Compound X

Identification can be far from simple when it comes to analysing extractable compounds migrating from packaging or processing components into drug products. A systematic approach, customised extractables database and human expertise are crucial to getting results

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Container closure systems for substances and components used in the drug manufacturing process may release unwanted chemicals into pharmaceutical products. These compounds can impact the safety of the product, affect its efficacy or stability, or even influence analytical tests used in its release. Therefore, determining the identity of these chemical entities and toxicologically assessing their impact on the patient is extremely important.

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Extractables and Leachables

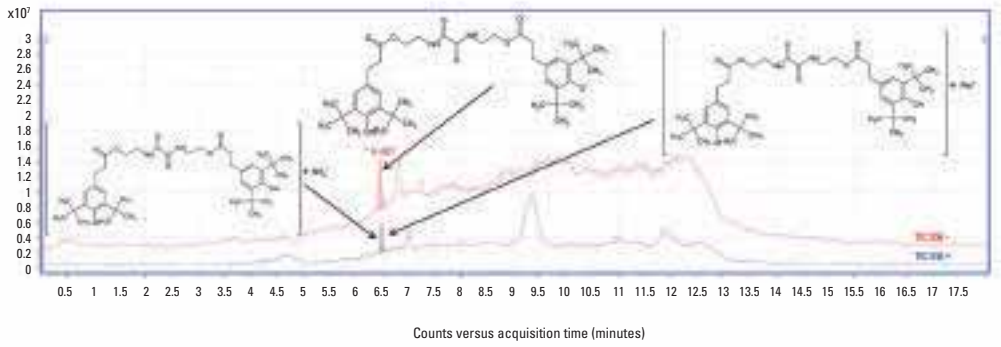
Extractables are compounds that can migrate from the material into a solvent under exaggerated conditions of time and temperature. They can result from the use of functional additives, solvent residues or catalysts utilised in the manufacturing process, and can also stem from the degradation of additives or polymers.

Leachables, meanwhile, are compounds which migrate into the drug product or bulk solutions under normal storage or processing conditions. Typically, they are a subset of extractables – however, not all leachables are extractables. They can be the outcome of an interaction between an extractable compound and the product, or be a degradation product from an extractable compound (1).

An extractable study should be performed on final packaging, delivery devices and manufacturing components that come into contact with the product formulation. This involves using stressing conditions which reflect a worst-case scenario – compared to typical conditions of use – in order to increase the rate of migration and facilitate detection and identification. Structural identification – and subsequent quantification – is crucial, since the actual toxicity of a molecule and its permitted daily intake amount can only be evaluated if the chemical structure is known.

Several analytical techniques can be applied to these studies as extractable compounds can be either organic or inorganic in nature. Typical instrumentation utilised includes liquid chromatography (LC) and gas chromatography (GC), both coupled with mass detectors – high-performance liquid chromatography-mass spectrometry (HPLC-MS) and GC-MS (2). These are used to monitor the presence of organic entities, while

Figure 1: Naugard XL-1 in ESI positive mode (blue line) and ESI negative mode (red line) as seen in a total ion chromatogram scan



inductively-coupled plasma and ion chromatography can be applied to determine the level of inorganic compounds present.

Overcoming Hurdles

LC-MS poses the greatest challenge in an extractables study as HPLC-MS libraries for the identification of unknown compounds are not commercially available. This is due to the fact that the generation of the mass spectrum is strictly dependent on the analytical conditions used. For this reason, the creation of a customised extractables database, which will permit identification of unknown compounds by retention time and mass, is critical to the success of these studies. The identification of extractable compounds is first required to build such a database.

Compound characterisation is undoubtedly a very complex analytical field that often requires data from several instruments such as high-resolution mass spectrometers (HR-MS), ion trap or triple quadrupole MS systems, ultraviolet absorption, nuclear magnetic resonance, infrared and elemental analysis. This thorough approach does not fit easily with the relatively high number of unknown chemicals frequently detected in an extractables study.

An alternative approach relies on HR-MS spectral data evaluation. Combining an analyst's expertise with state-of-the-art instrumentation, knowledge of the packaging materials and online compound databases – for example, Chemspider – often provides sufficient tools to allow for formulation of an identification hypothesis.

