Carbon-14 labelled API manufacturing.

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Carbon-14 labelled API and IMP requirements for Phase 0/I to Phase III mass balance and micro-dosing clinical trials can be met by contract manufacturing in compliance with MHRA and FDA Phase I cGMP guidelines. The short supply and escalating price of carbon-14 labelled building blocks has emphasised the demand for robust synthetic methodologies to incorporate the carbon-14 label efficiently into the API, and outsourcing to a CMO specialising in handling these challenges is a cost-effective approach. Further advantages accrue to clients by incorporation of these services within an overall drug development package, from discovery through to marketing.

The development of new drugs is a challenging compromise between time/cost projections and safety/efficacy profiles. Every day lost in the development of a successful drug leads to a direct loss in revenue for the pharmaceutical or biotechnology company. Every additional day worked on an unsuccessful drug sees unfulfilled investment (1).

One key element is development of an understanding of how the drug behaves in the human body, how it is absorbed, distributed and ultimately excreted2. Therefore a major challenge in the evaluation of an investigational drug is the early determination of its toxicological profile. Robustly designed A(D)ME (Absorption, (Distribution), Metabolism and Excretion) studies executed at an early stage in clinical development that yield useful information on the metabolism of test compounds can help avoid costly future clinical trials3.

ADME studies require use of a radiolabelled version of the investigational drug manufactured to a quality suitable for its intended use: to generate preclinical data evaluating the metabolic profile in animals, or early clinical data in a microdosing study using human volunteers Phase 0/I4. If the data

obtained from the study meets the drug's expectations it can then proceed to the Investigational New Drug (IND) approval process in the USA5, or submission of a national Clinical Trial Authorization (CTA) in the EU6. In this article, we consider how contract manufacturing organisations (CMOs) respond to these opportunities and challenges. Among the aspects considered are the radioactive element, the challenges of radiochemistry, the regulatory environments and critical success factors for a CMO offering these services.

Carbon-14 – the radioactive element of choice

The rationale for selection of carbon-14 in radiolabelling of drug substances lies in the ability to successfully substitute a particular carbon-12 atom in a molecule for carbon-14 to produce a chemically identical analogue. The introduction of this radiolabel in the carbon framework allows the fate of the drug to be traced in a biological system. This radiotracer approach stems from the work of Melvin Calvin when he used carbon-14 to determine how plants utilise carbon dioxide in the process of photosynthesis7.

One of the primary reasons for choosing carbon- 14 over other common radioisotopes such as tritium is that the exact position of the label can be selected based upon the synthetic route employed for labelling. Carbon occurs in the skeleton of nearly all drug molecules, and therefore allows choice of the radiolabelling site in a position more likely to be metabolically stable8. Additionally carbon-14 labelled compounds generally exhibit greater radiochemical stability than their tritium-labelled counterparts, owing to the higher specific activity of tritium-labelled material, which increases the risk of significant autoradiolysis during storage or use of the radiolabelled compound9. Carbon-14 is also detectable at very low levels using scintillation counting, which makes it an ideal choice for in vivo studies where doses close to the pharmacological threshold are frequently used10.

Radioactive carbon-14 was discovered by Martin Kamen from the bombardment of carbon- 13 with deuterons on February 27, 194011. This discovery led to the first reactor-produced radioisotope for clinical use, produced at Oak Ridge National Laboratory (ORNL) in 1946. Currently, carbon-14 is produced in a nuclear reactor by the continuous bombardment of an aluminium nitride target with a flux of neutrons (12 x 1014n/cm2/s). The transformation takes two years to ultimately provide (after processing) carbon-14 labelled barium carbonate at a specific activity 50- 60mCi/mmol12. Supply of barium [14C]-carbonate was dominated by two main suppliers until 2009 when production at the Canadian National Research Universal reactor, located in Chalk River, Ontario was halted14. Currently, the only nuclear reactor to commercially supply barium [14C]-carbonate is the Mayak facility in the Ural area of Russia15. Barium [14C]-carbonate, the primary carbon-14 labelling source, is converted to [14C]-carbon dioxide which can be reacted to generate a variety of aromatic and aliphatic carbon-14 building blocks (Figure 1)16.

