols may be required. If compounded, a prescription from a qualified physician and the IND or RDRC approval may suffice (for detailed definitions that differentiate these two processes refer to the USP General Information Chapter 1075; note that the preparation of radiopharmaceuticals is also categorized in USP <1075>).

Preparation of radioactive drugs for the clinical use involves a wide range of operations from chemical syntheses, radiolabeling procedures, compounding, quality controls and dispensing. All these steps contribute to the quality of the final product; therefore it is of the utmost importance that the equipment, the design of the working area, and the laboratory facility has been set up with the attention to the pharmaceutical and the radiation safety aspects. For example, radiolabeling procedures should take place in areas where the product can be guarded against microbial and particulate contamination and against any cross-contamination from other radioactive drugs or biological materials. In practice this is achieved by using sterile starting materials and supplies, and by applying aseptic techniques in work stations fitted with appropriate environmental filters, e.g., laminar flow hoods or similar enclosed work areas e.g., compounding aseptic isolators. In order to avoid any cross-contamination, only one labeling operation should be performed at a time, and other radioactive labeling or dispensing procedures should not take place simultaneously in the same area. Most radioactive drugs are prepared in small amounts, therefore special considerations are also necessary such as establishing documented evidence and standard operating protocols (SOPs), which provides assurances that products meeting predetermined specifications and quality attributes are reliably produced.

QUALITY ASSURANCE AND CONTROL PARAMETERS

Radioactive drugs must conform to all quality control measures required of nonradioactive drugs such as: sterility, apyrogenicity, chemical purity, absence of foreign particulate matter, appearance, particle size (this parameter will grow in importance with the increasing use of nanoparticles in drug delivery), pH and sometimes osmolality, and biological distribution. As a rule radioactive drugs must also conform to the following additional specifications: radionuclide purity and concentration; radiochemical purity; and specific activity.

In their Nuclear Pharmacy Compounding Guidelines [2], American Pharmaceutical Association (APhA) recommends that if the radiolabeled product from the reconstituted compounded reagent kit is listed in the United States Pharmacopeia (USP), the quality control parameters of such a compounded reagent or kit should meet all applicable USP monograph standards. On the other hand, if the radiolabeling kit or reagent is not listed in USP, the radiolabeled product is expected to meet professional standards of a similar nature appropriate for its safety and intended use. APhA Guidelines further recommend that radioactive drugs compounded under high-risk category II [3] or risk level 3 [4] should be subjected to quality controls described in the Finished Product Release Checks and Tests under USP <1206> Sterile Drug Products for Home Use [3].

Sterility

On January 1, 2004, a new General Chapter on compounding sterile products from the United States Pharmacopeia (USP) became effective [5]. This new standard has the weight of federal regulations and can be enforced by state boards of pharmacy and referenced by Joint Commission on the Accreditation of Healthcare Organizations™ evaluators. USP Chapter <797> details procedures and requirements for compounding sterile preparations. It also sets standards that are applicable to all practice settings in which sterile preparations are compounded, including radioactive drugs (proposed revisions for that chapter are currently under review).

Most of the manufacturing, compounding and dispensing of radioactive drugs can be undertaken in accordance with general GMP guidelines. However, some points are specific to the handling of radioactive products and must comply with the requirements of the radiation health protection. For example, radioactive drugs should be stored, processed, packaged and dispensed only in dedicated facilities. The equipment used for manufacturing or compounding operations should be reserved for radiopharmaceutical use only.

Special considerations for radioactive drugs have to be made. Due to their short half-life, some radioactive drugs are released before completion of the sterility testing. In this case, the continuous assessment of the effectiveness of the quality control systems is of special importance. Sterility or endotoxin testing procedures, when required as release criteria, should be conducted according to detailed written policies and procedures for specific radioactive drugs. The Code of Federal Regulations [1] (21CFR211.165) in sections related to the testing and release of drugs for distribution indicates that *"Where sterility and/or pyrogen testing are conducted on specific batches of short-lived radiopharmaceuticals, such batches may be released prior to completion of sterility and/or pyrogen testing, provided such testing is completed as soon as possible"*. In another words, the sterility and endotoxin tests should be initiated promptly after

preparing the product (21CFR 211.167(a)). In most instances, the sterility testing is done retrospectively, i.e., radioactive drug is released before the sterility testing is complete. For this reason, all microbial tests are conducted on randomly selected lots to check the adequacy of aseptic techniques. These tests should be done at regular intervals that are compatible with specific trends and sterility results in a given facility. When there are new personnel involved or new methods are implemented, a consideration must be given to a more frequent periodic testing. In some experimental protocols all batches of new radioactive drugs are retrospectively tested.

Tests for sterility can be carried out by either the method of *Membrane Filtration* or by the method of *Direct Inoculation* (also known as *Direct Transfer* or *Immersion*). In the latter method, the tested substance is inoculated directly into the test media. The membrane filtration method requires passing the tested substance through the size exclusion membrane capable of retaining microorganisms. An average pore diameter should not exceed 0.45 μm. After the aseptic filtration and removal, the membrane is rinsed and then transferred into the appropriate test medium. Oftentimes it is practical to divide each filter into two equal parts and transfer one part into medium supporting anaerobic and the other part to medium supporting aerobic growth. USP and 21CFR610.12 recommend using two media for both the direct transfer and membrane filtration methods. Most drugs are still tested using the direct transfer method. However, if any additives known or determined to be bacteriostatic or fungistatic are included in the final drug preparation, the membrane filtration method may be required. Sterility testing should be performed according to locally accepted standard operating protocols (SOPs), or procedures described in *USP <71> Sterility Tests* [6] should be adopted. Consideration for the volume of the radiopharmaceutical should be given regarding which method of sterility testing to use. USP29 NF 24 recommends that the preparation to be examined is directly inoculated into the culture medium so that the volume of the product is not more than 10% of the volume of the medium, unless otherwise prescribed. Most commonly, the sterility test is performed by inoculating radioactive drug samples in two or more of the specified media. The fluid thioglycollate growth medium test mixtures are incubated at 30°C-35°C and the soybean-casein digest medium test mixtures at 20-25°C. All tested radioactive drugs must be incubated for at least 14 days unless microbial contamination is detected at an earlier time. All media must also pass growth-promotion tests. When growth media are supplied by external vendors, this testing is not needed. However, a certification of the growth promotion test performed for each batch of media should be provided by the vendor and retained in the records.

The sterility of radioactive drugs is defined by the absence of viable and actively multiplying microorganisms when tested in the specified culture media. Appropriate negative controls should be used to identify a false-positive test result. Negative controls are usually products exposed to a terminal sterilization process and are of unquestionable sterility. If possible, negative controls should be tested during the same test session as the lot of radioactive drug.

The inclusion of positive controls in the sterility testing allows the identification of false-negative results that may arise if the growth conditions are not optimal. The absence of growth of challenge microorganisms yields a false-negative finding and invalidates results. It must be emphasized that because positive control tests use viable challenge microorganisms selected from reference strains, these tests should be conducted in an area separate from any areas where radioactive drugs are tested. Reference strains must be traceable to the recognized reference culture collection, such as the American Type Culture Collection (ATCC, Manassas, VA). Table **1** lists microorganisms typically used in the growth promotion, validation and stasis assays.

