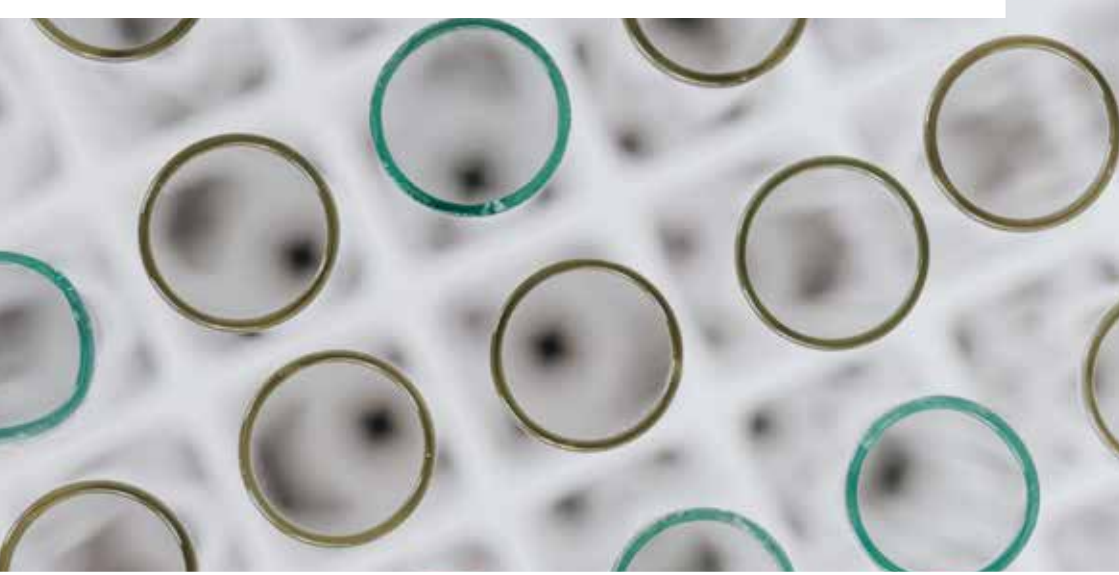
QUALIMETRIX
Analytical Laboratories - Quality Control

Migration studies in pharmaceuticals

by QUALIMETRIX

Contents

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.

"Analytical screening method" is a method whose purpose is to discover, identify and semi-quantitatively estimate the concentration of all relevant but unspecified, analytes in a test sample above an established reporting threshold (e.g. AET)

"Analytical targeting method" is a method whose purpose is to quantify, with an appropriately high degree of accuracy and precision, specified analytes in a specified test sample over a specified concentration range

"Targeted screening method" is a hybrid between the target and screening methods as it retains both the wide scope nature of a screening method and the more specific of a targeted for the individual migrants of interest.

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

Qualimetrix is a customer-driven Testing Laboratory that employs a structured and well-defined approach 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

The following figure presents a project setup; meaning the logical process by which a series of studies may be proposed to a sponsor.

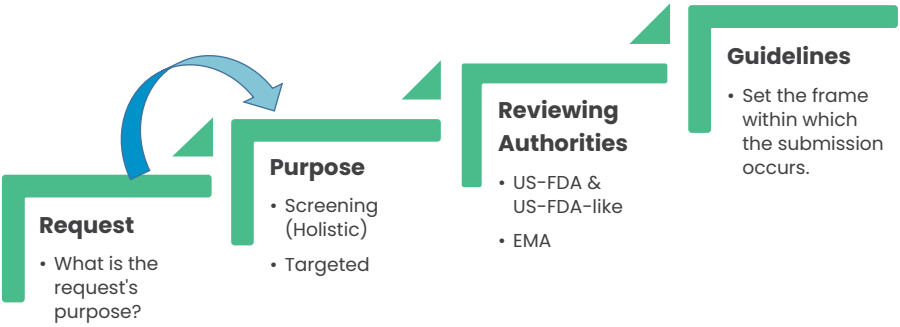


Figure 1: A diagram that depicts the logical process for arriving at a suitable study design based on the initial request and the information provided

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 2 and explained in more detail in the following paragraphs:

