LEACHABLES & EXTRACTABLES
IN PHARMACEUTICALS

QUALIMETRIX
Analytical Laboratories - Quality Control

Extractables and Leachables Testing

Definitions

"Extractables" are organic and inorganic chemical entities that can be released from a test article and into an extraction solvent under laboratory conditions. Test articles include packaging systems, delivery systems, manufacturing suites and/ or their associated materials or components of construction. Extractables themselves, or substances derived from extractables, have the potential to leach into a drug product under normal conditions of storage and use and become leachables. Thus, extractables are potential leachables.

"Leachables" are foreign organic and inorganic chemical entities that can migrate into the finished drug product from several potential sources, such as the finished drug product's manufacturing suite, packaging or delivery system and/ or their components, and construction materials under normal manufacturing conditions, storage and use.

QMX stepwise approach for the chemical assessment of Product / Packaging interactions

Qualimetrix is a customer-driven CRO that employs the Six Sigma philosophy in order to design and implement optimized processes with the aim of transforming customer inputs and requirements into "customer value". As such, the first and probably the most critical factor for a successful project is its proper definition in terms of both customer and technical requirements. To this end, a comprehensive study request form is provided to the customer with the following objectives:

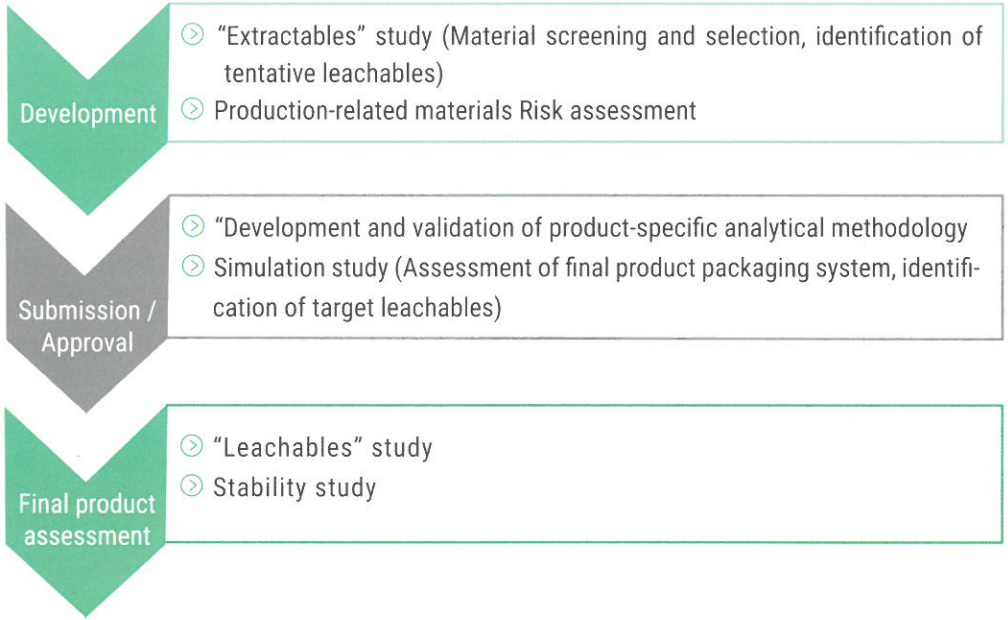
- The definition of the type and scope of the study
- The provision of critical product information
- The determination of the most suitable, expedient and cost-effective approach

Polymeric and elastomeric materials are commonly encountered during the manufacturing process of pharmaceutical products as well as components of the packaging / container closure system. During the product's expected shelf-life and use, the constant contact, as well as the stressing, may bring about a change in the composition of the product stored through interactions with its packaging.

Product packaging interaction studies focus on establishing this change in product composition brought about by the interplay of packaging and stored content through means of molecular exchange. This exchange involves the solubilization of compounds within the polymeric or metal matrix and their subsequent migration into the bulk of the stored product.

The main phases of interest in the product's life cycle that are relevant to drug-packaging interaction studies and hence to the safety assessment are schematically presented in Figure 1 and explained in more detail in the following paragraphs:

Figure 1: Product stage and associated studies / services provided by QMX



1. Development: The first operational step related to product-packaging interaction studies is the material screening process. During this process, all candidate materials are evaluated in terms of their available information. The means by which this initial evaluation is performed are compendial testing, which is usually conducted by the supplier and extraction studies in order to establish material composition.

