Impurity Isolation and Elucidation

Impurity isolation and elucidation is an essential part of the drug development process. The material isolated allows for structural determination and biological studies, which will contribute to the development and registration of new drugs.

An impurity is defined as 'Any component of the new drug substance that is not the chemical entity defined as the new drug substance.'[1] The International Council on Harmonisation (ICH) have produced guidelines surrounding the content and qualification of impurities in a new drug substance and have set minimum thresholds for reporting impurities. Reported impurities need full characterisation and biological activity studies. Impurities may arise during the synthesis or manufacture of a compound. They are often linked to the starting materials, by-products, intermediates, or degradants. For both drug effectiveness and patient safety, it is important to identify impurities. It must be ensured that impurities do not evoke any form of adverse response in patients.[2]

An impurity must be isolated to be characterised. At Reach Separations, a range of chromatographic techniques are employed to do this: reverse phase (RP) HPLC, normal phase (NP) HPLC, chiral SFC and achiral SFC. This wide scope of techniques allows orthogonal approaches to be investigated to exploit the physical and chemical properties of a molecule, to achieve the separation and isolation of a desired compound. By working intelligently, throughput, recovery, and purity can all be maximised.

Traditionally, RP HPLC would be employed to isolate a compound. In RP HPLC, Molecules are separated based predominately on their respective lipophilicities. RP HPLC is a universal and robust technique with a high success rate.

Recently, Reach Separations has been using SFC to aid impurity isolations. Separations are dependent on the compound's differing affinities with polar stationary phase and non-polar, slightly acidic supercritical CO_2. Chiral columns have been utilised to separate molecules which are closely related to each other. Separations using chiral stationary phases are a result of chiral recognition.[3] Often, SFC will be used to enrich a sample, followed by RP HPLC for the final purification. Separations based on differing chemical interactions can often result in elution order switches, which can be used to improve the purity of isolated material.

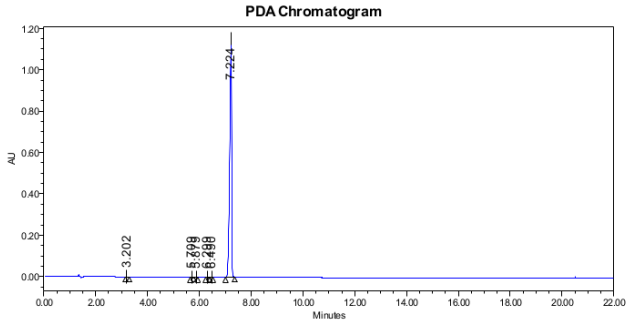

Figure 1. Chromatogram of incoming material with a <0.03% impurity (RT: 5.9 minutes)

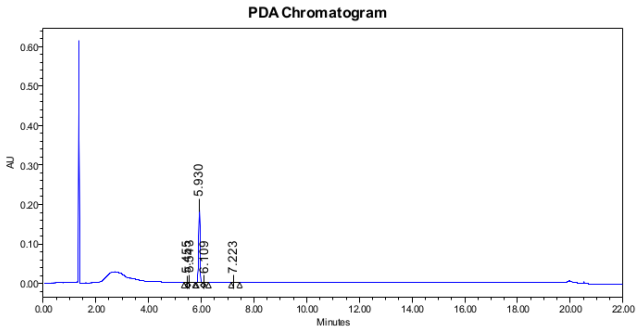

Figure 2. Chromatogram of isolated material (RT. 5.9 mins) at 98% chemical purity

Detection is a frequent challenge for impurity isolations on both an analytical and preparative scale, either due to the low levels of the impurity, the low extinction coefficients, or poor ionisation. For analysis, UV detectors, MS, CAD or ELSD can be exploited. On a preparative scale Reach is equipped with UV detection, MS and ELSD. This wide range of detectors maximises the scope of impurities that can be isolated.

The isolated material then requires structural elucidation. This is achieved using high-resolution MS-MS and NMR. Using preparative chromatography, isolated impurity can be returned to the client. This material is often used to assess its biological activity through assays.

Impurity isolations are an essential part of the drug discovery process. As an outsource purification provider, Reach Separations can offer expert advice and services in this field. Isolated material can be returned with high chemical purity and high recovery. Expert partners are able to provide elucidation via NMR and MS.

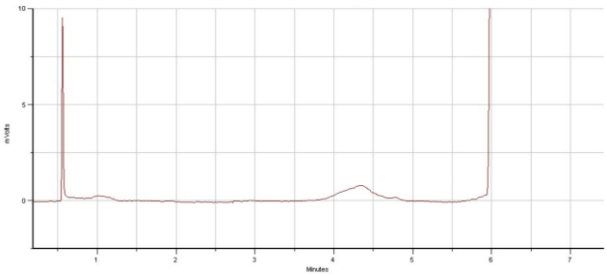

Figure 3. Highly developed isocratic RPHPLC was used to isolate the impurity prior to full structural elucidation by MS-MS and 2D NMR

(1)INTERNATIONAL COUNCIL ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE ICH HARMONISED TRIPARTITE GUIDELINE IMPURITIES IN NEW DRUG SUBSTANCES Q3A(R2) Current Step 4 version dated 25 October 2006

(2) A Systematic Approach to Impurity Identification, April 2007, Gary E. Martin, https://doi.org/10.1002/9780470988749.ch5

(3) Comprehensive Heterocyclic Chemistry III Volume 14, 2008, Pages 667-749 Pappalardo, M.F. Parisi, https://doi.org/10.1016/B978-008044992-0.01229-3

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