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Managing E&L Unknown Compounds

Eurofins BioPharma Product Testing, a leader in mass spectrometry, offers advice on extractables and leachables and explains why it is so important to know your compounds

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Unknown compound characterisation has always been one of the most challenging aspects of analytical chemistry. Despite the difficulties, this identification is critical for a meaningful extractables and leachables (E&L) evaluation of container closure systems; single-use systems; drug delivery and combination products; and medical devices.

Identification of unknown extractable compounds is key to a successful E&L study due to the fact that there is no universally established concentration limit for leachable compounds. As a result, a toxicological risk assessment based on the intended dosing regimen of the product is required to determine if a potential leachable compound poses a risk to patient safety. Unfortunately, accurate and meaningful toxicological assessments are not possible for unknown compounds. These are typically assessed using a worst case scenario, where they are evaluated as extremely toxic – this however often leads to applying an inaccurate level of risk. Therefore, a systematic approach for the characterisation of unknown extractable compounds is essential to enable scientists to assign the correct degree of toxicological concern to a given analyte.

So, how does one go about identifying an unknown compound? The use of the proper instrumentation is critical. High resolution mass spectrometers that enable an accurate mass determination to at least three decimal places – such as liquid chromatography-quadrupole time of flights (LC-QTOFs) and gas chromatography (GC)-QTOFs – are suitable for this purpose and their use generates a plethora of data that can often lead to the identification of an empirical formula or class of compound for the unknown. This information can then be further evaluated with specialised software that is able to quickly search historical data and databases to provide a preliminary identification of the unknown extractable compound.

The use of commercially available databases and the implementation and expansion of in-house LC and GC mass spectrometry (LC/MS and GC/MS) databases improve the chance of identifying an unknown compound. Eurofins continues to invest in databases in order to support its expertise and growth as a world-leading E&L service provider. For extractable compounds detected by LC/MS analysis, Eurofins maintains a proprietary database of more than 1,500 compounds.

MS is not your only option for identification of an unknown compound. Nuclear magnetic resonance spectrometry can also be a powerful tool in making the unknown known and is able to provide supporting data if identification by MS alone is not successful.

In any E&L study, the first step when evaluating the information is to perform a preliminary compound identification using the databases. Tentative identifications are assigned when matches are found within the database and, typically, the most effort is spent determining those peaks with the highest response or those that are repeatedly detected in other extractables studies. When a tentative identification is possible at this stage, confirmation through analysis of a certified reference standard should be performed. In case the standard is not commercially available, the use of a surrogate compound of similar chemical class and structure can be utilised, or it may even be possible to synthesise the compound of interest. If the compound is confirmed based on its mass spectrum and retention time, then its information is added to the database to aid in future testing.

For those compounds that are not identified through a database, a chemical class or potential molecular formula is assigned where possible. In these cases, the expertise of the scientist performing the work is critical. Advanced knowledge of MS, along with organic chemistry, can be used to piece together the data that are generated to provide as much information about the unknown as possible. If determined, the class of a compound can then be utilised by a toxicologist to provide an appropriate risk assessment.

When data from this preliminary assessment are not sufficient to propose identifications for all extractables detected, additional testing may be performed. If the quality of the mass spectrum of the unknown compound was poor due to low response or interference, then optimising the testing conditions – including adjusting mobile phase content to improve ionisation or changing columns to improve separation – may be required. Another option would be to isolate the peak of interest by fraction-collection, allowing concentration of the compound prior to evaluation by MS.

Identification of the unknown compound often involves evaluating both the nominal mass of the unknown along with fragmenting the compound to generate daughter ions. Fragmentation can be performed using a variety of instruments, including GC-QTOF, LC-QTOF, LC/MS-Ion Trap, LC-tandem MS or LC/MS-Orbitrap. These techniques can provide structural data based on evaluation of the spectra of the fragments or product ions. Determining how the compound fragments often offers insight into its structure.

If an unknown compound cannot be identified, it may be valuable to assess the type of study in which the compound was observed. For example, if it was detected during vigorous extraction conditions but was not seen during a simulation study, identification of the compound will not likely be required. Conversely, if the compound is observed in both studies, then it becomes critical to provide information on the compound, whether that be a positive or tentative identification, or assignment to a class of compounds.

Through the years, Eurofins BioPharma Product Testing has developed the expertise needed to support all phases of an E&L study, including the characterisation of unknown compounds. Eurofins is continuously investing in state-of-the-art equipment as well as ongoing advanced education and training for its elite team of scientists in order to support clients in this critical area on their path to regulatory approval.