Synthesis of Radiolabelled Compounds for Clinical Studies

Jens Atzrodt, Volker Derdau, and Claudia Loewe

Abstract

Regulatory requirements, quality-related measures as well as key manufacture, control, and release aspects for the synthesis of radiolabelled drugs for administration to human volunteers as part of clinical human ADME studies are discussed in detail. Additionally this review provides a general overview of synthetic, technical and methodological aspects to be considered for the synthesis of Tritium- and 14C-labelled compounds. Chemical and biochemical methods and new trends for isotope labelling are discussed based on published examples.

Contents

Introduction

During the development of new drugs, the candidate's pharmacokinetic (PK) properties and the absorption, distribution, metabolism, and elimination (ADME) characteristics have to be evaluated first in vitro, then in animals, and finally in humans (Caldwell et al. 1995; Roffey et al. 2007). The objectives of human ADME studies are to evaluate mass balance data and most importantly to confirm that the metabolism of the drug is similar to what was described in animal species (Deroubaix and Coquette 2004; Penner et al. 2009). In order to keep track of the drug molecules throughout the body and excreta even after their transformation into different metabolites, the

J. Atzrodt (✉) • V. Derdau • C. Loewe
Integrated Drug Discovery, Medicinal Chemistry, Isotope Chemistry and Metabolite Synthesis Departments (ICMS), Sanofi-Aventis Deutschland GmbH, Frankfurt a. M, Germany
e-mail: jens.atzrodt@sanofi.com

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administration of radiolabelled drugs is considered essential (Isin et al. 2012; Penner et al. 2012). Usually, ^{14}C is the label of choice for most drug candidates since it can be introduced into a metabolically stable position in the backbone of the compound, the detection is easy, and, in case of combustion of samples, the produced $^{14}CO_2$ can be nicely absorbed quantitatively (see Scheme 1) (Beumer et al. 2006). Generally ^{3}H-labelled drugs can be prepared more easily and more quickly than their ^{14}C counterparts. On the other hand, the ^{3}H-label is often less biologically stable, and it is more difficult to predict its metabolic stability, and therefore one always needs to bear in mind the potential risk of the in vivo formation of $^{3}H_2O$ (Lockley et al. 2012). The latter is highly toxic and can be distributed throughout the whole body which makes radioactivity measurement and quantification, even in animal studies, more difficult. Therefore, ^{3}H-labelled drug candidates are usually administered less frequently to humans and only if the specific activity of the ^{14}C compound is insufficient for the planned investigations, e.g., in case of high molecular weight and/or very low-dose drugs (Krauser 2013). For large complex biological molecules, like proteins or antibodies, ^{3}H- or ^{14}C-labelling via total synthesis may be extremely difficult or even impossible, and hence iodination with $^{125}I_2$- or a ^{125}I-precursor could be an alternative approach (Dewanjee 1992). However, the structural changes caused by an additional iodine atom in the molecule have to be considered and both materials (iodinated and non-iodinated) tested for bioequivalence. Other potential radioactive isotopes are ^{33}P and ^{35}S but compared to ^{14}C are much less frequently applied for labelling of drug candidates.

Short-lived radionuclides like ^{11}C and ^{18}F are used for PET (positron-emission tomography) (Cherry 2001) to study mostly in noninvasive experiments the drug passage over the blood-brain barrier, selective accumulation in critical organs, receptor occupancy, dose response or tumor metabolism, and proliferation rates (Rösch 2003). However, labelling syntheses using these short-lived isotopes require specific considerations which are not the subject of this chapter.

Microtracer Concept

Microdosing studies are commonly performed to collect pharmacokinetic data for drug substances following their in vivo administration at sub-pharmacological (trace) doses, a dose at which no pharmacological effect is predicted (Bae and Shon 2011). One method that has evolved significantly over the last few years for determining these data uses a labelled ^{14}C-tracer and a very sensitive detection instrument, the accelerated mass spectroscopy (AMS) technology (Vogel 2000; Vogel et al. 2007). AMS permits the option of decreasing the radioactive dose by a factor of 1,000 from around 50 μCi to about 50 nCi (Garner 2000). The very high sensitivity of AMS permits the evaluation of microdosing approaches including subtherapeutic doses (Lappin and Garner 2003). Smaller clinical doses and/or fewer radioactive administrations to humans may also change the regulatory view on the synthesis of ^{14}C-labelled compounds. The current downside of AMS is that all samples need to be converted into solid graphite, which is an expensive process and only a few laboratories are offering this service. Consequently AMS is not yet used as a standard analytical technique. At the moment, pharmaceutical companies conducting ADME studies apply AMS only when it appears to be absolutely necessary, e.g., in case of high potency drugs, large natural products, or compounds with a very long biological half-life (Lappin et al. 2006). An additional disadvantage is that the same human samples can't be used for AMS quantification and metabolite identification.

In a recent investigation, researchers determined the absolute oral bioavailability of AZD5122 (Hickey et al. 2016). To assess these values, an intravenous (IV) and oral administration study is mandatory. In the past, absolute bioavailability studies have been conducted by both arms, and for insoluble drugs especially tremendously, efforts have been made to develop a suitable IV formulation, which itself needed further toxicological testing. By applying AMS technology into this microdosing study, the therapeutic oral dose was coadministered with a radioactive IV tracer. Due to the low dose, the IV

formulation development was much simpler. By this method, very small differences between ^{12}C and ^{14}C can be quantified; however, the AMS analysis is still mainly outsourced to specialized CROs due to the high financial investment necessary. Nowadays, AMS technology is also used to study biomolecules such as insulins in human ADME studies (Salehpour et al. 2010).

In light of the recent FDA guidance "safety testing of drug metabolites," stable isotopically labelled (^{13}C, ^{15}N, ^{2}H) analogues can be used more frequently to obtain quantitative and qualitative information on drug metabolism in early human ADME studies (e.g., First in Man) even without specific studies administering radiolabelled drugs (Mutlib 2008). Modern LC/MS technologies and hyphenation of liquid chromatography with CRIMS (chemical reaction interface mass spectrometry) (Jorabchi et al. 2005) presents an opportunity to perform quantitative measurement of metabolites even in the absence of authentic standards or radiolabelled compounds (Abramson et al. 1996).

In these tracer studies, the stable isotopically labelled IV dose differs by mass from the parent drug. The plasma samples from these tracer studies are analyzed by standard HPLC-MS/MS instruments and an isotopically labelled internal standard. By this method, the levels of the labelled IV and of the orally administered drug are determined, and the absolute oral bioavailability can be calculated (Cannady et al. 2016).