Case Study

The transformation of an unknown extractable detected by HPLC-MS into an identified compound is demonstrated by the following example:

Drug Product in Polypropylene Primary Packaging

LC-MS data analysis (using MassHunter Qualitative Analysis Software) revealed an unknown compound detected at a significant concentration in an extraction solution placed in contact with the polypropylene primary packaging of a liquid drug product at 70°C for 24 hours.

Since the analysis was performed using a quadrupole-time-of-flight mass spectrometer, based upon its high-resolution performance, it was possible to determine the most probable molecular formula: $C_{40}H_{60}N_2O_8$ (MW 696.434). In particular, in electrospray ionisation (ESI) positive mode, the most representative positive-charged ions of $C_{40}H_{60}N_2O_8$ were the ($NH4^+$)-adduct (m/z 714.469) and (Na^+)-adduct (m/z 719.424). In ESI negative mode, the characteristic negative-charged ion was the corresponding deprotonated molecule $C_{40}H_{60}N_2O_8(-H^+)$ (m/z 695.427) (see Figure 1).

Unfortunately, it was not possible to identify the exact molecular structure of the compound, despite knowing the probable empirical formula. Searching the molecular formula in the Chemspider online database, a useful list of known molecules can be obtained; however, it is still unclear whether this unknown extractable was one of them (3).

Compound Identification

One candidate listed in the Chemspider search possessed functional groups related to common plastic additives from the Irganox family, and a quick search by

Figure 2: Naugard XL-1 product ion in ESI positive mode

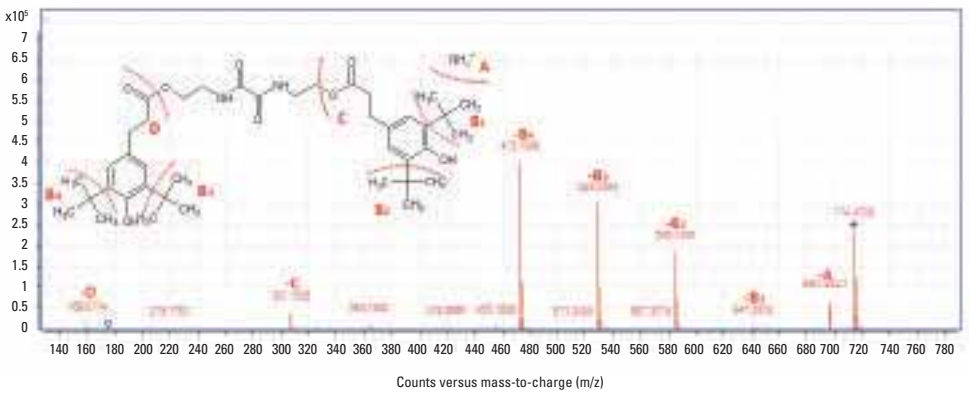
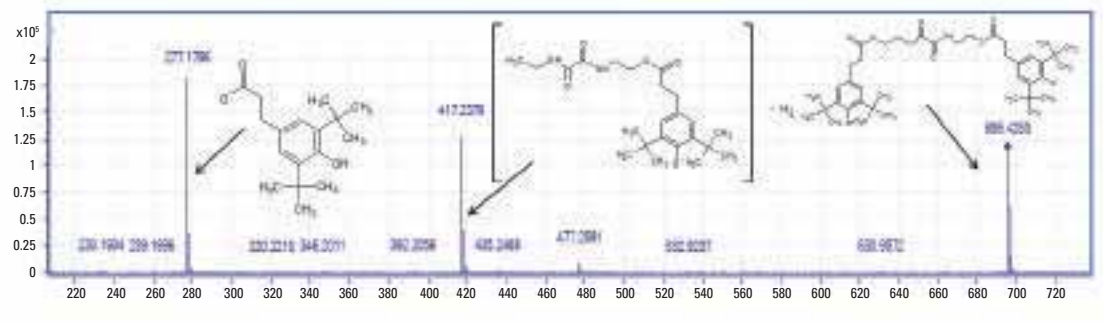


