

Accelerated Radiochemistry

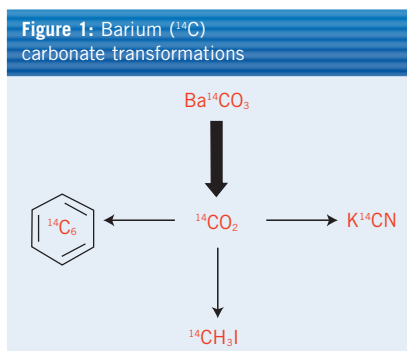
Excellence can be achieved through synergies between synthetic organic chemistry, key technologies and cGMP, explains Sean L Kitson at Almac

Pharma companies need to meet radiolabelling requirements in Phase I to Phase III clinical trials. Therefore, cGMP service providers must work closely to generate high quality carbon-14 labelled active pharmaceutical ingredients (APIs) and investigational medicinal products (IMPs) for preclinical and clinical studies on qualified person (QP) release. Radiolabelling services can benefit from active integration with other technology areas such as biocatalysis, physical sciences and peptide synthesis, producing technical excellence and a high quality service offering to customers involved in final drug candidates, peptides, small molecules and advanced intermediates for the drug development process.

ISOTOPIC LABELLING

The pharmaceutical industry can benefit from companies that offer synergies between the supply of New Chemical Entities (NCEs), their associated metabolites and the stable isotope and radiolabelled analogues that are a key component of the drug development process. The diversity handled by radiolabelling teams can fully reflect drug development pipelines. Radiolabelling imposes constraints and presents challenges beyond those found in normal chemical synthesis. This synthesis can be quite different to that employed for the API, and the radiolabelled API can possess a dramatically different stability profile due to radiolysis. The result is that both close support and guidance are required to ensure that customers receive fit-for-purpose carbon-14 labelled API materials, which are supplied using the most cost effective synthetic strategies.

Numerous labelled drugs and advanced intermediates have been generated successfully for CROs and pharma companies, using the stable isotope labels (^2H , ^{13}C , ^{15}N) to produce a wide array of metabolites and mass spectroscopy standards. There are no associated



radiation issues and isotopic enrichments are usually greater than 98 per cent. High sensitivity of detection is assured through state-of-the-art mass spectrometry, while multiple labelling can deliver complete mass resolution from an unlabelled analogue.

RADIOLABELLING

The major advantage of radiolabelling with carbon-14 is that it can be incorporated into the drugs' carbon framework without altering its chemical structure, producing an identical copy of the unlabelled drug. This approach reduces the risk of the carbon-14 label being scrambled compared to 'peripheral' tritium labelling. Consequently, during the design of the synthetic route, it is vital to locate a feasible, biologically stable position for the carbon-14 label and to identify suitable starting materials, which can be commercially available or easily made. Once the radiolabelled API has been synthesised, it can be used in preclinical studies to evaluate the absorption,

distribution, metabolism, excretion and toxicology (ADMET) profile.

There is also a requirement for radiolabelled API and IMP, prepared in a cGMP compliant manner for *in vivo* human apparent metabolisable energy (AME) studies. These needs are being met by several projects to synthesise small amounts of carbon-14 labelled compounds used in accelerator mass spectrometry (AMS) for first-in-man studies. This technique uses microdoses of labelled API to analyse the ratio of carbon-14 to carbon-12 to facilitate data on pharmacokinetics and metabolism profiling.

The low-energy beta emitter carbon-14 (half-life roughly 5,700 years) requires limited shielding and is the radiolabel of choice to obtain reliable quantitative mass balance data and tissue distribution studies in humans. This is essential for the determination of tissue half-life, clearance rates from the human body and the potential associated risks of toxicity after prolonged exposure. The primary source of the carbon-14 is barium [^{14}C] carbonate, which can be converted to [^{14}C] carbon dioxide to generate a variety of carbon-14 labelled starting materials (see Figure 1). The maximum specific activity of a single carbon-14 label cannot exceed 62.4mCi per millimole. This physical quantity is useful for tracing the radiochemical purity of the intermediates throughout a carbon-14 radiolabelling process.

Figure 2: Selective mild hydrolysis of ^{14}C -nitrile to ^{14}C -carboxylic acid using nitrilase

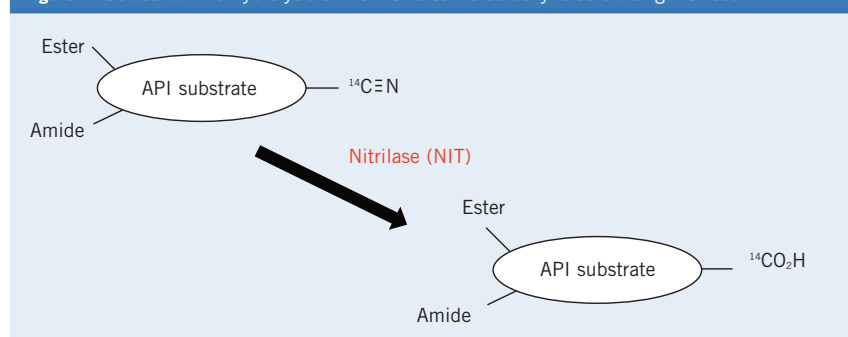
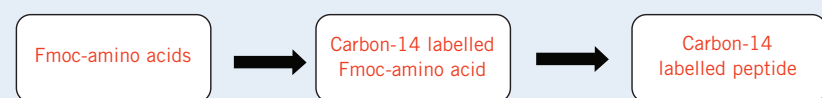