General Aspects to Be Considered for the Synthesis of ^{14}C-Labelled Compounds

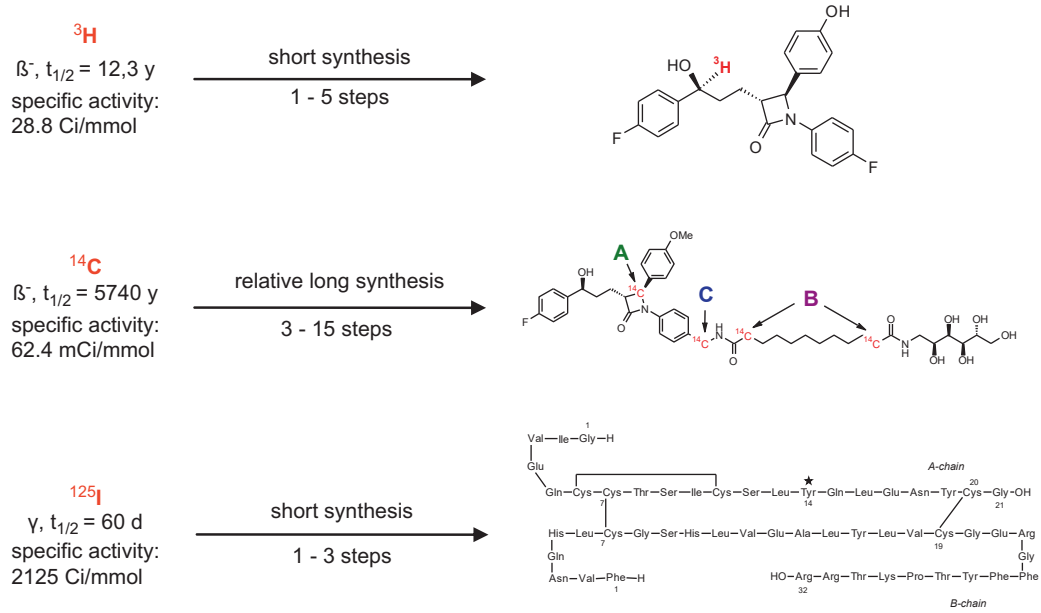
Technical Considerations for ^{14}C-Labelling

Carbon-14 has many of the properties of an ideal tracer nuclide for human ADME studies (Catch 1961). Because of its very long half-life (5,730 years), it is unnecessary to correct for decay, and ^{14}C-labelled compounds can be prepared and stored for a long period if radiolytical decomposition can be minimized (see also under "Synthetic Considerations for ^{14}C-Labelling"). Carbon-14 decays to nitrogen-14 with the emission of a β-particle (maximum energy, 156 eV; average energy, 49 eV). This emission is sufficiently energetic to make measurement at moderate specific activity fairly simple but weak enough to make shielding unnecessary. The range of these soft β-particles is about 15–16 cm in air and 0–2 mm in a solid medium. This means even at very high specific activities, ^{14}C-labelled compounds can be safely handled in standard glass vessels and conventional lab equipment if reasonable radiation safety precautions are taken. However, in most countries, working with radioactive materials requires strict reporting, licenses, and/or authorizations from authorities. Local regulations may be different or stricter with respect to the handling of radioactives, to containment equipment and to lab facilities.

Synthetic Considerations for ^{14}C-Labelling

In general, any organic compound that can be synthesized can be labelled, but specific labelling of more complicated molecules may be difficult and expensive. Usually, a target directed total synthesis approach is required for ^{14}C-labelling of drug development candidates. For selecting the right labelling position, several aspects have to be carefully considered. The position of the label should be away from sites which are chemically unstable and away from sites of metabolic attack in order to ensure the label is kept in the main metabolic fragment. In cases where high specific activities are required for the planned study, a double ^{14}C-labelling or alternatively ^{3}H-labelling needs to be considered because the maximum achievable specific activity for single ^{14}C-labelled organic compounds is 62.4 mCi/mmol (2309 MBq/mmol) (see also Scheme 1 (Schulte 1966)).

As well, the development of a labelling synthesis is dependent on the availability of suitable precursors, the length and complexity of the



Scheme 1 Radioactive isotopes used for labelling of drugs or drug candidates

synthetic pathway, the reliability of the process, as well as radiation safety aspects, e.g., avoidance of volatile reaction components if possible.

Planning of a ^{14}C-Synthesis

The reaction pathway developed for ^{14}C-synthesis should satisfy the following criteria (Raaen et al. 1968):

(a) Introduce the ^{14}C-label as near as possible to the last step of the synthesis.
(b) Introduce the ^{14}C-label after stereochemical resolution.
(c) Introduce the ^{14}C-label in a known position.
(d) Ensure adequate specific activity and high radiochemical purity of the product.
(e) Provide high radiochemical yields.
(f) Consider synthetic efforts for unlabelled precursor synthesis.

No special laboratory equipment is required, but preparation techniques may differ from conventional synthetic work especially when volatile radioactive precursors are handled or volatile intermediates and/or side products are expected. Further difficulties may increase as the scale of the reactions is reduced, e.g., yield, impurities, solvent content, crystallization.

A ^{14}C-labelled compound for which preparative methods are not well established should be synthesized as follows: First, the pathway is elaborated with non-radioactive material on the desired scale until a reliable process has been developed and the operator is adequately familiarized with the chemistry. Product purity should be checked by the usual chemical and physical methods. Then, the experiment is carried out at the tracer level to establish the nature of impurities and side products (e.g., volatility), to determine the yield of the desired product and to check for radio-accountability. Finally, the procedures developed are duplicated with limited amounts of the ^{14}C-labelled precursor.

Methods for ^{14}C-Syntheses

Reactor production of carbon-14 is achieved by neutron bombardment of solid beryllium nitride or solid aluminum nitride over a very long time

and subsequent transformation of all ^{14}C-compounds formed into $BaCO_3$ (Wilson 1966). This means that the preparation of labelled compounds is limited to using $^{14}CO_2$ as the only practical starting material. Certain key intermediates can be used for the preparation of a great number of labelled compounds, e.g., [^{14}C]potassium cyanide, [^{14}C]barium carbide, and [^{14}C]methanol (see Scheme 2). In principle, both chemical and biological methods can be applied for converting [^{14}C]barium carbonate and its simple derivatives into more complex labelled compounds, with chemical synthesis the most generally used.