Carbon-14 is a low energy beta emitter (maximum 156keV) with a half-life of 5730±40 years, compared to that of tritium at 12.3 years. This means that there is no need for correction for radioactive decay during the course of a drug development programme. As it requires limited shielding (maximum beta range in air: 24cm, water/tissue: 0.28mm and plexiglas/ lucite/plastic: 0.25mm) it is relatively safe to handle, and this helps to confirms it as the radiolabel of choice for quantitative mass balance and tissue distribution studies17.

Challenges of radiochemistry

Design of a radiolabelling synthesis depends on a number of interdependent factors, which ultimately determine the labelling position within the molecule. These factors must be carefully considered along with any prior knowledge of the molecule to ensure that a metabolically-stable labelling position is achieved:

• Identification of a synthetic pathway: a limited range of carbon-14 starting materials is available, and existing synthetic procedures are often not appropriate or efficient with expensive labelled reagents.

• Selection of appropriate starting materials: beginning a synthesis from barium [14C]-carbonate may help to reduce material costs, but can lead to increased labour cost due to a longer synthesis. Use of a more advanced starting material is often cost effective since smaller amounts and fewer steps to completion are required.

• Other factors, such as the amount of material and the specific activity required, may also influence the synthetic design.

The exceedingly slow radioactive decay of carbon- 14 to nitrogen-14 leads to negligible decomposition of material on a human timescale, however the ionising radiation generated may lead to secondary chemical reactions resulting in very significant radiochemical decomposition18. This effect cannot be predicted with certainty, and may result in changes of less than 1% over several years, up to several per cent in a week. Common precautions to minimise the effect include diluting the material with unlabelled active pharmaceutical ingredient (API) or a solvent, and storing the labelled API at cryogenic temperatures (typically <-70°C)19.

Carbon-14 labelling has recently become an even more useful tool as a result of advances in detection technology20. Accelerated Mass Spectrometry (AMS), although currently expensive, is a very sensitive technique for the detection of carbon-14 and other radiolabels21. The high sensitivity of AMS allows human micro-dosing to be carried out with sub-therapeutic doses and much lower levels of radioactivity22. This means significantly decreased exposure and waste issues, as well as reduced material requirements. Supply of small amounts of carbon- 14 labelled API for first-in-man AMS studies demands the ability to work on extremely small scales from the CMO. This expertise is exemplified by the recent synthesis of just 2mg of a carbon-14 labelled peptide API consisting of 84 amino acid residues, and representing 42μCi of activity. AMS microdosing studies typically use in the order of 10kBq per study to analyse the ratio of carbon-14 to carbon-12, and provide information on drug metabolism and pharmacokinetics (DMPK). In comparison, human mass balance studies require doses in the order of 4MBq of carbon- 14 labelled investigational medicinal product (IMP) per individual23.

Regulatory environment

Further challenges to a successful radiolabelling project are presented by the strict regulations governing the use of ionising radiation, for example the Ionising Radiations Regulations 1999 in the UK24. These regulations establish a framework to ensure that exposure to ionising radiation arising from work activities is kept as low as reasonably achievable (ALARA principle) and does not exceed dose limits specified for individuals. In particular, these regulations require employers to appoint one or more suitable Radiation Protection Supervisors (RPS) to supervise the work within radiation-controlled areas according to a set of Local Rules, which describe the implementation of the regulations specific to the CMO site. The CMO has also a legal duty to provide appropriate training for all employees working with ionising radiation. In addition to this, the various UK Environment Agencies issue certificates or permits to store, handle and dispose of radioactive materials. The details of legislation differ between countries, but all impose restrictions on who may handle radioactivity, how it may be stored, used and disposed of, and how these activities are recorded and the fate of materials tracked. Air transport of radioactive materials is regulated by the International Air Transport Association (IATA), which imposes further restrictions on the amounts, nature, packaging, labelling etc of radioactive materials that may be carried on aircraft.

A significant investment in infrastructure and quality systems is required in order to efficiently comply with all of these requirements, and few CMOs are able to offer radiolabelling services as part of a full pharmaceutical development programme, to the current Good Manufacturing Practise (cGMP) standard required for human studies. Carbon-14 labelling to cGMP standard requires a robust quality system developed to be compliant with Section 19 of the internationally harmonised ICH Q7A ‘Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients’ as it relates to the production of API and IMP25. Although ICH Q7A, which outlines cGMP regulations for APIs, refers to commercial APIs, it also provides an excellent general description of cGMP principles. In particular, Section 19 lists the requirements for early phase investigational APIs such as radiolabelled APIs. The principles outlined in Q7A cover process validation, complete method validation, annual product reviews, vendor qualification, change control etc.

