

CUSTOM CARBON-14 RADIOLABELLING

investing to meet new challenges

One consequence of an increasing demand from regulators for robust quantitative data on the behaviour of new drugs in man and for information on the fate of pharmaceuticals in the environment, has been an increase in demand for carbon-14 labelled compounds. This article focuses on the new uses for C-14 compounds, factors impacting on their preparation and how C-14 custom labelling suppliers are responding to meet new demands.

While traditional drug metabolism studies continue to play a key role in understanding the pharmacokinetics, metabolism and ultimate fate of small molecules, new technology has opened the door to study compounds directly in man. Use of accelerator mass spectrometry (AMS) enables detection of very small levels of C-14 allowing its use in humans at very low doses that pose no radiation risk. This, in part, has led to new initiatives by the FDA and EMEA encouraging fast track first-in-man studies. Recent FDA guidance on the safety testing of potentially toxic metabolites has provided another growth driver for C-14.

Regulatory authorities require that studies follow official guidance and meet statutory quality standards such as GLP, GCP and GMP. When using a C-14 compound it is vitally important to ensure there are no impurities present that may give the appearance of persistent drug residues, lead to artefacts or spurious results. Such adverse data may impact on a drug's future marketability and could lead to costly additional studies.

With increasing demand for quality C-14 compounds suitable for human studies, several of the leading custom labelling suppliers have invested in additional capacity, facilities, processes and analytical capability.

Radiolabelled compounds are considerably

more expensive than their non-labelled analogues for the reasons set out below. Taking short cuts in the radiolabelled synthesis may result in savings, however, these are often insignificant compared to the cost of a study and may compromise the ultimate value of the information and data generated.

Carbon-14 for quantification

Carbon-14 remains the isotope of choice as a tracer being biologically equivalent to carbon-12, having a low background in the biosphere (1ppt: 1 atom in 10^{12}) and importantly facilitating easy quantification. Its soft β -emission does not require shielding but is easily detectable allowing mass balance determination of the parent compound and importantly its metabolites (even of unknown structure) and gives good definition for autoradiography. Its half-life of 5,760 years means it is unnecessary to correct for decay during the experiment and provides good counting efficiency using liquid scintillation counting (LSC). Other advantages include minimal chance of isotope exchange and minimal isotope effects on metabolic transformations.

The new accelerator mass spectrometer (AMS) technology relies upon the ratio of C-14/C-12 for quantification. AMS enables detection of very low levels of C-14, several orders of magnitude below that of LSC, requiring a typical dose of

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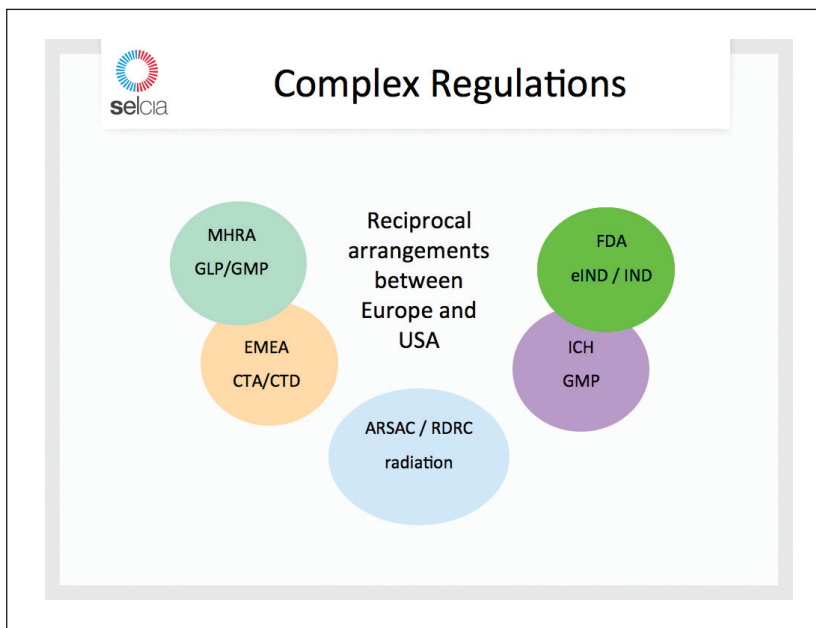


Figure 1 270nCi (10kBq) per individual. With such low levels it is very important to avoid any risk of C-14 contaminants. With this in mind, test materials are best prepared at high specific activity (~50mCi/mmol; 1.85GBq/mmol) with full analysis including structure confirmation prior to dilution with unlabelled material.