Chemical Methods

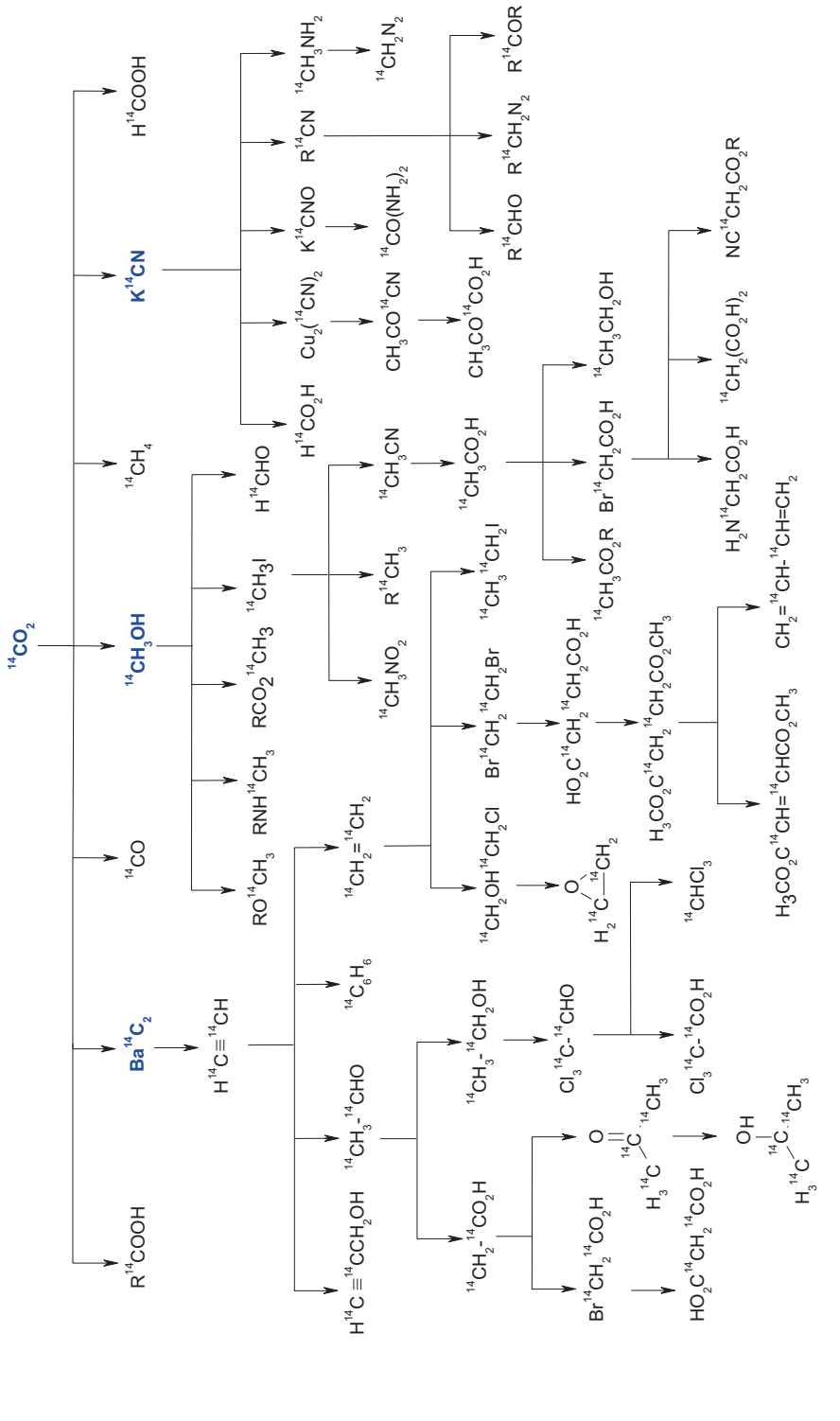
Many good standard procedures for the chemical preparation of the more common ^{14}C-labelled compounds have been summarized in literature reviews (McCarthy 2000) and in books or in book chapters (Murray and Williams 1958; Heys et al. 2009) (see also Scheme 2). Today, many specialized supply companies provide a variety of these basic ^{14}C-labelled precursors or even more complex molecules on a custom synthesis basis. Usually, the structural complexity of modern drug development candidates requires multistep labelling syntheses. Therefore, a common strategy in the pharmaceutical industry is to purchase or outsource the preparation of basic labelled organic compounds required as starting materials for an in-house synthesis of labelled drug development candidates. If applicable, a late-stage introduction of the ^{14}C-label into an unlabelled advanced intermediate can be highly efficient (see also "Planning of a ^{14}C-Synthesis," criteria (a)). For example, the Grignard reaction (Knochel 2005) of *organo*-magnesium halides with $^{14}CO_2$ or a transition metal catalyzed cyanation (Yu et al. 2017) with metal cyanide and subsequent saponification are convenient methods for introducing labelled carboxyl functionalities (Cao et al. 2007). Other small building blocks frequently used for ^{14}C incorporation into organic molecules are [^{14}C]formaldehyde, [^{14}C]methyl iodide, [^{14}C]thiocyanate, and ^{14}C-labelled acetic acid derivatives (McCarthy 2000; Heys et al. 2009).

A very good example of the advantage of a late-stage labelling strategy is the synthesis of ^{14}C-labelled celivarone. Initially, the synthesis was performed according to pathway **A** (see Scheme 3) starting with a cyanation of benzofuran derivative and subsequent synthetic construction of the molecule. However, as an alternative pathway, an approach applying lithiation and subsequent $^{14}CO_2$ addition was utilized, resulting in a longer synthesis but with improved overall yield (pathway B). Finally, by a late-stage ^{14}C-carbonylation of an unlabelled precursor (pathway **C**), the number of radioactive steps could be reduced from three to four to only a single step (Whitehead et al. 2013).