QMX services: "Extractables" study

Controlled Extraction studies are of paramount importance in order to:

- ✔ Characterize candidate materials and assess their suitability for use.
- ✔ Cover the safety gaps resulting from the lack of compendial testing or other material information that, in many cases, the suppliers are not eager to provide.
- ✔ Identify "tentative" leachables that could be employed as target analytes for the development and validation of a "product-specific" methodology for the determination of leachables.

The applied semi-quantitative generic methodology has been designed to cover representative leachables, designated by extraction studies of packaging materials available and which are commonly used in plastic manufacturing. The purpose of the initial screening of extractables is mainly to establish worst-case potential leachables profile for the product-specific packaging materials and facilitate the establishment of qualitative and quantitative leachable-extractables correlations.

Extraction techniques commonly employed for this initial step include but are not limited to the following:

- ✔ Maceration (solvent soaking)
- ✔ Reflux
- ✔ Soxhlet
- ✔ Sonication
- ✔ Sealed vessel

The profile of the extractable components is acquired by the use of leading edge, hyphenated, orthogonal **analytical techniques**, required to cover their significant chemical diversity (i.e. HRAM LC-MS/MS, GC/MS, GC/FID, ICP/MS, FTIR). Extractions that are not solvent-mediated can also be performed through the use of Headspace Gas Chromatography (HS-GC/MS) and Solid phase Microextraction Gas Chromatography (SPME-GC/MS).

QMX services: Production-related materials risk assessment

Preliminary assessment of the extend of component testing necessary in order to establish the suitability of plastic components involved in the manufacturing process stream (e.g. tubings, filters, connectors, etc.). The assessment is based on risk factors related to the nature and conditions of the contact between the product stream, the extraction propensity of the solvents used and the nature of the plastic materials.

2. Submission and Approval: This phase reflects a product that is fully defined and completely characterized with respect to leachables. This practically means that a leachables study has already been performed on the final product by employing a validated method in order to establish the product's leachables profile.

QMX services: Development and validation of product-specific analytical methodology

Based on the results obtained from the extraction study (or simulation study) previously performed, the generic methodology, comprised of the sample pre-treatment and analysis stages, is properly adjusted in order to become a "tailor-made" product-specific methodology targeting only the analytes / potential leachable species, identified during the extraction study that exceed or have the potential to exceed the product's Analytical Evaluation Threshold (AET) during the actual leachables study. The next step is to make this "tailor-made" method also "fit for purpose" by means of method validation according to the principles set by ICH Q2 (R1) guideline.

The validated product-specific methodology is subsequently applied in order to perform the actual "leachables" testing and provide reliable quantitative results for the leachables of interest.

QMX services: Simulation study (Assessment of final product packaging system, identification of target leachables)

However, since the leachables assessment should cover the product's shelf-life, it is rather hard to have relevant data available at the time of submission. To this end, simulation studies can be performed as a "surrogate" by submitting the final product to elevated temperature conditions in order to simulate the anticipated stressing effect at the end of shelf-life.

Moreover, simulation studies where the actual drug product is replaced by a solvent of equal or similar propensity can be performed in the following cases:

- ✔ Drug products with an extremely complex and challenging matrix (e.g. lipid emulsions) where a more "analytically expedient" sample needs to be produced for the evaluation of "leachables"
- ✔ Identification of "probable" leachables that could be employed as target analytes for the development and validation of a "product-specific" methodology for the determination of leachables. The advantage versus the extraction study is that the long list of "extractables" is significantly reduced and the target analytes are much more relevant since the simulation study mimics the conditions experienced by the final drug product

3. **Final Product Assessment and Maintenance:** This phase mainly comprises of the final and definitive assessment of the product at the actual end of shelf-life as well as issues that may arise from vendor-related raw material or compositional changes that may have an impact on the leachable species profile (change control).