Table 1. Bacteria and Fungi Strains Used as Positive Controls in Sterility Testing

Challenge Microorganism	
Species	**ATCC Strains**
Clostridium sporogenes anaerobic bacteria	19404
Staphylococcus aureus aerobic bacteria	6538
Bacillus subtilis aerobic bacteria	6633
Pseudomonas aeruginosa aerobic bacteria	9027
Candida albicans fungi	10231 2091
Aspergillus niger fungi	16404

It is important to determine if the substance tested for sterility contains components that interfere with the growth of microorganisms (*stasis test*; *Bacteriostasis/Fungistasis test: B/F*). This testing is typically required only once for a given drug formulation, however, if any changes in formulation or manufacturing processes have occurred, the B/F test must be repeated. Moreover, the B/F testing should be performed periodically to confirm that no significant undefined (or unknown) changes have occurred in the product or process that may affect the sterility assays. Although this test is not mandatory, it is often suggested as a routine procedure for new investigational radioactive drugs because it can confirm the inactivation of antimicrobial substances by the innately antimicrobial radioactive drugs. Sometimes the sterility test and B/F are conducted simultaneously (21 CFR 610.12). In the B/F test, if noticeable growth does not occur within 3 days for bacteria and 5 days for fungi, the test procedure is not valid and must be modified until obvious growth does occur when the positive control or validation tests are carried out. Usually, test samples are examined for evidence of microbial growth at intervals of 2-3 days. Samples producing a suspension or deposits that interfere with the detection of the microbial growth should be transferred in smaller portions (2-5% of the original sample) to a fresh

sample and the incubation should be continued for 7 days. An invalid sterility test of the radioactive drug itself may be repeated only when it can be demonstrated that the results were unacceptable for reasons unrelated to the product tested.

A full description of the interpretation of the sterility test results can be found in the USP <29> NF25 on page 2513. Briefly, under normal test conditions, no growth should appear in samples inoculated with the radioactive drug and therefore the sample tested is considered to comply with the test for sterility, provided of course that growth of challenge microorganisms has been demonstrated in the B/F test or in the method validation tests. This interpretation applies even if growth occurs in negative control cultures. If microbial growth is evident in product samples, the test can be invalidated and samples retested only if the review of procedures reveals a clearly identifiable error or the microbial growth is found in negative controls. Finally, the test can be nullified if after determination of the identity of the microorganisms isolated from the test, the growth of these species may be attributed unequivocally to faults with respect to the material and/or techniques used in conducting the sterility test and not the tested product itself. Careful records of these cultures must be maintained in order to detect a pattern of recurring impurities in the product.

Records of all sterility testing should contain the following information: the name of the radioactive drug product tested; the lot number; name(s) of personnel performing tests; dates of testing; methodology (volume tested, diluents/solvents used, media, media lot numbers, temperature and time of incubation); and full results. Additionally all details of validation tests, periodic stasis testing; results of any contamination irrespective of whether the test was valid or invalid; and finally the negative control contamination rate.

Apyrogenicity: Bacterial Endotoxin Testing

Pyrogenic substances are produced by gram positive bacteria, mycobacteria, fungi and viruses. Pyrogens produced by gram negative bacteria, i.e., endotoxins, are of significance to the pharmaceutical preparations. Endotoxins are high molecular weight complexes associated with the outer membrane of gram-negative bacteria. Most often they are the primary cause of fever induced by contaminated drugs. Endotoxins are stable at elevated temperatures and can survive the sterilization process. In their structure endotoxins contain lipid A which is responsible for the pyrogenic activity. This lipid has a spectrum of biological activities much like the endotoxin itself.

The endotoxin testing of radioactive drugs is performed to ensure their apyrogenicity. The *Limulus Amebocyte Lysate* (LAL) test is the most often used method to detect the presence of gram negative bacterial endotoxins, the most common source of the pyrogen contamination. LAL has been shown to be more sensitive in the detection of endotoxin than the USP Chapter <151> *Pyrogen Test (rabbit fever test)*. The specific bacterial endotoxin test procedures can be adapted from the *USP <85> Bacterial Endotoxins Test* [7]. Like sterility testing, this assay should be conducted at regular intervals and more frequently when new personnel or new procedures are involved. For parenteral radioactive drugs prepared on site or used in the experimental clinical protocols, each new lot of the drug must undergo the LAL testing prior to administration unless the short half life of the radionuclide prohibits the delayed release of the formulation.

The use of LAL for endotoxin detection was derived from the observation that infections of *Limulus polyphemus* (a horseshoe crab) induced by gram-negative bacteria resulted in extensive intravascular clotting and death. Levin and Bang [8] demonstrated that the extracellular coagulation of Limulus' hemolymph (blood) was caused by the reaction between endotoxin and a coagulative protein in amebocytes circulating in hemolymph. A few years later, Levin *et al*. [9, 10] developed a sensitive assay for endotoxin in human plasma using the material lysed from Limulus amebocytes. LAL reacts with bacterial endotoxin or lipopolysaccharides (a cell wall component of gram negative bacteria). This reaction is the basis of all LAL test methodologies including the gel-clot, turbidimetric, and chromogenic assays. Gram-negative bacterial endotoxin catalyzes activation of the proenzyme in LAL [10]. The initial rate of activation is determined by the concentration of endotoxin present. The activated enzyme, coagulase, hydrolyzes specific bonds within the clotting protein, coagulogen, also present in LAL. Once hydrolyzed, the resulting coagulin self-associates and forms a gelatinous clot.

The endotoxin standard expressed in Endotoxin Units (EU) per milliliter was instituted to normalize the definition of the endotoxin concentration. FDA initially defined EU as the endotoxin activity of 0.2 ng of Reference Endotoxin Standard (RSE) EC-2 or 5 EU per ng EC-2 [7, 11]. The conversion from endotoxin units to ng varies depending on the source of endotoxin. To convert the current FDA RSE EC-6, from EU into ng, the conversion is 10 EU per one ng EC-6.

Plasma of healthy humans contains about 0.07 EU/mL, this corresponds to approximately one-half of the endotoxin blood concentration during a pyrogen reaction [11]. European Pharmacopoeia 2000 [12] sets the maximum endotoxin dose in a parenteral solution of the radioactive drug for the intrathecal administration at 0.2 EU/kg body weight/h and for the intravenous application at 2.0 EU/kg bw/hr. Limits for intrathecally administered radioactive drugs, (maximum allowable administration of 14 EU) are significantly less than these for intravenously administered radioactive drugs, which have a maximum administration limit of 175 EU. Testing procedures should take these differences into consideration and appropriate positive controls should be prepared and tested alongside of the doses of radioactive drugs.

FDA-approved commercially available methods for the end-product release testing include: (i) the gel-clot; (ii) the turbidimetric (spectrophotometric); (iii) the colorimetric (Lowry protein); and (iv) the chromogenic assay. All LAL assay products are licensed by FDA and are submitted to FDA for testing. Only FDA approved lots are released for sale.

The gel-clot method is one of the most often used in testing of radioactive drugs. However, one must bear in mind

that certain product characteristics may interfere with LAL-based methods, e.g., DTPA, EDTA and similar chelating agents can also chelate divalent cations necessary for the LAL gelation reaction. The pH values outside of the 6.0-7.5 range also inhibit the LAL gelation reaction with endotoxins. In most instances these issues can be resolved by using appropriate dilutions of the tested radiopharmaceutical. Only endotoxin-free acid or base should be used to adjust pH of buffered solutions, if necessary. In the gel-clot methods, the type of glassware used can also adversely affect the LAL test. Siliconized tubes and some types of plastic inhibit the gel-clot formation. Therefore, the importance of carefully designed and conducted LAL validation methods for each radioactive drug cannot be overstated. If any change in reagents or their source is made, the test must be re-validated.