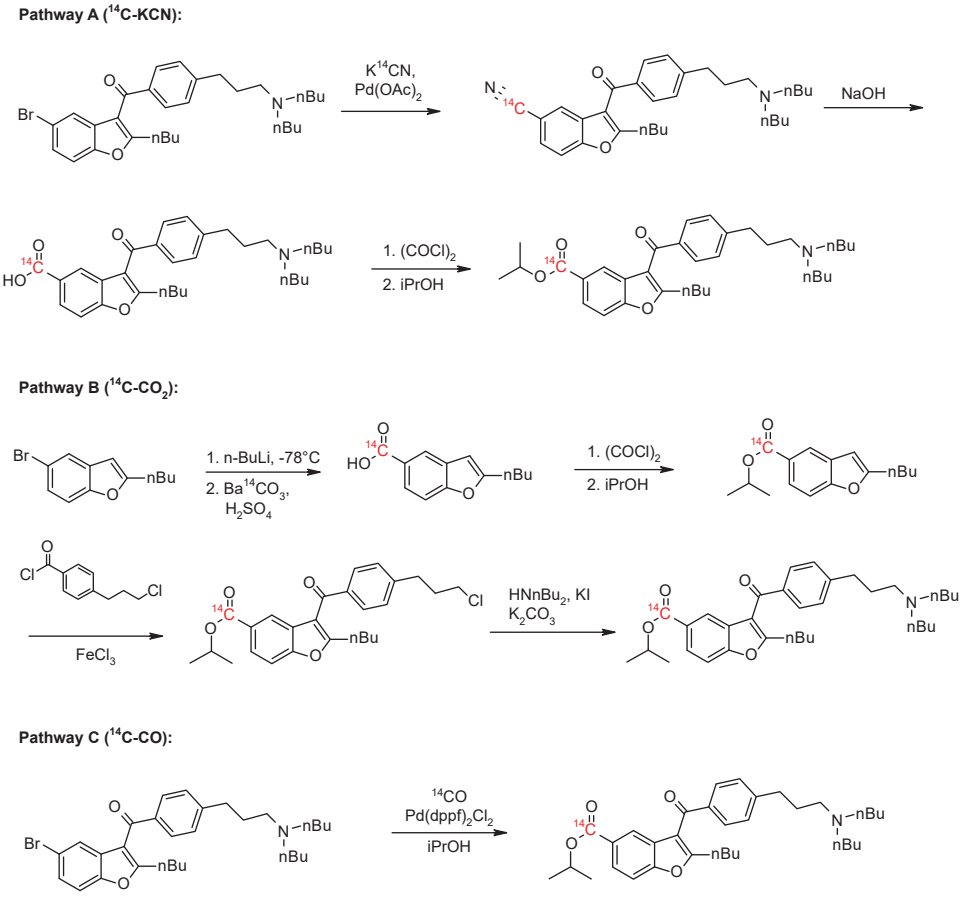
Often reducing the number of radioactive steps is a major objective of route development activities because the amount of radioactivity employed, the radioactive waste produced, and thus also the costs of a synthesis can be reduced dramatically. Besides the recognition of available labelled reagents, the accessibility of unlabelled precursors via reasonable synthetic efforts has to also be considered when planning a ^{14}C-labelling synthesis (see also "Planning of a ^{14}C-Synthesis," criteria (e)). One synthetic strategy applied to precursor synthesis is via a degradation reaction either by Hunsdiecker decarboxylation (Kurosowa et al. 1997) or oxidative cleavage (Shu and Heys 1994) as shown in Scheme 4. Subsequent labelling affords the ^{14}C-labelled version of the starting material using this protocol.

Other synthetic strategies for late-stage labelling include directed metalation or halogen-metal exchange by aromatic substitution and ^{14}C-introduction as demonstrated in the synthesis of [$^{14}C_2$] WIN-63394 ((Burgos et al. 1996); see also Scheme 5).

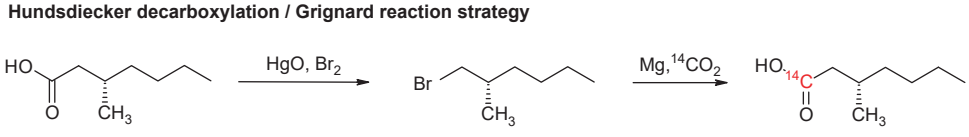
After successful introduction of the ^{14}C-label, all further chemical transformations can be performed applying classical or modern organic chemistry approaches as described for unlabelled compounds including asymmetric synthesis (Voges 2002) or cross-coupling reactions (Derdau et al. 2003).

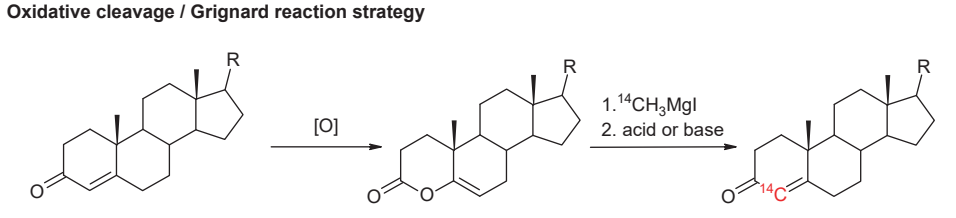


Scheme 2 Synthetic routes to ^{14}C-labelled compounds (Raaen et al. 1968)

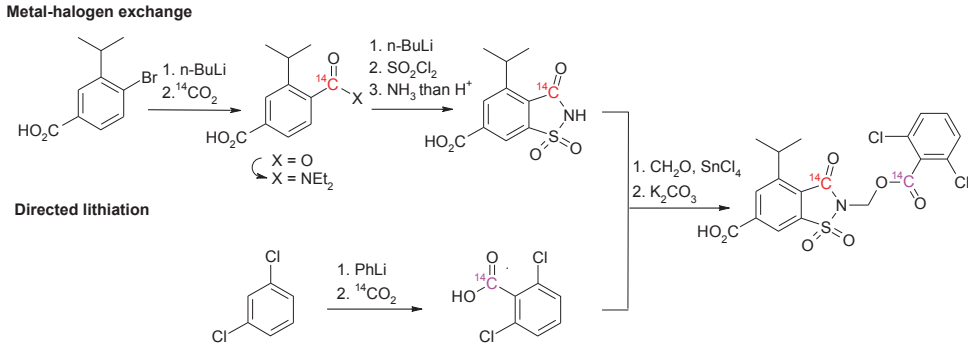


Scheme 3 Synthesis of [^{14}C] celivarone

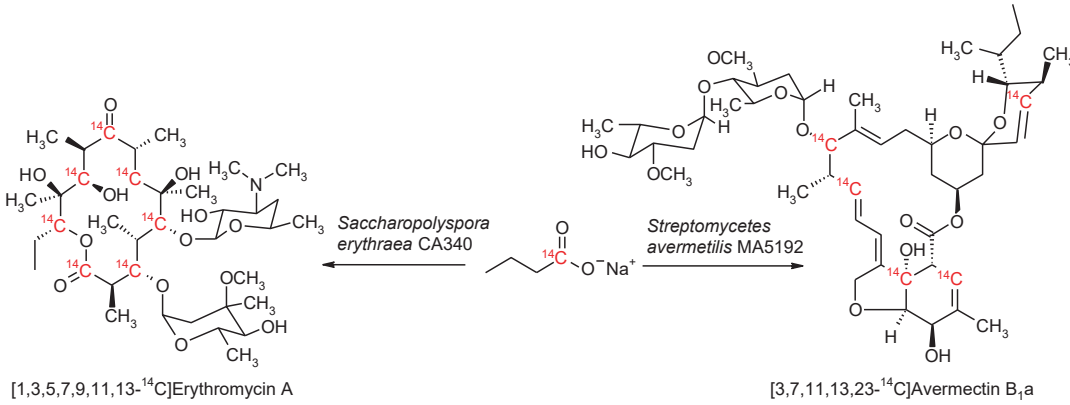




Scheme 4 Degradation/labelling strategy



Scheme 5 Metal-halogen exchange and directed metalation strategy for ^{14}C-labelling of WIN-63394

