JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 241–257.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.922

Review Article

Manufacturing I-123-labelled radiopharmaceuticals. Pitfalls and solutions

Jos L.H. Eersels[1,*], Michael J. Travis[2] and Jacobus D.M. Herscheid[1]
[1] *Department of Nuclear Medicine and PET Research, VU University Medical Center, Amsterdam, The Netherlands*
[2] *Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK*

Summary

A variety of radioiodination methods is described in the radiochemistry literature, however, only a few can fulfil manufacturing requirements. In this article we provide an overview of the process of preparing a radiopharmaceutical to inform the general reader in their everyday use of these products.

According to molecular structure of the precursor/ligand, the first decision to be made is whether a nucleophilic or electrophilic approach should be used. Both are suitable for obtaining a high specific activity, as well as for integration in production processes. Feasible reaction conditions and reliability in terms of labelling yield and recovery are further relevant parameters.

Recent changes and strengthening of the pharmaceutical regulations mean that I-123 radiopharmaceuticals are often autoclaved. In order to maintain the radiochemical purity during this process, as well as during storage/transport, radical scavengers or antioxidants have to be added.

Key Words: single photon emission tomography; I-123; radioiodination; formulation; sterilization

Introduction

Due to its attractive physical properties, iodine-123 (I-123) (EC, $E_\gamma = 159\,\text{keV}$, $t_{1/2} = 13.2\,\text{h}$) plays a pivotal role as a radionuclide for a variety of radiopharmaceuticals. The medium energy is ideal for planar imaging and for single photon emission tomography (SPET). The intermediate half-life permits this isotope to be imaged for more than 2 days, which makes it

*Correspondence to: J. L. H. Eersels, Department of Nuclear Medicine and PET Research, VU University Medical Center, Location Radionuclide Center, de Boelelaan 1085c, 1081 HV Amsterdam, The Netherlands. E-mail: jeersels@rnc.vu.nl

Received 5 August 2004
Revised 21 October 2004
Accepted 2 December 2004

possible to study the pharmacokinetics of a given ligand over a longer time period than can be done with the short-lived PET radiopharmaceuticals.[1] This, together with the development of better imaging devices, the omnipresence of SPET cameras in peripheral hospitals and the lower cost when compared to PET, increases the market and utility for I-123-labelled radiopharmaceuticals. In addition, the shelf life of these Pharmaceuticals is long enough for distribution over a wide geographical area.

In the last decade the applicability of I-123-labelled radiopharmaceuticals has been changed from functional imaging of metabolism, e.g. ^{123}I-fatty acids for studying β-oxidation of the myocardial cells or ^{123}I-hippuric acid for renal clearance studies, to receptor (neuroreceptor and hormone receptor) and transporter imaging.

^{123}I-FP-CIT is a very promising ligand for the dopamine transporter, allowing the early diagnosis and staging of Parkinson's disease.[2]

Worldwide there is an increasing morbidity burden from Alzheimer's disease and depression which has provided a critical impetus for the study of these two disorders.

For the former, a new approach is the development of ligands with a good brain penetration to detect or to label amyloid plaques.[3] In affective disorders serotonergic mechanisms appear to be involved in the core psychopathology; thus, much effort has been invested in the development of specific ligands, especially for the serotonin transporter (SERT). For the SERT, ^{123}I-ADAM might be a promising ligand[4] and a variety of analogues of cocaine (tropane-structure) is also under investigation.[5] The development of these, and other new ligands will lead to the emergence of new perspectives in nuclear medicine. These will include an increasing role in acquiring clinical data for neurology and psychiatry, as well as for general medical conditions. In addition, nuclear medicine will assume a greater role in basic molecular research, drug discovery and drug evaluation.[6]

Advances in technology will be increasingly implicated in the development of new tracers. To be successful these developments must be shared with clinicians from nuclear medicine and other disciplines. It is crucial to the endeavour of manufacturing new radiopharmaceuticals that the pitfalls and their solutions be understood by all involved.

It is important to remember that even after a new radioligand has been developed, many problems can arise during 'up-scaling' to produce high radioactivity quantities of tracer, and in obtaining a formulation which is stable during autoclaving and storage. Herein, we wish to report on the pitfalls that might be encountered during such efforts. We will suggest pragmatic solutions for these. In addition, we will outline the use of feasible reaction conditions and work-up procedures that can be integrated to circumvent other problems in the manufacturing of I-123 radiopharmaceuticals.

Production of I-123

One of the prerequisites in performing labelling reactions on a 'no-carrier-added (nca)' scale is the high purity of each reagent. Impurities which in normal chemistry might lead to minor side products can disturb a radiochemical reaction completely. Furthermore, the concentration of the radioiodine is an important parameter, governing the exchange rate in the labelling reaction. Thus, it is clear that the production route for I-123 can have a high impact on the applied chemistry.

Former production methods followed a ^{124}Te(p,2n) ^{123}I reaction on solid TeO_2 from which the iodine could be distilled off under an inert gas flow at high temperature. Besides the presence of small amounts of I-125, depending on the enrichment factor of ^{124}Te, there is always some I-124 formed due to the concomitant (p,n) reaction. In addition, the obtained radioiodine solution is contaminated with traces of Te or TeO_2, which might influence the labelling yield sometimes dramatically. As we have described in our production of ^{124}I from gaseous TeF_6.[7]

Currently the best source of ^{123}I, having the least contamination with other radioiodides, is the proton bombardment of highly enriched ^{124}Xe (Figure 1).

