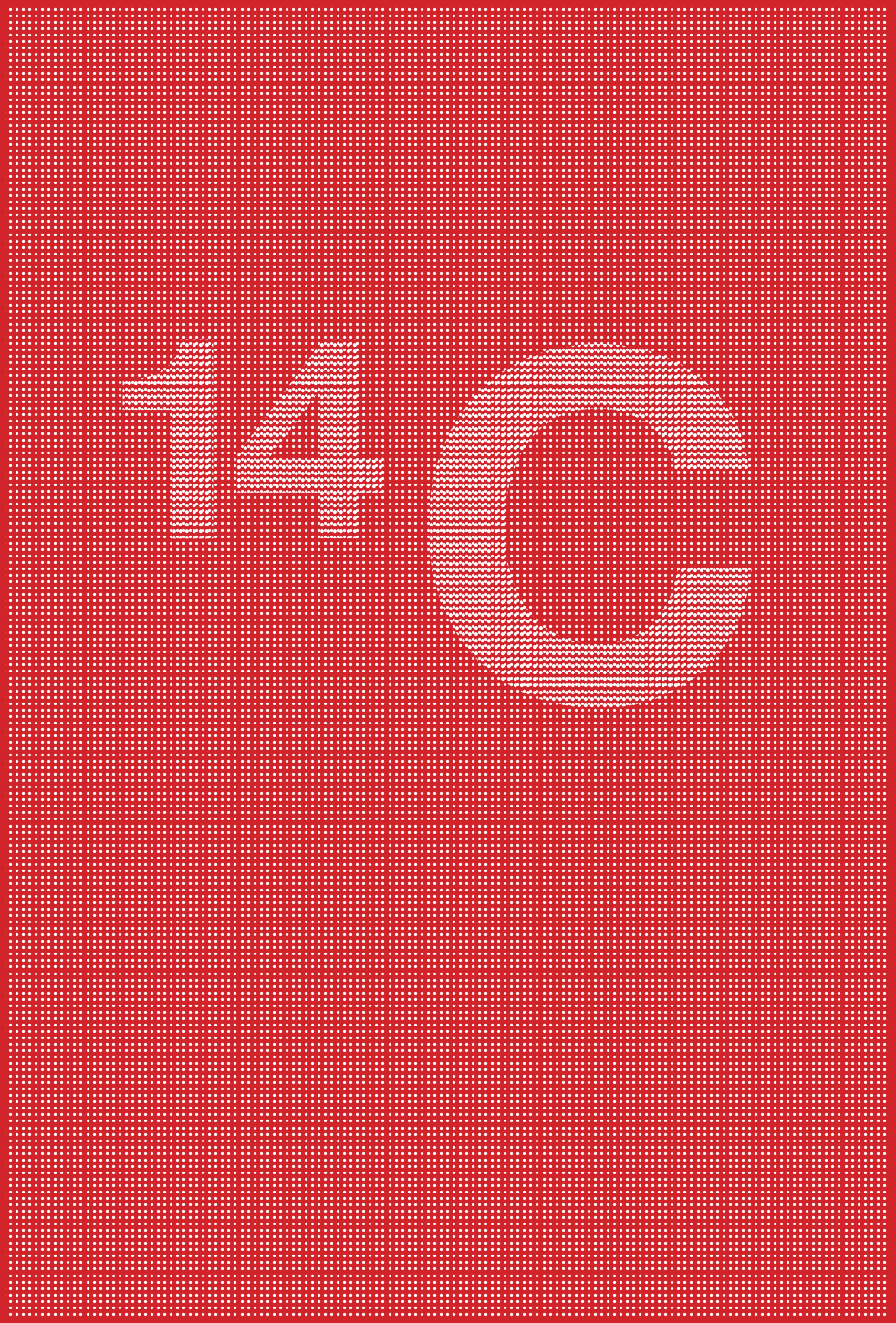
selcia

Selcia Radiolabelling

^{14}C Custom Radiosynthesis
^{14}C GMP API for Clinical Trials
GLP Analytics-GLP NMR
Metabolite & Impurity Synthesis

www.selcia.com



Carbon-14 is a natural isotope of carbon and its decay is the basis of radiocarbon dating. When produced artificially it provides an essential tool to determine the behaviour and fate of chemical compounds in organisms and the environment.