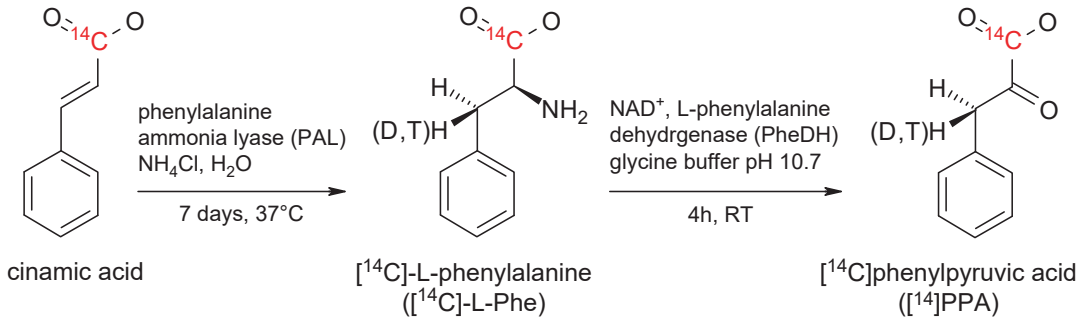


Scheme 6 Biosynthetic labelling of erythromycin A and avermectin B_1a

Biochemical Methods

Besides chemical methods, biochemical synthesis offers the ability to obtain labelled natural products and organic compounds not always accessible by conventional synthesis (Evans 1981; Benakis 1994) Oligopeptides, proteins, antibodies, as well as a large number of pharmaceutically relevant compounds, e.g., antibiotics synthesized by fermentation with the aid of yeast, bacteria, or fungi, can be labelled using biochemical methods. Corresponding ^{14}C-labelled compounds can be prepared if the relevant ^{14}C-labelled starting materials, e.g., ^{14}C-labelled amino acids, are employed in the fermentation process. Yields are as important as in purely chemical syntheses, but unfortunately, only a few biochemical processes provide a single ^{14}C-labelled product in high yield (Wallace et al. 1995). Decarboxylation and the production of large quantities of $^{14}CO_2$ might be another drawback that needs to be considered when planning fermentation for labelling purposes.

For example, [1,3,5,7,9,11,13-^{14}C]erythromycin A was produced in liquid fermentation broths of *Saccharopolyspora erythraea* CA340 in shake flasks after the administration of [1-^{14}C]sodium propionate. The labelled erythromycin A was separated by extraction of the fermentation broth and purified on Sephadex (see Scheme 6 (Walker et al. 1996)). A similar fermentation process with MA5192 (*Streptomyces avermitilis*) and [1-^{14}C] sodium propionate as precursor was also used for the synthesis of ^{14}C-labelled avermectin B_1a (Ku et al. 1984). For both compounds, a total synthesis



Scheme 7 Biochemical ^{14}C-labelling of phenylpyruvic acid

approach for ^{14}C-label would have been very difficult, time and resource consuming, or even impossible.

On the other hand, enzymatic reactions may also be applied for specific chemical transformations, e.g., saponification, oxidation, and hydroxylation, as part of multistep conventional chemical synthesis pathways for the preparation of ^{14}C-labelled drug development candidates and corresponding ^{14}C-labelled relevant metabolites (Allen et al. 2007). As an example of biochemical labelling, the enzymatic synthesis of ^{14}C-labelled phenylpyruvic acid ([^{14}C]PPA) is shown in Scheme 7.

The addition of ammonia to [^{14}C]cinnamic acid catalyzed by the enzyme PAL (phenylalanine ammonia lyase) carried out in ammonia buffer at pH 9.8 led to the formation of [^{14}C]L-phenylalanine ([^{14}C]-L-Phe). Subsequently, this intermediate was converted to [^{14}C]PPA employing the enzyme PheDH (phenylalanine dehydrogenase) in the presence of NAD^+. As is normal for enzymatic processes, the yield of this reaction strongly depends on the incubation time, concentration of L-Phe, buffer choice, pH, and enzyme quantity. Similarly, conducting the first step with fully deuterated or tritiated ammonia buffer gave [(*3S*)-^{2}H]-L-Phe and [(*3S*)-^{3}H]-L-Phe, respectively, which offered the opportunity to get access to the corresponding deuterium and tritium-labelled PPA as well (Skowera and Kanska 2008).

Another biochemical method for preparing labelled compounds is photosynthesis in which $^{14}CO_2$ is assimilated by, for example, algae such as *Chlorella vulgaris* (Godward 1960), cyanobacteria such as *Anacystis nidulans* (Tovey et al. 1974), or plants such as tobacco (*Nicotiana sp.)* or *Canna indica* (Putman and Hassid 1952), or by detached or full-sized plants grown in a $^{14}CO_2$ atmosphere in sealed greenhouses or plastic bags (Benkis et al. 1985). This technique called "isotope farming" can provide high specific activities and good yields of, for example, ^{14}C-labelled glucose, starch, nucleosides, amino acids, and lipids.

Stability of ^{14}C-Labelled Compounds

Compounds labelled with carbon-14 decay to nitrogen-14 with the emission of a β-particle. Since the energy of these β-rays by far exceeds bond energies of organic molecules, structural damage can occur. If the radiation energy is absorbed by the compound itself, the excited molecule may break up and/or react with other molecules. The activated decomposition fragments may also react in a sort of chain reaction with other molecules producing impurities (Bayly and Weigel 1960; Rochlin 1965). Typical reactions resulting from irradiation are dehydrogenation, oxidation, decarboxylation, deamination, condensation, and polymerization, in many cases through radical reactions (Sheppard 1972). In some cases, the shelf life is reduced from years to weeks or even days.

The rate of decomposition depends on storage conditions, specific activity, and chemical

structure. Though it is not yet possible to foresee exactly the behavior of each compound, a thorough stability study may result in storage conditions that provide reasonable shelf life of the compound and thus reduce purification efforts and secure rapid availability of stock material. The following storage rules have proved to be successful and can be applied to reduce decomposition (Evans 1976; Bayly and Evans 1966): optimize storage conditions as regards chemical stability, store at the lowest practical temperature, dilute the specific activity, avoid high amounts of activity, store labelled compounds in solution, add radical scavengers or other stabilizers (Fredenhagen 2002), and avoid unnecessary reopening of vials and warming/cooling cycles.

In spite of all potential precautionary measures, the shelf time of labelled compounds is always limited and requires repurification of the material more or less frequently (Bayly and Evans 1968).

Purification

The development of a suitable purification method for ^{14}C-labelled compounds can be crucial because typically a radiochemical purity of >98% is necessary for the planned studies (for specific applications up to 99.8% are required). Additionally, the limited shelf life of ^{14}C-labelled compounds may require repurifying the ^{14}C-labelled compound from time to time. Since synthetic impurities and degradation products can be completely structurally different, it might be necessary in some cases to develop different purification methods as well. Typically, ^{14}C-labelled compounds are purified by column chromatography, semi-prep reversed-phase HPLC or crystallization (Evans 1981).

Dilution

After purification, the ^{14}C-labelled drug development candidate often needs to be diluted to obtain the specific activity required for the planned studies. To this end, the highly radioactive compound is homogeneously mixed with unlabelled material of the same compound by dissolving both in a suitable solvent. Subsequently, the solvent is removed by evaporation, the product crystallized, precipitated, or lyophilized to afford the diluted ^{14}C-labelled drug development candidate ready for administration or further formulation to the drug product.