In fact, the iodine we use in the production of our radiopharmaceuticals (as described below) is produced by this method four times a week using conditions outlined below.

A target chamber is filled with ^{124}Xe under high pressure and irradiated with 25 MeV protons (region 23–29 MeV has a nearly constant cross-section[8,9]) and a beam current of 25 μA, resulting in irradiation yields up to 160 MBq/μA h, which is comparable with other values from the literature. After irradiation, the gas is kept for several hours in the target to allow for the radioactive decay of ^{123}Xe to ^{123}I, after which the enriched ^{124}Xe is recovered by cryogenic transfer to the storage vessel.

To remove ^{123}I in a nca state, the target chamber is flushed several times with a halogen free, diluted sodium hydroxide solution (some manufacturers use a small amount of caesium iodide for a better wash-out efficiency, which

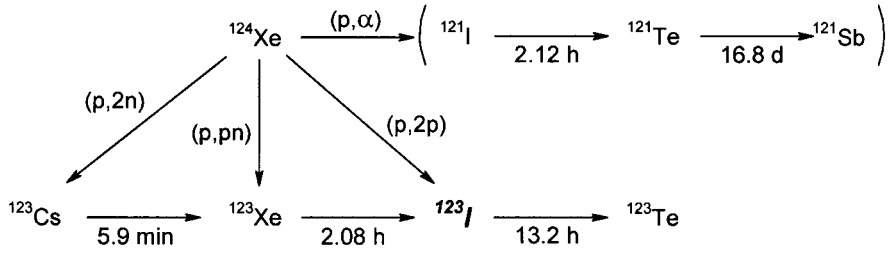


Figure 1. Production of ^{123}I on enriched ^{124}Xe

lowers the specific activity). The final-collected solution is transferred to an anion-exchange chromatography module, where the radioiodide is concentrated and harvested in 1 ml of sodium hydroxide (10^{-2} M). The merits of this procedure are that it enables us to recover the radioiodide in an almost carrier-free state (SA of 8695 GBq/μmol), which is necessary for the radiosynthesis of receptor-ligands.

Control of the carrier-free state (cq. specific activity) of the produced I-123 can be performed by neutron activation, X-ray fluorescence or it is even more accessible with UV-absorption.[10] Indeed, the high UV sensitivity of the iodide anion (4 ng sodium iodide at λ_{max} 230 nm, AUFS 0.01 with HPLC; ion-pair chromatography), enables to determine the specific activity of I-123 in an accurate manner. Controls of our commercially produced I-123, revealed the absence of traces of cold iodide. The guaranteed carrier-free state of our I-123, as such, permits us to produce in a reliable manner radiopharmaceuticals with a high and reproducible specific activity (in contrast with PET-radioisotopes, like fluorine-18 or carbon-11).

Hazardous radioiodide impurities (oxygenated species), formed during irradiation, are not always completely removed by the anion-exchange column in the concentration procedure. Fortunately, the appliance of our new concentration procedure, a platinum-filled mini column with *in situ* reducing properties, can alleviate these problems, without loss of activity.[11] This new process also offers the opportunity to recover ^{123}I in anhydrous organic solvents, which can reveal new labelling methods.[12]

Radionuclidic impurities like ^{125}I and ^{121}Te are below 0.05%. The first, induced on ^{126}Xe, can be limited using highly enriched ^{124}Xe (isotopic purity $>99.9\%$). The amount of ^{121}Te, which is generated through a (p,α)-reaction with a much smaller cross-section, is further decreased in the concentration procedure. Previously described alternative methods for the production of ^{123}I through ^{123}Xe like a (p,5n)-reaction on ^{127}I,[10] have, more or less, ceased to be used. This method requires an intricated chemical work-up process and a high-energy cyclotron for generation of 65 MeV protons. Nevertheless, the supply of the radioiodide in a glass ampoule, anhydrous ^{123}I deposited on the interior walls, gave the opportunity to the radiochemist to perform and search radioiodination reactions in anhydrous conditions.

The use of the (p,2n)-reaction to produce ^{123}I can be implemented by medium-sized cyclotrons and has been possible due to improvements in technical knowledge of handling reusable gas targets. This is indispensable for the expensive ^{124}Xe target gas.

This production method, in combination with chemical and simple work-up processes, is reliable in obtaining radiochemical and nuclidic pure ^{123}I, at a reasonable price.

Production of ^{123}I-labelled radiopharmaceuticals

The aliphatic carbon–iodine bond is relatively weak (222 kJ/mol). This results, especially *in vivo*, in a fast deiodination, either by nucleophilic substitution (S_N2) or β-elimination. So, when a radioiodine atom has to be incorporated in a radiopharmaceutical, the radioiodine is preferentially attached to a sp^2 carbon atom, in a vinylic or aromatic moiety, in which the carbon–iodine bond strength is higher (268–297 kJ/mol, respectively). Therefore, the radioiodination is often implemented by nucleophilic or electrophilic aromatic substitution and is more or less predicted by the structural feature of the molecule. As an example, for each method, two radiopharmaceutical preparations will be given (see below), which are used in routine or commercial productions. In conjunction with simple purification-steps, the described work-procedures can cope with pharmaceutical purity recommendations.

Nucleophilic labelling reactions

In organic synthesis, nucleophilic aromatic iodination reactions are often carried out on diazonium salts (Sandmeyer reaction, Figure 2).

The inconvenience of an unstable intermediate can be overcome by starting with shelf-stable triazenes, synthesized by amination of the diazonium salt.[13] However, the extensive procedure of pre-synthesizing such a modified precursor, the critical reaction conditions as well as the use of relatively high amounts of precursor,[14] has made this method a non-standard procedure in radiolabelling.