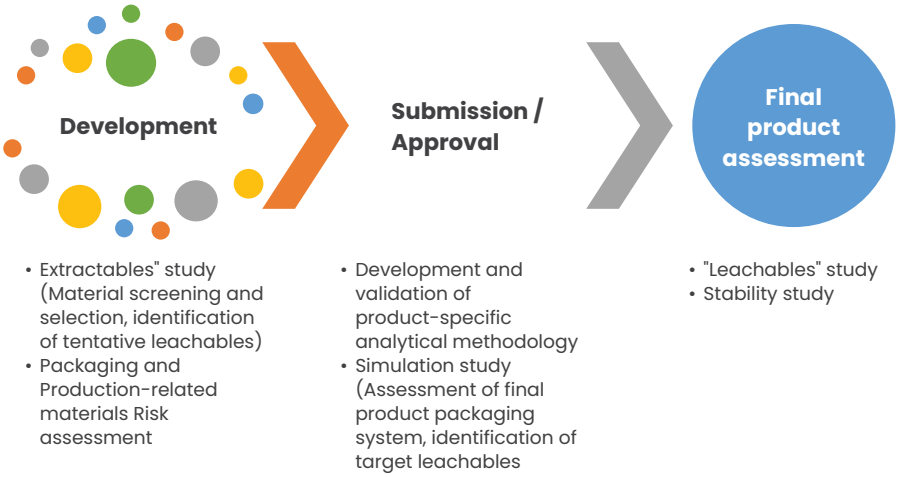


Figure 2: Product stage and associated studies / services provided by QMX

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 risk assessment and gap analysis, compendial testing, which is usually conducted by the supplier and extraction studies in order to establish the material composition and mitigate any information / data gaps.

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 a "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 (e.g. LC-HRMS, GC/MS, GC/FID, ICP/MS etc.). Extractions that are not solvent-mediated can also be performed through the use of Headspace Gas Chromatography (HS-GC/MS).

QMX services: Packaging and Production-related materials risk assessment

A preliminary assessment of the extent of component testing is necessary in order to establish the suitability of plastic components involved in both packaging and 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.

Recently, a framework for the risk assessment conducted for leachables in pharmaceuticals has been introduced. The Extractables and Leachables Safety Information Exchange (ELSIE) group has proposed such a framework, based on the concepts of the ICH Q9 Quality Risk Management guideline, while the general chapter of USP–NF 2022, Issue 1, <1665> proposes a framework for the risk evaluation of production related materials prior to the design of

studies that address this risk or gaps in data that hinder the risk evaluation process.

An evaluation strategy and a risk rubric proposed in relevant scientific literature has been reviewed, evaluated and properly amended by our scientific team, in order to form the "backbone" of risk assessment for a pharmaceutical product's leachable species profile.

The actions that need to be taken for risk mitigation / reduction and acceptance are presented within the concluding section of the risk assessment report.

The Risk Assessment exercise for Leachable Species in Pharmaceutical Products" is a very demanding process considering that it is not restricted to data presentation but also inference based on existing data. Depending on the reliability of the existing data and/ or constraints regarding the conditions/ processes/ materials that allow safeguarding quality, the risk assessment process may limit or even waive the required testing. For example, a low maximum dose pharmaceutical product, with a composition that does not exhibit a high propensity for leachable species solubilization, is likely to arrive to a "leachable species profiling" waiver through the risk assessment process – making the risk assessment procedure very cost-efficient. A high dose liquid injectable product on the other hand may even require testing through its shelf-life for determining the kinetics of migration and modifying the shelf-life appropriately so as to mitigate the risk of critical exposure.

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.

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 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. On occasion, the authorities may express a request that is actually targeted on a specific substance or a group of substances. Based on the request, this may fall either under screening studies or targeted studies.

Typical examples include the following:

- **OVIs – Organic Volatile Impurities:** This is usually triggered by the use of a rubber material in the product. It is addressed through a screening study but, as implied from the nomenclature, it is limited to volatile organic species.

- **Bis(2-ethylhexyl) phthalate (DEHP):** A known endocrine disruptor for which a high exposure of the general population is suspected. The request is usually triggered by the use of PVC materials in either manufacture, storage or even dilution of a pharmaceutical product. This is a targeted request.

- **Benzophenone:** A known potent photosensitizer species. The request is triggered by the submission of data on labeling to the authorities, that implies or states the use of a benzophenone or acetophenone photoinitiator. Depending on whether the data declare the exact compound used or not, the study may be treated as a targeted study or a screening study.

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)

QMx approach for the Identification and Quantitation of E&L species

The two main aspects that are crucial for conducting a compatibility assessment are the detected species identity, since their chemical structure is linked with biological activity, including toxicity and concentration, as the magnitude of any biological effect is directly related to the patient's exposure.

The task of identifying an extractable / leachable species is hindered by the background response. This is especially true in leachable species profiling studies in the presence of product components and their degradation products. The selection of a proper control for discrimination between product-related substances and leachables is thus critical to the process of identifying the latter. Another issue is the proper attribution of the species to a source material. While leachable species profiling could proceed with a cumulative profile, the ability to identify the source, as noted in earlier sections, provides opportunities for addressing the risk associated with them. In this scenario, leachables arising from a previous step need to be disregarded when evaluating the profile at subsequent stages of the product cycle i.e. disregarding production line leachables in the process of determining product packaging related species.