Analysis

After repurification and dilution of the radioactive drug substance, generally an intensive analytical release testing program according to specifications is followed to guarantee consistent product quality (Filer 1988; Dueker et al. 1998). Typically, the identity of the compound is confirmed by NMR (Schenk et al. 2015) and LC-MS; the radiochemical, chemical, and stereochemical purities are checked by analytical HPLC; and the specific activity is measured by LSC and/or high-resolution MS (Schenk et al. 2016). In addition, specific applications or specific compound-related properties may require supplementary analytical tests, e.g., ion chromatography, water content determination, polymorphism, or particle size investigations (Braun et al. 2004).

General Aspects to Be Considered for the Synthesis of Tritium-Labelled Compounds

Synthetic and Technical Considerations for ^{3}H-Labelling

Tritium-labelled compounds are much less frequently applied for human ADME studies (see also "Introduction") because an essential problem is the integrity of the carbon-tritium bond and the specificity of the labelling. There are however rare examples for clinical trials with tritiated compounds (Straznicky et al. 2014). The high specific activity of 28.8 Ci/mmol of tritium which is approx. 500 times higher than achievable with a single carbon-14 label (62.4 mCi/mmol) is an

advantage (Evans 1974). This property makes tritium irreplaceable for labelling of large molecules, particularly biomolecules such as peptides, proteins, oligonucleotides, and antibodies, as well as for early labelling strategies to support discovery purposes.

Tritium labelling is often much simpler than ^{14}C-synthesis and labelling can often be accomplished very late in the overall synthesis. The typical sources for the tritium label are tritium gas or specific tritiated reagents and sometimes tritium water. Modern stainless steel manifolds allow a safe handling and storage of tritium gas (Benakis 1994). However, as for ^{14}C-synthesis, a number of specialized supply companies offer custom tritiation services. In principle, similar points as mentioned under "Methods for ^{14}C-Syntheses" should be considered for the planning of a ^{3}H-synthesis. Compared to ^{14}C-synthesis, the scale of tritiations is even further reduced. Often, only a few milligrams of material is handled, which requires specific preparation techniques, operator training, and elaboration work. The shelf life of ^{3}H-labelled compounds is usually decreased compared to ^{14}C; however, it can be improved by storage in ethanol solution at low temperatures (<-80 °C). However, purification, dilution, and analysis aspects are similar to those already mentioned for ^{14}C-labelled materials (see also "Purification," "Dilution," and "Analysis").

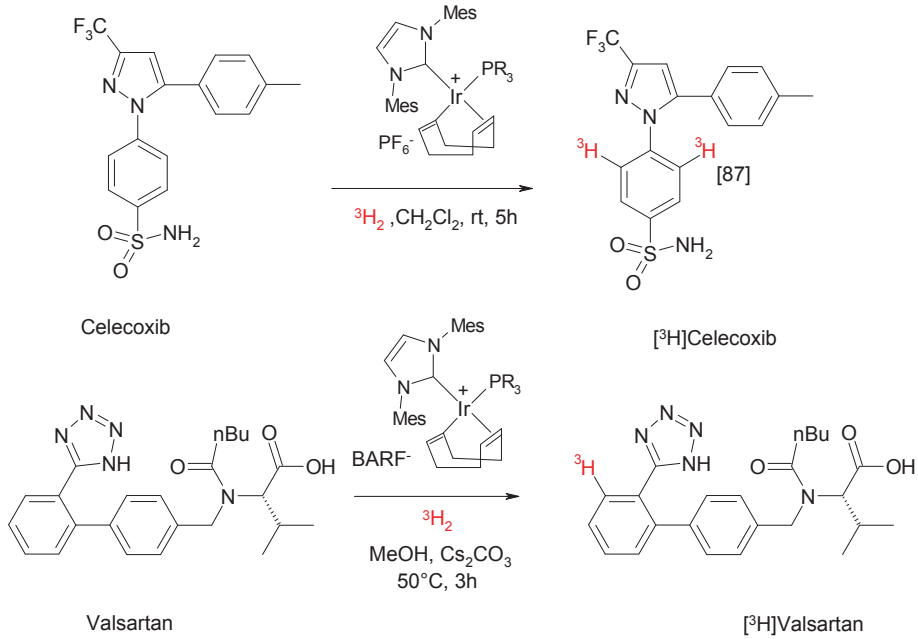
Chemical Methods for ^{3}H-Labelling

Comprehensive literature reviews summarizing synthetic techniques developed for tritium incorporation have been published recently (Heys et al. 2009; Saljoughian and Williams 2000), and therefore, only the main principles will be briefly discussed. There are two basic approaches for introducing tritium into organic molecules: exchange labelling (Atzrodt et al. 2007; Heys 2007; Lockley 2007) and synthetic tritiation methods (Evans 1981; Saljoughian 2002). While there have been several drawbacks applying exchange labelling methods in the past (e.g., lower tritium abundance or the isotope being widely distributed over the molecule), nowadays, newly developed catalysts have proven their unique efficiency for selective tritium introduction (Allen et al. 2010; Nilson and Kerr 2010). As exchange labelling can be highly cost and time efficient when carried out directly on the target molecule, it is a standard method in pharmaceutical laboratories today; however, a suitable catalyst directing group remains essential.

Excellent examples for the successful application of exchange labelling are the syntheses of tritiated celecoxib (Brown et al. 2014) and valsartan (Kerr et al. 2016). The tritium introduction was accomplished by a single, iridium-catalyzed hydrogen isotope exchange reaction with tritium gas starting directly from unlabelled celecoxib or valsartan, respectively (see Scheme 8).

In case of synthetic tritiations, the tritium is inserted into specific positions in the molecule resulting in high tritium abundances and mostly with high specific activities. Basically, beside H/T exchange reactions, four other chemical methods can be used to introduce tritium into the target molecule: (1) reduction of reducible functions (e.g., unsaturated CO, CN functions) with tritiated reagents, (2) metal catalyzed exchange of halogens by tritium, (3) hydrogenations of double or triple bonds with tritium gas, and (4) application of tritiated small molecule precursors such as methyl iodide in the labelling synthesis. Examples of the different labelling strategies are depicted in Scheme 9.