The method of choice in nucleophilic radioiodination is the well-established Cu(I)-catalysed halogen–halogen exchange reaction in an acidic, aqueous medium.

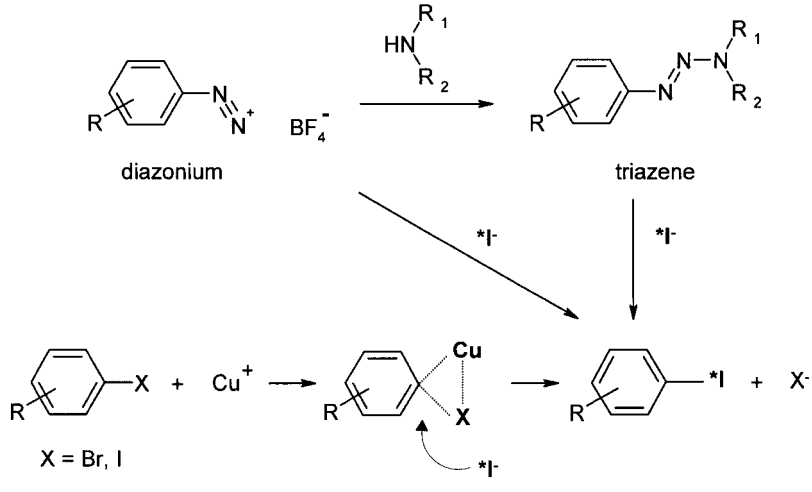


Figure 2. Nucleophilic radioiodination

The transition state involved is initially a complex intermediate, which is arranged between the aryl halide and Cu(I).[15] The Cu(I) species is formed *in situ* by reduction of Cu(II), with the presence of reducing agents (like gentisic acid) in the reaction mixture.

The complex entity (Figure 2) facilitates a nucleophilic attack of the entering radioiodide on the carbon–halogen, and effects a regio-specific exchange, leading to a single radiochemical pure product, without degradation products, and in a high labelling yield.

In an isotopic exchange, (*I/I), this often becomes a quantitative labelling. Further purification steps are superfluous, thus, also reducing radioactive waste, which is convenient and highly affordable for the production process.

The versatility of this nucleophilic Cu(I) method is further proved, by the possibility of non-isotopic exchange (*I/Br). This enables the synthesis of radiopharmaceuticals with a high specific activity, essential for receptor-imaging.

Before using the Cu(I)-nucleophilic exchange as a labelling method, one has to consider initially, the structural features of the (precursor)molecule. In general, it can be stated that a nucleophilic exchange can be successfully applied on activated (presence of electron-deficient substituents, e.g. carbonyl group) or non-activated (e.g. alkyl group) aromatic compounds. However, in organic media, electron-donating substituents are also well-tolerated.[16] In this latter case, the iododebromination presumably follows a radical mechanism. Secondly, it is important that the precursor does not contain any other moieties functionally susceptible to iodination or reaction with the added reducing agents, such as 2-nitroimidazole amino acid conjugates.[17] In addition, the presence of a thiourea group, which complexes copper(I,II)-species, inhibits the labelling completely.[18]

Other relevant experimental parameters involving the exchange reaction are the Cu^{1+}-concentration and the amount of precursor.

Cu^{1+}-concentration. The optimal substrate to Cu^{1+} ratio lays between 5 and 10, for a usual precursor amount in the μmol range.[15] However, the huge amount of radioactivity, and as such the iodine mass concentration, forces us to adapt the Cu^{1+}-concentration slightly, in order to prevent formation of insoluble $Cu^{123}I$. This is despite the fact that our ^{123}I is almost carrier free. Therefore, a smaller amount of Cu^{+} was used and a substrate to Cu^{+} ratio of 11 was found to be optimal, without interfering the labelling yield. For pharmaceutical reasons and for convenience in routine productions freeze-dried reaction-mixtures are often used instead of freshly weighed. However, in our experience, freeze-dried batches are more susceptible to the formation of insoluble $Cu^{123}I$, which is probably due to the presence of tracer amounts of disproportionated copper species during the freeze-drying process. To prevent

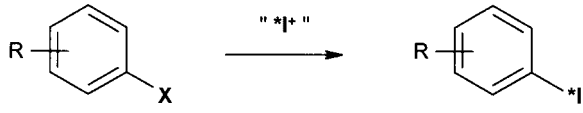
this problem, a shelf life of 3 months and a maximum amount of radioactivity per reaction mixture of 11 GBq for each time of synthesis was introduced.

Amount of precursor. A similar phenomenon to organic synthesis, in which the reaction rate is proportional to the amount of substrate, is noticed in labelling reactions. Higher labelling yields are obtained, with higher amounts of precursor. Precursors, with limited solubility in aqueous solutions, like fatty acids,[19] or ketanserine,[20] can be labelled in mixed solvent conditions, with the addition of ethanol or glacial acetic acid. In such circumstances, a smaller amount of the reducing agent $SnSO_4$ is necessary to prevent precipitation of the hydrolytically formed insoluble $Sn(OH)_2$. Although the presence of $SnSO_4$ in the reaction mixture is additional, it offers the opportunity to implement radioiodinations without a decrease in labelling yield, for example in the presence of radioiodinated impurities, like $*IO_3^-$ (up to 60–70% $*IO_3^-$, gave still quantitative exchange). The other reducing agent, gentisic acid or 2,5-dihydroxybenzoic acid, is essential and needed for the *in situ* reduction of Cu(II) to Cu(I).