QMX services: "Leachables" study

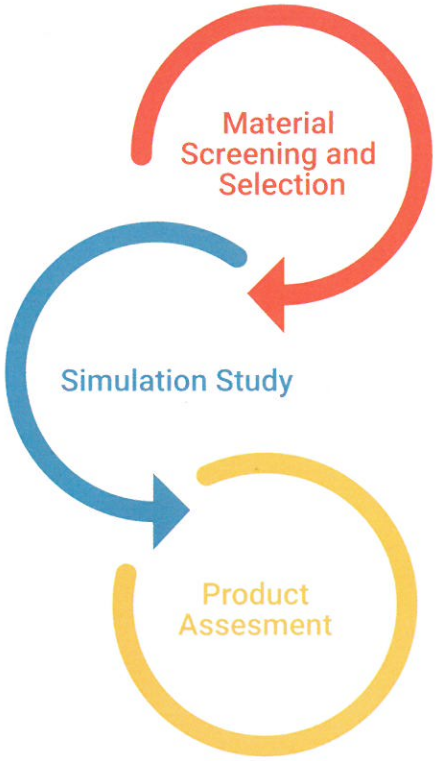
Application of the validated "product-specific" analytical methods for the quantification of leachables in the final drug product, stored under normal and accelerated storage conditions (e.g. ICH conditions) at the end of the product's shelf-life. Both target analytes, previously identified from extraction / simulation studies, and secondary leachables are monitored and determined.

QMX services: **Stability study**

Application of the validated methodology for the determination of leachables when on-going testing is required due to potential safety issues related to "leachable" species (e.g. inhalation aerosols and other OINDPs, for which leachables testing should be an integral part of the larger ICH registration stability program).

The different types of studies performed in order to evaluate the impact of interactions between a final product and its packaging across its lifecycle are schematically presented in Figure 2.

Figure 2: Steps employed in the chemical assessment of the interactions between a final product and its packaging

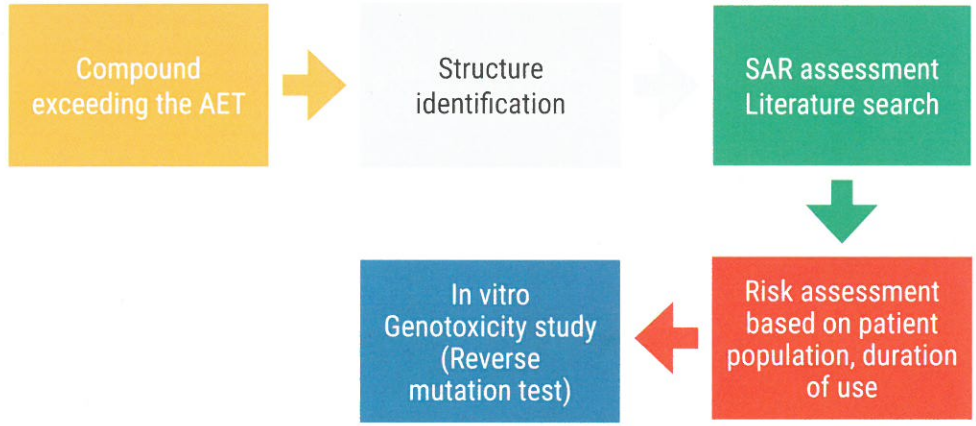


Safety assessment of E and L compounds

In the frame of "Extractables" and "Leachables" studies the major aspect of concern with respect to the "suitability for use" is the impact that the related compounds may have on patient safety. To this end, all compounds detected that exceed the Analytical Evaluation Threshold – AET (deriving from the Safety Concern Threshold – SCT) should be identified and toxicologically assessed.

Toxicological assessment is an optional complementary service offered both at the initial stage of "extractables" testing and at the stage of definitive product assessment during "leachables" testing. The advantage of performing the assessment prior to the "leachables" study is that the final list of "target compounds" will include only potential leachables that could adversely affect product safety. The steps following the detection of a compound that exceeds the AET are depicted in the following Figure.

Figure 3: Safety evaluation steps



More specifically, in case a compound exceeds the AET the following action plan is implemented:

- ✔ The compound's structure is elucidated to an extent that literature and Structure – Activity Relationship assessment can be performed.

