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ANALYTICAL METHODOLOGY FOR PHARMACEUTICAL EXTRACTABLES AND LEACHABLES: A COMPREHENSIVE APPROACH FOR CONTAINERS AND CLOSURES

Running title: Analytical E&L Methodology in Pharmaceuticals

Rajesh Kumar Chawla[1]*, Vinay Richards Rapaka[2], Naresh Ambekar[1]

[1] Brio Pharmaceuticals Inc., 10863 Rockley Road, Houston, Texas, USA 77099

[2] Kevis Pharma LLC, 12720 Dairy Ashford Road, Sugar Land, Texas, USA 77478

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Abstract: The control of extractables and leachables (E&L) from pharmaceutical packaging and delivery systems is paramount to ensuring patient safety and product quality. This manuscript provides a detailed, comprehensive analytical methodology for the identification, qualification, and quantification of E&L substances from containers and closures used across a wide range of pharmaceutical formulations, including oral liquids, nasal sprays, and injectables. The methodology is meticulously aligned with key international regulatory guidelines from bodies such as the International Council for Harmonization (ICH), the U.S. Food and Drug Administration (FDA), and the United States Pharmacopeia (USP). The core framework encompasses a robust risk assessment, systematic controlled extraction studies, and the application of highly sensitive and orthogonal analytical techniques, including Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). A critical component of the methodology involves the toxicological evaluation of identified compounds and the establishment of science-based acceptance thresholds, such as the Safety Concern Threshold (SCT) and the Qualification Threshold (QT). A detailed case study on a nasal spray formulation highlights the practical application of this methodology from initial sample preparation to data interpretation and regulatory submission. The manuscript also addresses common challenges faced during implementation, such as the analysis of low-level impurities and the absence of commercial reference standards, and proposes practical solutions. Finally, it explores future perspectives for E&L analysis, including the integration of non-targeted screening and in silico toxicology.

Keywords: Extractables, Leachables, Pharmaceutical Packaging, Analytical Methodology, Risk Assessment.

1. Introduction:

The primary packaging system for a pharmaceutical product serves as the first line of defense against physical damage, microbial ingress, and environmental degradation. However, the materials from which these systems are constructed—such as polymers, elastomers, and glass—are not inert. They can release chemical substances into the drug product over its shelf life, a phenomenon that poses significant risks to patient safety, product efficacy, and regulatory compliance. These impurities are broadly classified into extractables and leachables [1, 2].

Extractables are compounds that can be forced from a material using aggressive solvents under exaggerated conditions of temperature and duration. They represent a "worst-case scenario" profile of all potential impurities that could migrate into the drug product [3]. Leachables, on the other hand, are compounds that migrate from the packaging system into the drug product under normal storage conditions and are, therefore, a more direct measure of the actual risk to the patient.

The importance of E&L analysis is underscored by numerous regulatory guidelines. The U.S. FDA, European Medicines Agency (EMA), and ICH have issued comprehensive guidance [4, 5], while the United States Pharmacopeia (USP) provides specific general chapters (e.g., <1663>, <1664>, <661.1>, and <661.2>) that define the requirements for E&L assessment [6, 7]. The primary objective of a robust E&L program is to identify and quantify these compounds and to ensure that they are present below a predefined, toxicologically acceptable level, a concept central to the Safety Concern Threshold (SCT) [8, 9]. This manuscript provides a detailed analytical methodology for the E&L analysis of pharmaceutical products, with a particular focus on the identification, calculation of acceptance thresholds, and a practical case study.

2. Methodologies

A comprehensive E&L program is a structured, multi-phase process. It is a risk-based approach designed to efficiently identify and control potential impurities. The workflow can be broadly divided into four key stages: risk assessment, extractables study, leachables study, and toxicological evaluation.

***Corresponding Author**
Rajesh Kumar Chawla*
E-mail: rbchawla07@gmail.com

2.1. Risk Assessment and Study Design

A thorough risk assessment is the foundational step in any E&L program. It involves evaluating the potential for E&L based on:

- Material of Construction: Certain materials, such as plastics, elastomers (rubber stoppers), and multi-layered packaging, are considered higher risk than glass.
- Route of Administration: Injectables, nasal sprays, and inhalation products pose the highest risk due to direct contact with sensitive tissues.
- Drug Product Formulation: The polarity and pH of the drug product can significantly influence the leaching process.
- Storage Conditions: Higher temperatures and longer shelf lives increase the potential for leaching.

Based on this assessment, the scope and intensity of the subsequent analytical studies are determined. A high-risk scenario, such as a multi-dose nasal spray, would necessitate a more rigorous study than a low-risk scenario, such as a solid oral tablet in a blister pack.

2.2. Extractables Study: The "Worst-Case" Profile

The extractables study is designed to deliberately release the widest possible range of compounds from the packaging material.