Furthermore, the amount of precursor is often ruled or limited by the acquired specific activity. For instance, carrier-free preparations necessitate non-isotopic exchange, and the amount of precursor should be as small as possible, favouring a better separation in the HPLC-purification between the brominated precursor and the radioiodinated product (see below). In case of isotopic exchange a relative high specific activity can be reached by lowering the amount of precursor, however, with a decrease in labelling yield.[15]

Electrophilic labelling reactions

In contrast to the above-described methods, electrophilic labelling reactions can often be performed fast and under mild reaction conditions. The electrophilic species (HO*I, H_2O*I), generated from radioiodide and the oxidant, react directly with the aromatic moiety of the compound to be labelled (Figure 3). Electrophilic substitutions can often be carried out on a non-derivatized substrate. However, in case of low reactivity or lack of regio-selectivity, radioiodination might proceed via iododemetallation or iododesi-lylation. The tougher cleavage of a carbon–silicon bond in the latter implies a



X = H, HgCl, $Tl(OCOCF_3)_2$, $Sn(Me)_3$, $Si(Me)_3$

Figure 3. Electrophilic radioiodination

labelling procedure with heating.[21] Radioiododestannylation has become more and more the method of choice. The weakness of the carbon–tin bond readily gives regio-specific radioiodination, even at room-temperature, while the precursors can easily be made from the bromo- or iodoaryl compound.[22]

Frequently used oxidizing agents are peracetic acid and the *N*-chloro compounds, such as chloramine-T, iodogen and succinimides. The *N*-chloro compounds are by far the most popular oxidants, however, their relatively strong oxidizing properties often induce by-products.

In order to limit these oxidative side reactions, chloramine-T is immobilized on spherical polystyrene particles (iodobeads®, while iodogen, which contains four functional chlorine atoms, is coated as a thin layer on the walls of a reaction vessel. Of both immobilized oxidants, which are mostly used for protein-labelling, iodogen is the best to prevent loss of the immunoreactivity after labelling.[23] The *N*-chloro/bromo-succinimides are less frequently used, due to possible radical side reactions,[24] as well as the use of aggressive solvents. However, for the labelling of small organic molecules, peracetic acid is often preferred due to its mild oxidizing properties. It is a useful agent for sensitive substrates,[25] and chlorination of the aromatic moiety is excluded. Due to the instability or the limited shelf life of peracetic acid, it is advised to prepare the reactant *in situ*.

Molecular radioiodine, *in situ*, generated from radioiodide and oxidants (like hydrogen peroxide, or ferric sulphate) is not recommended due to the volatility of the reagent, furthermore, the radiochemical yield will never exceed 50%. Enzyme-mediated radioiodinations, for instance via lactoperoxidases, are critical and self-radioiodination is not precluded.[26]

Discussion

As previously stated, a radioiodination method of choice does not exist. Different parameters have been taken into consideration, as summarized in Table 1.

Key to these is the substituted R-group on the aromatic ring of the precursor. This activates (deactivates) towards nucleophilic or electrophilic substitution. Further, in both cases, the stability of the precursor needed to be verified. For instance, nucleophilic substitution requires higher reaction temperatures (100–180°C) and longer reaction times (20–60 min), while in electrophilic conditions the precursor is exposed to oxidative agents, but mostly in favourable, short reaction times (5–20 min), often carried out at room temperature.

In nucleophilic exchange, lipophilicity of the precursor can be a limiting factor for the amount of precursor needed to obtain a good labelling yield, and in its consecutive, critical HPLC-separation in non-isotopic exchange.

Table 1. Overview of radioiodination methods

	Oxidative radioiodination	Nucleophilic isotopic exchange	Nucleophilic non-isotopic exchange
Type of precursor (substitution on aromatic)	Electron-rich substituent, and/or modification of precursor: -$SnMe_3$, -$SnBu_4$, -$SiMe_3$, ...	Electron-deficient substituent, preferable Cu^{1+}-catalysis	Electron-deficient substituent, mostly Cu^{1+}-catalysis
Specific activity	NCA (8.695 TBq/mmol) or carrier-added	Low/moderate specific activity	High/NCA (traces of precursor might act as pseudo-carrier)
Reaction time	5–20 min	20–60 min	20–60 min
Reaction conditions	Mostly room temperature	Elevated temperature (100–180°C)	Elevated temperature (100–180°C), often higher (compared with isotopic exchange)
	Presence of oxidative agents	Presence of reducing agents (Cu^{1+}-catalysis)	Presence of reducing agents (Cu^{1+}-catalysis)
	Amount of precursor; μg-scale	Amount of precursor; mg-scale	Amount of precursor; mg-scale (preferable small amount)
	Labelling yield less proportional with amount of precursor	Labelling yield proportional with amount of precursor	Labelling yield proportional with amount of precursor
Purification step	Facile HPLC-purification (solid-phase extraction possible)	Often little, or no purification (in case of quantitative labelling)	Sometimes difficult, HPLC-separation to remove precursor

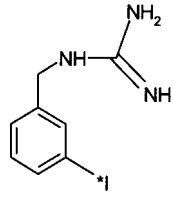
Electrophilic conditions require only a small amount of precursor (μg-scale) and offer more versatility in solvent choice.