Incorporation of ^{14}C into a molecule of interest creates a radiotracer that is chemically and biologically identical to its non-labelled counterpart.

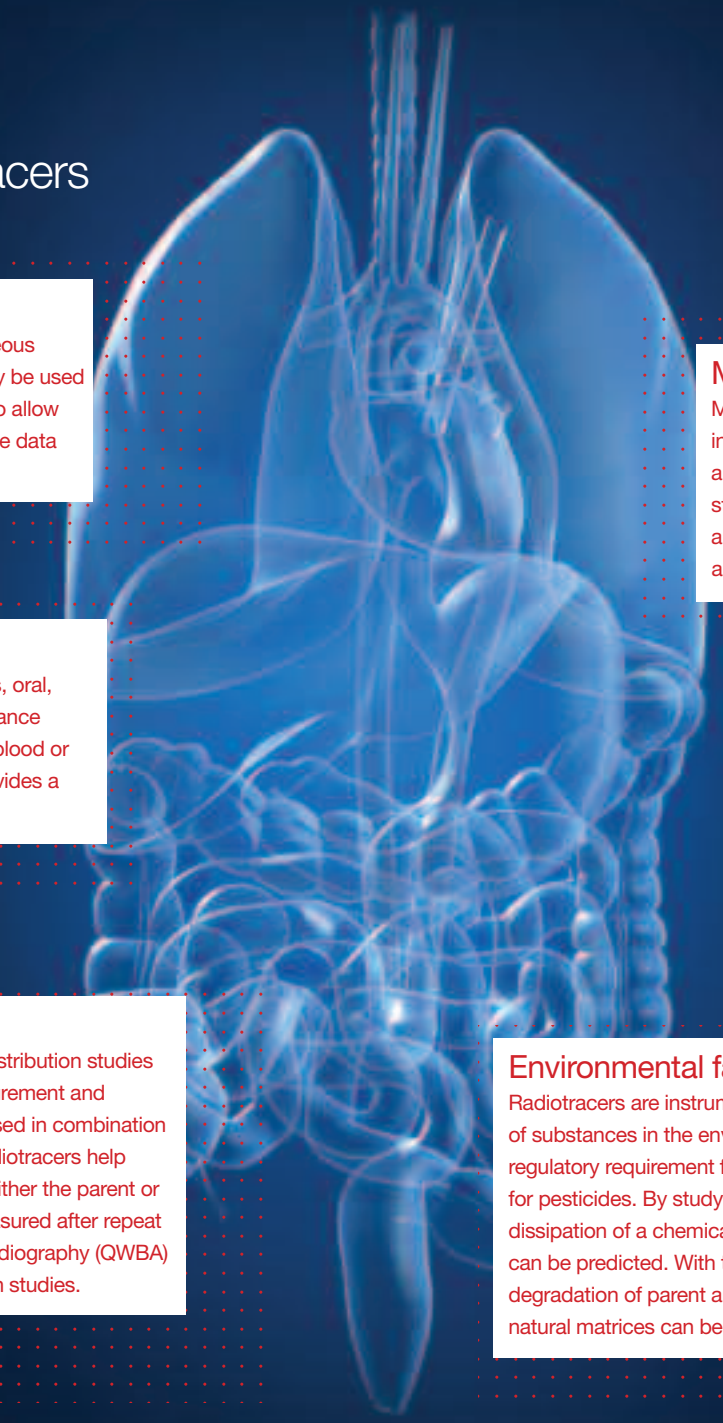
A ^{14}C-radiolabel allows easy detection with high sensitivity in complex biological matrices, due to the low natural background of carbon-14. This enables monitoring of both location and speed of processes.

If the radiolabel is in a metabolically stable position it remains associated with the compound and its metabolites.

The synthesis of a radiolabelled compound is a good investment especially when taking into account the relative ease of analytical work and the quality, reliability and suitability of the data generated for regulatory submissions.

selcia.com/radiolabelling

Typical studies using ^{14}C-radiotracers



Penetration

Radiotracers facilitate measurement of percutaneous penetration across membranes (i.e. skin) and may be used in a diffusion cell, such as a Franz diffusion cell, to allow determination of dermal absorption and to provide data on skin penetration for risk assessments.