Figure 3: Naugard XL-1 product ion in ESI negative mode



Counts versus mass-to-charge (m/z)

CAS number identified it to be Naugard XL-1. Further evaluation showed that this is used in plastic manufacturing, serving as an antioxidant. In order to strengthen the case for identifying the unknown compound as Naugard XL-1, further fragmentation studies need to be performed using LC-MS/MS. This technique allows for generation of daughter ions of the unknown, providing information on the fragmentation pattern of the parent compound. Assessment of this information allows an experienced analyst to determine if the fragmentation pattern observed, along with the accurate mass of the parent ion, is consistent with the tentatively identified compound.

Figure 2 (page 11) shows the MS^2 spectra generated in ESI positive mode. The ($NH4^+$)-adduct of $C_{40}H_{60}N_2O_8$ was the most prominent positive-charged ion. When fragmented using two different collision energies (CE 15-25V), it showed a characteristic fragmentation pattern based firstly on the loss of $NH4^+$ ion ($C_{40}H_{60}N_2O_8(+H^+)$ (m/z 697.442)) and then on the progressive loss of the four tert-butyl functional groups (C_4H_8; m/z 56.062).

This resulted in the following consecutive fragments:
$C_{36}H_{52}N_2O_8(+H^+)$ (m/z 641.381);
$C_{32}H_{44}N_2O_8(+H^+)$ (m/z 585.318);
$C_{28}H_{36}N_2O_{8(}+H^+)$ (m/z 529.256);
$C_{24}H_{28}N_2O_8(+H^+)$ (m/z 473.193).
The remaining positive-charged molecular fragment $C_{24}H_{28}N_2O_8(+H^+)$ (m/z 473.193) was further reduced by the loss of an hydroxyphenyl-propionate functional group ($C_9H_9O_3$-) to $C_{15}H_{18}N_2O_5(+H^+)$ (m/z 307.130).
In the end, another covalent bond in the molecule was broken, and the loss of $C_9H_8O_2$ produced the last characteristic fragment of Naugard XL-1 ($C_6H_{10}N_2O_3(+H^+)$; m/z 159.077).

Figure 3 displays the MS^2 spectra generated using ESI negative mode. The deprotonated Naugard XL-1 $C_{40}H_{60}N_2O_8(-H^+)$ (m/z 695.425) was fragmented into two characteristic molecules due to the loss of a 3-[4-Hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoate group ($C_{17}H_{26}O_3(-H^+)$; m/z 277.179), which left the remaining molecule: ($C_{23}H_{35}N_2O_5(-2H^+)$; m/z 417.237). This characteristic fragmentation was confirmed by using two different

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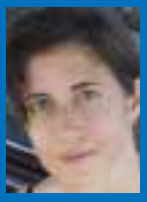

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collision energies (CE 15-25V). The fragments detected theoretically agree with the potential daughter ions expected from Naugard XL-1.

Final Confirmation

In addition, MS^2 spectra – both in ESI negative and positive mode – were selected and transferred from MassHunter Qualitative Analysis Software into MassHunter Molecular Structure Correlator (4). By using this software, it is possible to obtain all structures that match the selected formula – parent compounds plus confirmed fragments – from a selected database (like Chemspider), with all candidates being sorted by a percentage score. The correlation result with the highest score matched the Naugard XL-1 compound.

The last step in confirming the extractable was, in this case, purchasing the reference standard to allow for analysis by LC-MS to check the identification based on both retention time and mass spectra. If not commercially available, synthesis of the compound or isolation by preparative HPLC would have been necessary.

Utilising Expertise

Identification of unknown extractable compounds is usually not as simple as in the above example, with investigations often being time-consuming and potentially having an important economic impact on the overall project cost. Drawbacks and challenges that may be encountered during the process include compounds not fragmenting into significant daughter ions (for example, fatty acids), or the Chemspider output list showing hundreds of compounds matching an accurate mass.

In conclusion, these all underline the necessity of a systematic analytical approach; the need for a customised extractables database that houses a collection of all acquired knowledge; and human expertise and intuition – which often represent the most precious and efficient of resources.

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