A few years ago all the focus for AMS was on phase zero microdosing with drug levels at 1/100th of the therapeutic level (cold drug) with a micro-tracer level of C-14 to provide a tool for quantification. Advocates of the technology now focus on promoting the use of AMS to address specific problems where the added value is clear. For example, as an add-on to a traditional Phase I study where a microtracer of C-14 is used with a therapeutic dose. This can provide information on the metabolic profile in man at a very early stage and confirm that the pre-clinical data from other species is representative.

Recent literature has described use of sensitive laser-based isotope analysis to count [C-14] CO₂. However, it remains to be seen if this offers an alternative to AMS for analysis of biological samples.

As part of the IND package there is a requirement to perform a traditional human mass balance study. This will usually be performed on six healthy male volunteers using ~100µCi (3.7MBq), per individual, of C-14 radiotracer.

Pre-clinical studies using C-14 are performed by many of the leading contract research companies such as Charles River, Covance, XenoBiotic

Laboratories and Harlan, using C-14 compounds supplied by external suppliers or internal isotope groups. AMS analysis is performed, for example, by Xceleron and Vitalea.

Pharmaceuticals in the environment

Given that many pharmaceuticals need to be robust enough to pass through the stomach it is not surprising that they find their way into the environment via excretion and the public sewage system. This has led to increased attention on the environmental fate of pharmaceuticals (and other chemicals) from regulators to identify and mitigate possible adverse environmental effects prior to commercialisation. Most major pharmaceutical companies now model the environmental behaviour of their development compounds. It has long been a requirement to provide environmental fate data as part of the regulatory process for pesticides. The experimental protocols used to generate environmental fate information such as biodegradation/accumulation are robust and well-validated and are set out in regulatory guidance documents, for example OECD 308. In this guidance C-14 radiolabelled compounds are the preferred tools to study the degradation pathway and establish a mass balance. More recent guidance specifically covers chemicals found in the aqueous and sludge components of sewage (OECD 314).

The EMEA issued a guideline on the environmental risk assessment of medicinal products in 2006. It sets out a tiered approach: Phase I requires an estimate to be made of the environmental risk in the aquatic component using a predicted environmental concentration (PEC) formula. If this is triggered then Phase IIA requires basic environmental fate data in which C-14 radiolabelled compounds are used in studies, which should be performed to Good Laboratory Practice (GLP) in line with OECD 308. These then may trigger a Phase IIB data requirement.

Specialist CROs such as Brixham Environmental and Battelle UK Ltd provide guidance and perform regulatory studies using carbon-14 on pharmaceuticals.

Basic considerations on radiolabelling with C-14

Direct irradiation of suitable nitrogen containing precursors was ruled out as a possible short-cut to carbon-14 compounds following extensive research, which found that the C-14 atom was inserted randomly, in low yield, with formation of extensive impurities. Thus the only practical method for introduction of a C-14 label is by total

synthesis of the desired radiolabelled compound starting from a single C-14 atom precursor.

C-14 is a reactor-produced isotope requiring lengthy exposure of a nitrogen precursor to high flux neutron beam ($^{14}\text{N}(n,p)^{14}\text{C}$ reaction) followed by isolation and purification to barium [C-14] carbonate for distribution.

Given valuable C-14 starting material and the high cost of licensed radioactive waste disposal, it is imperative to carry out a practical trial run of the entire synthetic route with unlabelled reagents (the overall radiochemical yield can be reduced dramatically even if a small quantity of impurities are present) prior to performing the radiosynthesis. This demonstrates that the synthesis is practical and minimises radioactive waste production. The requirement for high specific activity often precludes dilution with unlabelled material and thus the radiochemist is typically working to prepare ~100mg of final product.