Figure 3: cGMP peptide API profile



INTEGRATION OF RADIOCHEMISTRY

The integration of radiochemistry with other technologies has the aim of finding fit-for-purpose solutions beyond the traditional realms of radiosynthesis. This often gives new and attractive synthesis options and shorter synthetic routes. Furthermore, these integrations ensure that best-in-class technical solutions can be found to the many challenges that contract radiosynthesis brings.

FOUR AREAS OF INTEGRATION

Radiochemistry and Biocatalysis

A nitrilase enzyme can be used to convert a carbon-14 labelled nitrile group through to the corresponding radiolabelled carboxylic acid under neutral conditions that were sufficiently mild, so as to leave a sensitive moiety elsewhere in the structure unchanged (see Figure 2). An initial biocatalysis screen of nitrilase enzymes can be carried out to identify the most effective enzyme. The application of the right enzyme can produce the desired labelled product in good yield and excellent purity. Interestingly, the best traditional synthetic alternative failed to give the same intermediate in acceptable purity.

Figure 4: XRPD instrument



Radiochemistry and Peptide Synthesis

The preparation of a number of carbon-14 labelled peptides can be achieved by using solid phase peptide synthesis techniques (see Figure 3). The key to the design of a labelled peptide synthesis is to select a suitable amino acid to label. Radiochemists are able to leverage considerable expertise in the area of synthetic organic chemistry to develop a robust peptide synthesis by designing a viable synthesis strategy, and then run with the initial trials. Some in-house solid-phase technologies can manufacture pharmaceutical-grade peptides of up to 200 amino acid residues.

Radiochemistry and Physical Sciences

One format for a radiolabelled IMP is powder in capsule, in which the radiolabelled IMP is typically hand-weighed into a suitable capsule without the addition of any excipients. However, one potential issue with this approach is the possibility that the radiolabelled IMP has a different polymorphic form to the bulk API. To counteract this, one powerful technique is to monitor polymorphic form using x-ray powder diffraction (XRPD), but this technique is only available in a limited number of service companies (see Figure 4).

Figure 5 shows the XRPD spectrum of a bulk API, while Figure 6 shows the spectrum of the corresponding radiolabelled IMP. In this case, we were able to use this data, expertly interpreted in-house, to confirm that both materials contained the same polymorph.

Labelling and Metabolite Synthesis Biocatalysis groups can

offer access to oxygenated metabolites using biotransformation, while custom synthesis groups offer expert access to other complex metabolites, such as glucuronides and steroids. This expertise can therefore be applied to the synthesis of a labelled metabolite, thereby increasing the synthetic access options that can be offered.

Effective and active technology integration of this manner is proving to be an efficient strategy to identify cost-effective synthesis solutions for pharma.

REGULATORY SUPPORT & QUALITY SYSTEMS FOR RADIOLABELLING SERVICES

It is important for service companies to understand the regulatory requirements for each phase of clinical development and country of filing. This must be demonstrated by providing data and support for the European, US and Japanese regulatory submissions – for Chemistry, Manufacturing and Controls (CMC), Investigation New Drug Application (IND), New Drug Application (NDA), and Investigational Medicinal Product Dossier (IMP).