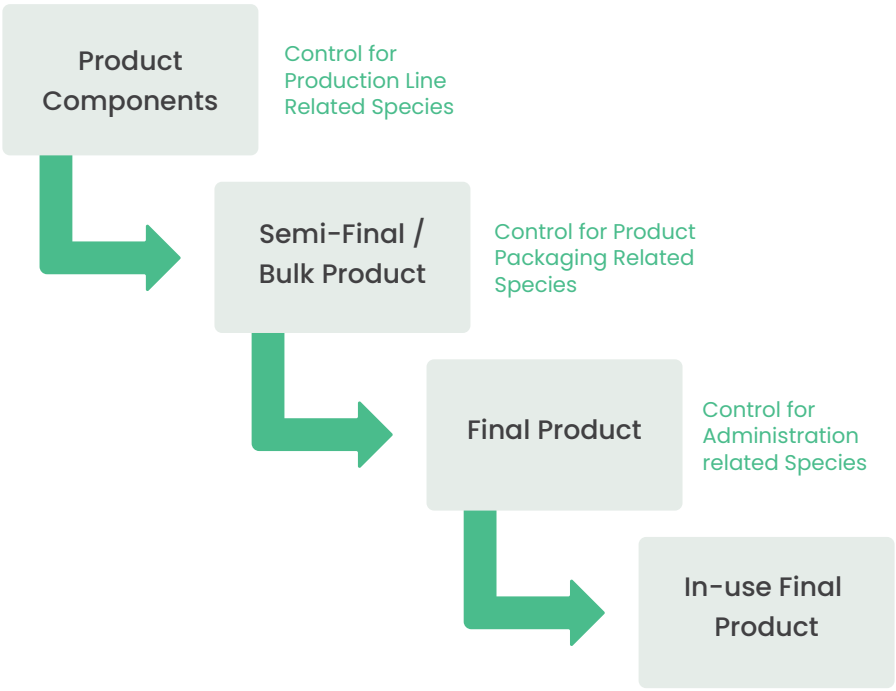


Figure 3: Scheme for the selection of proper control

Based on the above, one challenge that needs to be effectively tackled prior to proceeding with identification is to isolate and focus on the species of interest, especially during leachables testing due to the complexity of the final product matrix. The latter may produce a multitude of analytical responses that interfere with, mask, or obscure the analytical responses associated with leachables. Data acquired in mass spectrometry, when working in full scan analysis are a lot more complex than data acquired by most other detectors. This is because the chromatogram is composed of a plethora of overlaid chromatograms – which in the case of a high-resolution mass analyzer they could amount to millions. Human-based evaluation is observation dependent. However, it would be very difficult, if not impossible, for a person to observe minor differences in the TIC (total ion chromatogram) so as to discover something present, and it would be impossible to go through all possible ion

chromatograms. As a result, a reliable process of ppm-level analyte detection should be software driven.

Qualimetrix employs a highly sophisticated software algorithm for processing that encompasses all processes that may take place to lead from alignment, to feature detection, consolidation and identification. The different steps of processing workflows aim to successively reduce the complexity of analytical data. The algorithm has been fine-tuned and evaluated in terms of "sensitivity", "specificity" and "overall concordance" so as to establish its suitability for performing the **differential analysis** between the profiles of the control samples and the respective samples.

When an extractable or leachable species is detected, the next step is to assess its potential safety impact. To this end, it is necessary to properly establish its identity and concentration with the lowest degree of uncertainty. Of course, not every species eliciting a response needs to be assessed in terms of its safety impact but only those exceeding a dose-based threshold (DBT) such as the ones established by the PQRI in order to cover both carcinogenic and non-carcinogenic effects (i.e. **Safety Concern Threshold – SCT and Qualification Threshold – QT**). In order for those DBTs to be meaningful from an analytical perspective, a conversion to a concentration-based threshold is necessary. The latter is known as the **Analytical Evaluation Threshold (AET)** and defines the level below which the analyst needs not identify or quantify leachables or extractables or report them for toxicological assessment.

Quantitation in extractables or leachables testing can be performed by means that exhibit different degrees of uncertainty with regards to the estimated concentration depending on the selected approach. The quantitation exercise in the frame of an analytical screening or targeted screening method is a two-step process. The aim of the first step (i.e. preliminary quantitation) is to conclude on the species that need to be further processed and identified while the second step focuses on providing a more accurate quantitative estimate.

A commonly employed strategy is that of a surrogate standard that is used to normalize the responses obtained for the detected species and estimate their concentration in the test sample. This approach, however and despite the fact that it compensates for recovery losses and instrument response variability, is

based on the simplifying assumption that all analytes respond similarly among themselves and with respect to the surrogate standard. Unfortunately, this is far from the actual reality since the analytical response significantly varies across the universe of the chemically diverse E&L compounds. A consequence of this variation is that the AET becomes potentially less "protective" for compounds with low response factors considering that these would be falsely estimated to lie below the AET and therefore not reported and submitted to the toxicological evaluation process.

To this end, the AET should be properly adjusted in order to mitigate the risk emanating from these "low-responding" species. This adjustment is achieved by means of the **Uncertainty Factor (UF)** which is included in the calculation of the AET to account for the analytical uncertainty associated with the variable responses. However, the default UF of 2, recommended by the PQRI, is not sufficiently protective, especially for non-volatile compounds detected by means of HPLC – MS, given the significant variability in their ability to elicit a response.