2.2.1. Sample Preparation and Extraction Conditions

Packaging components are prepared by cutting them into small pieces to maximize the surface area-to-volume ratio. Extractions are performed using a range of solvents with varying polarities to simulate the drug product and to cover all potential extractables.

- Aqueous solvents: Water for Injection (WFI), pH-buffered solutions.
- Polar solvents: Ethanol, Isopropyl alcohol (IPA), or acetonitrile.
- Non-polar solvents: Hexane, Toluene, or other hydrocarbons.

The extraction is carried out under elevated temperatures (e.g., 50-70°C) for an extended period (e.g., 72 hours). A negative control (blank solvent) is always prepared under identical conditions to identify any background impurities.

2.2.2. Analytical Techniques

The extracts are analyzed using complementary analytical techniques to ensure a comprehensive profile.

- Gas Chromatography-Mass Spectrometry (GC-MS): This is the gold standard for volatile and semi-volatile organic compounds. GC provides excellent chromatographic separation, and MS provides a unique mass fragmentation pattern that can be used for identification.

  Principle: The extract is injected into the GC, where compounds are vaporized and separated based on their boiling points and affinity for the stationary phase. The eluting compounds then enter the MS, where they are ionized, fragmented, and detected based on their mass-to-charge ratio (m/z).

- Liquid Chromatography-Mass Spectrometry (LC-MS): This technique is essential for non-volatile, polar, and thermally labile compounds. High-resolution mass spectrometry (HRMS) is often coupled with LC to provide accurate mass and fragmentation data.

  Principle: Compounds are separated on the LC column based on their interaction with the mobile and stationary phases. The eluting compounds are then ionized (e.g., Electrospray Ionization, ESI) and analyzed by a mass analyzer (e.g., TOF, Orbitrap), which provides highly accurate mass measurements.

- Inductively Coupled Plasma-Mass Spectrometry (ICP-MS): Used to identify and quantify elemental impurities. This technique is particularly important for detecting heavy metals like lead, cadmium, and arsenic, as per ICH Q3D [5].

2.3. Leachables Study: The Real-World Profile

The leachables study is performed on the final drug product packaged in its intended container system. This study provides a direct measure of what compounds actually migrate into the product over time.

2.3.1. Study Design

The study is conducted under real-time (long-term) and accelerated storage conditions (e.g., 25°C/60% RH and 40°C/75% RH). Samples are analyzed at multiple time points, typically 0, 3, 6, 12, 24, and 36 months, to establish a leaching profile. The analytical methods used are the same as those in the extractables study but are validated for the specific drug product matrix.

3. Explanations, Calculation, and Acceptance Thresholds

3.1. Compound Identification and Quantification

Once an analytical signal is obtained, the process of identification and quantification begins.

- Tentative Identification: For GC-MS, identification is typically achieved by matching the mass spectrum of the unknown compound with a vast spectral library (e.g., NIST, Wiley). For LC-MS, accurate mass and isotopic pattern analysis are used to determine the molecular formula.
- Confirmation: The identity of a compound is confirmed by comparing the retention time and mass spectrum with a certified reference standard.
- Quantification: A calibration curve is generated using a series of known concentrations of the reference standard. If a reference standard is unavailable, a surrogate standard with a similar chemical structure and response factor can be used for semi-quantitative estimation.

3.2. Calculation of Acceptance Thresholds

The core of E&L risk assessment is the calculation of acceptance thresholds. The Safety Concern Threshold (SCT) is the level of daily exposure below which a leachable is considered to have a negligible safety risk [8]. The SCT values vary by route of administration:

Route of Administration	SCT (µg/day) [8, 10]
Injectables (Parenteral)	0.15
Nasal Sprays	5
Orally Inhaled	0.15
Oral Liquids	120

From the SCT, the Analytical Evaluation Threshold (AET) is calculated. The AET is a concentration level in the drug product.

AET (µg/mL) = SCT (µg/day) / MDD (mL/day)

Where MDD is the maximum daily dose of the drug product. If a compound is detected at a concentration above the AET, it must be identified and quantified.

The Qualification Threshold (QT) is a higher threshold that, if exceeded, requires a formal toxicological qualification of the leachable, such as a review of existing toxicological data or a new safety study [11].

3.3. Toxicological Evaluation

The final step is to assess the toxicological risk of the identified leachables. A toxicologist reviews the daily exposure level and compares it to the compound's Permitted Daily Exposure (PDE) or other established toxicological data.

4. Practical Applications and Case Study

- Case Study: Nasal Spray with a Polypropylene Spray Pump
- Background: A new nasal spray formulation, with a three-year shelf life, is being developed. The primary container is a glass bottle with a rubber stopper and a polypropylene (PP) spray pump with a metal spring. The risk is high due to the route of administration and the use of a plastic pump and elastomer stopper.