[^{3}H]Vardenafil was synthesized by reduction of a specially synthesized amide precursor with freshly prepared lithium aluminum tritide (Pleiss 2003). For the synthesis of [^{3}H]ragaglitazar at high specific activity, a suitable dihalogenated precursor was synthesized and then the tritium incorporated by a catalytic dehalogenation reaction in the presence of tritium gas (Kristensen et al. 2003). The tritiation step for the synthesis of [^{3}H]Mecillinam was performed by treating the corresponding dehydro-mecillinam, itself prepared via a six-step synthesis, with tritium gas in the presence of 10% Pd/C as the catalyst (Frederiksen and Sörensen 2003). [^{3}H] Ansamitocin P-3 was prepared by alkylation of



Scheme 8 Synthesis of [^{3}H]celecoxib and [^{3}H]valsartan by iridium-catalyzed hydrogen isotope exchange labelling

ansamitocin PDM3 with [$^3H_3$]methylnosylate under basic conditions. Ansamitocin P-3 is a precursor of LDM4 which is used to prepare drug-antibody conjugates (Sun et al. 2011). In all cases, specific unlabelled precursor molecules had to be synthesized, and therefore synthetic tritiations are often limited by the chemistry required both prior to and during the labelling process.

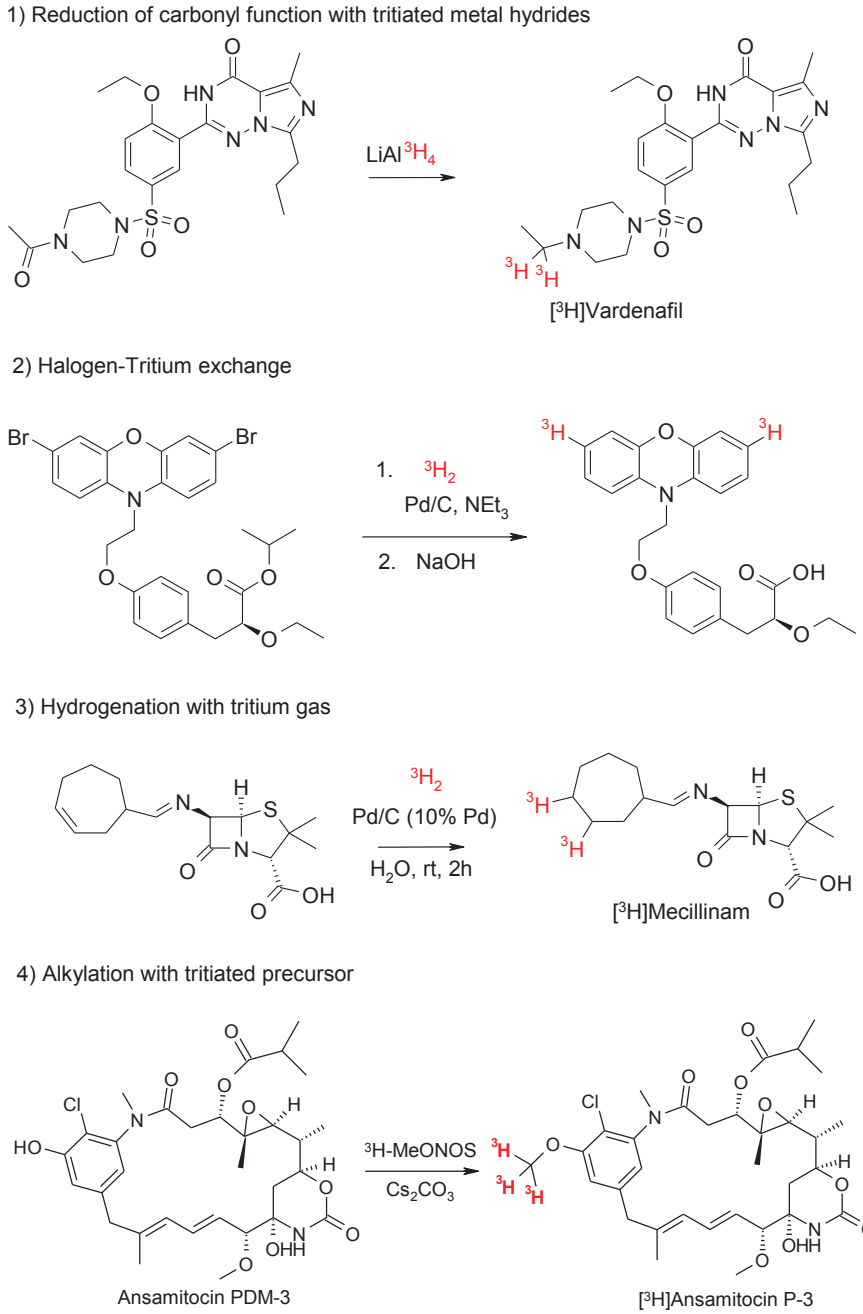
Regulatory Requirements for Application of Radiolabelled API to Humans

General Study Requirements

Administering radioactivity to human beings raises general ethical questions due to the well-established carcinogenic and/or teratogenic potential of radioactive compounds (Dain et al. 1994). Radiation exposure should be "as low as reasonably achievable" (ALARA concept), and even when administering small doses of radioactivity, given the residual risk, exposure should be minimized. Therefore, clinical studies involving the administration of radioactive drugs to humans need to be approved by a special ethical committee. Additionally, submission and approval of an Investigational New Drug Application (IND) for the USA or, if the study is carried out in the EU, an Investigational Medicinal Product Dossier (IMPD) is required. These documents include detailed information on the synthesis, medication, and analytical release, as well as a supportive stability study. The regulatory requirements for both the manufacturing process and study design are subject to various local and national authorities and strongly depend on the location and specifics of the study site and the country of the manufacturing facility.

Impurities

Based on the ICH Guidelines Q3A *Impurities in New Drug Substances* and Q6A *Specifications in New Drug Substance*, the limit for an unspecified impurity in drug substances for phase I/IIa/IIb is set at 0.1%. This threshold (see Scheme 10) is also valid for the diluted radioactive drug substance



Scheme 9 Examples for synthetic tritiations: (1) [^{3}H]vardenafil, (2) [^{3}H]ragaglitazar, (3) [^{3}H]mecillinam, and (4) [^{3}H] ansamitocin P-3

Maximum Daily Dose:	Reporting Threshold	Identification Threshold	Qualification Threshold
<= 2g/day	0.05%	0.10% or 1.0 mg per day*	0.15% or 1.0 mg per day*
		(* whichever is lower)	

Scheme 10 Thresholds of impurities in new drug substances (U.S. Department of Health and Human Services Food and Drug Administration Guidance for Industry Q3A Impurities in New Drug Substance 2008)

(rDS). For the highly radioactive drug substance (hrDS), the usual internal release criteria require a radiochemical purity of at least 98% or even higher. This material (hrDS) usually undergoes at least a tenfold dilution with GMP-produced cold material to afford the rDS, and hence the radioactive impurity over all content is reduced below the threshold.