Each labelling project is unique and while ideally the radiolabel would be introduced in the last synthetic step, in practice, as the label position is often required in the core of the molecule, the label often has to be introduced early in the synthetic sequence. The challenge for the radiochemist thus becomes one of synthetic chemistry: to devise the most efficient route in terms of radiochemical yield and number of synthetic steps from barium carbonate. Radiochemists start much further back in the synthesis than traditional medicinal chemists as basic building blocks must be freshly prepared and in any event must derive from C-14 barium carbonate. For example, synthetic reasons often dictate use of simple single carbon precursors such as potassium [C-14] cyanide as starting material.

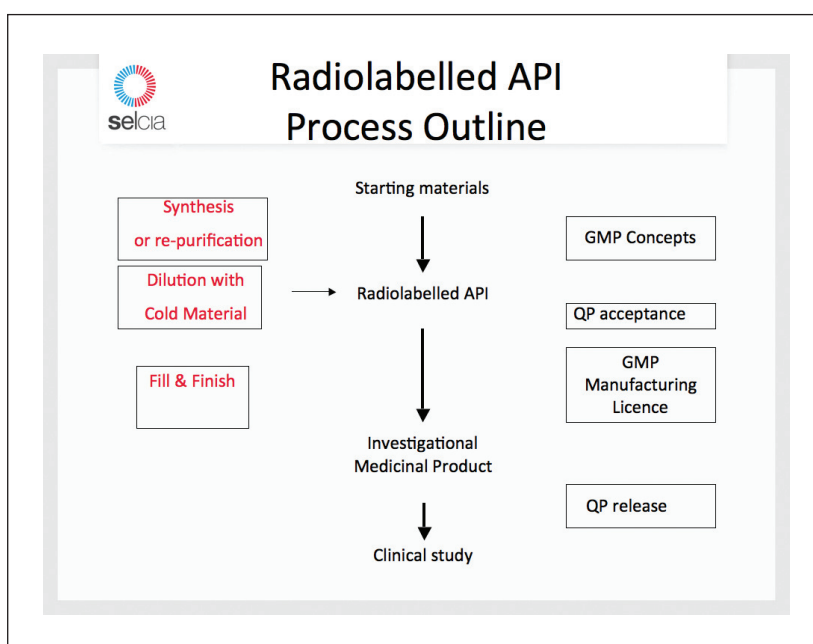
The radiochemist may employ process type chemistry, for example, when preparing aromatic moieties. Consider the sequence to a fluorobenzene starting with barium carbonate \Rightarrow barium carbide \Rightarrow acetylene \Rightarrow benzene \Rightarrow nitrobenzene \Rightarrow aniline \Rightarrow fluorobenzene: six steps each involving handling volatile radioactive materials. Further complexity occurs when considering the additional issues of isomerism arising from the position of the C-14 atom. In the case above the aromatic moiety may have molecules with differing numbers of C-14 atoms, depending upon at which stage the carbon-14 is diluted with unlabelled material. As a result this uniformly labelled material will exhibit multiple mass ions in the mass spectrometer. Detection sensitivity may require a single mass ion and in this case an alternative route must be followed to produce singly labelled material.

The synthetic route provides control over the specific labelling position allowing study of the metabolic fate of different parts of a molecule, providing confidence in the fate of the parent and facilitating metabolite identification.

C-14 compounds may need repurification

Compounds labelled with C-14 have a liability to decompose under the influence of their own radiation; termed radiolysis, this effect is independent of chemical stability. The primary effect when the disintegrating radioactive atom transmutes results in total disintegration of a single molecule and may produce reactive fragments. The secondary effect is from the β -radiation that may produce ions and radicals, which can react with other molecules or interact with solvent if present. Knowledge of radiolysis is empirical and cannot be predicted with confidence prior to synthesis of the compound. However, the effect of radiolysis can be mitigated, but not eliminated, by storage at low temperature under an inert gas. Storage in a solvent in solution, such as ethanol, may help. Thus, there may be a need for regular purity checks to ascertain if repurification is required prior to the study. Purity checks are especially important if studies are planned over several months to ensure the material is within specification prior to each study. Discolouration is not necessarily an indication of decomposition as free radicals may get trapped in the crystal lattice leading to marked colouration with no impurities detectable.

