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Strengthening Extractable & Leachable Study Submissions: Best Practices to Avoid Regulatory Deficiencies

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Abstract

The assessment of extractable and leachables (E&L) has become a cornerstone of pharmaceutical product safety, yet no unified global standards currently exist to quantify or report E&L from container closure systems. While initially limited to primary packaging, E&L evaluations now encompass secondary packaging, manufacturing equipment, and medical devices. Key milestones include the FDA's 1999 guidance on container closure systems and more recent frameworks from USP chapters <1661>, <1663>, and <1664>, as well as PQRI recommendations. These guidelines have set the foundation for conducting E&L studies, especially for high-risk dosage forms, and are complemented by the forthcoming ICH Q3E, which aims to standardize E&L considerations across dosage forms. This article outlines a structured procedure for E&L assessments, integrating regulatory expectations with practical insights.

Keywords

Extractables and Leachables, Pharmaceutical Container Closure Systems, Manufacturing Equipment, Guidance, Regulatory Standards, Prototype Procedures

1. Introduction

Pharmaceutical packaging systems are integral to maintaining drug products' safety, efficacy, and stability throughout their lifecycle. These systems safeguard pharmaceutical products from environmental factors, contamination, and physical damage during storage, transportation, and use [1]. In addition to providing

protection, they facilitate precise dosing, support efficient drug administration, and ensure compliance with regulatory standards. Advancements in technology have enabled the development of innovative packaging materials and designs, enhancing drug stability, promoting patient adherence, and reducing the environmental impact of pharmaceutical packaging [1].

The complexity of packaging systems lies in their multifaceted roles, which include protecting and preserving drug products over extended periods [2]. Each component is selected based on its material properties and functional requirements and can be classified as primary, secondary, tertiary, or ancillary, as detailed in **Table 1** [2]. Primary packaging, which directly contacts the drug product, is vital for maintaining stability and efficacy. Secondary packaging provides an additional layer of protection, while tertiary packaging is used for bulk transportation and storage. Though not formally categorized, ancillary items, such as desiccants and adhesive tapes, can still influence drug product quality and safety [2] [3].

Table 1. Packaging levels and examples.

Packaging Level	Description	Examples
Primary	Direct contact with the drug product ensures stability and efficacy.	Bottles, blister packs, vials, ampoules, syringes, closures, stoppers, pouches
Secondary	Provides additional protection; outer layers not in direct contact with the drug product.	Cartons, boxes, shrink wrap, labels, inserts
Tertiary	Used for bulk handling and transportation; ensures safe delivery.	Pallets, crates, shipping containers, drums
Ancillary	Additional items are necessary to store or administer the drug product that does not fall into the other categories.	Scotch tape, desiccants, humidity indicators, handling tools

The Center for Drug Evaluation and Research (CDER), under the 21 Food, Drug, and Cosmetic Act (FD&C Act), mandates that drug product containers and closures must not interact with the drug in ways that compromise its safety, identity, strength, quality, or purity [4]. These materials must also shield the drug product from environmental factors that could lead to contamination or degradation. However, the chemical composition of packaging materials can sometimes interact with drug formulations, potentially affecting their stability, compatibility, and therapeutic effectiveness [4].

One of the primary safety concerns related to packaging is contamination caused by extractable and leachables (E&L) [5]. These substances include organic and inorganic chemical entities that may migrate from packaging or manufacturing materials into the drug product [4]. Extractables are typically released under

laboratory conditions that simulate extreme interactions, while leachables migrate under average storage or use conditions. For instance, leachables from secondary packaging materials, such as adhesives and inks, can accumulate in drug products, compromising their safety, efficacy, and stability [5].

Qualifying a pharmaceutical packaging system involves demonstrating its protective, functional, and compatible properties while ensuring it does not pose safety risks [5]. Early-stage evaluation of container closure systems during drug development is strongly recommended. By the later stages, comprehensive studies should validate the packaging system's safety and compatibility, supporting regulatory submissions [5] [6].

Although no globally harmonized guideline for E&L assessments exists, organizations like the Product Quality Research Institute (PQRI) have published vital recommendations, particularly for high-risk dosage forms such as inhalation and parenteral products [6]. These recommendations, alongside guidance from the FDA, EMA, ICH, USP, and ISO, provide valuable frameworks for conducting E&L studies throughout the drug development lifecycle [7].

2. Existing Guidelines and Standards

No universally enforceable monographs specifically address assessing extractable and leachables (E&L) in pharmaceutical packaging and delivery systems [5]. However, regulatory authorities such as the FDA, USP, ICH, and Health Canada have established comprehensive guidelines to ensure that packaging systems comply with relevant compendial standards. For example, the FDA emphasizes detailed Chemistry, Manufacturing, and Controls (CMC) documentation in its guidance on container closure systems and associated devices, as summarized in **Table 2** [8].

Table 2. Key FDA guidance publications.

Guidance Published	Year
FDA guidance for industry: container closure systems for packaging human drugs and biologics. Chemistry, manufacturing, and controls documentation.	1999
FDA draft guidance for industry: Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) drug products. Chemistry, manufacturing, and controls documentation.	1998
FDA guidance for industry: nasal spray, inhalation solution, suspension, and spray drug products. Chemistry, manufacturing, and controls documentation.	2005
FDA reviewer guidance for nebulizers, metered dose inhalers, spacers, and actuators.	1998
FDA guidance for industry and FDA staff: technical considerations for pen, jet, and related injectors intended for use with drugs and biological products.	2013

In addition to FDA regulations, the USP monographs provide critical insights into the material properties of packaging systems, ensuring their safety and suitability. For instance, USP <661> outlines guidelines for assessing plastic materials, focusing on their composition, chemical properties, and suitability for pharmaceutical use [8]. Similarly, USP <662>, currently under development, will address the safety and suitability of metal materials in pharmaceutical packaging. Each enforceable monograph is typically paired with an informational counterpart to provide additional guidance. The key USP chapters for various materials of construction are listed in **Table 3** [5] [8].

Table 3. USP monographs for materials of construction in pharmaceutical packaging and manufacturing.

