Extractable & Leachable studies – Meeting regulations by LC-MS

White Paper

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Introduction

The materials used throughout the life sciences industry range widely, but include metals, plastics, and glass. One can get the impression these materials are inert and play no part in the integrity of the pharmaceutical products. The reality is the interaction of any of these materials with the product allows for the possible movement species from that material to migrate into the product, raw material, or reagent. As these migrating species can have a detrimental impact on patient safety, they must be evaluated through a risk assessment and potentially through analytical testing as well.

Extractables and leachables (E&L) are potentially harmful species that have the potential be administered to a patient with a drug. It is therefore vital that extractable and leachables are tested for and controlled. These species may be either introduced during the drug manufacturing process (during interaction with specialised equipment such as reactors, mixers and filtration systems) or during packaging (interaction of the drug product with the primary packaging such as bottles, blister packs and vials or secondary packaging such as labels, ink etc).

Plastic is frequently used in the pharmaceutical and medical device industry due to the wide variety of polymers that can be molded into an infinite number of shapes and sizes. The polymers most frequently used for these applications are made of polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyamide (PA), polyethylene terephthalate (PET), and ethylene vinyl alcohol (EVOH)[1].

All materials used during the production of a drug product or medical device must meet strict quality standards and regulatory requirements to ensure the safety and efficiency of the final product.

Extractables are defined by the US Pharmacopeia (<USP 1663>) as organic and inorganic chemical species that can be released from a pharmaceutical packaging/ delivery system, packaging component, or packaging material of construction under laboratory conditions including extraction solvent, technique, stoichiometry, temperature, and duration. Extractables themselves, and/or substances derived from extractables, have the potential to leach into a drug product formulation under normal conditions of storage and use[2].

Leachables are defined by the same pharmacopeia (<USP 1664>) as foreign organic and inorganic chemical entitles that are present in a packaged drug product because they have leached into it from a packaging/ delivery system, packaging component, or packaging material of construction under normal conditions of storage and use or during accelerated drug product stability studies. Leachables are present in a packaged drug product because of the direct action of the drug product on the source of the leachable[3].

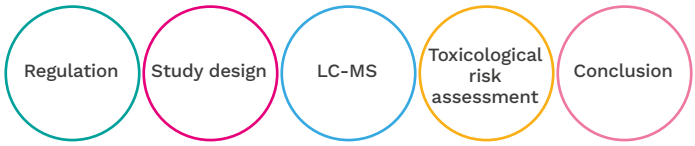
Currently, no internationally harmonised guidance exists on E&L assessment and control that specifically outlines the identification, qualification, and reporting thresholds for E&Ls in multiple therapeutic modalities and dosage forms, including addressing safety assessment. It is therefore important to follow guidelines developed by the the US Food and Drug

Administration (FDA), the US Pharmacopeia (USP – <1663>, <1664>, <1664.1>), the (EMEA – ICH-M7) and organisations such as the Product Quality Research Institute (PQRI), to support pharmaceutical industries to make their regulatory submission a success. For medical devices, ISO 10993-1:2018 (Biological Evaluation of Medical Devices) guidelines are followed to ensure all aspects of the required analytical testing is performed and contextualised regarding the risk to the patient.

E&L studies utilise analytical techniques such as liquid chromatography coupled high resolution mass spectrometry (LC-MS) for the detection and identification of polar and semi-polar species, Gas Chromatography-Mass Spectrometry (GC-MS) for volatile species and Ion Couple Plasma-Mass Spectrometry (ICP-MS) for elemental impurities. These orthogonal techniques provide the ability to detect a spectrum of organic and inorganic species. Ion chromatography for the analysis of anions and cations, can be included if a specific risk if highlighted.

This white paper outlines the study design framed by the regulation and the importance of employing LC-MS to determine unknown organic species towards the successful toxicological risk assessment of these species. The aim of an E&L study is to assess the safety of the packaging or manufacturing materials used in the production of a drug product or medical device and answering an ultimate question – how safe is the material?

The main sections of this white paper are highlighted below:



Regulation

In May 1999 the FDA released the guidance "Container Closure Systems for Packaging Human Drugs and Biologics", addressing the review and evaluation of packaging requirements. According to this document, each New Drug Application (NDA) or Abbreviated New Drug Application (ANDA) should contain enough information to demonstrate that a proposed container closure system and its components are suitable for its intended use[4].

Two factors are described that aid in determining the E&L risk for a packaging, with the table below illustrating their correlation. They include the degree of concern regarding the route of administration and the likelihood of packaging component-dosage form interactions, listing examples of different classes of drug products.