In order to mitigate the above risks and establish the appropriate level of protection, our approach in terms of this preliminary quantitation includes the following:

- The establishment and proper selection of one or more representative and "protective" (in terms of response) internal standards that are introduced in order to perform the initial estimation.

- The establishment of a value for the uncertainty factor, based on the database of internal standards to ensure adequate coverage (i.e. flagging of the vast majority of compounds whose true concentration is above the AET) and minimize the risk of "missing" potentially toxic compounds.

This stage concludes on the species that need to be further processed in terms of identification and semi-quantitation.

Identification is typically an integral part of a chromatographic screening process employing mass spectrometry, aiming to recognize any unknown compound that could present a potential safety risk. In general, a compound

present above a specified threshold is considered confidently identified when it can be assigned:

- a proper chemical name
- an appropriate identifying number (e.g. a CAS registry number)
- a structure with an acceptable degree of confidence

A compound's identity is based on the interpretation of available analytical data. By increasing the amount of corroborating data as well as the relevant information available, the confidence in identification increases. To this end, different identification classes have been described in the USP monograph on extractables, as shown in the following figure.

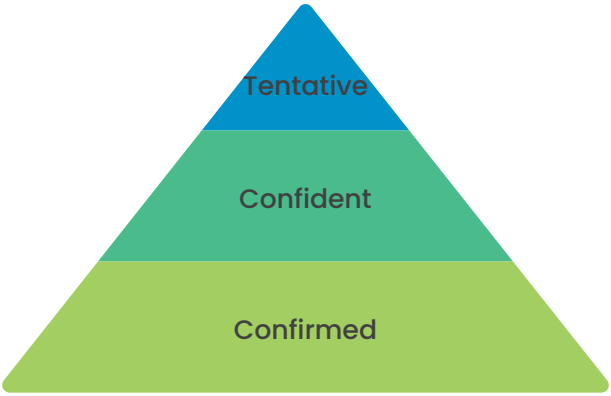


Figure 4: Identification levels

The identification categories are assigned based on data obtained by MS, as well as other analytical techniques, expert assessment and available reference of information (i.e. spectral libraries).
More specifically, the information that can assist on the identification process include, but are not limited to the following:

a. Comparison with a reference standard, in terms of spectral (ion mass) similarity index and retention time match. MS/ MS fragmentation profile is also considered, when available.

b. Molecular weight

c. Elemental composition (molecular formula)

d. Fragmentation pattern/ mass spectral interpretation data

e. Mass spectral matching using automated library or literature spectrum

f. Supporting spectral information (e.g. NMR data)

g. Information regarding the composition of the test article

Each level of identification builds upon the last level by providing more confirmatory information as the level increases. The three main categories as described in the USP <1663> are the following:

Tentative identification: The data obtained are consistent with only a class of molecule. This identification category can be assigned when only a few pieces of information are available that allow for recognizing critical chemical moieties/ substructures. Due to the expected variability in the freedom for interpretation of existing data, the "content" of a tentative identification may vary between cases.

Confident identification: Sufficient chromatographic and/ or spectroscopic data exist to infer a specific identity, which are supported by confirmatory information. This would be the case of a tentative identification (by means of a and / or d) augmented by (b), (c), or (f), resulting in a two-dimensional identification that provides a specific structure and CAS number, where available. The degree of confidence increases according to the confirmatory information obtained, including relevant data as described in the scientific literature or by material supplier.

Confirmed identification: The compound in question can only be the one identified. It is based on corroborating data that are sufficient to infer

confident identification, which are also supported by comparison to an authentic reference standard (a). This identification level is considered as three-dimensional. A specific structure and CAS number, where available, is provided.

From the point of patient safety, a confirmed identification is the most desirable outcome and the ideal basis to proceed upon for qualification. Desirable, however, does not coincide with necessary or feasible. There are multiple cases of chemical substances that have been evaluated as a mixture, a group of similar species, etc. The reason that such practices are acceptable from a toxicological standpoint is that a relatively limited number of substances to which the human body is exposed are capable of toxicity based on a specific mechanism of action. Most effects are the result of the propensity of a substance to interact with a biological system in a specific manner; which entails only the presence of one or a few specific chemical moieties and chemical characteristics. Therefore, a confident identification may suffice for qualification purposes as long as it can be properly justified that the amount of freedom in structure that remains unaddressed bears no negative impact on the validity of the assessment.

Analysis based on **GC-MS** or **Headspace GC-MS (HS-GC-MS)** methodology employing an electron impact (EI) source takes advantage of the source's high reproducibility of fragmentation, for the identification. This ionization source employs the standard ionization energy of 70 eV, in order to fragment ions present in the gas phase in a reproducible way. The fragments produced have a certain relative abundance. The pattern produced is compared to the spectra contained in NIST spectral library, following the process termed *mass spectral matching.*

When the spectrum is submitted for similarity matching the following aspects are noted:

- The %Similarity Score between the query and the reference.
- The contents of the subtraction spectrum and their intensity relative to the peaks of the query spectrum.