Material of Construction (MOC)	Enforceable Monograph	Informational Monograph
Plastic	USP <661> (Plastic Packaging Systems)	USP <1661> (Evaluation of Plastic Packaging for Pharmaceutical Use)
Glass	USP <660> (Containers Made of Glass)	USP <1660> (Evaluation of the Inner Surface Durability of Glass Containers)
Elastomeric	USP <381> (Elastomeric Closures for Injections)	USP <1381> (Evaluation of Elastomeric Components Used in Pharmaceutical Packaging/Delivery Systems)
Plastic Manufacturing Components	USP <665> (Plastic components used to manufacture pharmaceutical products)	USP <1665> (Assessment of Polymeric Components Used in Pharmaceutical Manufacturing Systems)
Metallic	USP <662> (under development)	Not applicable

Note: Chapters below 1000 are enforceable, while those above 1000 serve as informational guidelines.

The USP provides specific guidance for E&L assessments in chapters <1663> and <1664> [6] [7]. USP <1663> focuses on evaluating extractable, offering methodologies to identify potential chemical entities that could migrate from packaging materials. USP <1664> complements this by detailing procedures for assessing leachables under average storage and use conditions [9]. Although these chapters are not enforceable, they are vital for manufacturers aiming to develop robust E&L testing strategies that ensure product safety [6] [7].

3. Testing Strategy and Stepwise Approach for Conducting E&L Studies

Extractables and leachables studies are generally conducted during the later stages

of drug development. However, initiating the assessment of container closure systems at earlier stages can significantly enhance product safety and regulatory compliance [10]. Due to the inherent complexity of packaging systems, multiple testing steps are required to ensure compatibility between the drug product and its packaging [10].

The evaluation process follows a **three-tiered approach** to packaging system qualification (Table 4), comprising:

Table 4. Three-tiered approach to packaging system qualification.

Section	Activity	Description
Characterization of Materials of Construction	Ingredient Testing	Evaluate the composition and properties of packaging materials.
Evaluation of the Packaging System	Controlled Extraction Studies (CES)	Simulate worst-case conditions to identify potential extractables.
Assessment of the Packaged Drug Product	Leachable Studies	Monitor the real-time migration of compounds from the packaging into the drug product.

4. The E&L Study Process

4.1. Material Assessment

This preliminary step evaluates packaging components' material properties and chemical composition to identify risks and confirm suitability for pharmaceutical applications [11].

4.2. Controlled Extraction Studies (CES)

These studies simulate extreme conditions to extract and identify potential chemical compounds that may migrate from packaging materials. CES data provide the foundation for further toxicological and safety assessments [11].

4.3. Leachable Studies

In the final stage, the packaged drug product is evaluated under normal storage conditions to detect and quantify any leachables that migrate from the packaging over time.

Manufacturers can address potential risks early in development by employing this structured approach, ensuring compliance with regulatory requirements and safeguarding patient safety (see Figure 1). The following section outlines detailed methodologies for implementing these E&L studies effectively [11].

5. Risk Assessment Based on Route of Administration

The design of an extractable and leachables (E&L) study is significantly influenced by the dosage form and its route of administration, as these factors determine

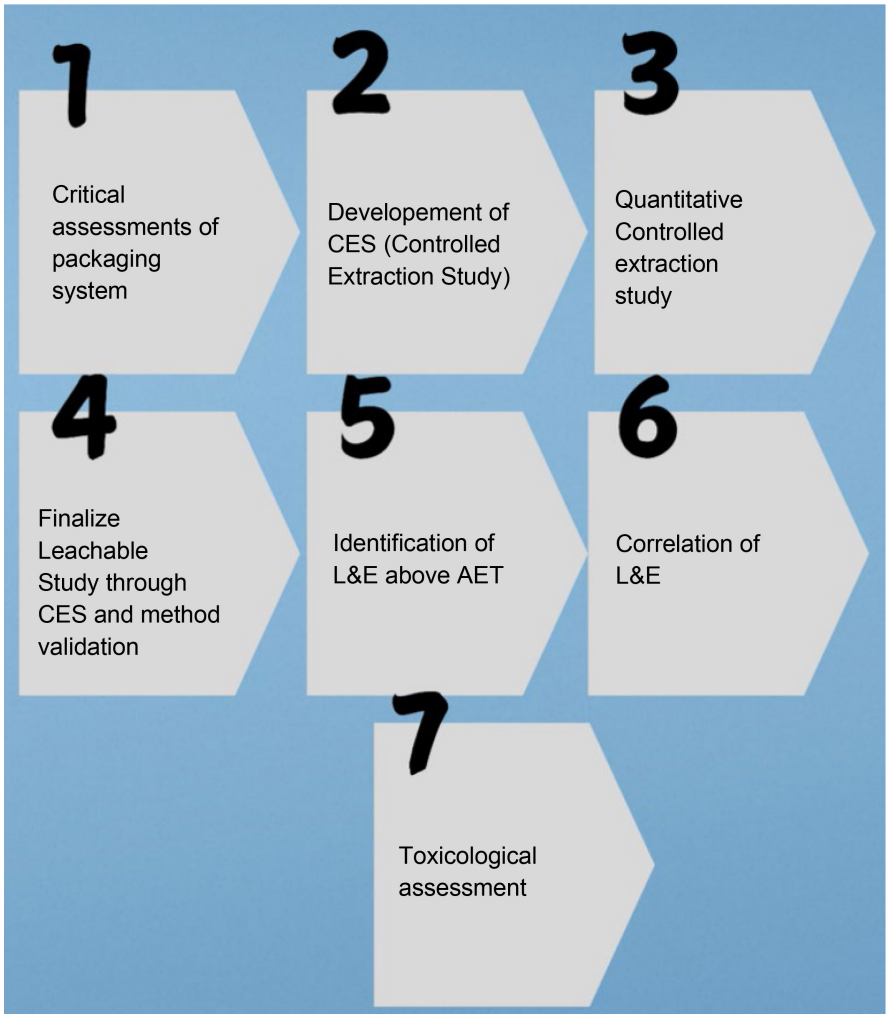

Figure 1. Stepwise approach to conduct EL studies.

the extent of interaction between the drug product and its packaging materials [5]. Dosage forms vary widely in their potential for such interactions, necessitating a tailored approach to E&L risk assessment for each type [5].

The **FDA's container closure guidance** identifies inhalation dosage forms as the highest risk, requiring extensive evaluation due to their direct interaction with sensitive biological systems, such as the lungs [6]. Similarly, injectable solutions are classified as high risk because they come into direct contact with the bloodstream, and the solubility and reactivity of liquids can facilitate the migration of leachables [5] [6].