Several LAL gel-clot assays are commercially available. It is important to note that component parts for the assays are not interchangeable from supplier to supplier. The LAL reagent is available in single test vials or multiple test vials. In concert with each LAL assay of the radiopharmaceutical product, a standard series with positive and negative controls is analyzed. The standard series is prepared from the lyophilized standard endotoxin that is provided with the LAL assay kit. The endotoxin is reconstituted and then diluted with the LAL reagent water (endotoxin-free water usually provided with the LAL assay kit) to a concentration of the stock solution of 1 EU/mL. Successive two-fold dilutions are prepared. The selected series of dilutions should bracket the sensitivity, lambda, (λ) of the LAL reagent. As an example, if the sensitivity, λ, of the reagent is given as 0.125 EU/mL the series should include 0.5, 0.25, 0.125, 0.06 and 0.03 EU/mL. The endotoxin concentration indicated as the release limit for the tested radiopharmaceutical should be established based on limits for radioactive drugs. The maximum allowed dilution of the tested radiopharmaceutical is determined using the maximum valid dilution (*MVD*) formula. This formula is based upon the final dosage, endotoxin tolerance limit, lysate sensitivity and the route and method of administration (e.g., intravenous bolus, infusion, intrathecal, etc.). Dilutions beyond MVD will render any negative result meaningless because harmful endotoxin concentrations may be diluted below the detectable range. To find MVD for products with the specified USP endotoxin limit, typically expressed as EU/mg, the value expressed in EU/mg is multiplied by the concentration of the undiluted solution to be tested. In the chart provided with the assay, the calculated EU/mL value is located. If the exact value is not listed in the table, the endotoxin limit, which approaches but is not greater than the EU/mL limit calculated above, is used to find MVD. The endpoint dilution is determined as the last dilution of endotoxin which still yields a positive result. The lysate sensitivity is calculated by determining the geometric mean of the endpoint. Acceptable variations are within ½× to 2× the labeled lysate sensitivity. There are two methods that can be used to determine the Maximum Valid Dilution [13]. The first method is used when there is the official USP limit and is calculated using the formula: $MVD = (Endotoxin\ Limit \times Potency\ of\ Product)/\lambda$. For drugs administered on a weight-per-kg body weight (bw) basis, the drug concentration is expressed in weight units/mL. For drugs administered on a volume/kg bw basis the potency is equal to 1.0 mL/mL. In the case of radioactive drugs the dosing expressed as mCi (MBq)/kg bw is applicable. In the second method typically used for all new and developmental drugs when there is no official USP limit, MVD is calculated in two steps: (1) $Minimum\ Valid\ Concentration\ (MVC)=\lambda \times M/K$; and (2) $MVD=Potency\ of\ Product/MVC$; again λ is the labeled sensitivity in EU/mL in the gel-clot method or the lowest point used in the standard curve in the chromogenic, turbidimetric, kinetic-turbidimetric and colorimetric assays; M is the maximum human dose/kg bw that would be administered in a single one hour period; and K is the Tolerance Endotoxin Limit ($EU \times h^{-1} \times kg^{-1}$), e.g., 5 $EU \times h^{-1} \times kg^{-1}$ for parenteral drugs except those administered intrathecally, for which K = 0.2 $EU \times h^{-1} \times kg^{-1}$. Typically for radioactive drugs, M is the maximum human dose in mCi (MBq)/kg bw at the product expiration date or time.

Almost all drugs exhibit some inhibition or enhancement of the LAL assay. To overcome this problem, the least complicated method involves the use of serial dilutions of the final drug formulation. Similarly, to determine the unknown endotoxin concentration in a solution of the radiopharmaceutical, serial two-fold dilutions of the sample are prepared and tested until the endpoint is reached. The geometric mean dilution is calculated ($geometric\ mean=antilog[(\Sigma e)/f]$; where Σe=sum of log endpoints, and f =number of replicate endpoints) and multiplied by the lysate sensitivity to find EU/mL in the tested sample. Negative controls in the LAL assay usually consist of the LAL reagent water provided with the kit. Negative results for samples, which contain substances that may inhibit the LAL test, such as chelating agents, some detergents, or pH values outside of the 6.0-7.5 range, etc., do not necessarily indicate the absence of endotoxin. Therefore, initially each new radioactive drug preparation should be screened for its potential to inhibit the gel-clot assay. Typically, a series of two-fold dilutions of the endotoxin standard in the LAL reagent water is prepared alongside the identical endotoxin dilutions prepared with the solution of the tested radioactive drug as the diluent. At the end of the incubation period, positive and negative results are recorded and the geometric mean endpoints are calculated for both series of endotoxin dilutions. The product inhibition of tested radiopharmaceutical is considered absent if the geometric mean endpoint of endotoxin in this radiopharmaceutical is within ½× to 2× the labeled lysate sensitivity. Tables **2** and **3** give examples of the pyrogen test reports.

Chemical Purity

For many radioactive drugs, it is difficult or nearly impossible to measure and quantify their chemical purity without loosing the entire dose in the process. USP requires the control and measurement of any potentially toxic substances in the synthetic methods, including radiochemical syntheses, which may generate known chemical impurities, or contain unlabeled ingredients, reagents, and by-products. Conveniently purification processes that provide drugs of high radiochemical purity also tend to give products of high chemical purity. The difficulty in measuring chemical purity of radioactive drugs can be best appreciated by means of the following example: ^{90}Y-dodecanetetraacetic acid-Phe^{1}-Tyr^{3}-octreotide (SMT487), a somatostatin receptor-seeking radiopharmaceutical currently under investigation as a therapeutic

Table 2. Example of the Test Report for the Inhibition and Enhancement Screening of a New Radiopharmaceutical

LIMULUS AMEBOCYTE GEL-CLOT METHOD
INHIBITION/ENHANCEMENT SCREEN

Product Name: 5-[^{125}I]Iodo-2'-deoxyuridine **Lot:** 09-15-05A

LAL Reagents:
Manufacturer: Endosafe **Lot**: X807
Validation Sensitivity: (EU/mL): 0.12 **Expiration date**: 01/06
Date reconstituted: Sept. 15, 2005

MVD Calculations:

(1) $MVC = \lambda \times M/K = 0.12\ EU/mL \times 0.02\ mg/175\ EU = 1.37 \times 10^{-5}\ mg/mL$

λ = 0.12 EU/mL
M = 0.02 mg
K = 175 EU

(2) $MVD = Potency\ of\ Product/MVC = 0.05\ mg/mL/1.37 \times 10^{-5}\ mg/mL =$ ***3,650***

Potency of Product = 0.05 mg/mL

Calculated MVD 3,650
Calculated by: JBK Date: 09-15-05

Product dilutions:
Controls: Positive water: 4 + 2 + 1 + 0.5 - 0.25 - ×λ
Negative water: -

dilutions	1 : 0.5	1 : 2	1 : 4	1 : 8	1 : 16
spiked product (2 λ)	+	+	+	+	+
	+	+	+	+	+
unspiked product	-	-	-	-	-
	-	-	-	-	-
pH	7.35	7.28	7.3	7.34	7.4

Intepretation:
Product shows (*indicate one*):

1. No inhibition, no enhancement.
2. Enhancement, no inhibition.
3. Inhibition, no enhancement.
4. Inhibition, enhancement.