Absorption

Various routes of administration (e.g. intravenous, oral, etc..) of a radiotracer allow monitoring of appearance and disappearance of the test substance in the blood or plasma. Simultaneous dosing by oral and IV provides a measure of absolute bio availability.

Distribution

Radiotracers are invaluable for tissue distribution studies facilitating detection, separation, measurement and isolation of the test substance. When used in combination with mass spectroscopy techniques radiotracers help determine if a peak is associated with either the parent or a metabolite. Accumulation can be measured after repeat doses. Quantitative Whole Body Autoradiography (QWBA) is especially useful for tissue distribution studies.

Metabolism

Metabolism studies using radiotracers can be performed in vitro and in vivo and require the radiolabel to be in a stable position within the test molecule. Metabolism studies require sensitive detection, efficient separation, and accurate quantitation as well as isolation of the parent and the metabolites in biological matrices.

Excretion

Without a radiolabelled compound an excretion mass balance study is extremely difficult. Formal mass balance studies are required for all New Chemical Entities as part of a New Drug Application.

Environmental fate

Radiotracers are instrumental in the study of the fate of substances in the environment. Such studies are a regulatory requirement for product registration, in particular for pesticides. By studying metabolism, degradation and dissipation of a chemical, its persistence in the environment can be predicted. With the help of radiotracer the rate of degradation of parent and metabolites in water, soil and other natural matrices can be measured accurately.

Quality Radiochemicals: Essential Tools in Life Science R&D

^{14}C Custom Radiolabelling

"We are radiosynthesis experts with many years of industrial experience"

Selcia is the leading *independent* carbon-14 radiolabelling company

Our independent position and established relationship with leading CROs gives you maximum flexibility when choosing the appropriate contract laboratory for your ^{14}C study

- ^{14}C Radiosynthesis including *de novo* development of synthetic routes
- Synthesis of radiolabelled API to GMP for clinical trials
- Chiral separation and salt formation
- Rapid re-purification service
- Storage and stock management of radiolabelled compounds
  Long term storage at +4°C, -15 °C or -80 °C.
- Safe and fast shipment worldwide to multiple destinations
- QC with full characterisation (HPLC, NMR, MS). Gravimetric analysis for specific activity, radiochemical purity by HPLC. Chemical purity and structural identity by HPLC and NMR and LC-MS
- GLP certification for regulatory submission

All our compounds are delivered with an industry leading data package, including full spectroscopic analysis and experimental documentation. A Certificate of Analysis specifying chemical as well as radiochemical purity by NMR, MS, radio-HPLC and radio-TLC accompanies each compound. The quality and detail of the data given in the data package make it suitable for regulatory submission.

Carbon-14: The isotope of choice in the life science industry

Even minute amounts of radioactive material can be detected easily, which makes it ideal for use in tracing the movements of highly diluted substances in complex matrices, such as the environment or living organisms

Characteristics of carbon-14 and ^{14}C-radiotracers

- Low natural background
  Natural abundance: 1.18 x 10^{-12} in atmosphere
- Universal quantification
- No standard required
- Identity of analyte is not required for quantitation
- Rate of decay is constant and independent of pressure, temperature, pH and of chemical structure

Carbon-14, the isotope of choice in the life science industry

- Biologically equivalent to carbon-12.
- Introduction of ^{14}C label does not change physico-chemical properties or biological activity of molecules
- No shielding necessary; max. penetration in air: 20cm
- High detection sensitivity in biological matrices by LSC
- Long half life: 5730±40 years
  / No correction for decay necessary

^{14}C is introduced into a defined position within the molecule through total synthesis

Selcia Synthetic Expertise

At Selcia we are at the forefront of technological development in our sector. Constantly reviewing our skill portfolio we apply the latest techniques in organic synthesis and purification such as microwave-assisted reactions and chiral preparative HPLC to achieve the best possible results for our clients.

Carbon-14 labelling of organic molecules requires total synthesis of the target structure from a basic single carbon precursor such as [^{14}C]cyanide or [^{14}C]carbonate. The synthetic expertise of Selcia scientists enables us to prepare almost any chemical compound including complex natural products.