4.1. Extractables Study

- Preparation: The PP pump and rubber stopper were extracted separately with water, 50% ethanol, and hexane at 60°C for 72 hours.
- Analysis: GC-MS and LC-MS analysis of the extracts revealed several compounds. From the PP pump, BHT (Butylated Hydroxytoluene) and Irganox 1010 (an antioxidant) were identified. From the rubber stopper, a vulcanization accelerator, Zinc Diethyldithiocarbamate (ZDEC), was identified.

4.2. Leachables Study

- Study Design: The finished nasal spray product was stored at 25°C/60% RH (long-term) and 40°C/75% RH (accelerated). Samples were taken at 0, 3, 6, 12, 24, and 36 months.
- Analysis: A validated LC-MS/MS method was used for Irganox 1010 and ZDEC, and a validated GC-MS method was used for BHT.
- Findings (Hypothetical):
  - BHT concentration at 36 months (long-term): 0.05 µg/mL.
  - ZDEC concentration at 36 months (long-term): 0.008 µg/mL.
  - Irganox 1010 was not detected.

4.3. Toxicological Evaluation

- MDD: The maximum daily dose for this nasal spray is 0.5 mL/day.
- SCT for Nasal Sprays: 5 µg/day.
- AET Calculation:

  AET=5 µg/day/0.5 mL/day=10 µg/mL
- Leachable Daily Exposure:
  - BHT: 0.05 µg/mL×0.5 mL/day=0.025 µg/day
  - ZDEC: 0.008 µg/mL×0.5 mL/day=0.004 µg/day
- Comparison and Conclusion:
  - Both the BHT and ZDEC concentrations (0.05 µg/mL and 0.008 µg/mL, respectively) are well below the calculated AET of 10 µg/mL.
  - The daily exposure levels (0.025 µg/day and 0.004 µg/day) are also far below the SCT of 5 µg/day.
  - Based on these findings, the E&L from the nasal spray packaging do not pose a significant safety risk. This conclusion is documented and included in the stability section of the regulatory submission.

5. Challenges and Solutions in Implementation

5.1. Matrix Effects

The drug product matrix can suppress or enhance the analytical signal of leachables, leading to inaccurate quantification.

Solution: Use of matrix-matched calibration standards or the method of standard addition. Robust sample preparation techniques like solid-phase extraction (SPE) can also help isolate analytes from interfering compounds.

5.2. Lack of Reference Standards

Many E&L compounds, particularly degradation products or proprietary additives, lack commercially available reference standards. This makes accurate quantification difficult.

Solution: Employ a surrogate standard approach where a structurally similar compound with a known response factor is used. For identification, advanced mass spectrometry techniques like HRMS with fragmentation analysis are critical for proposing a chemical structure and performing a toxicological assessment.

5.3. Non-Targeted Analysis

The number of potential leachables can be vast, making it impossible to create a targeted method for every single compound.

Solution: Use of non-targeted HRMS screening. This involves collecting comprehensive data on all detected ions, which can then be retrospectively analyzed against a database of known compounds or for unknown compounds using sophisticated data processing software.

6. Future Perspectives

The field of E&L analysis is continuously advancing with technological innovations and a growing understanding of drug-container interactions.

- Integration of Data Science: The future of E&L analysis will heavily rely on data science and machine learning to analyze the massive datasets generated by HRMS. These tools can help identify patterns, predict leaching behavior, and link chemical structures to potential toxicological concerns.
- In Silico Toxicology: Computational models are being developed to predict the toxicity of E&L compounds based solely on their chemical structure. This can significantly reduce the need for costly and time-consuming in vivo and in vitro safety studies, especially for new or unknown leachables.
- E&L in Biologics: The focus is expanding to include biopharmaceutical products. The challenges are more complex due to the inherent sensitivity of proteins to impurities and the potential for leachables to cause aggregation or degradation of the active molecule.
- Green Chemistry: There is a growing trend towards the use of more inert, sustainable, and less-leaching materials in pharmaceutical packaging to reduce the E&L burden from the outset.

7. Conclusion

The analytical methodology for pharmaceutical extractables and leachables is a critical component of product development and quality assurance. A comprehensive approach, starting with a robust risk assessment and leveraging a suite of modern analytical techniques, ensures that packaging systems do not compromise patient safety or product integrity. By adhering to international guidelines and applying sound scientific principles, companies can effectively identify and control E&L, ultimately securing regulatory approval and ensuring the safety of their products throughout their lifecycle. The systematic framework and practical case studies presented in this manuscript provide a clear roadmap for implementing a successful E&L program.

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9. Conflict of Interest:

The authors declare no conflict of interest.

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