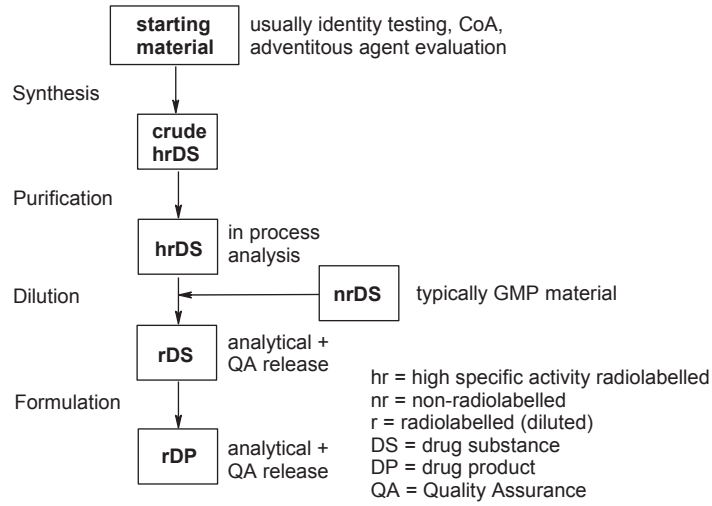
Regulatory Requirements

The GMP guide ICH Q7A *Good Manufacturing Practice for Active Pharmaceutical Ingredients* (*API*) does not apply to manufacturing/control aspects specific to radiolabelled compounds with long-lived isotopes (e.g., ^{14}C, ^{3}H). However, Chap. 19 contains guidance for the manufacture of APIs used in clinical trials (APIs for investigational use during early phases of development) in general. For EU countries, the EU GMP Guideline Part II *Basic Requirements for Active Substances used as Starting Materials* including Chap. 19 *APIs for Use in Clinical Trials* is recommended to be followed although not required by community legislation. During the manufacturing process, the highly radioactive drug substance is diluted with unlabelled API (manufactured according to GMP requirements) to achieve the specific radioactivity required for the planned study. Although typically only very small amounts of radioactive material are incorporated in the final drug substance, it could be considered a radioactive Active Pharmaceutical Ingredient (rAPI). Therefore, in some countries, the manufacture of radiolabelled APIs is covered by national drug laws and ordinances, whereas in others, this has not come within the scope of regulatory GMP inspections and does not require certification. These inconsistent interpretations may result in different levels of GMP (Good Manufacturing Practice) being requested for the synthesis of radiolabelled API's by different national health authorities.

Consequently, depending on national regulatory requirements, pharmaceutical companies have developed different creative approaches that combine quality principles, radiation safety aspects, and the other challenges inherent in radiosynthesis (Lloyd et al. 2003; Fontana et al. 2000). Approaches including full GMP compliance for the whole synthesis or only parts of it (e.g., only purification and dilution, including in some cases full environmental control), different GMP or GLP-like (Hong et al. 2008), or non-GMP classified processes (Bonacorsi et al. 2007) have been applied. Generally, the local authorities are allowed to inspect the manufacturing facilities to review compliance with the appropriate regulations.

General Quality-Related Measures that Should Be Applied for the Synthesis of Radioactive APIs

Independent of whether GMP is formally required or not, the synthesis of ^{14}C-labelled drug development candidates that are to be administered to humans should follow higher-quality standards than those for in vitro or animal study applications. Thereby, the stringency of quality standards should increase as the process proceeds from the early to final synthetic steps, purification, and



Scheme 11 Typical process steps for the synthesis and manufacture of radiolabelled drug product

dilution. Compared to orally administered compounds, the synthesis of radiolabelled drug substances that will be formulated for parenteral application should be subjected to more stringent control. At least the final steps, such as purification and dilution, or even the last covalent bond modification step, should be performed as a kind of validation to confirm the correctness and quality of the applied methods. Usually all synthetic, manufacturing, analytical, and release activities are reviewed by an independent quality assurance organization to ensure the compliance of all process steps with regulatory and internal company quality requirements. SOPs (standard operation procedures) are established to define the details of all quality-related operations and processes.

Although in principle the labelled material could be any proportion of the administered drug substance (rDS), in practice, it is normally small, typically forming less than 5% of rDS.

Assuming that the hrDS forms less than 10% of the rDS, even in a worst-case scenario with a single 1% impurity in hrDS, after dilution this would end up in only 0.1% impurity of the final rDS (see also Scheme 11). Therefore, it might be appropriate to focus increased quality requirements on the purification and dilution steps only.

Adventitious agents evaluation and a complete review of the synthesis concerning BSE/TSE and viral safety is requested by several authorities. In addition, raw materials, intermediates, solvents and reagents, as well as materials that will come into direct contact with the radiolabelled drug (dry reagents, charcoal etc.) often have to be evaluated by testing or received with a supplier's Certificate of Analysis (CoA) and subjected to at least identity testing.

The retrievable and traceable recording of all process- and testing-related information (including signatures) in legible documents should be in place. In addition, several affiliations to combined manufacture units require written production instructions and records as well as a full quality assurance review of the synthesis documentation.

All equipment of the radiosynthesis laboratories that are critical to product quality (balances, preparative HPLCs, pH-meters) should be calibrated and serviced at appropriate intervals according to written procedures. Furthermore, the equipment used has to be qualified by a certain formal methodology in order to attest its proper technical status. This is supplemented by the regulations for computerized systems. Hence, for example, the hardware and software of an HPLC has to be evaluated regarding data security, robustness, validated operating procedures, etc.

Glassware and other equipment that will come into direct contact with the radiolabelled compound for clinical use should be new or cleaned according to standardized procedures. Stirrer bars, syringes, needles, and glass pipettes should be new.

The manufacturing facilities including fume hoods and HPLC cabinets should be dedicated and cleaned. Appropriate measures must be taken to prevent product contamination or cross-contamination. In general, a microbiological environmental monitoring of the work surfaces is required. Often due to the contradictory nature of radioprotection and GMP clean room regulations, establishing a GMP clean room facility is challenging (Loewe et al. 2016).

Another significant consideration in the manufacture of API for clinical use is the level of GMP expertise of all staff members. Their knowledge and awareness of GMP compliance needs to be kept to a high standard by continuous training on SOP and guideline compliance, in transparency and accurate documentation, and in hygiene or bio burden. For each employer, specific duties and responsibilities should be recorded in written job descriptions. Finally, a document management system has to be established including deviation and change control reporting, and last but not least, a regular interval of inspections should take place (Filer et al. 2016).

Conclusion and Outlook

Suitable quality measures should be in place in order to ensure that the manufacture, control, and release of radiolabelled compounds administered to human volunteers will satisfy quality requirements and guarantee both patient safety and the reliability of study results. In the last couple of years, the regulatory stringency for the synthesis of radiolabelled compounds for clinical use has increased, and certainly this will continue in the future. Whether radioactive tracers or the AMS technology will be used to study proteins, peptides, and other macromolecules on a frequent basis remains unclear. Up until now, there has been no requirement from the authorities to perform human ADME studies with biologicals in order to gain regulatory approval. However, this translational information might be valuable from a scientific point of view to help support milestones decisions in pharmaceutical drug development.

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