A testimony to Selcia's synthetic expertise

"First Total Synthesis of ^{14}C-Labelled Procyanidin B2"

A Milestone Toward Understanding Cocoa Polyphenol Metabolism

European Journal of Organic Chemistry, **2008**, 36, 6069-6078

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Radiolabelled API to GMP for Clinical Trials

"Selcia is certified by the UK Medicines and Healthcare products Regulatory Agency (MHRA) for the preparation of ^{14}C labelled active pharmaceutical ingredients (API) for clinical trials"

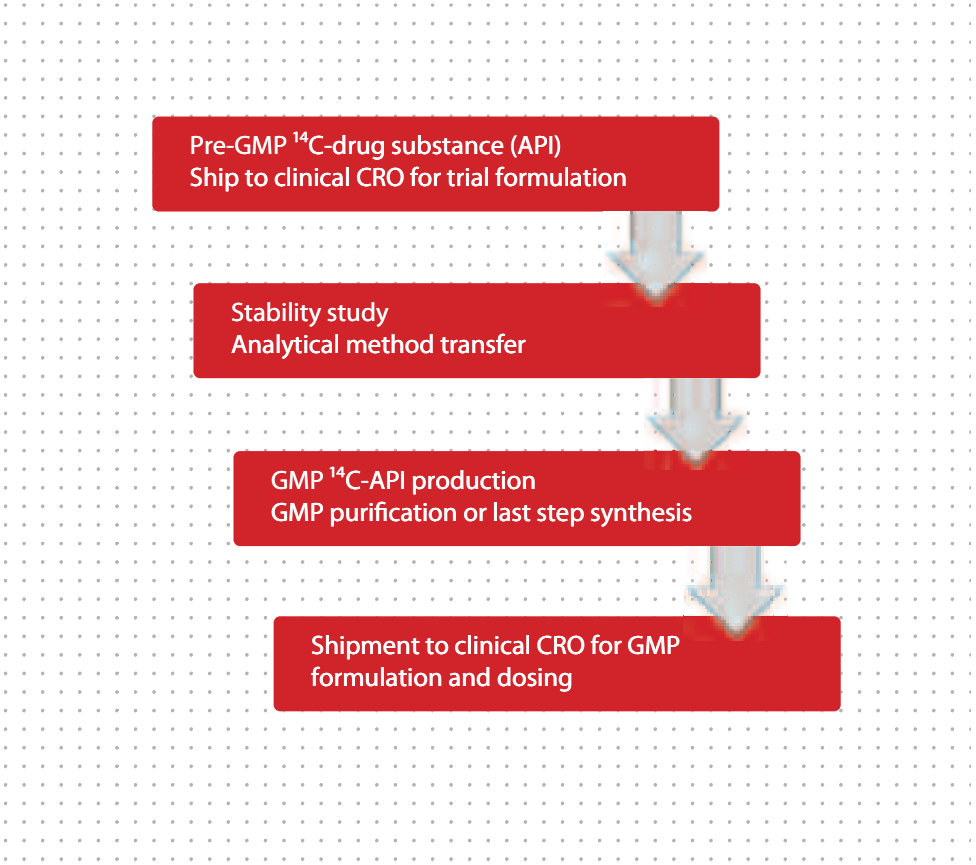
Whether you require a synthesis or a repurification under GMP, we perform both in compliance with EMEA and the FDA Phase I GMP guidance (ICH Q7A Section 19: Single batches for investigational drugs).

The radiolabelled API is analysed in Selcia's MHRA certified GLP analytical laboratory and supplied with a complete analytical data package. Selcia will also assist you in providing CMC information for your clinical trials application.

GMP API Preparation at Selcia:

- Quarantine and identity testing of raw materials
- Verification of production area clean down
- Accurate recording of experimental procedures and witnessing of critical processes
- Rigorous analytical procedures to establish product identity and quality
- Radiochemical stability data
- Quarantine and release of API by our internal QA

The process of manufacturing a ^{14}C API for clinical trials



GLP Analytical Services and Metabolite Synthesis

Our analytical staff have a strong background supporting regulatory studies acquired over many years in the life science industry

We provide specialist analytical services tailored to individual needs to support regulatory studies and submissions.