Conversely, **solid dosage forms**, such as tablets and capsules, are considered low risk because they lack solvents and moisture that typically enhance the extraction of packaging components. These forms exhibit minimal interaction with their packaging materials [8]. **Semi-solid and liquid forms**—such as solutions, ointments, and creams—pose moderate risk levels due to solvents, increasing the likelihood of extracting chemical entities from packaging materials [8].

Even low-risk dosage forms, such as oral or topical products, require a docu-

mented material risk assessment to evaluate the suitability of container closure systems. This risk-based prioritization helps allocate resources effectively for E&L testing, ensuring patient safety without overburdening the evaluation process (refer to Table 5) [8].

Table 5. Risk factors for extractables and leachables based on formulation and route of administration.

Risk Level	Route of Administration/Formulation	Likelihood of Package-Drug Interaction
Highest	Inhalation aerosols and solutions, injections	High
	Sterile powders, powders for injection, inhalation powders	Medium
High	Ophthalmic solutions, transdermal ointments and patches, nasal sprays	High
Low	Topical solutions, topical/lingual aerosols, oral solutions	Medium
	Topical powders, oral powders, oral tablets, and capsules	Low

This **risk-based approach** ensures that E&L testing is targeted appropriately, focusing on the likelihood and severity of package-drug interactions [8].

6. Critical Assessment of Packaging Systems

The selection of packaging materials is an integral part of drug development, closely tied to the dosage form and its route of administration. A systematic evaluation ensures that the packaging system aligns with the specific needs of the drug product, considering its complexity and functionality [12].

1) Material Suitability and Initial Assessments

In the initial stages, the focus is on assessing the suitability of packaging materials using vendor-supplied data and literature sources. At this point, extractables studies are not conducted. Instead, the evaluation centers on understanding the material's composition and identifying critical components most likely to interact with the drug product [12].

2) Key Steps in Material Assessment

6.1. Data Collection

Obtain detailed information about each packaging component's composition and manufacturing processes.

Identify critical components, such as elastomeric seals, canisters, mouthpieces, and plastic containers for inhalation solutions.

6.2. Vendor Information

Request comprehensive chemical formulation and manufacturing process details from component suppliers.

If available, reference the vendor's Drug Master File (DMF) in regulatory submissions to streamline the approval process [11] [12].

6.3. Addressing Gaps in Information

When vendors provide incomplete information, mainly if their materials were initially designed for non-pharmaceutical uses, the manufacturer must generate the necessary extractable data [13]. This often involves additional testing or supplementary documentation to address regulatory requirements. For example, resins and other materials not intended initially for pharmaceutical applications may require further scrutiny to ensure their safety [13].

6.4. Material Risk Assessment

A thorough risk assessment identifies potential leachables and their degradation products, which could compromise drug stability and efficacy [14]. Toxicologists should be engaged early to assess the suitability of packaging components for their intended use. This evaluation provides the foundation for designing controlled extraction studies that comply with regulatory standards [15].

6.5. Key Information Required from Vendors

The data collected from suppliers is pivotal in guiding the development of controlled extraction study plans. Vendors must provide detailed information on:

- Chemical composition and potential extractables.
- Manufacturing processes, including raw material sourcing.
- Compatibility of packaging materials with pharmaceutical applications.

A well-documented material assessment minimizes risks and ensures that the selected packaging system supports product safety and regulatory compliance [14] [15].

6.6. Next Steps

Building on the material risk assessment, the next section will detail the development of **Controlled Extraction Studies (CES)** and their role in characterizing potential extractable under worst-case conditions [16]. These studies serve as the basis for understanding material interactions and guiding leachable evaluations during product development [17]. Key information required from the manufacturer to support these studies is outlined in **Table 6**.

7. Introduction to Safety Thresholds

Although ICH Q3B(R) guidelines address drug-related impurities, they do not cover extractables and leachables (E&L) arising from non-drug components like packaging and delivery systems [7]. Industry groups like the PQRI leachables and

Table 6. Critical information to be obtained from the manufacturer.

Category	Details Required
Chemical Composition	Complete formulation of the material, including all additives (e.g., plasticizers, stabilizers, fillers, pigments).
Manufacturing Process	Description of the processes used to create the material, including information about polymerization, curing, and any secondary treatments.
Drug Master File (DMF)	Availability of a DMF with the regulatory authority and the scope of the data included, such as extractable studies or toxicological assessments.
Potential Extractables	Identification of potential extractable substances based on material composition or prior testing data.
Material Testing Data	Analytical data from prior studies (e.g., migration studies, extractables profiles) performed by the supplier.
Raw Material Sourcing	Information on the origin and quality of raw materials used in manufacturing the packaging component.
Regulatory History	Previous regulatory approvals or rejections for the material or similar components in pharmaceutical applications.

Extractables Working Group have established thresholds for E&L risk evaluation to address this gap. These thresholds provide a framework for identifying, assessing, and mitigating the risks associated with leachables, ensuring regulatory compliance and patient safety. The definitions and PQRI recommendations for these thresholds are detailed in **Table 7** [11].

Table 7. Key thresholds for E&L assessments.

Threshold	Definition	PQRI Recommendations
Safety Concern Threshold (SCT)	Daily exposure level below which a leachable is unlikely to pose a safety risk.	- Individual organic leachable: 0.15 µg/day - Solid and oral solutions: 1.5 µg/day
Qualification Threshold (QT)	The level below which leachables are not considered for toxicological evaluation unless structural concerns arise.	QT: 5 µg/day
Threshold of Toxicological Concern (TTC)	Estimate of health risks based on structural properties, used when toxicity data are unavailable.	Not product-specific
Analytical Evaluation Threshold (AET)	The level at or above which extractable or leachables must be identified and evaluated for toxicological risks.	AET is calculated specifically for each product.

Analytical Evaluation Threshold (AET) Calculation

The **AET** is derived from the SCT and incorporates adjustments for product-specific parameters. The process involves: [11] [14].

1) Converting the SCT to an estimated AET.
2) Adjusting the AET for extractables and leachables.
3) Mapping the AET to extractable or leachables profiles.
4) Factoring in analytical uncertainties to determine the final AET.

If a drug product is intended for short-term use (less than lifetime exposure), thresholds described in **ICH M7** may also be applied. Regulatory submissions must document the selected safety-based threshold (SCT or TTC) along with the rationale for its selection [11].