Assay performed by: JBK Date Sept. 15, 2005
Assay approved by: PB Date Sept. 15, 2005

option for neuroendocrine tumors [14], is administered at a dose of 120 mCi (3.24 MBq) in three cycles of therapy. The drug is prepared using a no-carrier-added $^{90}YCl_3$. The therapeutic dose of ^{90}Y-SMT487 corresponds to approximately 2.46 nmole ^{90}Y. Published reports indicate the ratio of reagents varying from 0.2 nmole to 3 nmole SMT487 per 1 mCi (0.02 nmole) ^{90}Y and specific activities of the final product of ~1 Ci/μmole. At these ratios under the most favorable of circumstances, only approximately one in 10 to 50 molecules of the peptide is radiolabeled. Typically, the chemical purity of any compound is determined by UV, IR, mass spectrometry, quantitative gas or high-performance liquid chromatographic (HPLC) procedures, or elemental analyses, as appropriate. Only rarely any of these techniques can be successfully applied to determine the chemical purity of radioactive drugs without destroying the entire therapeutic dose to meet the detection limits of most analytical techniques. The separation of radiolabeled compounds from the unlabeled starting material is frequently impossible. On the other hand, on occasion addition of a specified concentration of the unlabeled drug is required to achieve the desired biological activity. For example, the Bexxar® therapeutic regimen, which consists of Tositumomab and [^{131}I]Iodine-Tositumomab is supplied at protein concentrations of 1.1 mg/mL and activity of 5.09 mCi/mg protein; i.e., approximately one in 20 molecules of Tositumomab is radiolabeled. In this and similar cases, the chemical purity of the radiolabeled material although not immaterial, it is certainly not quantifiable. Consequently, the chemical purity of all starting materials is of the utmost importance in the synthesis of radioactive drugs. To assure that only compounds of the highest standards of purity are used, it is sensible to select and use materials listed

Table 3. Example of the Test Report for the LAL Assay of a New Radiopharmaceutical

LIMULUS AMEBOCYTE GEL-CLOT METHOD
PRODUCT TEST REPORT

Product Name: 5-[^{125}I]Iodo-2'-deoxyuridine **Lot:** 09-15-05A **Date:** Sept. 15, 2005
Prepared by: JBK **Concentration:** 0.008 mg/mL (40 mCi/mL)

LAL Reagents:
Manufacturer: Endosafe **Lot**: X807
Validation Sensitivity: (EU/mL): 0.12 **Expiration date**: 01/06
Date reconstituted: Sept. 15, 2005

LAL Reagent Water:
Manufacturer: Abbott **Lot**: 47-201-XK **Expiration date**: 05/06

CSE:
Manufacturer: Endosafe **Lot**: E012 **Expiration date**: 10/05
Date reconstituted: Sept. 15, 2005

RESULTS:

CONTROLS	**1**	0.5	0.25	0.125	0.06
positive	+ +	+ +	+ +	+ +	+ +
negative	- -	- -	- -	- -	- -

PRODUCT	**Sample 1**	**Sample 2**
spiked product (2 λ)	+ +	+ +
unspiked product	- -	- -
pH	7.4	7.38

Comments: Test Sample 2 was diluted to 0.004 mg/mL. Both samples were run in duplicate and were negative for endotoxin at 0.12 EU/mL.

Assay performed by: JBK Date Sept. 15, 2005
Assay approved by: PB Date Sept. 15, 2005

in FDA Drug Master Files, whenever possible. Starting materials, buffers, solvents, etc., are listed in Type II Drug Master Files (DMF) and other supplies such as filters, vials, etc., are listed in type III DMF. DMF is submitted to FDA and provides confidential detailed information about facilities, processes, or materials used in the manufacturing, processing, packaging, and storing of drugs (21 CFR 314.420; online [15]). Information contained in DMF is often used to support Investigational New Drug Applications, New Drug Applications, Abbreviated New Drug Applications, other DMFs, biologics license applications, Export Applications, or amendments to any of these.

Occasionally the chemical purity of radionuclides can also be in question. When a ^{99}Mo/^{99m}Tc generator is eluted, it is possible to elute Al ions along with ^{99m}Tc. USP allows ≤10 μg Al ions/mL ^{99m}Tc eluate. Levels of Al ions can be measured with a commercial colorimetric test kit. In the event that even with the purification (or because of it) any residual solvent is suspected to remain in the drug solution, potential toxic, physiologic, or pharmacologic effects of this solvent must be considered. Radioactive drugs are often purified using HPLC with acetonitrile or ethanol as one of the solvents. USP and the Chemistry, Manufacturing, and Controls (CMC) issued by FDA stipulate that no more than 0.04% acetonitrile or 0.5% dehydrated alcohol is allowed in injectables [16].

Absence of Foreign Particulate Matter

Particulate matter in injectable solutions is defined as the unintentional presence of foreign insoluble materials. Insoluble particles can be injurious and are of deep concern to all involved in the drug regulation, preparation and administration. In a period of three years between 1996 and 1999, the FDA instigated nearly 30 recalls (Class II and Class III) of sterile products because of the presence of foreign substances, particulate matter, or precipitates. Animal studies have demonstrated that the tissue distribution of inert infused particles is related to their diameter. Particles in the range 10-12 μm are stopped in the pulmonary capillaries, those in the range 3-6 μm are usually lodged in the spleen and hepatic lymph nodes, and 1 μm particles stay in liver. The presence of such materials is best evaluated by visual inspection of the compounded radioactive drug before its release. The particulate matter may arise from various sources such

as the drug itself, solvents, or the production process including the production environment, equipment, and personnel. Occasionally rubber septa, plastic vials, powdered gloves, the environment in which the dose is prepared, if not carefully controlled, can be an unintended source of insoluble contaminants [17]. Filters with a maximum pore size of 0.45 μm are recommended during the dose preparation. Appropriate filters can also be used throughout the administration of radioactive drugs. For example, Bexxar® must be administered *via* the IV tubing set with an in-line 0.22-μ filter. Likewise, the package insert for Zevalin® (Ibritumomab Tiuxetan) labeled with ^{111}In (diagnosis) or ^{90}Y (therapy) indicates that a 0.22-μ low-protein-binding filter must be in-line between the syringe and the infusion port prior to the injection. A good example of the unintended particulates is the formation of macroaggregates of antibodies during radiolabeling. It is a known problem and an appropriate chemoprotection during the labeling process can considerably reduce the aggregate formation. For example, a high dose ^{186}Re labeling of monoclonal antibody K928 with 300 mCi ^{186}Re-MAG3-TFP ester, which corresponds to a dose of 200 krad×h^{-1}, resulted in ~30% aggregate formation. Application of the appropriate protection reduced this amount to <5% [18].

Particle Size

Appropriate particle size allows the desired biodistribution while minimizing the patient risk. Several radioactive drugs are deliberately injected in the form of particles in both diagnostic and therapeutic procedures. For example, radionuclide synovectomy is used as the alternative therapy for rheumatoid arthritis. This treatment involves the injection of the suspension of ^{166}Ho-macroaggregates directly into the joint to destroy the inflamed synovium [19]. Macroaggregates of ^{99m}Tc-labeled human serum albumin are a standard in the lung perfusion studies. A recent report outlines the use of these macroaggregates injected directly into the lesion for lymphoscintigraphy [20]. There are also recent attempts to treat unresectable hepatocellular carcinoma with ^{90}Y-resin particles [21]. In these cases, the verification of the macroaggregate size is essential for particle-based radioactive drugs. Values must be within the acceptable range, as defined by the USP monograph, the package insert, or appropriate literature, prior to use. The control of particle size is essential in all these applications. For example, a lung scanning agent containing particles which are too small will be trapped in the liver. The presence of large particles in lymphatic agents can result in poor clearance from the injection site resulting in poor visualization of lymph nodes. Particle numbers should also be verified to be within the prescribed range. Standard particle size and number measurement techniques, including microscopy, laser-light scattering, Coulter counter and filtration can all be applied. In some cases the sample can be analyzed using a simple hemocytometer under the microscope.