- GLP NMR for structural confirmation and comparison with standards and full interpretation and structural elucidation
- GLP analysis including mass spectrometry and infra-red spectroscopy
- Custom synthesis of metabolites, impurities and reference standards
- Custom synthesis of compounds labelled with stable isotopes such as Carbon-13, Nitrogen-15, Deuterium
- All custom prepared products can be provided with a GLP certificate of analysis
- Non-routine analytical studies including structural elucidation and impurity profiling at microgram levels using capillary NMR

Our analysts work closely with our synthetic chemists to resolve the chemical structures of unidentified process impurities, metabolites or degradation products. Our analytical department is accredited by the MHRA and we are a full member of the Good Laboratory Practice program in the UK and specialise in GLP NMR to support regulatory submissions.

Selcia can act as Study Director or Principal Investigator to support your GLP regulatory study

We specialise in mass spectrometry and particularly NMR spectroscopy. Instrumentation in our GLP laboratory includes:

- High Field NMR, 300MHz, 400MHz and 500MHz spectrometers
- Flow and capillary flow LC-NMR
- ^{2}H, ^{13}C, ^{15}N, ^{19}F and ^{31}P probes
- Mass Spectrometry LC-MS/MS
- Analytical and Preparative HPLC
- FT-IR

Working with Selcia

Credibility

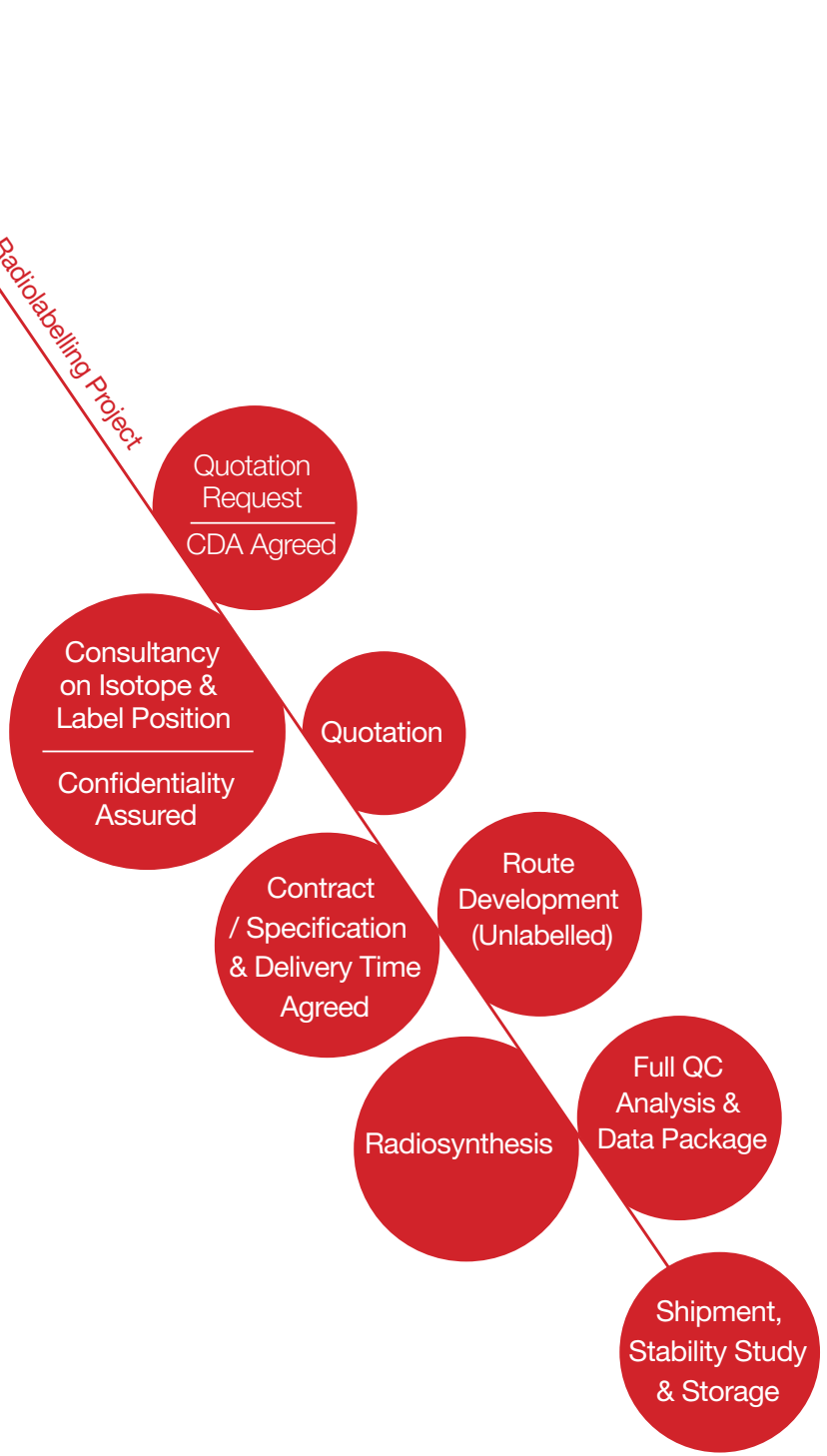
We have a tradition of applying scientific excellence to satisfy the stringent quality criteria of our clients. We fully understand the importance of delivering a high quality test substance on time for the study. The success of your study is our primary concern.