- Literature search through all available databases (e.g. Toxnet) is performed in order to gather all information necessary for the assessment. SAR evaluation is performed "in-silico" by the use of the Cramer Classification Scheme (i.e ToxTree) and other available software.
- In case the assessment results in safety concerns with respect to potential carcinogenic toxic effects, a risk-based approach is applied based on the available data to evaluate the safety impact by considering the patient population and the duration of use.
- In case the risk exceeds the acceptable level an in-vitro bacterial reverse mutation test is performed as a minimum screen for the assessment of the compound's genotoxic potential.

Instrumentation / Software

Laboratory Infrastructure and Equipment

High Resolution Mass Spectrometer

The CRO possesses a cutting edge HRMS instrumentation by Thermo Scientific; Orbitrap Elite. It is a hybrid Ion Trap- Orbitrap Mass Spectrometer, with very high resolving power up to 240,000 FWHM, high speed, sensitivity and advanced fragmentation information.

Based on advanced signal processing on the detector and high velocity during scanning, it provides full advantage of a UPLC system, as well as advanced detection system and quantitation capabilities over a wide linear range. Multiple fragmentation techniques, including the possibility for MS^n fragmentation, can give a boost in the identification of unknown compounds, in the minimum analysis time.

Accurate mass measurements, together with distinct isotopic profile and fragmentation information can provide the means for structural elucidation and identification of possible leachable and extractable compounds.

Orbitrap Elite is employed in either ESI or APCI ionization mode and it is connected to a UPLC-PDA chromatographic system.

In order to fully take advantage of the great possibilities of this instrumentation, powerful software are employed for the detection, identification and structural elucidation of analytes.

- Mass Frontier (Thermo Scientific):

It is a spectral interpretation software, that provides structural elucidation based on in-silico fragmentation rules and spectral annotation.

SIEVE (Thermo Scientific):

A highly sophisticated software (e.g. mZCloud and XCMS) is employed, based on the needs of each study, as well as mass spectral libraries (MassBank).

✔ UPLC-MS/MS (triple quadrupole, QqQ)

A triple quadrupole combined with a UPLC chromatographic system, together with a PDA detector, by Shimadzu, is also employed within the CRO.

Triple Quadrupole is the technique of choice for a reliable identification and quantitation of already known analytes. Through the Multiple Reaction Monitoring (MRM) mode, it provides higher Signal-to-Noise, allowing thus selective and sensitive identification and quantitation, as well as wide linear range.

This technique is widely applied for the determination of polar and semi-polar analytes.

Chromatographic separation is achieved with a wide variety of analytical columns, based on different interactions, which is wisely selected according to the nature and the needs of the study.

✔ GC-MS

Gas chromatography combined with a quadrupole mass analyzer (GC-MS) is available within the CRO. It is applied for the determination of volatile & semi-volatile compounds. An electron ionization source is employed (EI) in order to achieve the fragmentation of the eluted compounds producing characteristic patterns used for the tentative identification of analytes by NIST similarity matching.

Substitution to a headspace autosampler unit allows for the profiling of highly volatile species.

In the case of Solid phase Microextraction Gas Chromatography, desorption of the analytes takes place and analysis is carried out. SPME is a time- and solvent- saving analytical technique for liquid and gas matrices, for the determination of both volatile and non-volatile analytes.

✔ ICP-MS

The NexION 350 of Perkin Elmer, employed at our CRO, provides exceptional stability and productivity, as it includes an array of technical innovations that reduce background and interferences, optimize signal stability, minimize maintenance requirements and downtime and generate better results.

The "Triple Cone Interface produces a focused ion beam and prevents sample deposition on internal components. Quadrupole Ion Deflector turns positively charged ions 90° into the Universal Cell and filters off neutrals.

The biggest advantage of NexION is the possibility of 3 different operational functions, depending on the nature of the analysis and the matrix interferences.

Standard mode: The system works like a non-cell instrument.

Collision mode: A non-reactive gas is introduced into the cell to collide with interfering ions and remove interferences through Kinetic Energy Discrimination.

Reaction mode: A highly reactive gas is introduced into the cell to create predictable chemical reactions. Any side reaction is removed by the scanning quadrupole, so that only the target-element is reaching the detector.

Sample pre-treatment, which is a critical step in elemental analysis is performed in a Laminar Flow Work Bench, Heraguard ECO by Thermo Scientific, while for sample digestion, a Closed-vessel microwave system is employed by Milestone, ETHOS UP.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