Degree of concern associated with the route of administration	Likelihood of Packaging Component–Dosage Form Interaction		
	High	Medium	Low
Highest	• Inhalation aerosols and solutions • Injections and injectable suspensions	• Sterile powders and powders for injections • Inhalation powders	
High	• Ophthalmic solutions and suspensions • Transdermal ointments and patches • Nasal aerosols and sprays		
Low	• Topical solutions and suspensions • Topical and lingual aerosols • Oral solutions and suspensions	• Topical powders • Oral powders	• Oral tablets • Oral capsules (hard and soft gelatin)

Table 1: Examples of packaging concerns according to the FDA.

As a response to the FDA, several industry guidance documents have since been issued by bodies such as the USP (<1663> and <1664> and <1664.1>), the EMEA (ICH-M7) and industry groups such as the PQRI, providing valuable information when carrying out extractable and leachable studies.

Recent regulatory developments in medical devices, namely the publishing of ISO 10993-1:2018 (Biological Evaluation of Medical Devices) has been created with the aim of protecting humans from potential risks arising from the use of these products.[5]

Considering all the above regulations, a risk-based approach in the design of the E&L study allows the most appropriate analytical data to be generated, ensuring a successful drug product launch.

Setting the threshold

A significant aspect in designing an E&L study is the determination of the level, or threshold, that underlines the risk of species migrating and their toxicological impact on the patient. The next step is for the concentration detection limit to be calculated ready for analytical assessment. The analytical evaluation threshold (AET) is defined as the threshold at or above which a component should be identified and toxicologically assessed[6].

The AET is calculated using the following equation:

$$\text{AET} = \frac{\text{TTC or SCT or QT}}{\text{Maximum daily dose}}$$

The acronyms are defined below:

TTC: Threshold of Toxicological Concern. The total duration of exposure is a key factor impacting on the probability of any carcinogenic outcome. The ICH M7 recommended limits for daily intake of mutagenic impurities are shown below[7]:

Duration of treatment	<1 month	>1 to 12 month	>1 to 10 years	>10 years to lifetime
Daily intake (TTC) (µg/day)	120	20	10	1.5

Table 2: Acceptable intakes for an individual impurity.

SCT: Safety Concern Threshold is defined as the threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and noncarcinogenic toxic effects. The SCT of 1.5 µg/day for parental drug products is recommended by the PQRI.

QT: Qualification Threshold is defined as the threshold below which a given leachable is not considered for safety qualification (toxicological assessment) unless the leachable presents structural activity relationship concerns. The QT of 5 µg/day for orally inhaled and nasal drug products has been also established by the PQRI.

The TTC, SCT or QT are utilised to establish the AET concentration when conducting E&L studies. Species that are determined to be below the AET are considered of having no potential to affect the quality of the product and are not interrogated further.

Species that are analytically determined to be equal or above the AET concentration are specifically evaluated and investigated. The species require identification and merit further toxicological assessment. Figure 1 below illustrates the chromatographic peak above and below the AET concentration.

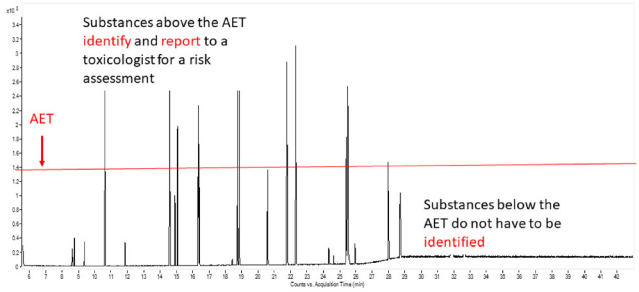


Figure 1: The AET is the limit at or above which extractable and leachable species require toxicological assessment.

The **AET** is a critical parameter in both extractable and leachable studies as it helps to ensure that potential migrating species are accurately identified and quantified. If the AET is too high, there is a risk that a low level leachable may be missed, potentially leading to safety issues. On the other hand, if the AET is too low, there can be a high level of data analysis with the risk of false positives, unnecessary investigations, and less focus on those species that pose the highest risk. Therefore, extra precaution should be given when setting the AET.

Study approach

a. Designing the study

Extractables studies and leachables studies are traditionally intertwined, however they are very specific studies.