Personal service

Our business is built on fostering long-term relationships with our clients. We work hard to get to know our customers and the specific requirements of their business. We are in favour of face-to-face meetings and regular visits to help sustain these ongoing relationships. Equally we encourage clients to see our facility first hand. Informal visits are as welcome as audits.
We strive to create transparent and open business relationships built on trust to meet clear objectives.

Communication

The dialogue begins from the moment we receive your enquiry. We take time to discuss your requirements to ensure the radiolabelled product will be of suitable specification for its intended use. Where necessary we can advise on label position, quantity, specific activity, synthesis route and other factors as part of the quotation process and at no extra charge. Once we have agreed the proposal and the project is underway, you will be able to communicate directly with the project scientist to follow progress. Our scientists report pro-actively on the status of your project with a minimum of bi-weekly written progress reports with further updates as soon as significant developments arise.



Working with Selcia

At Selcia we work to a rigorous quality program designed to ensure that the specifications of our products and services meet the client's requirements in all respects

Personal service with regular communication scientist-to-scientist

- Minimum of bi-weekly progress reports
- Access to project chemist at any time

Exceptional synthetic chemistry expertise

- Track record of successfully completed complex syntheses

Second-to-none documentation and reporting

- Industry leading data package
  - / Analytical report including NMR, HPLC, LC-MS data and interpretation
  - / Synthetic report
  - / Certificate of Analysis

Scientific excellence

- Highly qualified chemists
- 60% of employees at PhD level
- Many years of industrial experience

The key elements of the Selcia Quality System

- Project Quality plan for each project defining
  - / Product specifications as agreed with the client
  - / Procedures and duties of each staff member working on the project to ensure delivery
- Training carried out and recorded to demonstrate that our scientists are qualified to carry out all tasks required by the project
- Standard Operating Procedures (SOP) for all key activities
- Product release by Quality Assurance Unit, independent of production

Radioactive units

Characterisation of ^{14}C labelled compounds

Primary measure of the quality of a radiolabelled compound is the “radiochemical purity”, which is determined by HPLC in conjunction with a radiodetecor (liquid scintillation flow counter). The radiochemical purity is directly obtained by integrating the radio-HPLC trace. No calibration against a standard is required.

Chemical purity is determined by conventional HLPC methods and NMR.

In order to use the radiochemical in quantitative experiments its specific activity, i.e. the radioactivity per mass unit has to be determined. This is typically achieved by gravimetric analysis, the direct measurement of the radioactivity in a known amount of radioactive sample.
The result obtained is MBq/mg or µCi/mg.

This can be converted into molar specific activity by the formula shown in the box on the next page.