A %Similarity Score of NLT 80%, in most cases, is accurate enough for the

tentative identification of a substance, however, on the simultaneous absence of non-attributed major ions, the fragments that are present in the query spectrum but absent in the reference should always alert the user; especially if the non-attributed ions exhibit a relative intensity of ≥ 10% to the prime ion or there are multiple of them.

The latter may also provide up to a confident identification level, depending on the similarity match and the quality of the data. Electron Impact is a highly potent ionization process. In all but the most rigid structures, ionization proceeds through multiple pathways of fission starting from the pseudomolecular cation radical towards cations and cation radicals of higher stability. This means that it is actually not possible to observe the pseudomolecular cation radical for most compounds; and, thus, knowledge of the molecular weight is not usually attained. The use of chemical ionization (CI) can supplement the data of electron ionization procedures in gas chromatography-coupled methods since it allows for the determination of the pseudomolecular cation or an adduct with the reagent ionization gas. This in turn facilitates the determination of the compound's molecular weight.

The acquired data are most likely to cover for requirements: (a) fragmentation pattern interpretation and/ or (d) similarity matching against a library. Some information can be attained on (b) molecular weight and (c) elemental composition through inference. With regards to (b) this is usually achieved through the correlation between volatility and MW, while inference pertaining to (c) is circumstantial and entirely dependent on the fragmentation data acquired and the chromatographic profile of the peak i.e. silicon, phosphorus (but not phosphates) and sulfur (but not sulfates) for example do provide distinct fragments for evaluation.

When the similarity matching process provides suboptimal results, an expert review allows for increasing confidence in the identification through review of the data and evaluation of the "gaps" and "shortcomings" of the process.

LC-MS applications almost exclusively use soft ionization procedures (electrospray or atmospheric pressure chemical ionization), producing protonated [M+H] + or deprotonated [M-H]- molecular ions, according to the polarity employed. Adduct ions such as [M+Na] + or multiple others can also be detected due to the use of glassware, buffers etc. When high resolution MS

is employed, accurate mass information is acquired, which is a key element for molecular formula generation.

During an **LC-HRMS** analysis, the instrument detects the compounds eluting from the liquid chromatographic system upon their ionization in the ion source. The error in mass accuracy of the ions is not more than 20 ppm. In screening methods, a data dependent fragmentation method can be implemented, in order to collect MS and MS/MS data simultaneously. During specific compound identification, targeted MSn experiments can be performed.

Similarly, to GC-MS procedures, identification is based on the data acquired through the respective system. The quality of the data, thus, is critical for the identification process. During identification, a clean MS spectrum should be acquired, which should be, to the extent possible, representative for the respective species and free from ions corresponding to other analytes.

Accurate mass measurements, due to the high resolving power of the HRMS, provide ions that enable the task of molecular formula attribution. Furthermore, the isotopic pattern can be easily discerned and may contain useful information on the formula attribution. The isotopic abundance of different elements affects the abundance/ relative intensity of isotopic peaks. As a result, the pattern can be used to establish whether specific elements are included in the molecular formula/ composition or not. Once molecular formulas fitting into the exact mass and an isotopic pattern with an acceptable error have been selected with confidence, additional actions are initiated that pertain to the identification of substructures. The MS/MS spectrum is acquired and is compared to both in-house and commercially available libraries.

The possible paths for proceeding further with the identification process are two, namely, **compound identification & substructure identification**. Substructure identification proceeds on a wider scope evaluation than compound identification because it acknowledges that the compound to which the spectrum belongs may not actually be present in the library, so instead it scans through MSn data in order to evaluate whether the spectrum could support partial similarity. Evaluation of the data is performed in order to conclude on whether a clear or partial substructure match can be supported.

Partial substructure matching relies on the confirmation for the existence of more than one fragment ions annotated to belong to the same structure (e.g. anthraquinone core, corticosteroid core, phthalate core, etc.)

The above processes may provide up to a confident identification level depending on the quality of data and the information available. LC-HRMS methods are capable of providing a wealth of information. The attained data then require rigorous evaluation by an expert in the process of structural elucidation.

It can be inferred from the above that one of the key aspects of identifying unknown compounds using mass spectrometry is the expertise and ability to interpret mass spectra. More specifically, the following factors are prerequisites for properly and successfully conducting an identification exercise:

- **Expertise in Mass Spectrometry:** Mass spectrometry is a complex analytical technique that requires specialized knowledge and skills to operate and interpret the results.

- **Accurate and effective interpretation of Mass Spectra:** Identification of the peaks corresponding to the molecular ions, fragment ions, and other characteristic ions, and interpretation of the fragmentation patterns to deduce the structure and composition of the unknown compound.

- **Structural Elucidation:** Deduction of the structural information of unknown compounds from mass spectra, including determining the molecular weight, elemental composition, and functional groups present in the compound.