Currently there are no testing requirements that are specific to nanotechnology products, however, despite the fact that the FDA's current requirements for safety testing of products are rigorous, if any additional toxicological risks unique to nanomaterials are identified, additional testing may become necessary. As more new drug delivery methods start to utilize nanotechnology safety concerns will increase because as the particles gets smaller, size-specific effects on the drug activity may emerge or nanoparticles may gain access to tissues and cells that are normally bypassed by larger particles.

pH, Osmolality

Buffers, reagents, and various solvents used in the preparation of radioactive drugs have a certain pH range that must be strictly followed to assure that pH of the final compounded radioactive drug is within prescribed limits. In the case of new radiolabeled drugs and in the absence of the USP or equivalent standards, pH specifications must be precisely established prior to any clinical use. pH may affect drug solubility, stability and efficacy. When grossly outside of the physiological range, it may also contribute to the discomfort and injury during and after injection. There is a normal pH value in each body compartment, i.e. extracellular fluid, plasma, intracellular fluid, etc., for example, the average pH of plasma is 7.4 within a narrow range of 7.35-7.45. Acidic and alkaline solutions evoke pain at a pH value less than 4 or greater than 11.

According to USP <791>, the pH test paper may be suitable for the measurement of the approximate pH value [22]. For some radioactive drugs the range of pH limits is relatively broad, e.g., pH of ^{18}F-fluorodeoxyglucose dose is stipulated by USP from 4.5 to 7.5. In such cases, the use of a pH paper with a color change for each 0.5 pH unit is adequate. The accuracy and traceability of pH paper must be verified with standard pH buffers. It is useful to remember that values obtained using the pH paper are only approximate. Ideally, pH is determined using a suitably calibrated pH-meter in conjunction with traceable pH reference standards. The pH test must be completed before release of the drug product.

Osmolality is the concentration of a solution, expressed in osmoles (Osm) of solute per kg of solvent. Extremes of osmolality in injectable solutions can produce intense pain during injection. Peripheral veins are innervated with polymodal nociceptors [23], which mediate the response to the injection. Klement and Arndt [24] discovered that with the osmolar stimulation, pain occurs at 1.0 Osm×kg^{-1} during perfusion and 3.0 Osm×kg^{-1} with rapid bolus injection. Normal range for serum osmolality is between 285 and 295 mOsm/kg. An ideal well-tolerated solution of the radioactive drug should have osmolality closely matched to this of serum, i.e., be isotonic.

Biological Distribution

Presence of any impurities, inferior chemical purity, significant departures from the prescribed pH range should be considered not only as a potential sources of the unwanted direct effects on the patient but also for their possible unfavorable influence on the biodistribution of the product. Altered biodistribution caused by an improperly prepared drug can destroy the image quality. Moreover, it can have a significant impact on the internal radiation dose to the patient. Delivery of drugs to a specific site relies on specific mechanisms and any deviation from the approved formulation or presence of adventitious agents can produce undesirable ra-

dionuclide localization masking pathological conditions and leading to image misinterpretation. Imperfect purification of radiolabeled drugs may cause clinically detectable pharmacological effects. Inasmuch as these factors can influence biological distribution of radioactive drugs, their effect can only be measured and controlled prior to the clinical use of the drug. Fig. (**1A**) and (**1B**) show biodistribution of a new agent developed to measure the liver function. Both images are acquired in healthy animals with normal liver function. While the Fig. (**1A**) depicts acceptable distribution of a high quality radiopharmaceutical, Fig. (**1B**) exemplifies a poor quality drug that causes the unexpectedly low accumulation in the liver with the evidence of unusual accretion of radioactivity in kidneys giving the appearance of poorly functioning liver. When such radiopharmaceutical formulation is released to the clinic, only the subsequent evaluation of images may provide some clues as to the source of the altered biodistribution.

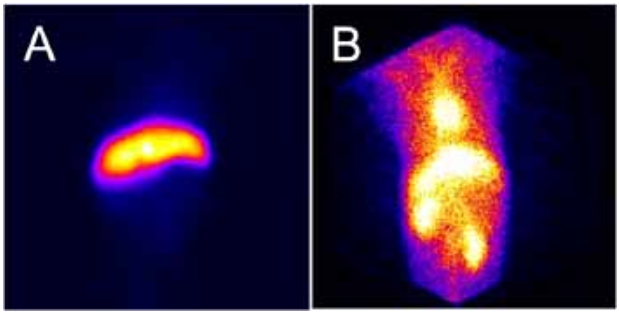

Fig. (1). Whole body planar images of cynomolgus monkeys 15 min after administration of 3.5 mCi ^{99m}Tc-labeled liver function agent. Image **A** shows distribution and liver uptake of a high quality radiopharmaceutical; image **B** shows distribution of the same liver function tracer that for unknown reasons lacked the desired radiochemical purity.

Radionuclide Concentration

The radiopharmaceutical dosage must be determined prior to patient administration and must be consistent with that ordered by the physician to within 10% and 5% of the prescribed dose or the dosage range, for diagnostic and therapeutic drugs, respectively. Radioactivity should be expressed in Bq at a given date and hour. Other units such as mCi may also be used. The calibration time must also state the time zone.

The most universal instrument used to assay positron, γ and high energy β emitting radionuclide concentrations is the dose calibrator, i.e., a sealed, well-type ionization chamber that can be used for assaying relatively large quantities of gamma ray emitting radionuclides (weak β-emitting radioactive drugs may require liquid scintillation counting; in these cases, an appropriate aliquot of the drug must be counted in a properly calibrated liquid scintillation counter). Most radionuclide calibrators contain pressurized gas, e.g., the chamber of CRC-15R is a sealed thin wall, deep well filled with Argon gas under high pressure to achieve best possible speed of response without having to make temperature or pressure adjustments (Capintec, Inc. Ramsey, NJ). Under these conditions the concentration of gas molecules capable of ionization is increased, making the instrument more sensitive for most gamma-emitting radionuclides. Virtually all commercially available dose calibrators have ionization chambers containing pressurized Argon gas usually at ≥12 atmospheres. The detector shielding is lead on all sides except the top well opening with the Pb thickness varying from 3 mm to >6 mm. Workings of the gas-based dose calibrators are simple: a photon interacts with the gas and produces ion pairs in the electric field. The charge is collected by electrodes and the activity is measured by electrometers located within the assembly of the ionization chamber. These are usually controlled by a microprocessor. The measured current is proportional to the energy deposited. The ionization chamber dose calibrators determine the amount of activity by integrating the total amount of ionization produced by the sample, therefore the calibration is required for each radionuclide because radionuclides do not generate the equivalent number of photons per decay. Most dose calibrators are subject to the sample geometry effects, i.e., the calibration geometry will affect the reading of unknown samples [25]. The Capintec dose calibrators use the activity in 5 g liquid (~ 5 ml) sealed in a borosilicate glass ampoules (Ø 17 mm; height 40 mm with a wall thickness 0.6 mm) as the calibration geometry. If the calibration is done with a point source, the activity of diluted samples will be underestimated due to the self-absorption in the sample. Conversely, if the dose calibrator is calibrated with diluted sources, the activity of a point source will be overestimated. Low energy radiation is significantly attenuated before reaching the pressurized gas. For example, the use of dose calibrator to assay the ^{125}I activity always gives inaccurate measurements. The activity of high-energy β-emitting radionuclides can be measured in the ionization chamber-based dose calibrators but such measurements are made indirectly using the Bremsstrahlung radiation produced. The sensitivity of dose calibrators to β-emitters is significantly lower than sensitivity to γ-emitters [26]. Correction factors for certain isotopes are usually supplied by the dose calibrator manufacturer. For ^{125}I a correction factor of two is not unusual if this radionuclide is assayed in a glass vial and due allowances must be made for the volume and geometry of the sample.