As mentioned above, radioiodide can be produced on a no-carrier-added manner, resulting in a high specific activity of 8.695 TBq/mmol. However, such a huge specific activity of the radioligand is not always a prerequisite for successful receptor imaging. Indeed, measurements in routinely performed clinical PET studies (carbon-11 or fluorine-18 labelled) reveal a negligible receptor occupancy of 1–2% of the administered radiopharmaceutical, with a specific activity of 100 TBq/mmol, at the time of injection,[27,28] which is also illustrated in the following paragraph 'Examples'; regular-prepared receptor imaging radiopharmaceuticals (^{123}I-iomazenil, ^{123}I-R91150) are synthesized by the addition of a small amount of carrier, resulting in a specific activity of $\geqslant$70 TBq/mmol. However, the necessity of preparation of

the radiopharmaceutical on a no-carrier-added level has to be investigated in each particular case, *in vivo*.

Examples

Nucleophilic isotopic exchange



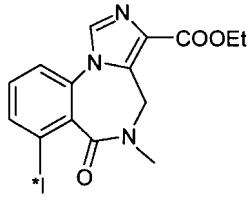
^{123}I-MIBG. Routine productions start with approx. 11 GBq ^{123}I on time of synthesis ($n > 1000$).

To a freeze-dried reaction mixture (kit) containing 2 mg of MIBG.1/2 H_2SO_4, 0.5 mg of $SnSO_4$, 5 mg of 2,5-dihydroxybenzoic acid, 11 mg of citric acid and 0.1 mg of $CuSO_4$ is added nca $^{123}I/H_2O$ to an end volume of 1 ml.

This reaction-mixture is flushed with N_2 and heated at 121°C during 25 min. The quantitative labelling ($\geqslant 99\%$) allows the labelled pharmaceutical to be diluted, without purification. So addition of an isotonic citrate buffer (pH 4), gives an activity concentration of 74 MBq/ml ART(activity reference time – about 30 h after synthesis) and a specific activity of 40 GBq/mmol. This diluted ^{123}I-MIBG is dispensed through a 0.22 μ filter, in a shielded laminar down flow box, into clean vials and sterilized by autoclavation.

The radiochemical purity of the finally autoclaved radiopharmaceutical is higher than 97% (at ART) and its overall yield more than 95%.

Nucleophilic non-isotopic exchange



^{123}I-iomazenil. Routine productions start with approx. 11 GBq ^{123}I on time of synthesis ($n > 500$).

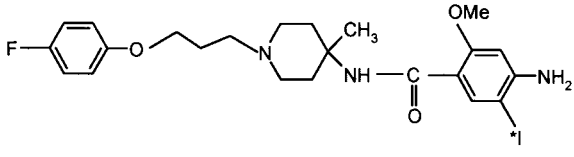
To a freeze-dried reaction-mixture (kit) made up of 1 mg bromo-mazenil, 0.2 mg $SnSO_4$, 4 mg 2,5-dihydroxybenzoic acid, 5 mg citric acid and 0.1 mg $CuSO_4$ is added nca ^{123}I (+carrier; 0.41 nmol sodium iodide/37 MBq ^{123}I)/ H_2O/EtOH (v/v = 70/30), to an end volume of 1 ml. This reaction-mixture is flushed with N_2 and heated at 121°C during 25 min. The labelling yield

is 80–90% and in order to obtain a radiochemical pure product, with high specific activity ($\geqslant$74 TBq/mmol ART), the labelled pharmaceutical is purified by a HPLC-separation (HPLC-conditions: NovaPak RP-18, 6μ, 300 × 7.8 mm – with an isotonic EtOH/acetate 25/75-eluent, flow 2 ml/min, R_t 30–35 min).

The collected ^{123}I-iomazenil (HPLC-fraction) is further diluted with an isotonic citrate buffer (pH 4), filtrated, dispensed and sterilized by autoclavation. The radiochemical purity of the autoclaved ^{123}I-iomazenil is higher than 97% (at ART) with an overall yield of 70–80%.

Due to conjugation of the electron-withdrawing carbonyl-group in iomazenil, nucleophilic exchange is advisable rather than an electrophilic route. Even, with a tributyltin compound as precursor, and with adapted but not-suitable reaction conditions in electrophilic substitutions (reaction-times up to hours,[29] or with heating[30]), low yields were obtained, while reproducible and high labelling yields are possible with the nucleophilic exchange, as described above.

Direct electrophilic route



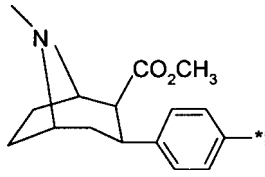
^{123}I-R91150. Routine productions start with approx. 4 GBq on time of synthesis ($n > 100$)

To a V-vial, containing 40 μg of R91150 in 300 μl of HOAc (99–100%) is added 500 μl nca Na^{123}I (+carrier; 0.532 nmol sodium iodide/37 MBq ^{123}I). To this is added four times 25 μl of H_2O_2 (30%-solution – at 0, 10, 20 and 25 min). The reaction is stopped at 30 min with 0.5 ml of sulfite in alkaline solution (0.5 M in 4.5 M).

After labelling (70–80% yield), the ^{123}I-R91150 is purified by HPLC (RP Select B; 250 × 4 mm, 10 μ – with an isotonic EtOH/acetate-eluent, 40/60 – flow 0.8 ml/min – R_t 28 min). Therefore, the reaction-mixture is on-line concentrated on a guard-column (10 × 10 mm) mounted on the HPLC injector (see below).