Figure 5: XRPD spectrum of the unlabelled bulk API

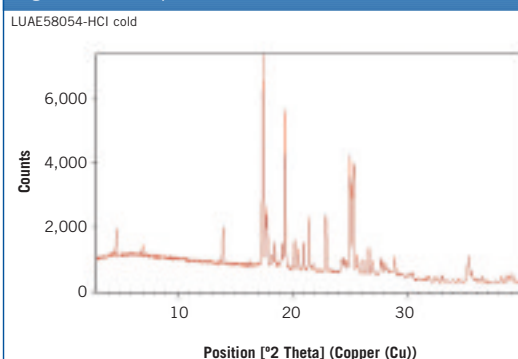


Figure 6: XRPD spectrum of the carbon-14 radiolabelled IMP

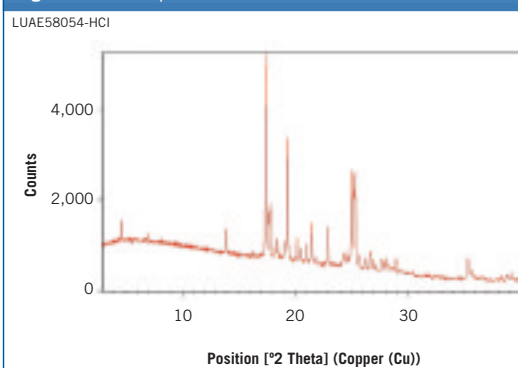
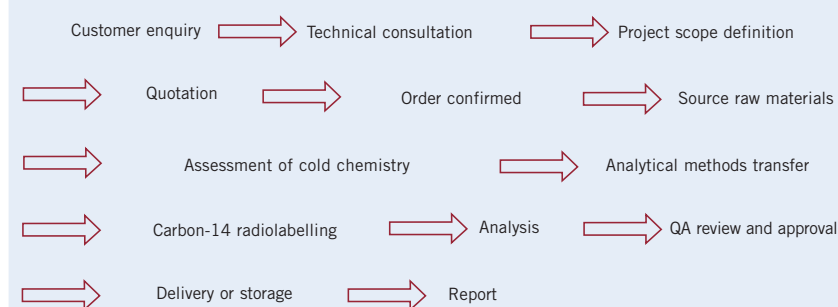


Figure 7: A typical cGMP process flow



As the market moves increasingly towards cGMP requirements for carbon-14 labelling at the level of both API and drug product for investigational use, the demand for cGMP radiolabelling continues to escalate. The quality system must be an integral part of any service business and therefore can be specifically developed to be compliant with Section 19 of the internationally harmonised ICH Q7 'Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients', as it relates to the production of API and API intermediates.

cGMP radiolabelling is provided by trained chemists and analysts using validated equipment in dedicated facilities, real-time documentation and is reviewed by an independent Quality Assurance (QA) officer. Finally, a Certificate of Analysis (CoA) for the API is issued by the QA department (see Figure 7).

Radiolabelling chemists and analysts should be rigorously trained in cGMP procedures. These cGMP needs are met in the specially designed cGMP suites, which facilitate the repurification and radiosynthesis of preclinical to Phase I materials under strict cGMP controls.

The quality system must apply across all areas of cGMP activities, including process development, manufacturing, analytical, stability services and isotope labelling. This system is driven to maintain continuous improvement supported by internal and external audits, and all cGMP activities are closely monitored by the Quality Unit consisting of quality control (QC) and QA departments. This assures customers continued adherence to cGMP specifications and regulatory compliance at all times. All cGMP batches are released by QA.

Many companies provide employees with regular training, ensuring that developments and improvements are applied consistently throughout the organisation. The Quality System and Analytical Services can be regularly audited by the Medicines and Healthcare products Regulatory Agency (MHRA). These external accreditations can lead to service companies being listed as a contract laboratory on a number of manufacturers authorisations for IMPs issued by the MHRA.

Figure 8: Isotope chemistry services

Chemistry	cGMP
<ul style="list-style-type: none"> • Synthesis route design • Definition of labelling strategy • Stability, storage and repurification facilities 	<ul style="list-style-type: none"> • cGMP synthesis • Stability storage and purification • Radiolabelled IMP manufacture and QP release facilities
Analysis	Labels
<ul style="list-style-type: none"> • Full product analysis • Analytical method development • Radiochemical stability studies validation and transfer 	<ul style="list-style-type: none"> • Radioisotope: ^{14}C • Stable isotopes: ^2H, ^{13}C, ^{15}N • Related non-isotopic labels: Alexafluor, BODIPY

About the author



Sean L Kitson is an Investigator of radiochemistry and holds a PhD in Synthetic Organic Chemistry, and an MSc and

diploma in Medical Physics. He joined the Almac radiolabelling team in September 2008 from GE Healthcare and received the 2006 Wiley Journal of Labelled Compounds and Radiopharmaceuticals Young Scientists Award for his work on the carbon-14 radiosynthesis of the Parkinson's drug apomorphine. He is the Editor-in-Chief of the peer review journal, *Current Radiopharmaceuticals*, and a committee member of the International Isotopes Society (UK Group).

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A key milestone in carbon-14 radiolabelling services is the approval by the MHRA to be granted an IMP licence. For instance, radiolabelling laboratories can include workstations with specifically designed fume-hoods for carbon-14 radiochemistry and fume-hoods for cold chemistry and analysis.

API & IMP MANUFACTURING FINISHING SUITE

In addition, a dedicated manufacturing finishing suite can be utilised for the development of IMPs to be used in clinical trials and the final steps in API manufacture. These provide formulations of powders and capsules, and sterile filtered solutions on QP release of an IMP to allow a clinical laboratory to administer the drug product to humans.

CONCLUSION

Radiolabelling facilities must provide a service to meet the highest standards of the pharmaceutical industry. These can come from recognition by the MHRA, in-house technical ability and QA systems. Carbon-14 radiolabelling can assist pharma companies in meeting the requirements for Phase I to Phase III clinical trials, ensuring they remain at the forefront of drug development and testing in the pharmaceutical sector.