Figure 2



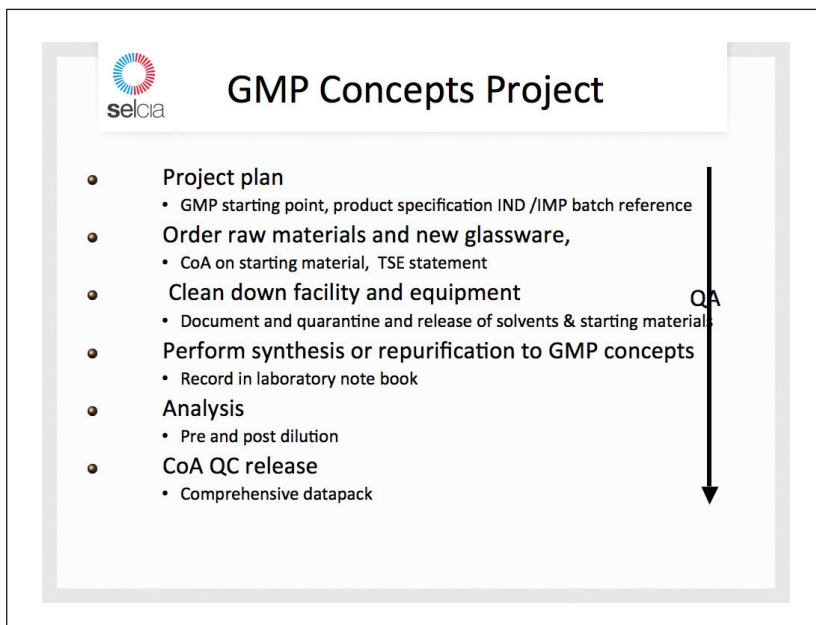


Figure 3 Radiolabelled API: GMP concepts

The objective of human ADME studies is to determine the mass balance of a drug and to evaluate its metabolism. Such studies require administration of a radiolabelled compound and are subject to a number of regulations summarised in Figure 1. The exact applicability depends on the nature of the study, for example administration of microtracer quantities do not require ARSAC ethics approval.

This process for preparation of radiolabelled material suitable for administration to humans is shown in Figure 2.

Figure 4



The radiolabelled component is usually prepared only once, on a very small scale (less than one gramme) and in a single batch. It is also subject to significant dilution with unlabelled material. The preparation of the radiolabelled component, the radiolabelled API, is thus not subject to full GMP manufacture but to Section 19 of the GMP guidance which states that the controls should be consistent with the stage of development of the drug product and that appropriate GMP concepts should be applied.

The relevant guidance has been issued in Europe as Eudralex volume 4 and recently in the USA by the FDA under their Guidance for Industry CGMP for Phase 1 Investigational Drugs.

This guidance places the emphasis on avoiding cross contamination, on accurate record keeping and ensuring that the material is well characterised. Process validation is not appropriate for a single batch so less emphasis is placed reproducibility or consistency and environmental controls.

Preparation of the radiolabelled API may involve a new synthesis or repurification of DMPK radiolabelled material (Figure 3) under GMP concepts. It is preferable to carry out the synthesis at high specific activity to provide certainty on the position of the C-14 atom and to ensure knowledge of C-14 impurities thereby minimising any problems that might arise during the analysis of the clinical samples.

Once the radiolabelled API has been prepared and fully characterised, the clinical laboratory performing the study accepts the material and prepares the final dosage form using a GMP-licensed manufacturing process under the control of a Qualified Person (QP) immediately prior to administration. It is good practice to test the final preparation using LSC and to keep a parallel sample as a check.

If a radiolabelled intravenous dosage form is required (ie for absolute bioavailability studies) then an additional step is needed. As terminal sterilisation, using irradiation or heat treatment is not suitable for sensitive C-14 compounds, a GMP validated aseptic process involving filtration is employed to prepare the sterile dosage form (the investigational medicinal product (IMP)). Examples of companies which are licensed by the MHRA to perform this in the UK are SCM Pharma and HMR.