The collected ^{123}I-R91150 (HPLC-fraction) is diluted with an isotonic citrate buffer (pH = 6, containing 1 mg hippuran per ml) and filtered through a Millipore LG-filter and subsequently dispensed and sterilized by autoclavation. The formulated ^{123}I-R91150, with a high specific activity (70 TBq/mmol), has a radiochemical purity higher than 96% (at ART), and an overall yield of 55–60%.

Electrophilic labelling using a Sn-precursor



^{123}I-β-CIT. Routine productions start with approx. 3 GBq on time of synthesis ($n > 100$).

To a V-vial, containing 20 µg of trimethyltin-β-CIT in 300 µl of HOAc (99–100%) is added 100 µl nca Na^{123}I and successively, three times 15 µl of H_2O_2 (30%-solution – at 0, 10 and 15 min). The reaction is stopped at 20 min by adding 0.5 ml of sulfite in alkaline-solution (0.5 M in 4.5 M).

Purification is done by HPLC (RP Select B; 250 × 4 mm, 10 µ – with an isotonic; EtOH/acetate-eluent, 40/60 – flow 0.55 ml/min – R_t 15 min). The reaction-mixture is therefore on-line concentrated on a guard-column (10 × 10 mm). The ^{123}I-β-CIT (HPLC-fraction) is diluted with an isotonic citrate buffer (pH 5), dispensed and sterilized by filtration.

The labelling yield is higher than 95%, and its radiochemical purity more than 98% (at ART).

In the case of R91150, the presence of two electron-donating groups favours electrophilic labelling and directs the radioiodide specifically. A nucleophilic, non-isotopic exchange (*I/Br) resulted in a poor labelling yield, due to deactivation by the two mentioned groups.[31]

For the labelling of β-CIT a demetallation method is necessary to prevent formation of other isomers. On the other hand, nucleophilic exchange is not recommended, because of hydrolysis of the ester function. In our experience, however, in sufficient acidified conditions (pH ⩽ 3), hydrolysis (by heating in autoclave; 25 min at 121°C) can be limited to less than 1%, while higher pH-values favour hydrolysis considerably.

Work-up procedures

As one can imagine, the work-up procedure is greatly dependent on the applied labelling method. Due to the quantitative labelling in nucleophilic, isotopic exchange (e.g. MIBG), dilution, filtration and autoclavation of the diluted reaction-mixture after labelling might be enough. However, when the amount of precursor is reduced in order to obtain a higher specific activity, the radiochemical yield drops, leaving free iodide in the final product. In these special cases, trapping of the free radioiodide by means of a silver membrane[32] might be a possible solution.

In general, preparative high performance liquid chromatography (HPLC) is needed to obtain a (radio)chemically pure compound. In order to obtain a

labelled compound for human use, a water/ethanol mixture is used as elution solvent in reversed-phase HPLC. Since under these conditions the brominated precursor, of which a few milligrams have been used, comes off first, it might occur that the iodinated product is collected while the precursor peak is still tailing. This amount of pseudo-carrier, which mostly also has affinity for the receptor under investigation, reduces the effective specific activity. Methods to prevent this are, firstly, a second HPLC separation or, secondly, using a smaller amount of precursor to obtain an initial better separation. The disadvantages are obvious. The first requires more time and thus leads, in addition to the loss of the second separation, to a lower overall yield due to decay. On the other hand, a lower amount of precursor also often leads to a lower (radio)chemical yield.

The above problems do not occur when an electrophilic route on a Sn-precursor has been chosen. Normally these precursors have a longer retention time than the iodinated products. Unfortunately, although the amount used is only a few micrograms and even given the possibility of using an analytical column for a preparative separation, often the reaction volume is too large to inject it directly into an HPLC column.

A preconcentration is then necessary to obtain a good separation. The most convenient way to do this is by mounting a small column filled with reversed-phase material (we prefer Waters Oasis®) on the injector instead of a sample loop (Figure 4).

To this end, the reaction medium is diluted with water and loaded onto the preconcentration column, which is then subsequently washed with another 4 ml of water to remove all the polar contaminants. On switching the injector, the preconcentration column is eluted in the reverse way and the sample is loaded onto the preparative HPLC column.

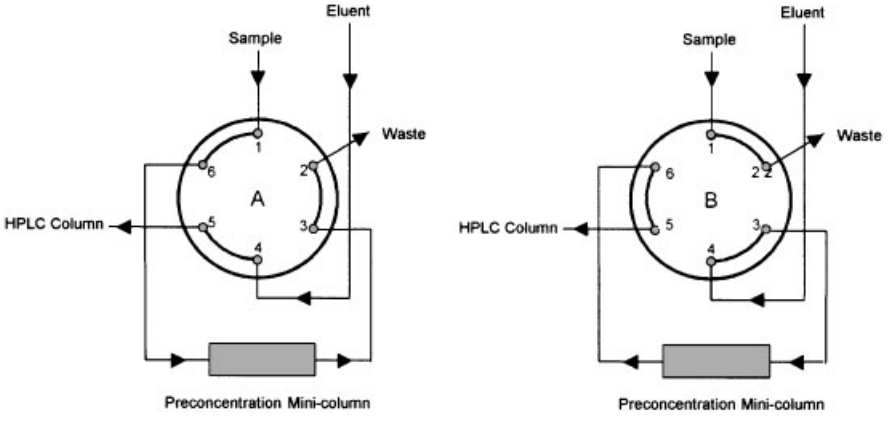


Figure 4. Preconcentration before HPLC separation. (A) Load, (B) inject

Formulation and sterilization

After reaction (isotopic exchange) or after the product has been collected from the preparative HPLC run, the solution is diluted with an isotonic citrate buffer (pH 3–4) and dispensed in sterile vials in a radioactive concentration of 1–1.5 mCi at ART.