8. Staging an E&L Study

E&L studies should follow a staged approach to evaluate packaging systems systematically. This ensures data aligns with regulatory expectations and supports product safety across the drug lifecycle [5] [8].

By adhering to this systematic approach, as described in Table 8, manufacturers can generate robust data sets, support regulatory submissions, and ensure patient safety.

Table 8. Key stages in E&L study.

Stage	Objective	Key Activities
Preliminary Material Assessment	Evaluate packaging materials for suitability and identify critical components.	- Collect data on material composition from vendors. - Use literature and regulatory references for screening.
Controlled Extraction Studies (CES)	Simulate worst-case conditions to identify potential extractables.	- Select extraction solvents based on material properties. - Characterize chemical entities through advanced analytical techniques.
Leachables Assessment	Monitor leachables in the packaged drug product under storage conditions.	- Evaluate leachables over time using thresholds (SCT, QT, TTC).
Toxicological Risk Assessment	Assess the safety impact of identified leachables.	- Compare findings with SCT, QT, and TTC thresholds. - Engage toxicologists to ensure compliance with safety margins.

9. Controlled Extraction Study

A controlled extraction study is a laboratory investigation designed to qualitatively and quantitatively analyze the extractable profile of critical components in a container closure system. It marks the first step in the chemical characterization

of packaging material and provides crucial insights for selecting appropriate materials [18]. It helps meet regulatory expectations and ensures the safety of materials to control leachables in the final dosage form. The experimental protocol for a controlled extraction study should be meticulously crafted to establish a meaningful correlation between packaging materials and drug product interactions. Several factors must be considered in designing a controlled extraction study [11] [14].

- Details about the packaging system
- Selection of components for extraction study
- Selection of solvent system
- Selection of extraction techniques
- Selection of instrument techniques
- Selection of standards for screening non-targeted extractable (qualitative analysis)
- Identification of structure based on extractable level
- Quantitative controlled extraction study

The most practical way to begin a controlled extraction (CE) study is by identifying and selecting the critical components of the container closure system, such as vials, stoppers, seals, and syringes, especially those in direct contact with the drug product [15]. Evaluating the chemical composition of these packaging components can provide potential clues for toxic elements such as nitrosamines and PANs, which can often arise from materials like carbon black or sulfur curing agents [10]. It is recommended that vendor chemical composition information be obtained during the material screening process before initiating extraction studies. The effect of manufacturing processes, such as heat sterilization, must be carefully examined during the study design [16] [18].

When selecting components for a controlled extraction study, it is crucial to prioritize primary packaging materials, given their direct contact with the drug product. The extent of extractables is influenced by the material type, including plastics, elastomers, glass, metals, and coatings [19]. While primary packaging is prioritized due to its potential for extractable, secondary packaging (e.g., labels, adhesives) and functional systems (e.g., inhalers, pre-filled syringes) are also assessed for compatibility. The study's focus also varies with the type of dosage form: for parenteral products, the emphasis is on rubber stoppers, vials, and IV bags; for ophthalmic products, on dropper bottles and their caps; and for inhalation products, on inhaler devices and associated components [6] [10]. This comprehensive approach ensures patient safety, material suitability, and compliance with regulatory requirements [20].

Once the components and their chemical profiles have been evaluated, the next critical step is selecting appropriate extraction solvents to ensure comprehensive analysis [21]. The extraction solvents should be based on USP <1663>, and the selection of proper solvents must encompass a wider polarity range that can extract various chemical compounds that could become potentially leachable (**Table**

9). The solvent range should consider the drug product composition and be able to bracket the pH range of the formulation that could extract organic extractable. The study should also include additional solvents for extractable elements [18].

Table 9. Possible extracting media relative to particular packaging components.

Packaging Component	Possible Extracting Media
MDI valve elastomer seal (MDI formulation contains 1,1,1,2-tetrafluoroethane and ethanol)	Nonaqueous solvents (e.g., Dichloromethane, Isopropanol, Hexane)
Dry powder inhaler mouthpiece	Water (unbuffered), Isopropanol
Small-volume parenteral vial rubber stopper (aqueous formulation buffered at pH 6.5)	Water (pH 5.2), Water (pH 9.5), Isopropanol (50:50)
Large-volume parenteral plastic bag (aqueous formulation buffered at pH 7.2)	Water (pH 5.2), Water (pH 9.5), Isopropanol (50:50)

To maximize extraction efficiency, the components can be introduced into the selected solvents by pressing, grinding, or cutting them into small pieces to increase the contact surface area of the component in the solvent [19]. Controlled extraction studies must be performed on individual components, although elements of the same material can be combined. The major drawback of combining components is the inability to track the source of impurity during leachable study [19] [20].

Multiple extraction techniques like reflux, Soxhlet, microwave digestion, etc., should be employed to extract a wide range of compounds, including volatile organic compounds [19]. The extraction ratio between solvent and material is guided by the Analytical Evaluation Threshold (AET). The ratio must be high enough to achieve the AET level with the applicable analytical methods. Further, the selection of extraction time, extraction temperature, and extraction methods that will mimic or amplify the clinical conditions of contact between drug product/packaging system configurations should aim to derive maximum extracts without compromising the packaging components' integrity or degrading the extractable compounds that it does not provide adequate, useful information [21].

Once an extract is generated, the extracted sample solutions are prepared for analytical testing, with adjustments such as dilution or concentration based on AET requirements [21]. The next step is its chemical characterization, which involves qualitative and quantitative assessments across four key stages: scouting, discovery, identification, and quantitation. Scouting provides a preliminary screening to detect potential extractables, followed by discovery to uncover unexpected or unknown compounds. Identified compounds are matched with reference standards or spectral libraries, and advanced techniques like MS/MS or NMR are used when needed [21].

Appropriate analytical methods are selected to detect various volatile, semi-volatile, and non-volatile organic and inorganic compounds. However, it is impossi-

ble to validate these methods in advance, as the structures of the extractables are unknown [20]. Instead, methods are chosen for their ability to provide broad coverage and sensitivity, ensuring comprehensive detection and characterization of extractables. Various analytical techniques are employed to detect different types of extractables, as outlined in **Tabe 10** [18].

Table 10. Analytical techniques used in E& L studies.