Properly functioning dose calibrators are vital to the operation of any radiopharmacy or radiochemistry laboratory. Each dose calibrator must be regularly tested in accordance with applicable regulations. The ***constancy*** check should be performed daily prior to the use of the dose calibrator. The reference source is usually long lived Cs-137. This test measures precision and is designed to show that using a long-lived source reproducible readings are obtained continually on all the settings that are likely to be used. The instrument constancy means that the reproducibility within ± 5% is observed over time. Any values outside of these limits indicate that the dose calibrator may not be functioning properly and it must not be used for any clinical applications until adjusted or repaired. Appropriate linearity of a dose calibrator indicates its proper response over a wide range of radioactive drug activity or the calibrator is linear over a given range of radioactivity (e.g., μCi – Ci). The ***linearity*** test is verified during the installation and then the dose calibrator is tested at least every three to six months [27] (Kowalsky and Phalen in *Radiopharmaceuticals in Nuclear*

Pharmacy and Nuclear Medicine, APhA, 2004 state quarterly pp. 435). As in the constancy testing, any values outside the ±5 % limits will render the dose calibrator not operational and in need of repairs. Measurement of the radioactivity requires that the measurement be as close to the true value as can be achieved. Since the radioactivity of the labeled drug may vary depending upon the energy of the radionuclide, a dose calibrator energy response must be tested. Low, medium, and high energy sealed sources, usually ^{57}Co, ^{133}Ba and ^{137}Cs or ^{60}Co, respectively, are measured in the dose calibrator using appropriate settings. Standard and measured values are compared. The ≥ ±5 % departure from the expected reading is not acceptable. The test of ***accuracy*** is conducted annually [28]. The final requirement verifies efficiency of dose calibrator in reading sources with varying ***geometry***. This is done one time during the installation of the instrument and only as needed after maintenance. The records of these tests are usually retained for the life of the equipment (constancy check records can be discarded after two years) and must specify the model, the serial number, the identity of the reference source, the date of the check, the activity measured and the name (or initials) of the individual performing tests [29].

Radiochemical Purity

Radiochemical purity, usually expressed as a percent, identifies the proportion of the radionuclide present in the desired chemical form. All radiochemical purity specifications should be established beforehand and must be measured before administration to patients. In the absence of the USP monograph or equivalent standards, appropriate radiochemical purity testing methods should be based on literature evidence, scientific data, and the professional judgment of the radiochemist or radiopharmacist. High levels of radiochemical purity are important to ensure correct biodistribution of the radiopharmaceutical (Fig. (**1B**)). When some proportion of the radionuclide is in the undesirable chemical form, possible errors in diagnosis are likely. Unpredictable irradiation of untargeted organs can also be expected. Radioactive drugs by and large contain only very small amounts of the radioactive component - in terms of the mass - therefore as in the case of the chemical purity, the chemical analysis is impractical and often impossible. For example, 555 MBq (15 mCi) of ^{90}Y-labeled IgG-size protein, a therapeutic dose in certain clinical trials of ^{90}Y-labeled conjugates, is equivalent to approximately 0.05 mg protein. To determine the radiochemical purity, the entire dose would have to be used to meet the detection limits of conventional analytical methods. Therefore, the determination of the radiochemical identity must rely on comparing the chromatographic, electrophoretic, etc., behaviors of the radioactive drug with that of chemically validated reference compounds under identical set of conditions. Radiochemical purity tests must be performed on the actual batch delivered to the clinic. Added complications include timing restrictions imposed by radionuclide delivery schedules, tight drug administration schedules, and the rate of decay of the radionuclide. Consequently, suitable analytical methods should be simple, rapid, nondestructive, and undemanding in terms of the specialized equipment, and must be able to separate and to quantify various radiochemical species that may be present. Radiopharmaceutical formulations can comprise a radiolabeled drug, a ligand, a reducing agent, and various additives, e.g., buffers, sometimes antioxidants. Chemical identification of these components, although certainly possible, is so demanding in terms of the equipment, time, and the amount of material, as to render any such analyses impractical. For this reasons whenever appropriate only the radioactive components are measured. Techniques include various types of chromatography, gel filtration and electrophoresis. One of the most convenient methods is the thin layer chromatography (TLC). TLC is a chromatographic system based on the fact that given a proper selection of the solvent system, the radiochemical species will migrate with unique R_F values (R_F = *Relative Front = distance migrated by the drug divided by distance migrated by the eluting solvent*). These values are helpful in establishing product identity provided that the test is validated by comparing the migration of the tested radioactive drug with that of a chemically identified reference substance, which can be located colorimetrically or fluorimetrically. A rapid test of radiochemical purity readily adopted by quality control programs is the Instant Thin Layer Chromatography (ITLC). ITLC alone is not considered appropriate as a method to establish the chemical identity of radioactive drugs as it can only isolate and quantify impurities rather than the main radiopharmaceutical component. R_F values tend to be either 0 or 1 and are generally not unique to a particular product. Nonetheless once validated, this technique can be applied to a host of radioactive drugs. The method is simple and requires very little material. After radiolabeling, a small aliquot of the reaction mixture is removed and approximately 1 μL without any further dilution is applied at the origin of the ITLC plate (glass fiber strips impregnated with silica gel, usually 1cm ×10 cm, PALL Life Sciences, Ann Arbor, MI). To minimize any potential oxidative and other chemical changes, ITLC is performed as soon as possible after removal of the sample. The choice of eluting solvent depends of the tested radioactive drug. When the migrating solvent front reaches within 1 cm of the top of the ITLC strip, the plate is removed from the developing chamber, cut in several pieces and each section is counted in a gamma scintillation counter or dose calibrator. If the radiochromatogram analyzer is available, the entire (uncut plate) is scanned. The percent radioactivity in each band or section is calculated. In most cases, R_F of the desired radioactive drug is 0, whereas free radionuclide migrates with the solvent front, i.e., R_F=1. The radiochemical purity in such cases is calculated as: *%bound=radioactivity in bottom half of the strip×100 divided by the total radioactivity on strip*. Fig. (**2A**) illustrates ITLC analysis and shows a typical distribution of the radioactive species after 5 min of ^{111}In radiolabeling of CC49 antibody modified with a derivative of diethylenetriaminepentaacetic acid. The radioactivity associated with the origin indicates that ~48.8% of ^{111}In was bound to CC49 5 min into the reaction. The fraction of radioactivity which migrated with the solvent front was in the form of unreacted free $^{111}In^{+3}$. In the Fig. (**2B**), the same reaction mixture is analyzed after 30 min. All radioactivity is associated with the origin indicating that ^{111}In is protein-bound. This is verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [30] shown in the inset in Fig. (**2B**). Two lanes represent two detection methods ap-