The acidic conditions are often advisable in order to minimize deiodination during autoclaving (121°C for 25 min).

However, for some compounds, e.g. R91150, this is not enough. In customized procedures, radiochemical purities of 92–94% were obtained after autoclavation at ART (only deiodination was observed), even in the presence of classical radical scavengers, like ascorbic acid or gentisic acid, which are often omitted in the autoclaving processes anyway, due to their colouration. Also adaptations, like a change in acidity or solute, gave no significant improvement. Therefore, other additives were sought. To study a statistically relevant effect, we enhanced the radiolysis by exposing the samples to a ^{60}Co-γ-source.[33,34]

At 22°C, ^{123}I-R91150 and ^{123}I-MIBG samples (resp. pH 6 and 4), containing different scavengers were irradiated for 1.5 h at a dose-rate of 0.1 Gy/s. The total dose delivered to each exposed sample was 540 Gy. The specific activity at the time of study was 1500 TBq/mmol.

As published,[33] we found that on exposure to the γ-source only deiodination was induced from the radioligand, without degradation products (Figure 5), which proves that the carbon–radioiodine bond is the weakest. Since under these conditions ^{123}I-MIBG (with the same specific activity) deiodinates much more slowly, we anticipate that conjugation of the electron-donating groups in ^{123}I-R91150 favours deiodination by stabilization of the intermediate cation. Radical scavengers, like thiourea (or its analogue DMTU, dimethylthiourea). NAC (*N*-acetyl-D-cysteine, a muco-regulator) and OIH (ortho-iodo-hippuric acid, used as pseudo-carrier or competitor) all gave satisfactory results.

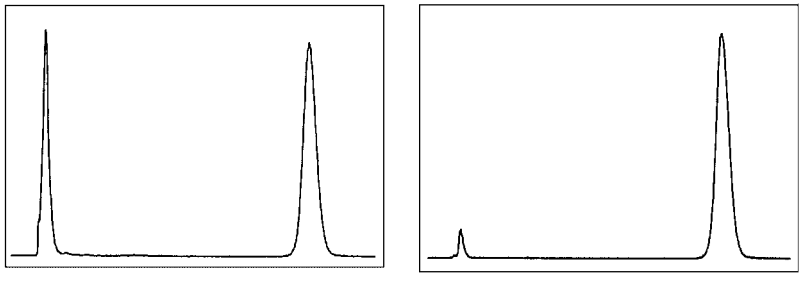


Figure 5. Radio-chromatograms ^{123}I-R91150

But for reasons of toxicity only OIH was allowed by the hospital pharmacist. A low amount of 1 mg/ml OIH, in the formulated ^{123}I-R91150 doses, was sufficient to obtain radiochemical purities higher than 97% (24 h after synthesis and autoclavation).

Sterilization of the end product by membrane-filtration can sometimes be troublesome. The lipophilicity of the radioligand can lead to retention or stickiness of the radioligand on the membrane.

Addition of ethanol, to a limited extent (e.g. less than 12 vol %), pH-adjustment of the diluent, or pre-saturation (in case of low specific activity) with carrier of the filter-membrane, can lead to an improvement. However, an adapted choice of membrane material can be more useful. In most cases, usage of a PVDF-membrane (Millex-GV, low protein binding) gives satisfactory results. However, in the case of R91150, none of the above-stated adaptations resulted in any improvement, and losses of radioactivity (on the membrane) still remained high (20–30%).

Therefore, other membrane filters were tested, and usage of filters with a PTFE-containing membrane (hydrophilic; Millex-LG or hydrophobic; Millex-FG; with pre-treatment with ethanol and diluent) gave a reproducible radioactivity loss of less than 5%.

Conclusions

Administration of radiopharmaceuticals requires a certified sterile and pure product, complying with the radiochemical and chemical purity as specified. Taking a broad view, this concept should not only be applied at the end of the process or for the end product, but rather, each step of the whole process should be evaluated as part of a contribution to the purity of the end product. This is, in fact, one of the basic principles of good manufacturing practice (GMP).

For these reasons, the processes we have described above were developed and assessed using simple parameters, like feasibility, reliability and purity. As shown in the 'Nucleophilic and Electrophilic Methods' sections, pragmatic and non-complicated reaction-conditions and work-up procedures are utilized. All radioiodinations are accomplished, as much as possible, with the aid of non-toxic chemicals (even in HPLC-purification), with a good labelling yield, and a low spread in the variation of labelling yield, making the whole production process more reliable.

A labelling method of choice does not exist for all putative ligands. First the structural requirements of its precursor or ligand must be evaluated, as well as the required specific activity.

The nucleophilic Cu(I)-method, in isotopic exchange even offers the possibility to perform labelling without purification, at least in case of

quantitative labelling, due to the use of non-toxic chemicals. Electrophilic labelling can be carried out quickly and in mild reaction conditions.

Sterilization of radiopharmaceuticals is usually performed by membrane filtration or by autoclaving. The former method is applicable to heat-unstable molecules (e.g. ^{123}I-β-CIT), while autoclaving is more secure and can often be applied for radioiodinated ligands, with minor adjustments of the solute or with the addition of stabilizers.

In the interface between the clinician, clinical scientist and radiopharmacist there is increasing recognition of the common aims of research and development in the field of nuclear medicine. An understanding, in general terms, of the pitfalls and solutions in the development and large-scale production of radiopharmaceuticals across disciplines is essential. This understanding will inform the further assessment of these pharmaceuticals in relevant experiments and ultimately their appropriate use, *in vivo*, in humans.