The various regulations are complicated, and continuously being updated, so in addition to supplying fit-for-purpose carbon-14 materials, made using the most cost-effective synthetic strategies, a CMO must be able to support and guide study sponsors through the entire process, up to and including Qualified Person (QP) release of IMP, to help ensure a successful outcome for their project. This is particularly important for smaller biotech or virtual companies who may not have previous experience with radiolabelling studies.

CMO radiolabelling facility

A radiolabelling facility (Figure 2) must be designed with quality and regulatory requirements in mind, and must additionally be able to address the full range of materials encountered in drug development pipelines, which may include heterocycles, aromatics, chiral compounds, peptides and proteins,

steroids, volatile compounds, sugars, PEGylated materials, cytotoxic and highly potent compounds16,26. By its nature, radiolabelling imposes constraints, and presents challenges beyond those found in medicinal synthesis laboratories. The synthetic route can be quite different to that employed for unlabelled API, and the carbon- 14 labelled API may possess dramatically different stability and impurity profiles. Radioactive materials must be fully tracked from their arrival on-site, until their eventual dispatch as either products or waste, which imposes further conditions on the manner in which chemistry may be carried out.

All handling of radiolabelled materials is carried out within fume hoods, both to protect personnel from exposure, and also to protect the quality of the material itself. Equipment for manipulation of volatiles and for purification and isolation of final materials (eg semi-preparative HPLC, freezedrying and milling apparatus) is required, as well as the analytical capacity to determine the quality of labelled compounds. Dedicated GMP suites contain an extensive range of validated analytical equipment, such as scintillation counters, high performance liquid chromatography (HPLC) systems (including radioactivity detectors as well as traditional detection methods), dissolution and disintegration apparatus, titrators and meters. Larger items of apparatus such as nuclear magnetic resonance (NMR) spectrometers, high resolution mass spectrometers and x-ray diffractometers may not be available within dedicated radiolabelling facilities, and in this instance a CMO must have access to these services elsewhere on-site. Determination of stability of radiolabelled materials is frequently of pivotal importance to the success of a radiolabelling study, and the capacity to carry out GMP stability studies to ICH standards (including the commonly used <-70°C conditions used for radiolabelled materials) is therefore vital.

GMP carbon-14 labelling process

Carbon-14 labelling to cGMP standard is carried out by trained chemists using validated equipment in dedicated facilities, working under a comprehensive quality system which covers all aspects of the manufacture, from the facility itself (eg air quality, cleanliness of surfaces, microbial monitoring), the equipment used (eg validation, cleaning, maintenance, usage logbooks), to the actual synthesis (eg written manufacturing directions, control of materials and processing aids used). An independent analytical group carries out quality control on starting materials, intermediates and final compounds, issuing certificates of analysis for API and IMP. Oversight, review and final approval is in the hands of a separate Quality Assurance department, which may include QP release directly to a clinic in the case of IMP manufacture.

Regular training ensures that developments and improvements are applied and recorded consistently throughout the CMO organisation. Process development is typically carried out using unlabelled materials (cold trials) and/or highly diluted radioactive materials (warm trials) to optimise the parameters required for the labelled API synthesis. Vital information regarding reaction step yields, impurities and purification of the intermediates and API is obtained, and reagents to be used in the labelled cGMP synthesis are tested for fitness – especially important when using expensive carbon- 14 labelled starting materials. Other benefits of carrying out cold or warm trials is to investigate the effects of scale (typically hundreds or thousands of times lower than plant procedures) and to validate analytical methods to be used with the final manufacture. A hot trial, using undiluted radiolabelled material is often carried out as a final verification of the process parameters, since differences are occasionally observed between labelled and unlabelled materials, particularly with regard to stability. Indeed, an important aim of the hot trial is often to provide materials for determination of stability, to ensure that under the study conditions the API will stay within specification.