Analytical Technique	Application
Gas Chromatography (GC)	Separation and analysis of volatile and semi-volatile organic compounds
Gas Chromatography-Mass Spectrometry (GC-MS)	Identification and quantification of volatile and semi-volatile organic compounds
Liquid Chromatography (LC)	Separation of non-volatile and thermally labile organic compounds
Liquid Chromatography-Mass Spectrometry (LC-MS)	Identification and quantification of non-volatile and thermally labile organic compounds
Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)	Detection and quantification of inorganic compounds (metals)
Fourier Transform Infrared Spectroscopy (FTIR)	Identification of organic compounds and polymeric materials based on functional groups
Ultraviolet-visible spectroscopy (UV-Vis)	Preliminary screening and quantification of compounds that absorb UV and visible light
High-Performance Liquid Chromatography (HPLC)	Separation and quantification of complex mixtures of organic extractables
Nuclear Magnetic Resonance (NMR) Spectroscopy	Structural elucidation and identification of organic compounds

Depending on the container closure system (CCS) material, additional analytical methods may be required to target specific extractables of concern [14]. For example, when analyzing extractables from elastomers, methods capable of detecting low levels of mutagenic compounds, such as nitrosamines and 2-mercaptobenzothiazole, may be necessary. Similarly, if the CCS contains carbon black, analytical methods sensitive to low levels of polycyclic aromatic hydrocarbons (PAHs) must be employed [16].

Standard reference materials are selected to represent common extractable compounds found in polymeric materials. For LC-UV/MS analysis, at least one standard must be detected in each mode (positive ionization, negative ionization, or UV) [13]. MS standards may show different response levels, so their concentrations should be adjusted to ensure accurate quantitation.

It is optional to identify all peaks observed during extractable analysis. Only peaks observed above AET should be identified using a clearly defined identifica-

tion. Also, only peaks not observed in the extraction blank are labeled as extractables [18]. Hence a blank solvent extract must be prepared in the same manner as the sample and analyzed before sample analysis. Suppose a peak in the blank is also observed at a significantly higher response level in the sample extract. In that case, the peak should also be labeled as extractable, and the background corrected for the area in the blank during the semi-quantitation step [18].

The controlled extraction study provides critical data on potential extractables that may migrate into the drug product. Identifying and quantifying these extractables against safety thresholds allows toxicologists to assess risks and determine which compounds to monitor during stability studies, forming the basis for the leachable study [18].

10. Simulation Study

Another way to support the extractable-to-leachable risk assessment is through a simulated leachable study. A simulation study is a Controlled Extraction Study designed to produce an extractables profile that mimics the worst-case leachables profile of a drug product [19]. This approach offers a powerful predictive tool that allows researchers to anticipate potential leachables in drug products without waiting for the full shelf-life, which is particularly valuable for products with extended shelf life [19] [20].

The extract generated in a simulation study should:

- Contain all substances that could potentially leach into the drug product at significant levels,
- Include these substances at concentrations equal to or higher than the maximum concentrations they may reach over the product's shelf life and
- Be generated more efficiently and in less time than a traditional drug product leachables study.

A simulation study involves placing the drug product or a placebo in contact with the packaging material under conditions that simulate actual storage [22]. Common temperature conditions for simulation studies include:

- 40˚C ± 2˚C for 6 months to simulate long-term storage.
- 50˚C to 60˚C for shorter durations (e.g., 1 - 3 months) for a more accelerated assessment.
- For extreme worst-case scenarios, 70˚C to 80˚C for a few days to a week can be used, although this must be carefully managed to avoid degradation of the packaging materials.

For products that require refrigerated or frozen storage, additional temperature conditions may include:

- 2˚C to 8˚C to simulate refrigerated conditions, or −20˚C for frozen storage, to ensure stability at lower temperatures.

Alternatively, an extraction solvent can be selected to closely match the pH and solvating properties of the drug product. A placebo can be used for simple formulations, provided it does not interfere with the analytical methods employed for

detecting leachables [21]. If neither the drug product nor the placebo is used, the extraction solvent must be carefully selected to ensure compatibility with the analytical techniques [22].

The simulation study should be conducted on packaging materials that closely resemble their final intended use. This ensures that the resulting data accurately reflects the potential migration of compounds into the drug product, aiding in a more focused toxicological assessment [14]. While simulation studies offer a faster and more targeted approach than traditional leachables studies, there is a risk that certain extractable identified in more aggressive controlled extraction studies might not appear under the milder conditions used in simulation studies. Thus, careful interpretation of the results is essential to ensure a comprehensive risk assessment [19] [20].

11. Leachable Studies

Leachable studies assess chemical entities that migrate from manufacturing and packaging components into pharmaceutical products under average storage and use conditions and during accelerated stability tests [21]. These studies also evaluate substances that may leach directly from medical devices during clinical use, potentially leading to patient exposure. Unlike extractables, which represent potential leachables and hypothetical risks, leachables migrate into the drug product, posing real safety concerns [21].

A scientifically sound leachables assessment must involve validated, quantitative analytical methods based on those used in Controlled Extraction Studies (CES) [15]. The following points are essential in leachable studies:

- **Analytical methods** used to detect leachables should be developed from CES-based techniques and fully validated for use.
- **Setting up an Analytical Evaluation Threshold (AET)** to define the minimum level of concern for safety risks.
- **Establishing a correlation** between extractables and leachables profiles to anticipate potential risks.
- **Considering the potential for special-case compounds** that may require additional scrutiny.

Storage conditions for leachable studies should cover normal conditions, reflecting typical usage, and include accelerated conditions to simulate aging, along with worst-case scenarios [23]. For instance, worst-case scenarios can include storing containers in inverted or sideways positions to maximize the contact between the drug product and the container closure system (CCS) [22]. While higher temperatures are typically expected to increase leachables, in some cases, elevated temperatures can reduce leachable concentrations by altering the partitioning between the CCS and the product. Sampling should occur at regular intervals such as 1, 3, 6, 9, and 12 months, with extended intervals if necessary [22].

The appropriate storage conditions, following **ICH Q1A(R2)**, are shown in the Table 11 below:

Table 11. Recommended storage conditions/duration for leachable studies.

Condition	Temperature (˚C)	Relative Humidity (% RH)	Time Points (months)
Long term	25 ± 2	60 ± 5	0, 6, 12, 24, 36
	30 ± 2	65 ± 5	
Intermediate	30 ± 2	65 ± 5	0, 6, 12, 24, 36
accelerated	40 ± 2	75 ± 5	0, 3, 6, 9, 12

Control samples stored in inert, non-reactive containers, such as glass bottles, are essential for accurate leachables studies. These control samples must be stored under the same conditions and durations as the stability samples, serving as a baseline to detect potential leachables [23]. Labels, adhesives, and inks should be avoided on control containers to prevent contamination. Comparisons between stability and control samples allow for the identification of leachable species. Including placebo samples stored in the CCS as part of the leachable study is strongly recommended. These samples can help confirm whether leachables are observed in the active [24].