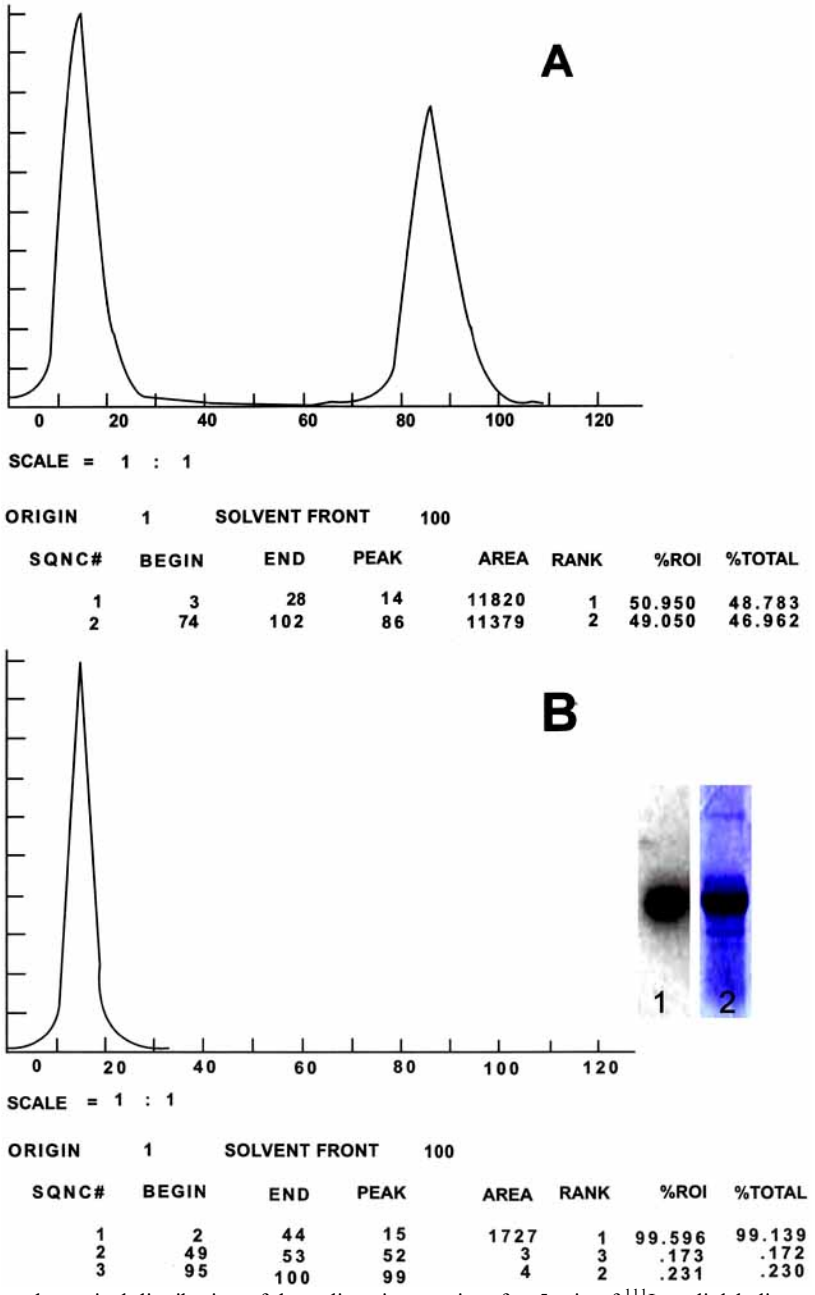

Fig. (2). **A**. ITLC analysis and a typical distribution of the radioactive species after 5 min of ^{111}In radiolabeling of CC49. **B**. The same reaction mixture analyzed after 30 min of the reaction. All radioactivity is associated with the origin indicating that all ^{111}In is protein-bound. This is verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Inset: lane 1 is an autoradiogram of ^{111}In associated with the IgG protein; lane 2 protein detected using the Coomassie Blue R-250 method.

plied to this gel: the lane 1 is an autoradiogram of ^{111}In associated with the IgG protein detected in lane 2 using the Coomassie Blue R-250 method. To detect the radioactivity and to verify that it coincides with the stained protein bands, gels are either placed on an autoradiography film (Kodak's BioMax XAR films can be used with all commonly used isotopes) or the phosphor screen (lane 1).

One alternative to ITLC is the high pressure liquid chromatography (HPLC). The accuracy and applicability of this method depends entirely on the proper choice of the column and the solvent system. HPLC in conjunction with properly selected standards and authentic, unlabeled reference compounds can provide practical data not only about the radiochemical purity but also the identity of the radioactive drug. The elution is monitored with the in-line UV and radioactivity detectors. Alternatively, fractions are collected and their radioactive content is determined in a gamma counter. Retention times of the radioactive peaks are compared with the parent unlabeled drug. Fig. (**3**) shows the HPLC trace obtained with a radioactivity detector of radioiodinated CC49 antibody. The same antibody was also analyzed using SDS-PAGE electrophoresis. The HPLC trace indicates the pres-

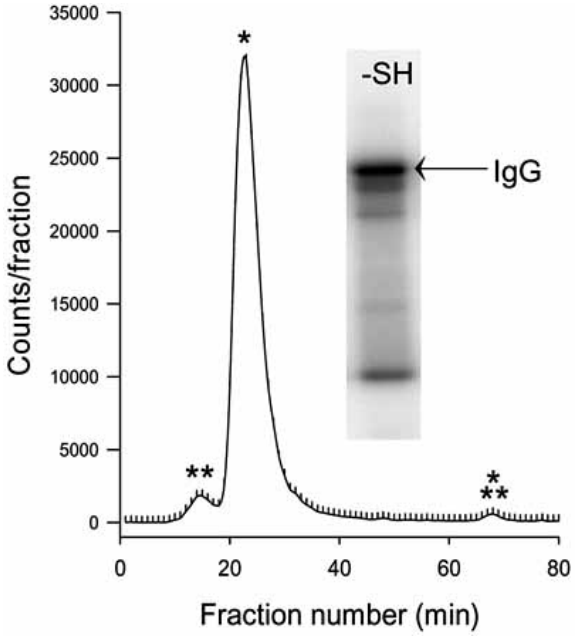

Fig. (3). HPLC trace of the reaction mixture from ^{131}I-radioidoination of a monoclonal antibody CC49. (*) Indicates intact, full size ^{131}ICC49; (**) corresponds to high molecular weight radioactive impurities; and (***) corresponds to trace amounts of low molecular weight impurities, possibly unreacted free ^{131}I. Inset shows SDS-PAGE (non-reducing) analyses of the same reaction mixture.

ence of high molecular weight impurities (**), almost certainly macroaggregated CC49, and a trace amount of impurities, possibly free ^{131}I (***).