Useful units and conversion for ^{14}C radiotracer work

Radioactive decay: **1Bq = 1 disintegration per second (SI unit)**

Old unit, still in use: **1Ci = 3.7 x 10^{10} disintegrations per second**

Conversion: **1Ci = 3.7 x 10^{10}Bq or 1mCi = 37MBq**

Specific activity (SA): **Radioactivity per mass unit**
Most commonly used in ^{14}C tracer work:
1µCi/mg or 1MBq/mg

In radiochemistry it is common to use molar specific activity:
mCi/mmol or MBq/mmol

The max. specific activity for ^{14}C , i.e. 1 atom of ^{14}C per molecule is 62.4mCi/mmol (theoretical maximum) in practice max. 50-60mCi/mmol

Conversion: 1mCi/mmol = 1mCi/mg x MW
(MW = molecular weight)

Example: $Ba^{14}CO_3$ (MW = 199.10)
55mCi/mmol is equivalent to 0.276mCi/mg or 10.2MBq/mg

Formula to calculate the adjusted molecular weight of a radiolabelled compound from its specific activity:

$$MW_{labelled} = MW_{unlabelled} + \frac{SA\ [mCi\ mmol^{-1}] \times 2}{62.4mCi\ mmol^{-1}}$$

or in MBq

$$MW_{labelled} = MW_{unlabelled} + \frac{SA\ [MBq\ mmol^{-1}] \times 2}{2309MBq\ mmol^{-1}}$$

Carbon-14 facts

Carbon-14 timeline

1934 Carbon-14 first postulated by Franz Kurie

1940 First detected and isolated by Martin Kamen and Sam Ruben

1942 First studies with ^{14}C-radiotracer: Incorporation of $^{14}CO_2$ into plants

1947 Libby states radioactive ^{14}C is found in all living creatures

1960 Libby et al. receive Nobel prize for carbon dating

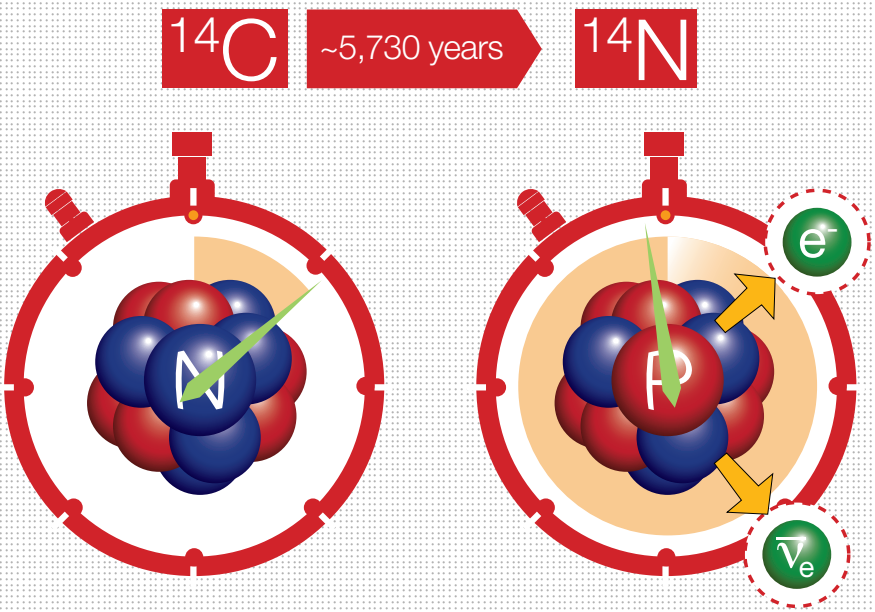
1961 Calvin wins Nobel prize for work on photosynthesis

Carbon-14 is a cosmagenic isotope produced in nature through cosmic ray bombardment of nitrogen at high altitudes in the atmosphere

- $^{1}n + {}^{14}N \rightarrow {}^{14}C + {}^{1}p$
- Natural abundance = 1.18×10^{-12}. Approximately 1 atom in 1 trillion

Carbon-14 decay

- ${}^{14}_{6}C \rightarrow {}^{14}_{7}N + e^{-} + \nu_e$
- Low energy ß—emitter (Emax=156keV)
- Half life $T_{1/2}$ = 5730±40 years
- Maximum molar specific activity SA_{max}=62.4mCi/mmol



About Selcia

A leading worldwide provider of integrated drug discovery and ^{14}C custom radiolabelling services. Selcia is a privately held company operating from state-of-the-art laboratories near London, United Kingdom.

Integrated Drug Discovery
Macrocycles & Natural Products
Medicinal Chemistry
PPIase Screening

selcia
drug discovery

selcia
radiolabelling

^{14}C Custom Radiosynthesis
^{14}C GMP API for Clinical Trials
GLP Analytics-GLP NMR
Metabolite Synthesis

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