- **Database and Spectral Library Utilization:** Availability and access to different databases and libraries, including in-house databases, as well as the ability to search and compare experimental mass spectra against these resources to confidently identify unknown compounds.

- **Experience in Compound Identification:** Track record of successful identifications that are confirmed by comparison with an authentic reference standard

- **Advanced Techniques:** Availability of state-of-the-art techniques, such as high-resolution mass spectrometry or tandem mass spectrometry (MS/MS), which can provide the necessary pieces of information and improve the accuracy of compound identification.

After the identification of the species of interest the next stage of processing focuses on providing a more accurate quantitative estimate of their concentration. Regarding the case of a targeted analysis, where authentic reference standards of the target analytes are available, the approach is straightforward and the result is characterized by the greatest degree of confidence considering that it is based on the response functions which are specific for the analytes of interest. However, this is rarely the case due to the following reasons:

- An authentic reference standard is, in many cases, not commercially available

- Screening or targeted screening methods aim to address numerous compounds whose presence and identity cannot be foreseen and therefore the inclusion of the corresponding reference standard is not feasible.

In these cases, one has to proceed by employing the surrogate standard (i.e. representative compound) that will exhibit a response that is as close as possible to that expected for the identified analyte in order to limit the risk related to quantitation uncertainty. A target analyte is then quantified by means of the most suitable representative analyte. The suitability is evaluated according to the pyramids depicted in Figures 5 and 6 (base to top: least to most important factor) for LC-MS and GC-MS analysis, respectively:

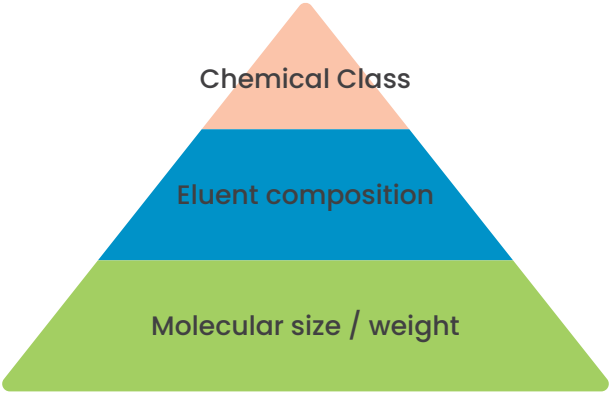


Figure 5: Factors of importance for the selection of a surrogate standard in LC-MS

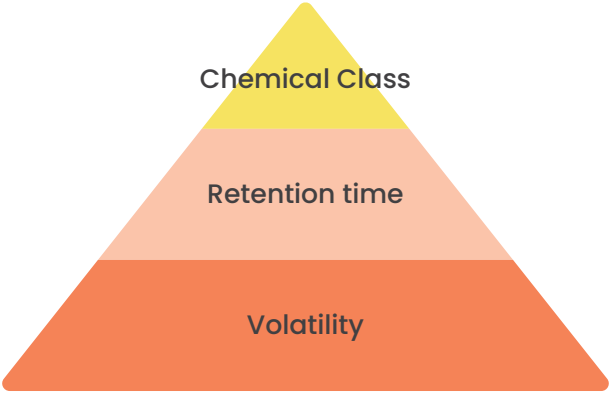


Figure 6: Factors of importance for the selection of a surrogate standard in GC-MS

In conclusion, when selecting representative analytes for quantitation, it is critical to consider the target analytes as well the as the expected species. The representative analytes should be compound(s) that are similar to the targets in aspects of: lipophilicity/ volatility, which determine the elution segment, and chemical class or structure, which determines the ionization propensity and

fragmentation pattern. Additional things to consider would be stability/ susceptibility to processes undertaken for test solution preparation, limited solubility, etc.

In an ideal situation the leachable species in a final product exceeding the AET should be discovered / revealed, their identification confirmed and their concentration accurately and precisely determined. However, considering the practical difficulties one has to deal with when performing such a demanding exercise, a realistic objective is to mitigate as much as possible (i.e. feasible) and practical, the errors related to the omission of analytes, the inexact identification and inaccurate / imprecise quantitation. To this end, the strategy employed by QMx can be summarized in the following Figure.