Successful C-14 GMP concepts projects rely upon a smooth exchange of documentation between the sponsor, the clinical unit performing the clinical study and the radiolabelled API contractor.

Custom C-14 radiolabelling companies

The suppliers of custom C-14 radiolabelled compounds have responded to the market for increased quality and demand for GMP concepts radiolabelled APIs. The following section provides some selected examples of how key suppliers have invested to meet this demand:

Selcia has grown dramatically since it began life as an internal isotope laboratory within May & Baker (Rhone-Poulenc). This radiolabelling group was part of a spin out from Aventis in 2001 and, following a management buyout in late 2005, Selcia has invested heavily in expanding its custom carbon-14 radiolabelling business with the result that it has grown to be one of the leading independent custom-14 radiolabelling groups. It recently announced the opening of a new radiosynthesis laboratory (Figure 4) in 2009 bringing its capacity to 29 radiochemists. Selcia specialises in preparing radiolabelled API in accordance with GMP concepts and supplies material to clinical Phase I units in Europe and the US which perform the final IMP production and QP release just prior to human administration. Selcia's analytical laboratory is GLP accredited by the MHRA and offers GLP NMR and GLP certification of its C-14 products and GLP certification of unlabelled material. One advantage of being an independent C-14 supplier is that clients are able to choose from the widest number of CROs and clinical Phase I units the most appropriate specialist for their study.

Quotient provided the main news in 2009 when it announced it would be acquiring the former Amersham custom carbon-14 radiolabelling business from GE Healthcare. Amersham had been the leading supplier of custom carbon-14 compounds with a long history going back more than 50 years. Quotient announced it would be building a new facility to house this business at a cost of £15 million within 10km of the old Cardiff facility. Quotient also plans to retain its existing radiolabelling group (previously known as Biodynamics) in Northamptonshire. Biodynamics was one of the first custom radiolabelling companies to be licensed to manufacture radiolabelled IMPs and has its own QP to perform release to the clinical lab. Quotient is now focusing on offering clients an integrated service. As well as radiolabelling it can perform Phase I clinical tests and perform non-AMS C-14 bioanalysis, albeit not at a single location.

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- EMA: Guideline for the Environmental Risk Assessment of Medicinal Products for Human Use. (1 June 2006).
- FDA: Guidance for Industry CGMP for Phase I Investigational Drugs (July 2008).
- FDA: Guidance for Industry Safety Testing of Drug Metabolites (February 2008) <http://www.fda.gov>.

Table 1: Large Pharma with internal isotope groups

Abbott
Astra Zeneca
Bayer Schering Pharma
Boehringer Ingelheim
Bristol-Meyers Squibb
Eli Lilly
GlaxoSmithKline
Johnson & Johnson
Merck & Co Inc
Merck Serono
Novartis
Pfizer
Roche
Sanofi-aventis

Another radiolabelling group to be acquired by a CRO is the former Eagle Picher group (ex Chem Syn) which is now part of **Aptuit**. Following the acquisition it has moved to new laboratories in Kansas City, Missouri.

Almac has more than 2,500 employees and added a radiolabelling group, based in Craigavon UK, to its CMC supply chain activity in 2005. Like Quotient it favours the integrated model and has recently announced a broader early stage drug development package by partnering with a CRO, Covance. In 2009 Almac announced it was expanding its radiolabelling team with three new laboratories and publicised that it has an MHRA GMP licence for radiolabelled IMP (Drug Product) manufacture and formulation including QP release to the clinical trials facility.

PerkinElmer acquired the former NEN (New England Nuclear) radiolabelling group based in Boston, Massachusetts (once part of DuPont) in 1996. In 2008 PerkinElmer announced it was expanding its radiochemical manufacturing facility and relaunched custom radiolabelling under the 'Under One Roof' label. It has been very active publicising its 'elite' custom synthesis service for

radiolabelled API to GMP in compliance with FDA Phase I guidance and has opened a second GMP facility to meet demand. Unlike the other suppliers covered here, PerkinElmer also offers C-14 catalogue items under its NEN-Radiochemicals brand.

These companies and a number of other suppliers are active participants of the International Isotope Society which promotes radiolabelling and use of isotopically labelled compounds.