The point at which cGMP controls are required in the synthesis must be agreed with the study sponsor, usually a covalent bond-forming step which results in formation of the major backbone of the molecule. Obviously fewer cGMP steps required leads to cost savings, and in cases where a significant dilution with unlabelled API is being carried out (≥1:100 typically), it may even be possible to use non-GMP radiolabelled API blended with GMP-grade unlabelled API (subject to an acceptable history of the nonGMP material, especially regarding potential exposure to transmissible spongiform encephalopathies (TSE). Detailed manufacturing directions are prepared, including details of reagents, reaction conditions, in process checks, purity limits and testing, etc. After Quality Assurance (QA) approval and issue of these directions, verification of equipment and facility cleaning, and once the required reagents and materials have been certified and released by the Quality Control (QC) and QA departments the cGMP manufacture may commence.

Full records of all equipment used in the manufacture are maintained, and an electronic stock control system tracks the movements and use of reagents as well as the intermediates and API as they are formed. In-process checks and analyses of intermediates and API are carried out against predetermined specifications, which are developed to cover the key quality determining parameters of the material. This typically includes confirmation of identity by methods such as mass spectrometry and NMR, as well as purity assessment (HPLC), and solvent content (GC). Solid APIs may exist in more than one polymorph, with serious implications for their physical behaviour, and confirmation of polymorphic form by x-ray powder diffraction (XRPD) is frequently required, a specialised technique offered by only a few CMOs (Figure 3a). Comparison of the measured XRPD spectrum to a reference material allows confirmation that the same polymorph has been obtained (Figure 3b)27. The quality system applies to all aspects of the cGMP activities and is subject to frequent audits which verify continuous regulatory compliance at every stage from receipt of materials through to the API manufacture and final release of API. In addition to internal audits, study sponsor audits and radiation compliance checks, CMOs may voluntarily request compliance checks by agencies such as the Medical and Healthcare Regulations Authority (MHRA, UK) or Federal Drugs Authority (FDA, US) as a further demonstration of their quality status.

Critical success factors – integration

A drive is on by pharmaceutical and biotechnology organisations to outsource more of their operations to service providers, especially for specialised services such as carbon-14 labelled API manufacture. Radiolabelling in a GMP setting can be costly (although it is a small fraction of the overall cost of launching a drug to market) but the information gained during the synthesis is necessary to support the drug in the later stages of clinical development, and in contributing data towards the drug's safety profile. A CMO must retain highly skilled personnel encompassing a range of expertise in the areas of synthetic organic chemistry, isotope labelling, solid state chemistry, analysis, product formulations and quality assurance. A changing marketplace and demands on materials and costs enforce a discipline of continuous improvement on CMOs in order to remain competitive, while maintaining high quality standards throughout the process. This is all governed by exacting regulatory requirements, and critical timelines to deliver high quality products for use in clinical trials.

A number of CMOs offer radiolabelling services, a more limited number cater for cGMP radiolabelling28- 29. Practical capabilities in radiolabelling and an established track record in regulatory compliance are strategically vital for these CMOs. Through integration of services some CMOs are able to offer even greater benefits: by providing inhouse biocatalysis, physical sciences and peptide synthesis departments, formulation specialists and clinical trial support a compelling service offering to customers involved in drug development pipelines is created. An integrated offering allows the CMO to better serve customers by taking advantage of the synergies that exist between the supply of API, their associated metabolites and labelled analogues. Intellectual property protection is also enhanced by performing all key stages of the drug development process under one roof, from preand formulation development, stability studies, method development, pre-clinical and Phase I clinical trial materials, late-stage clinical trial materials, to scale-up and registration batches, and commercial production.

Conclusion

Pharmaceutical companies need a reliable supply of isotopically labelled APIs. The isotope of choice is carbon-14 to produce API for Phase 0 to Phase III, ADME, mass balance and micro-dosing studies. A present challenge is the escalating price of carbon-14 labelled building blocks generated from carbon-14 labelled barium carbonate, which is at present in short supply. Consequently, there is even more pressure on the radiochemist to execute robust synthetic methodologies to incorporate the carbon-14 label efficiently into the API.

For human studies, this necessitates procedures to perform synthesis and repurifications under cGMP conditions, in compliance with ICH Q7A Section 19 guidelines (single batches for investigational drugs) and/or MHRA guidelines for the preparation of carbon- 14 labelled APIs for clinical trials. These carbon- 14 labelled compounds provide vital information for chemistry, manufacturing and controls (CMC) towards investigational medicinal product dossier (IMPD) submission.

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