The aim of an extractable study is to provide a comprehensive evaluation of the material or component, looking at which species have the potential to migrate. Therefore, the study design requires the inclusion of extraction conditions that ensure the species are extracted into solution without any material degradation. A failed extractable study may generate degradation products from the packaging (providing a fault alert for the extractable profile), or may not be aggressive enough (providing limited information on the extractable profile of the material).

The extractable study design determines the appropriate worst case extraction conditions that mimic the conditions under which the product is used. There are numerous factors taken into consideration, namely determining the extraction solvent, the extraction time, temperature, and the technique (i,e. reflux, sonication, incubation etc). Ensuring suitable conditions are set is key for a successful extractable study.

The mode of the extraction is dependent on use of the material. Normally, aggressive techniques such as reflux and sonication at elevated temperature are preferred for materials with long contact time and of rigid construction. Incubation is more likely to be suitable for materials with short contact time and a more flexible construction. The extraction solvent represents a worst-case scenario of the drug product and governs the analytical technique to be used. Two properties are mimicked with the choice of solvent, namely pH and hydrophobicity. Aqueous solvents (at neutral, low or high pH) and a mixture of solvent of different polarities (for example 50/50 % v/v water/isopropanol) are normally preferred for analysis by LC-MS. Figure 2 depicts the different extractable profile obtained after applying extraction solvents of different polarities.

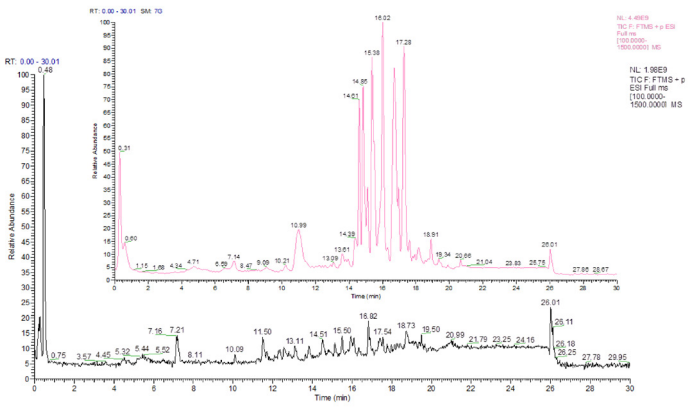


Figure 2: Total Ion Chromatogram (TIC) of a plastic material after being extracted with water (black) and water/isopropanol 50/50 (pink).

The leachable study is traditionally performed by the analysis of the finished product with the aim of determining what species has migrated. The finished product is then analysed over the duration of its agreed stability, using methods including LC-MS.

The calculated AET, using the equation in the 'Setting the Threshold' section, is used. Industry expectations appear to include an uncertainty factor, normally 50% when analysing by LC-MS. The reasoning is based on the phenomenon that the response of every molecule detected by LC-MS can vary. Whether a leachable or an extractable study, the aim is to detect unknown species and their response.

In this situation, the 50% uncertainty factor – which scientific evidence suggests to be most appropriate – appears to be lacking. Given the original calculated AET uses the maximum daily dose and a SCT (using a worst-case ICH-M7 exposure), an alternative approach would be to utilise a careful selection of known leachables within a threshold standard that would complement the process of identifying of the unknown migrating species.

Another aspect of a leachables study is ensuring the LC-MS analytical method is compatible with the finished product formulation. During this method verification process, a sample preparation protocol is developed that provides sufficient recovery for targeted known leachable species of multiple functionalities (for example: phthalates, plastic additives, sulphates, amines etc). Sample preparation depends on the technique and might include a simple dilution or precipitation (LC-MS) to complicated liquid-liquid extractions (GC-MS) or even digestion (ICP-MS analysis).

b. Employing LC-MS for analysis.

LC-MS has emerged as a technique of choice for polar and semi-polar extractables and leachables due to its high sensitivity for trace components within a mixture and its ability to unambiguously identify these trace components via accurate MS and MS/MS data.

The aqueous extractions (from the extractable study) or the stability samples (for the leachable study) will be tested by LC-MS against control samples (extractable study – a procedural extraction solvent, leachable study – the drug product that has not been in contact with the packaging). The importance of a control cannot be overlooked. An ideal control allows system interference and finished product degradation in a leachable study to be easily mitigated giving the analyst a filtered data set in which to probe.

A reversed phase chromatography is employed with a gradient elution program starting from a low organic range and reaching 95% organic at a total run time of 30 min. This ensures elution and chromatographic separation of polar smaller species and semi-polar heavier components.