References

1. Lassen NA. *Eur J Nucl Med* 1996; **23**: 1–4.
2. Booij J, Tissingh G, Winogrodzka A, Boer GJ, Stoof JC, Wolters ECh, van Royen E-A. *Eur J Nucl Med* 1997; **24**: 68–71.
3. Kung HF, Kung M-P, Zhuang ZP, Hou C, Lee C-W, Plössl K, Zhuang B, Skovronsky DM, Lee V, Trojanowski JQ. *Mol Imag Biol* 2003; **5**: 418–426.
4. Acton PD, Choi S-R, Hou C, Plössl K, Kung HF. *J Nucl Med* 2001; **42**: 1556–1562.
5. Davis HML, Ren P, Kong NX, Sexton T, Childers SR. *Bioorg Med Chem Lett* 2002; **12**: 845–847.
6. Costa DC, Pilowski LS, Ell PJ. *Lancet* 1999; **354**: 1107–1111.
7. Vis R, Mooij R, van Leuffen P, Pereboom K, van Langevelde F, Visser GWM, van der Jagt PJ. *Proceedings of 4th International Conference on Isotopes*, Cape Town, South Africa, March, 2002.
8. Kurenkov NV, Malinin AB, Sebiakin AA, Venikov NI. *J Radioanal Nucl Chem Lett* 1989; **135**: 39.
9. Quaim SM, Tarkanyi F, Stöcklin G, Sajjad M, Lambrecht RM, Schweickert H. *J Label Compd Radiopharm* 1991–1992; **29** (Suppl.): 103–104.
10. Mausner LP, Srivastava SC, Mirzadeh S, Meinken GE, Prach T. *Appl Radiat Isot* 1986; **37**: 843–851.
11. Herscheid JDM, Moet FP. Process for the purification and concentration of radioiodide isotopes. *Int Appl PCT/US* 2000; 00/01824.
12. Braker AH, Moet FP, van der Zwart RE, Eersels JLH, Herscheid JDM. *Appl Radiat Isot* 2002; **57**: 475–482.
13. Foster NI, Heindel ND, Burns HD. *Synthesis* 1980; **1**: 572–573.
14. Foster NI, Dannals R, Burns HD, Heindel ND. *J Radioanal Chem* 1981; **65**: 95–105.
15. Mertens J, Gysemans M. Cu(1+) assisted nucleophilic exchange; application and mechanistic approach. In *New Trends in Radiopharmaceutical Synthesis, Quality*

Assurance, *and Regulatory Control*, Emram AM (ed.). Plenum Press: New York, 1991; 53–65.
16. Moerlein SM. *Radiochim Acta* 1990; **50**: 55–61.
17. Henneken H, Krummeich C, Holschbach M, Stöcklin G. *J Label Compd Radiopharm* 1995; **37** (Suppl.): 208–210.
18. Wilkinson G, Gillard RD, McCleverty JA. *Comprehensive Coordination Chemistry*: *The Synthesis*, *Reactions*, *Properties* & *Applications of Coordination Compounds*, *vol. 2*, *Ligands*. Pergamon Press: Oxford, 1987; 639.
19. Mertens J, Eersels J, Vanryckeghem W. *Eur J Nucl Med* 1987; **13**: 159–160.
20. Mertens J, Gysemans M, Bossuyt-Piron C, Thomas M. *J Label Compd Radiopharm* 1990–1991; **28**: 731–738.
21. Wilbur DS, Anderson KW, Stone WE, O'Brien HA. *J Label Compd Radiopharm* 1982; **19**: 1171–1188.
22. Dawson MI, Hobbs PD, Rhee SW. *J Label Compd Radiopharm* 1992; **31**: 865–869.
23. Visser GW, Klok RP, Klein Gebbinck JW, ter Linden T, van Dongen GA, Molthoff CF. *J Nucl Med* 2001; **42**: 509–519.
24. Youfeng H, Coenen HH, Petzold G, Stöcklin G. *J Label Compd Radiopharm* 1982; **19**: 807.
25. Kung M-P, Kung HF. *J Label Compd Radiopharm* 1988; **27**: 691–700.
26. Bakker C, Kaspersen FM. *Appl Radiat Isot* 1979; **30**: 320.
27. Hume SP, Gunn RN, Jones T. *Eur J Nucl Med* 1998; **25**: 173–176.
28. Nordström A-L, Farde L, Pauli S, Litton J-E, Halldin C. *Hum Psychopharmacol* 1992; **7**: 157–165.
29. Mc Bride BJ, Baldwin RM, Kerr JM, Wu JL. *Appl Radiat Isot* 1991; **42**: 173–175.
30. Zea-Ponce Y, Baldwin RM, Zoghbi SS, Innis RB. *Appl Radiat Isot* 1994; **45**: 63–68.
31. Mertens J, Terriere D, Sipido V, Gommeren W, Janssen PMF, Leysen JE. *J Label Compd Radiopharm* 1994; **34**: 795–806.
32. Eersels JLH, Custers FGJ, Ensing GJ, Herscheid JDM. *Eur J Nucl Med* 1995; **22**: 1185–1186.
33. Eersels JLH, Herscheid JDM. *J Label Compd Radiopharm* 2001; **44** (Suppl.): S939–S941.
34. Werner IA, Altorfer H, Perlia X. *Int J Pharm* 1990; **63**: 155–166.