The analytical techniques used for CES serve as the foundation for developing and validating methods for leachables studies. Given that leachables may be present at very low levels and that drug matrices can interfere with detection, validated methods must meet stringent criteria, including accuracy, precision (repeatability and intermediate precision), specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity, range, and robustness [24]. System suitability tests should be integrated into each method to ensure performance during analysis. Once the CES results are finalized, appropriate standards must be procured to validate the analytical methods used in the leachables study [24] [25].

No further analytical steps are required if the leachable is identified in the extraction study and validated using the authentic material. However, if the authentic material is not used for method validation, it should be employed in additional validation experiments to confirm the method's suitability for the leachable [25]. These extra tests may involve calculating a response factor, which could alter the results. In such cases, only the results calculated with the response factor should be reported [24] [25].

Reporting leachables requires defining specific thresholds to determine the significance of the detected compounds. Based on total daily intake (TDI), these thresholds guide reporting and identification processes to ensure patient safety [11]. The following **Table 12** outlines the reporting and identification thresholds used for leachables:

A minimum of three batches of drug product must be considered for leachable study. If the product has multiple strengths or packaging configurations, each should be represented in the study. These batches should be studied throughout the proposed shelf life of the product [26] [27].

Table 12. Reporting and identification level of leachables.

	Reporting Threshold for leachables	Reporting Threshold for leachables (Tentative structures)	Identification threshold for leachables (confident structures)	Identification threshold for leachables (confirmed structures)
Leachable level based on total daily intake (TDI)	0.2 µg/day	0.2 µg/day	>0.2 µg/day	~0.2 µg/day

12. Challenges in Analytical Techniques for E&L Assessments

1) Sensitivity Limitations: Modern extractable and leachables (E&L) assessments face challenges in detecting compounds at trace levels, often in parts per billion (ppb) or lower, to ensure patient safety. Techniques like Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) are highly sensitive but can encounter difficulties with compounds that have poor ionization efficiency or require specific derivatization for accurate detection [28]. Differentiating genuine leachables from background noise further complicates the process and can lead to false positives or negatives. Addressing these challenges involves employing advanced instrumentation such as high-resolution mass spectrometry (HRMS) to enhance sensitivity and mass accuracy, utilizing multiple ionization modes like Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) to broaden the detection range for diverse compound classes, and developing robust signal-to-noise optimization protocols to ensure reliable quantification [12] [29].

2) Matrix Effects: Complex matrices, such as drug formulations or biological fluids, pose significant challenges in extractable and leachables (E&L) assessments by interfering with analytical methods and causing ion suppression or enhancement, particularly in techniques like Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). These matrix effects can compromise quantitation and reproducibility, especially when analytes are at low concentrations. Addressing these issues requires thorough matrix evaluations during method development to identify and mitigate potential interference, using isotopically labeled internal standards to compensate for matrix effects, and implementing sample preparation techniques like solid-phase extraction (SPE) to eliminate interfering substances before analysis. Similarly, selectivity challenges arise when differentiating target leachables from structurally similar compounds, mainly when using spectroscopic methods such as Fourier Transform Infrared Spectroscopy (FTIR) or Ultraviolet-Visible Spectroscopy (UV-Vis), where overlapping peaks or lack of unique features can hinder accurate identification [29] These challenges can be addressed by pairing complementary techniques like Gas Chromatography-Mass Spectrometry (GC-MS) with Nuclear Magnetic Resonance (NMR) for confirmatory identifica-

tion, applying chemometric tools and advanced spectral deconvolution algorithms to improve resolution, and employing targeted methods such as selected reaction monitoring (SRM) in LC-MS/MS to enhance selectivity [30] [31].

3) Quantitative Limitations:

Accurate quantification of compounds across a broad dynamic range presents a significant challenge in extractable and leachables (E&L) assessments. High concentrations of extractable in packaging materials can saturate detectors, while ultra-sensitive quantification is required for trace levels of leachables in drug formulations. Addressing these issues involves calibrating instruments with a wide range of standards to ensure linearity across the dynamic range, employing multiple detectors, such as UV for high and MS for low concentrations, within the same workflow, and regularly validating and verifying calibration curves to maintain precision and accuracy. Similarly, managing and interpreting large datasets generated by advanced analytical methods pose data integrity and processing challenges [10]. Variability in analyst expertise and differences in data processing protocols can further complicate results. These challenges can be mitigated by implementing robust software tools for automated data analysis to reduce human error, rigorously training analysts in data interpretation to ensure consistency across studies and using statistical tools to evaluate reproducibility and precision across replicates [24].

4) Method Validation: Addressing Challenges

Method validation ensures analytical techniques' accuracy, precision, sensitivity, and reproducibility in extractable and leachables (E&L) studies. To address challenges, it is essential to rigorously evaluate validation parameters such as specificity, linearity, limit of detection (LOD), quantification (LOQ), accuracy, and robustness. For instance, specificity testing with spiked and unspiked matrices is critical to address matrix effects effectively. Interlaboratory validation studies further enhance method transferability and reproducibility, ensuring consistent results, which are crucial for regulatory submissions. Adopting a risk-based approach prioritizes validation efforts by focusing on highly toxic compounds or critical materials that pose significant safety concerns and optimizing resources while addressing the most pressing risks [18]. Continuous monitoring and periodic revalidation are necessary to maintain method relevance and accuracy as materials or formulations evolve, particularly in long-term stability studies or post-market surveillance [21]. By understanding and mitigating limitations such as sensitivity constraints, matrix effects, and data processing issues, researchers can achieve robust and reliable E&L assessments. Employing advanced technologies, risk-based strategies, and ongoing monitoring further ensures reliability and compliance, supporting safer and more efficient pharmaceutical development [24].

13. Extractable and Leachable Correlation

Establishing a correlation between extractables and leachables is essential for interpreting, assessing, quantifying, and controlling the interactions between the

drug product and the container closure system (CCS) [32]. A robust qualitative and quantitative correlation between the extractable and leachables profiles helps ensure product safety and compliance with regulatory requirements. The correlation process should account for results from extraction studies conducted on multiple batches of packaging or manufacturing components and leachable studies performed in various batches of the drug product across different stability storage time points [33] [34].