Radionuclidic Purity

Radionuclidic purity is defined by the International Union of Pure and Applied Chemistry (IUPAC) as that fraction of the total radioactivity which is present in the form of the stated radionuclide, including daughter products [31]. The radionuclides obtained by the generator elution can be contaminated by the parent nuclide, e.g., ^{99}Mo in ^{99m}Tc. Sometimes the contaminant is the radioactive daughter. However, for most of radionuclides the radionuclidic purity is determined by the manufacturing process. Although the bombardment conditions, the energy of the proton beam and the timing are chosen to ensure ^{111}In of high radionuclidic purity, when the ^{112}Cd-enriched target is bombarded in a cyclotron to produce ^{111}In in the (p,2n) reaction, the measurable amounts of ^{114m}In are also produced. This radionuclide has a longer half-life (49.5 days) than ^{111}In (67.2 h) and will therefore make an increasing contribution to the radiation dose with time as ^{111}In decays. Because of its β-emitting component with a potentially high organ radiation dose contribution, levels of ^{114m}In in ^{111}In preparation must be carefully controlled. Most of ^{111}In-labeled radioactive drugs should not be administered later than 4 days from the reference date of $^{111}InCl_3$ to ensure that the level of ^{114m}In present are within the specified limits, usually less than 0.2%. Depending on the vendor, the radionuclidic purity for ^{111}In is specified at the time of calibration as not less than 99.925% ^{111}In and, not more than 0.075% ^{114m}In and ^{65}Zn combined. The specification also indicates levels of radionuclidic impurities at the time of expiration, for example, Indiclor® should contain not less than 99.85% ^{111}In and not more than 0.15% ^{114m}In and ^{65}Zn combined (Amersham Health, Medi-Physics, Inc. Arlington Heights, IL) [32]. These specifications apply to all radioactive drugs synthesized with Indiclor such as for example ^{111}In-ProstaScint®, (Cytogen Corporation, Princeton, NJ). The package insert for ProstaScint® provides much relevant information but it does not specify radionuclidic purity parameters. The view in this case is that the USP-defined parameters for the radionuclide also apply to all radioactive drugs derived from it. The contribution of ^{114m}In and ^{114}In to the radiation absorbed dose from [^{111}In]oxine has been evaluated from the imaging studies and it was estimated at an additional 0.16% to 12% on top of the ^{111}In dose, and in one case, that of the spleen from [^{111}In]oxine labeled erythrocytes, as much as an additional 33%. The authors concluded that for this indication it is advisable to avoid using ^{111}In products older than about 3 days after the time of calibration [33].

Sodium [^{123}I]Iodide is produced by bombardment of enriched ^{124}Xe with protons in the ^{124}Xe (p,2n) ^{123}Cs→ ^{123}Xe→^{123}I reaction. The radionuclidic purity at calibration time is specified as not less than 99.5% ^{123}I and not more than 0.5% all other nuclides, which include ^{121}Te, ^{125}I, ^{131}I, ^{126}I, ^{124}I, ^{130}I, ^{121}I and ^{24}Na. The radionuclidic composition at the expiration time is not less than 98.28% ^{123}I and not more than 1.72% all other nuclides. The radionuclidic purity of [^{125}I]Iodine produced in the nuclear reaction of ^{124}Xe(n,γ) ^{125}Xe(17 h)→^{125}I is > 99.99% with only one major contaminant ^{126}I at < 0.001% and two minor ones: ^{134}Cs and ^{137}Cs at levels < 0.0001%. [^{131}I]Iodine can be produced in either a nuclear reaction ^{130}Te (n, γ)→^{131}Te→^{131}I and isolated by the dry distillation of iodine from activated target into alkaline solution or as a fission product ^{235}U (n,f). The production of ^{99}Mo (for ^{99m}Tc generators) from enriched ^{235}U fission also produces fraction containing ^{131}I. MDS Nordion (Ottawa, Ontario, Canada) currently provides ^{131}I from both production methods. The radionuclidic purity for ^{131}I is determined by the high resolution gamma spectrometry at 364 keV. The reactor generated product has a radionuclidic purity ≥99.9% with less than 0.1% of ^{130}I. The specifications for the fission product state radionuclidic purity at ≥99.9% but do not identify any potential contaminants. Depending on the purification and isolation processes these could include ^{95}Nb, ^{95}Zr, ^{132}I and ^{133}I.

It should also be mentioned that for some radionuclides USP standards for radionuclidic purity do not necessarily imply the release criteria for the drug. For some radionuclides, it may not be possible to comply with the USP standard at the time of the drug release but only after a complete decay of the primary radionuclide [34].

A Germanium-Lithium, Ge(Li), detector coupled to a multichannel analyzer is the universally used instrument to assess the radionuclidic purity of various radionuclides. The same detector is also useful to verify or establish the identity of radionuclides. USP [34] recommendations are that a multichannel spectrometer coupled to a calibrated sodium iodide scintillation detector, or preferably with the higher resolution Ge(Li) compensated detector, should be used for the deter-

Table 4. Maximum Specific Activities for Several Commonly Used Radioisotopes

Radioisotope	Half-life	Theoretical Maximum Specific Activity GBq/μatom (Ci/μatom)
Iodine-125	60 d	80.7 (2.18)
Iodine-131	8.05 d	599.4 (16.2)
Iodine-123	13 h	8,916 (241)
Yttrium-90	64.2 h	1,806 (48.8)
Indium-111	67.4 h	1,720 (46.5)
Technetium-99m	6 h	19,321(522.2)
Copper-64	12.7 h	9,127 (246.7)
Fluorine-18	109.8 min	63,340 (1,712)
Carbon-11	20.4 min	340,092 (9,214)*
Nitrogen-13	10 min	695,477 (18,797)
Oxygen-15	122 sec	3,420,377 (92,443)

*^{11}C is typically produced in the chemical form of carbon dioxide, which is present in the atmosphere; therefore specific activities rarely exceed 0.1 Ci/nanoatom.

mination of radionuclidic purity and for the identification of the radionuclides. Detectors can be calibrated for energy and efficiency with standard ^{57}Co (122 keV), ^{137}Cs (661 keV) and ^{60}Co (1,332 keV) sources. The ^{152}Eu source can also be applied. The determination of α-emitter content can be performed using detectors such as the Ortec Model A-576 A-PAD or the Alpha Analyst Integrated Alpha Spectrometer from Canberra Industries, Inc. (Concord, Ontario, Canada) for α spectroscopy and counting of α-emitters in conjunction with the standard source such as the calibrated ^{241}Am source (Ortec, Oak Ridge, TN).

Specific Activity

Specific activity of radionuclide or radiopharmaceutical is defined as the amount of radioactivity per unit mass or per mole such as mCi/mg, MBq/mg or mCi/mole, MBq/mole. This value corresponds to drug product potency specifications and it should not be confused with the specific concentration, which is the ratio of radionuclide activity to the total volume (mCi/mL or MBq/mL). The maximum specific activity of the radionuclide is the "carrier-free" or "no-carrier-added" specific activity. Table **4** lists a few radionuclides and their theoretical maximum specific activities. Many radionuclides used in nuclear medicine are "no-carrier-added". However, this does not guarantee that the specific activity of the radioactive drug is at maximum. The existing methods of synthesis and purification predetermine and limit the final specific activities of radiopharmaceutical. Knowledge of the specific activity allows the administration of the exact amount (mass) of the drug. These data are critical if there is toxicity associated with the drug or if the pharmacokinetics and tissue uptake are dose-dependent. Diagnostic radioactive drugs that target specific receptors must have high specific activities to limit the receptor occupancy to <10% in order that the observed ligand-receptor interactions are the true phenomenon and not the perturbation of the natural equilibrium caused by the tracer. This cautionary note does not apply to radiotherapeutics. However, if the radiotherapeutic is based on a biologically active molecule and the consequences of the ligand-receptor interactions are not desirable, the need for high specific activities remain.

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