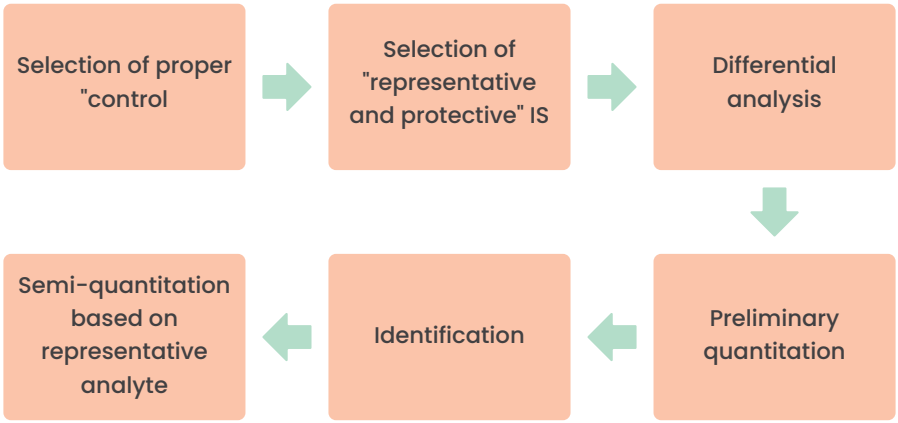


Figure 7: Discovery, Identification and Quantitation strategy

Method Validation / Suitability

The term method validation is used to describe the procedures that are followed in order to establish the suitability of a proposed methodology for its intended purpose. In the frame of migration studies method validation of either a **"reduced set-up"** (i.e. method suitability) or a **"full-scale setup"** (i.e. method validation) may be performed, depending on the scope of the study.

In the **"reduced set-up" (i.e. "method suitability")**, the approach is entirely focused on attesting that the method can provide data that allow discrimination between species below the analytical evaluation threshold and over the analytical evaluation threshold (or the J target concentration for elemental impurities). This is quite similar to the principles of "limit testing", with the limit being the AET or J value respectively; where it is necessary to ascertain whether an analyte is present beyond a certain concentration or not.

In the **"full-scale set-up" (i.e. "method validation")**, evaluation is more thorough. In this case, the procedures evaluate multiple sources of variability that may affect the quality of the data acquired, as well as the accuracy of data along a wider range of concentrations. In this manner, there is greater confidence in decision-making processes that are based on concentrations that are above or below the analytical evaluation threshold i.e. risk assessment processes, leachables qualification, etc.

The tables below summarize the proposed quality characteristics to be included in the evaluation for the qualification schemes described above. The characteristics and the respective acceptance criteria are indicative since they need to be adjusted and optimized based on the purpose of the method, the authority of submission and the client's requirements.

Table 1: Indicative Characteristics & Acceptance Criteria – Reduced Setup

Method characteristics	Indicative Acceptance Criteria
Sensitivity	LOD estimation
Specificity	Depends on the technique / The criteria for accuracy / precision should be met
Linearity/ Range	R^2 0.98
Accuracy at 80% AET / J and 120% AET / J	• 80 – 120% Recovery on organic species • 70 – 150% Recovery on elemental impurities
Repeatability at AET/ J	%RSD NMT 20%, n ≥ 6

Table 1: Indicative Characteristics & Acceptance Criteria – Reduced Setup

Method characteristics	Indicative Acceptance Criteria
Sensitivity	LOD estimation and LOQ establishment (see "Accuracy" and "Repeatability")
Specificity	Depends on the technique / The criteria for accuracy / precision should be met
Linearity/ Range	R^2 0.98
Accuracy at 3 levels (i.e. LOQ, AET, ≥ AETirritant effects)	• 80 – 120% Recovery on organic species at the AET and higher levels • 50 – 150% or 70 – 130% at the LOQ, depending on analytical concentration level and the associated variability • 70 – 150% Recovery on elemental impurities
Repeatability at AET/ J and LOQ / 20% J	%RSD NMT 20%, n ≥ 6
Intermediate precision at AET/ J and LOQ / 20% J	%RSD NMT 25%, n ≥ 12
Robustness	• Critical parameter effects • Stability of solutions

Chemical characterization of Medical Devices

According to ISO 10993 – Part 1, consideration of the chemical characterization of the materials from which a device is made is a **v** in assessing the biological safety of the device.

The extent of chemical characterization required should reflect the nature and duration of the clinical exposure and it should therefore be determined by the toxicological risk assessor based on the data necessary to evaluate the biological safety of the device.

Chemical Characterization comprises a variety of analytical techniques, in order to identify and quantify materials that may have migrated from the product contact material into the solution of interest. The purpose of this testing is to evaluate the biological effect that leachables attributed to a medical device could have on a patient. Different individual standards of the 10993 series, commonly known as "Parts", focus on different aspects of the biological evaluation of medical devices. Part 12 provides a detailed description of the "test article" preparation while Part 18 specifies the framework and a stepwise process for the identification and quantification of medical device constituents.

In order to perform an adequate risk and safety evaluation for the compounds resulting from chemical characterization studies, each compound's structure should be elucidated to an extent that literature and Structure – Activity Relationship assessment can be performed. 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.

A thorough **toxicological risk assessment** is performed on the notion that if all of the constituents of a medical device are known, then the safety of the device can be assessed based on the toxicology of those constituents. ISO 10993, Part 17, specifies a method for the determination of allowable limits for substances leaching from medical devices.

At QMx we combine state of the art instrumentation and extensive experience in the biological evaluation of medical devices that allow the undertaking of either "standalone" chemical characterization studies or studies that are guided by a more holistic biological safety evaluation scheme (defined through a comprehensive **biological evaluation plan**).

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 steps following the detection of a compound that exceeds the AET are depicted in the following Figure.