A **qualitative correlation** is established by demonstrating that compounds detected in the leachable study are also present in the controlled extraction study. A **quantitative correlation** is confirmed by showing that the levels of leachables identified in the leachable study are consistently lower than or comparable to the levels of extractables found in the quantitative controlled extraction study [23]. This correlation confirms that the extraction study accurately predicts the leachables that may migrate into the drug product under real-world conditions [35].

Extractable Leachable Studies from Manufacturing Equipment

Extractables can originate not only from a drug product's packaging system but also from the components and systems used in the manufacturing process that come into direct contact with the drug product before final packaging [33]. Production batches and process solutions, such as chromatographic eluents or cleaning agents, can come into contact with various manufacturing equipment, including tanks, single-use systems, tubing, or gaskets, potentially introducing leachables into the product stream. These interactions could affect the pharmaceutical product's quality and influence the effectiveness of subsequent processing steps [36].

To mitigate these risks, conducting extractable and leachables studies under conditions that reflect actual manufacturing processes is crucial. Variables such as the surface area-to-solvent ratio, contact time, temperature, and the formulation's composition play a significant role in determining the extent of leaching. Tailored studies are essential for tubing materials, such as silicone and Santoprene [20]. Silicone tubing has been shown to release compounds like dioctyl phthalate and dioctyl adipate, while Santoprene tubing releases phthalates, alkyl phenols, and Irganox-type antioxidants. Additionally, these materials may release inorganic extractables, including calcium (Ca), magnesium (Mg), zinc (Zn), and boron (B), necessitating comprehensive analysis [33].

A typical tubing leachables study is conducted by placing the product directly inside the tubing and allowing it to remain for various durations under controlled conditions. The product is periodically tested to detect potential leachables, simulating real-world scenarios of product-tubing interactions [25]. Analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) are employed to identify and quantify both organic and inorganic leachables. Blank controls are essential to ensure the accuracy and reliability of the data [27] [28] [32].

These studies help identify potential risks associated with leachables from manufacturing equipment and allow for the implementation of mitigation strategies to minimize contamination risks. Comprehensive extractables and leachables assessments for packaging and manufacturing systems are essential to ensure product safety, quality, and regulatory compliance [34].

14. Toxicological Risk Assessment

Toxicological risk assessment is the primary goal of extractable and leachables (E&L) studies. The involvement of toxicologists is crucial from the material selection stage, where they use vendor data to identify the presence of alert chemicals [15] [35]. Data from extractable studies help determine which compounds have the potential to become leachables, followed by evaluating their toxicity using available literature. Once a potential leachable is identified, further analytical testing confirms its presence and measures its concentration in the final drug product. The next step is calculating the leachables estimated daily intake (EDI), considering its concentration, the product's dosage form, and the maximum daily dose based on the route of administration [36]. Toxicologists then collect relevant toxicological data from scientific literature, databases, and regulatory resources to assess the potential risks. In cases where direct toxicological data is lacking, predictive methods such as read-across approaches or structural alerts are used based on chemical similarities, and allometric scaling may be employed to extrapolate toxicological effects from animal studies to humans [37]. A margin of safety (MOS) is then calculated by comparing the estimated exposure and the no-observed-adverse-effect level (NOAEL) or other toxicity thresholds. If the MOS is sufficient, the leachable is deemed safe; if not, mitigation strategies, such as changes in packaging or formulation, may be recommended. This comprehensive process ensures an accurate toxicological evaluation, minimizing risks to patient safety and meeting regulatory compliance [38].

15. Interpretation and Application of Toxicological Data in Risk Assessments

The interpretation and application of toxicological data are essential for conducting robust risk assessments in extractable and leachables (E&L) studies. This process begins with identifying compounds extracted from materials or leached into products during storage or use. Toxicological evaluation determines which compounds require further assessment based on their concentration, structure, and potential toxicity. For example, if benzophenone is identified as a leachable in a polymeric container through GC-MS analysis, its concentration and toxicological profile are evaluated to assess potential risk. Safety thresholds such as the Permitted Daily Exposure (PDE) or the Threshold of Toxicological Concern (TTC) guide these evaluations. PDE values are derived using toxicological data like the No-Observed-Adverse-Effect Level (NOAEL), adjusted by safety factors to account for variability. In contrast, TTC values offer a conservative benchmark for com-

pounds with limited toxicological data [27] [28].

Exposure assessments involve quantifying a compound's Estimated Daily Intake (EDI) based on product use and comparing it against these safety thresholds. For instance, if the concentration of benzophenone in a medication is 0.05 mg/L and a patient consumes 2 L/day, the EDI would be 0.1 mg/day, then compared to the PDE. Risk characterization assesses whether the EDI exceeds the threshold. If it does, mitigation strategies such as material substitution or process optimization are implemented. TTC is particularly useful for compounds with unknown toxicological data, providing a default threshold to guide decisions without requiring extensive testing. For example, a non-genotoxic packaging extractable with an exposure below 1.5 µg/day may not need further toxicological evaluation. PDE-based assessments, such as those for Bisphenol A (BPA) with a PDE of 4 µg/kg/day, compare patient exposure to the defined threshold to evaluate safety [29].

Exceptional cases, like genotoxic impurities, apply much stricter thresholds, such as the 10 ng/day concern level per ICH M7 guidelines, ensuring patient safety. Challenges in toxicological interpretation include data gaps, cumulative or synergistic effects of multiple leachables, and considerations for vulnerable patient populations like pediatrics, where additional safety factors are applied. Strategies to address these include using in silico predictions, conducting cumulative exposure assessments, and employing stricter thresholds for specific populations. Integrating toxicological data into validated workflows enhances reliability, with robust analytical methods confirming compound identity and exposure models ensuring real-world applicability [39]. Practical examples, such as evaluations of benzophenone or BPA, illustrate how safety thresholds guide decision-making. Addressing data gaps and cumulative toxicity further strengthens assessments, ensuring patient safety and compliance with regulatory expectations [40].

16. Common Deficiencies in E&L Studies

Ensuring the safety and efficacy of pharmaceutical products involves comprehensive Extractable and Leachable (E&L) studies. However, despite precise FDA requirements, many submissions fail to meet regulatory expectations due to gaps in study design, execution, and data interpretation. Addressing these deficiencies is critical to ensuring compliance, product quality, and patient safety.