The HPLC system is coupled with a UV unit to detect species that are strong UV responders and to either a high resolution MS (screening of unknown, components characterisation, and semi quantitation), or to a triple Quadrupole (targeted analysis).

A Thermo Q-Exactive Plus Hydrid Quadrapole-Orbitrap and an Agilent 6545 Quadrapole Time Flight are the employed MS instruments for screening of unknown species with both offering high mass accuracy – a powerful tool for the determination of the molecular weight of the unknown species. Both mass spectrometers are equipped with an an Electrospray Ionisation (ESI) or an Atmospheric Pressure Chemical Ionisation (APCI) source. Data is acquired in both ion polarities to enable the identification of components of non-volatile components with acid and/ or basic properties.

A full scan and/or a full scan-dd2 experiment are employed, covering a scanning range of m/z 50–1500. The wide scan range allows the detections of components of a various mass to charge (m/z), while the data dependent experiment enables the collection of fragment ions which will then aid the identification.

Identification by LC-MS

Data interpretation is the key and the most challenging stage for identifying extractable and leachable species. Any species determined to be on or above the AET merits careful investigation.

The use of processing software is customary, such as Thermo Compound Discoverer, which provides a wealth of features to ensure all mass ions uniquely

found in a sample compared to a control is extracted and its abundance compared to the calculated AET. Furthermore, the mass ions can be grouped as one species – multiple ions are commonly observed for one species – and compared to existing LC-MS databases.

A complete evaluation of the species migrating into a finished product requires the remaining observed species that cannot be identified via a LC-MS database to be manually investigated. This is more likely in LC-MS data within extractables and leachable studies, as LC-MS databases are still limited and the interaction of the material with the finished product does yield unique compounds – reaction with an excipient has been observed.

In cases wherein structural elucidation is required, LC-MS provides a considerable amount of information. Accurate mass and isotopic pattern, in conjunction with observed adducts, can give you a highly likely proposed molecular formula. Further, fragmentation of those mass ions frequently bears structural information. Taken into context of the sample analysed, namely the known API and excipients, and the packaging material, there is a likelihood that the unknown species can at least be partially identified.

This information may be sufficient for the toxicologist to evaluate the toxicity of that type of compound, thereby providing the necessary information to achieve the overall goal – the risk posed by the material use to the patient.

Toxicological assessment

It is important to note that not all leachable species detected above the AET are necessarily toxic or pose a significant risk to patient safety. The toxicity of a leachable depends on a range of factors including its chemical structure, dose and duration of exposure. Therefore, toxicological assessment should be conducted on a case-by-case basis, taking into account the specific characteristic of the leachable.

The structural information data derived from the leachable study is vital for a successful toxicological risk assessment. This explains why the high-resolution MS is a necessary analytical technique when conducting E&L studies. By utilising an LC-MS, the number of unknown leachable species is reduced. When a species remains unidentified, information such as the accurate mass, isotope pattern, fragmentation may be useful for the toxicological risk assessment

The goal of the toxicological assessment is to determine whether the levels of leachables detected above the AET pose a significant risk to patient safety. If leachables are deemed to be of concern, appropriate measures may be taken to mitigate the risk, such as modifying the manufacturing process or using a different material.

Conclusion

The credentials of packaging and manufacturing materials play a vital role in ensuring the safety and efficacy of a drug product or medical device.

Regulatory agencies around the world require E&L studies to be conducted as part of the drug approval process to ensure that the product is safe for human use.

LC-MS has been determined to be one of the imperative analytical techniques when conducting E&L studies. The high-resolution MS is the preferred instrument for quantification and identification of aqueous organic species. Its high sensitivity and good mass accuracy enables the structural elucidation of species that migrate from the packaging into the drug product or from the medical device into the human body. This is important for E&L studies as it allows the toxicological assessment of the compounds and their potential impact on human health.

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Vasiliki holds a Chemistry degree, in addition to a Master of Science degree in Analytical Chemistry-Quality Control from the National and Kapodistrian University of Athens, Greece.

Vasiliki is passionate about E&L studies and has more than five years' professional experience in designing and conducting E&L studies, including method development, validation and structural elucidation.

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Joining RSSL in 2013, Daniel has taken a leading role in the delivery of our quantitative and investigative LC-MS techniques, including extractable and leachables and nitrosamines testing services.

With an undergraduate degree in Chemistry with Medicinal Chemistry from the University of Surrey, Daniel is recognised as a technical specialist in key analytical fields, developing E&L approaches to meet the challenges of an ever-changing industry.

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