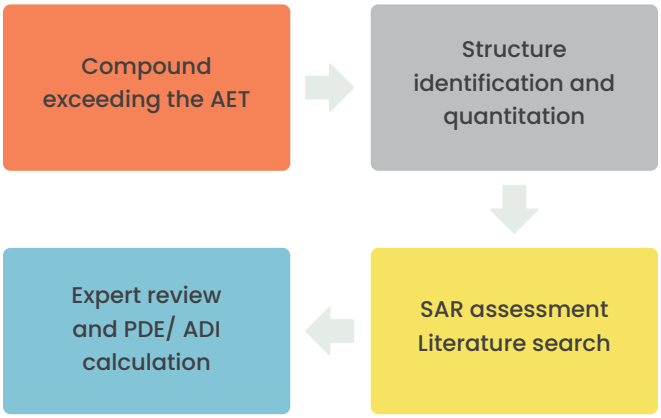


Figure 8: Safety evaluation steps

Qualification is the process of acquiring and evaluating data that establish the biological safety of an individual E&L species at the level(s) being considered. The assessment strategy for the qualification of the species in question consists of the following steps:

1. **Chemical Classification** of the target analyte(s);

2. **Gathering of toxicological data & Identification of data gaps;**

3. **Hazard Appraisal Process (HAP)** including an analysis of the species' toxicokinetic / toxicodynamic profile, and evaluation of their toxicological potential, based on data acquired from the scientific literature and in-silico data.

4. **Recognition of critical effects** within the pharmacodynamic and/or toxicological profile

5. **Selection of the "dose descriptor (DD)"** corresponding to the most "sensitive" effect; defined as the adverse effect that is observable at a higher relative probability in the population and/ or at the lowest exposure to the species.

6. **Calculation of a PDE** based on the "dose descriptor" value.

7. **Risk evaluation** based on comparison between permitted and actual patient exposure to the agent.

In the frame of the Hazard Appraisal Process, Quantitative structure activity relationship (QSAR) analyses or in silico predictions are commonly employed especially when toxicity data for a given compound and/or its mutagenic potential is not available. A QSAR analysis assesses a chemical structure, using software tools for structurally-similar compounds to leverage their toxicity data or for the presence (or absence) of structural alerts for mutagenicity. For mutagenic structural alerts, the analyses should apply both, knowledge-based and statistical computerized systems, following the recommendations of the ICH guideline M7 for limitation of DNA reactive (mutagenic) impurities.

The OECD has adopted five principles for establishing the validity of the (Q)SAR models for use in regulatory assessment of chemical safety. These are that there should be:

1. a defined endpoint

2. an unambiguous algorithm

3. a defined domain of applicability

4. appropriate measures of goodness-of-fit, robustness and predictivity

5. a mechanistic interpretation, if possible

QMx employs widely accepted and well-established in silico tools (e.g DEREK, Leadscope) that fulfil the above criteria together with expert assessment, in case of ambiguous or "out-of-domain" outcomes, by a registered toxicologist.

Instrumentation / Software Laboratory Infrastructure and Equipment

Ultra-Performance Liquid Chromatographic system with High Resolution Mass Spectrometer (UPLC - HRMS)

QMx possesses cutting edge Orbitrap HRMS instrumentation by Thermo Scientific. These are hybrid Ion Trap- Orbitrap Mass Spectrometers, with very high resolving power, high speed, sensitivity and advanced fragmentation information.

Based on advanced signal processing on the detector and high velocity during scanning, they take full advantage of a UPLC system and provide quantitative and qualitative analytical capability in a single platform and often in a single run over a wide linear range. Multiple fragmentation techniques, including the possibility for MSn 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.

Orbitraps are employed in either the ESI or the APCI ionization mode and they are connected to UPLC-PDA chromatographic systems.

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

Ultra-Performance Liquid Chromatographic system with tandem Mass Mpectrometer (UPLC-MS/MS)

Low-resolution mass analyzers are also employed in the frame of targeting methods.

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 are selected according to the nature and the needs of the study.

Gas Chromatographic systems with a single quadrupole or tandem Mass Spectrometer (GC-MS, GC-MS/MS)

Gas chromatographic systems combined with singe quadrupole (GC-MS) and triple quadrupole (GC-MS/MS) mass analyzers are available at QMx. They are employed 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. Additional information regarding the molecular ion can be extracted by exploiting the “softer” ionization conditions of the Chemical Ionization mode.

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

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The NexION 350 of Perkin Elmer, employed at our Testing Laboratory, 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 generate better results.

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.

Ion chromatographic system

The Dionex Ion Chromatographic Systems are powerful and versatile instruments designed for high-performance analysis of ionic compounds in various sample types. They are employed to effectively separate, identify, and quantify anions, cations, and polar molecules in complex mixtures with exceptional accuracy and precision.

The system also features a high-pressure gradient pump, a temperature-controlled column compartment, and a wide range of detector options, including conductivity and amperometric detectors.

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