16.1. Deficiencies in Study Design and Execution

Incomplete study design is a frequent issue in E&L submissions. This often results in the omission of critical tests or insufficient analysis of key components. Common gaps include:

Leachables Study:

Many submissions lack data on leachables originating from the primary packaging system. These studies should include a detailed analysis of impurities, additives, and degradants to assess their potential impact on the drug product.

Controlled Extraction Studies:

Rigorous extraction studies using solvents of varying polarity are essential for identifying a wide range of extractables. These studies are necessary to ensure the comprehensive evaluation of packaging materials.

Manufacturing Materials:

E&L studies for materials used during manufacturing, such as silicone tubing, should be considered. These components can introduce impurities during batch-filling operations and must be thoroughly assessed.

Specialized Components:

Unique materials like shower caps or other ancillary items in contact with the drug product are frequently excluded from compatibility assessments. Their omission creates potential safety risks that could otherwise be mitigated.

A robust E&L study requires including all critical components, detailed extraction protocols, and solvent systems capable of identifying diverse extractables.

16.2. Deficiencies in Data Interpretation and Justification

Data interpretation and justification gaps often lead to regulatory deficiencies even when studies are conducted. These include:

Correlation Between Extractables and Leachables:

A lack of comparative analysis between extraction profiles and leachables profiles from near-expiry drug products hinders understanding of long-term safety.

Leachables Stability Data:

Submissions frequently fail to include stability data showing that extractables do not appear as leachables in the drug product over its shelf life. In cases where leachables are observed, comparative studies with reference-listed drug (RLD) lots are often missing.

Clear, scientifically supported data interpretation is essential for establishing a link between extractables and leachables and product safety. This ensures regulatory confidence in the study results.

16.3. Bridging the Gap: Best Practices

To address these common deficiencies, manufacturers should adopt the following strategies:

- **Comprehensive Study Design:** Include all relevant components, prioritize critical packaging materials, and use extraction solvents with a broad polarity range.
- **Thorough Data Analysis:** Compare extractable profiles with leachables profiles over the product's shelf life and provide stability data to demonstrate safety.
- **Proactive Justifications:** Document the rationale for observed leachables or the absence thereof, supported by regulatory guidance and toxicological data.

By addressing these areas proactively, manufacturers can meet FDA expectations and ensure product integrity and patient safety throughout the product lifecycle. A meticulous and scientifically sound E&L study is not just a regulatory

requirement but a cornerstone of pharmaceutical quality assurance.

17. Innovations in E&L Assessments

In the evolving pharmaceutical and medical device industries, extractable and leachables (E&L) assessments are critical for product safety and regulatory compliance. A significant advancement in this field is integrating in silico predictive tools, which leverage computational models to estimate the likelihood of compound migration, toxicity, and interactions based on material composition and exposure conditions [30] [31]. These tools enable early-stage risk assessments, reducing reliance on extensive laboratory testing and providing a proactive approach to identifying and mitigating potential risks.

17.1. Predictive Tools and Advanced Technologies

Predictive platforms such as Quantitative Structure-Activity Relationship (QSAR) models simulate material behavior and identify toxicological risks, particularly in commonly used materials like polymers, coatings, and adhesives [12]. These tools, enhanced by comprehensive databases such as Tox21 and ECHA REACH, streamline the selection of safer materials by predicting migration behaviors and toxicity alerts [32]. Advanced machine learning (ML) algorithms further enhance these capabilities by analyzing historical E&L data, refining predictions, and ensuring alignment with evolving regulatory requirements. This adaptive approach improves the reliability of assessments for novel materials and supports a more dynamic evaluation process [34].

17.2. Integration with Regulatory Frameworks

In silico tools also align closely with regulatory frameworks, supporting compliance with the FDA and EMA agencies. The FDA encourages computational toxicology approaches for evaluating impurities and leachables, while EMA guidance highlights the utility of risk-based strategies to prioritize testing efficiently [27]. While in silico methods cannot replace experimental studies, they complement traditional techniques like LC-MS, GC-MS, and FTIR spectroscopy, enabling a tiered approach [28]. This includes using in silico tools for initial risk assessments and material selection, targeted analytical testing, and confirmatory toxicological evaluations for high-risk compounds. This integrated approach enhances the efficiency, cost-effectiveness, and safety of E&L assessments [33].

17.3. Challenges and Opportunities

Adopting in silico tools comes with challenges, including the need for high-quality input data, comprehensive material databases, and expertise in computational modeling. Aligning these methods with traditional testing protocols requires a cultural shift within regulatory agencies and industry [40]. However, the benefits are substantial: reducing reliance on extensive physical testing can significantly lower costs, early-stage predictive capabilities can accelerate development time-

lines, proactive risk management aligns with global regulatory trends favoring computational methodologies, and minimizing experimental testing supports sustainability goals by conserving resources. The future of extractable and leachables (E&L) assessments lies in integrating in silico tools with omics technologies such as metabolomics and proteomics, enhanced databases, and real-world data. These innovations will enable predictive models to capture complex interactions more effectively, broadening their applicability across product categories like biologics, gene therapies, and combination products [41]. In conclusion, in silico predictive tools represent a transformative shift in E&L assessments, offering faster, more accurate, and cost-effective evaluations while ensuring patient safety and meeting rigorous regulatory demands [42].

18. Conclusions

Maintaining pharmaceutical products' highest quality and safety standards is critical to protecting patient health. Impurities from packaging materials, though not direct degradants, can migrate into drug products and pose risks over time. Conducting well-designed Extractable and Leachable (E&L) studies minimizes these risks and ensures product purity.

While general guidelines like USP <1663> and <1664> provide a framework, E&L studies must be tailored to the specific drug product, packaging system, and storage conditions. Early assessment during development allows manufacturers to address potential challenges, select appropriate analytical methods, and evaluate vendor-provided data, reducing the risk of delays. Incorporating thresholds such as the Analytical Evaluation Threshold (AET) and Safety Concern Threshold (SCT) ensures scientifically sound evaluations.

Thorough E&L studies support regulatory compliance and patient safety and deliver cost efficiencies by identifying risks early, avoiding market delays, and ensuring competitive advantage. A proactive approach strengthens product development and ensures the timely delivery of high-quality pharmaceuticals to patients.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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