Clinical labs using C-14

The nature of this work lends itself to specialist companies which are expert in their field and licensed to work with C-14. The radiolabelled API can be sent to the most appropriate specialist to perform the Phase I clinical trial.

An example of a Phase I unit licensed to use C-14 is LCG Bioscience, other Phase I units licensed to perform the GMP manufacture and formulation of the radiolabelled IMP include: Hammersmith Medicines Research (HMR), housed in a new 100 bed clinic; PRA International based in Netherlands; SGS in Belgium and Covance and HLS in the UK.

Analysis of C-14 clinical samples can be performed, for example, by Charles River in Edinburgh.

Internal radiolabelling groups

Many of the large pharmaceutical companies have one or more internal radiolabelling groups (Table 1). They can benefit from outsourced overflow buffer capacity, which can be available immediately to meet urgent requests and ensure projects are not delayed by a lack of resources. When large pharma acquire a project from a biotech, their first request may be for GMP radiolabelled API and with no internal experience of the project, as would normally be the case for an internal project, an outsourcing company provides a very cost-effective solution. It is no longer unusual for internal groups to consider outsourcing GMP concepts radiolabelled API synthesis.

Many mid-size pharmaceutical companies regularly outsource their custom radiolabelling, sometimes using FTE arrangements to ensure resources are immediately available to work on their projects. One advantage of carrying out route development in advance is that radiolabelling can be removed from the critical development path.

The number of internal isotope groups has been in steady decline for decades. A consequence of industry consolidation has led to closure of isotope groups while formation of new groups has been hampered due to entry barriers such as the requirement for licences, decommissioning liability

and not least difficulty in acquiring trained radiochemists. Examples of internal radiolabelling groups closed over recent decades are shown in Table 2.

A complicating factor is that isotope laboratories do not always fit comfortably in an organisation as they cover research, pre-clinical and development functions, often with conflicting priorities.

COUNTRY	LOCATION	ONE-TIME OWNER
Fr	Romainville	Roussel Uclaf
ITA	Verono	Glaxo Spa
UK	Beckenham	Burroughs Wellcome
UK	Billingham	Zeneca Specialities
UK	Brockham Park	Beecham
UK	Dagenham	Rhone-Poulenc
UK	Great Burgh	Beecham
UK	Greenford	Glaxo
UK	Harlow	Smith Kline Beecham
UK	Horsham	Ciba Geigy
UK	Milton Keynes	Hoechst
UK	Nottingham	Boots
UK	Ware	Glaxo
UK	Welwyn	Roche
UK	Welwyn	Smith Kline French
USA	Ann Arbor, MI	Parke-Davis, Warner Lambert
USA	Cincinnati, OH	Hoechst Marion Roussel
USA	Chesterfield, MO	Pharmacia
USA	Kalamazoo, MI	Pharmacia Upjohn
USA	King of Prussia, PA	Smith Kline French
USA	Palo Alto, CA	Roche Bioscience (Syntex)
USA	RTP NC	Burroughs Wellcome
USA	Skokie, IL	Searle
USA	Summit, NJ	Ciba Geigy

Table 2: Pharma isotope groups closed since 1980

Also, isotope groups cannot be moved easily as laboratories have specific licences and if centralised cause added problems on divestiture.

Conclusion

So what of the future? Consolidation continues apace in big pharma with the mega-mergers of Merck-Schering Plough-Organon and Pfizer-Wyeth. Eli Lilly and GlaxoSmithKline have announced outsourcing initiatives and Sanofi-Aventis a reorganisation of its R&D. All have internal isotope groups so we wait to see what impact, if any, this has on their custom radiolabelling activities.

What is certain is that the custom radiolabelling companies have taken the view that outsourcing will continue to grow and have invested to ensure they remain ahead of the curve and are ready to meet the increased demands of their clients. **DDW**

Dave Roberts is Business Development Director with Selcia Ltd. Starting as a synthetic chemist he spent more than 20 years with Rhone-Poulenc and Aventis. In 2001 he was a founder of the spin-out which was to become Selcia in 2005 and has been closely involved in the expansion of their radiolabelling business.