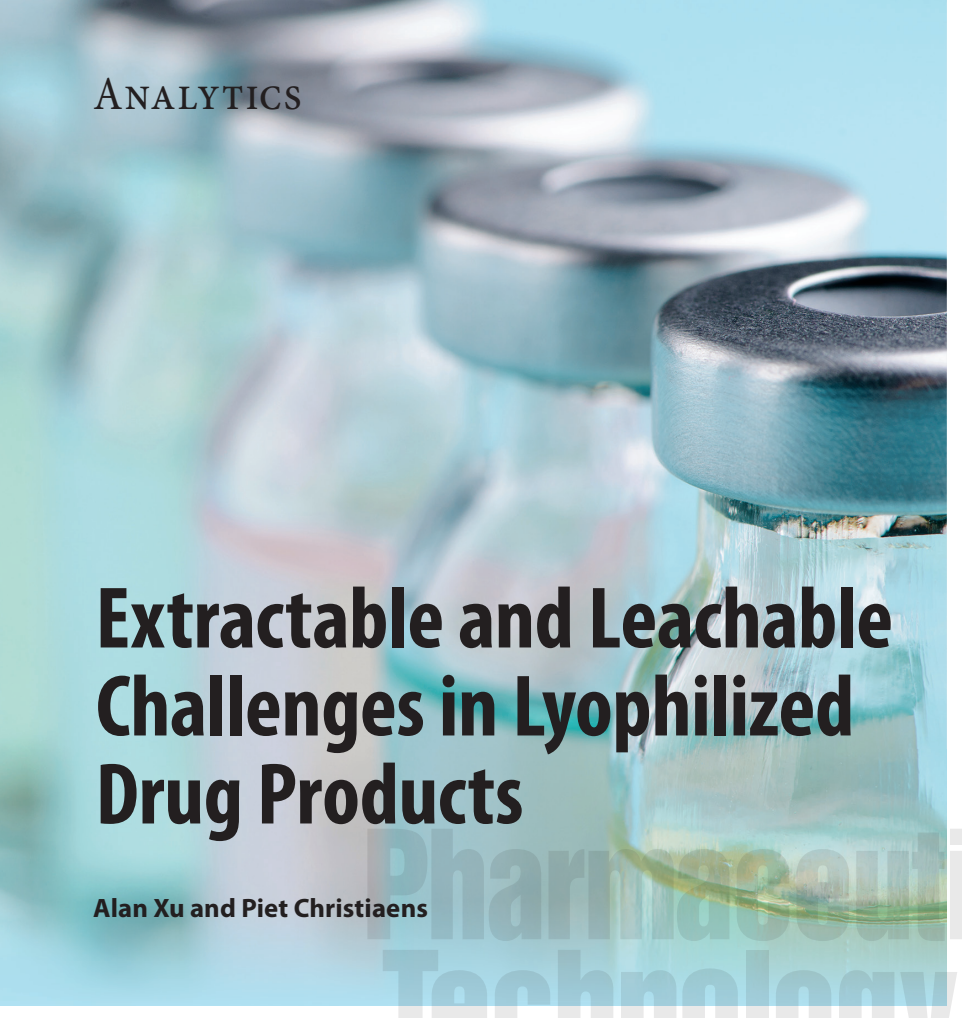

Extractable and Leachable Challenges in Lyophilized Drug Products

Alan Xu and Piet Christiaens

The authors examine the risks of extractables and leachables, and present solutions that emphasize the importance of a strategic, multi-prong approach.

Lyophilization is a sophisticated preservation technique designed to stabilize sensitive pharmaceutical compounds by removing water through a meticulously controlled process of freezing and sublimation under vacuum conditions. This method is indispensable for the stability and long-term efficacy of medications that otherwise would have excessively stringent requirements for temperature and humidity control. Despite its advantages, the lyophilization process introduces a complex interplay between the drug product and its packaging materials. Exploring the challenges inherent in testing and mitigating extractables and leachables (E&L) risks require innovative solutions grounded in regulatory guidance as well as advancements in analytical methods, primary packaging, and systemic collaborations.

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Key benefits and associated challenges of lyophilization

Lyophilization involves transforming liquid formulations into stable, lightweight, and portable solid forms known as lyo cakes. The process consists of three primary stages: freezing, primary drying (sublimation), and secondary drying (desorption). This transformative process not only ensures the preservation of the drug's chemical and physical integrity but also allows simpler storage and transportation such at room temperature. However, the absence of water and the unique characteristics of the resulting solid-state product amplify certain E&L contamination risks due to interaction with packaging materials.

Primary packaging materials, including glass vials and rubber stoppers, are integral to maintaining the stability of lyophilized drug products. However, these materials can release volatile and semi-volatile compounds through mechanisms such as outgassing, which accumulate on the highly absorbent surfaces of the lyo cake. Secondary packaging systems, such as multilayer films and aluminum pouches, though designed to shield against external contaminants, may also contribute to E&L risks under varying environmental conditions such as temperature and humidity fluctuations. These interactions necessitate rigorous testing and validation protocols to mitigate potential contamination and ensure the drug product's long-term stability.

Understanding the fundamentals

Extractables are chemical entities that can be released from packaging materials under controlled but often extreme conditions, such as exposure to solvents, elevated temperatures, or pressure. Leachables are substances that migrate into the drug product under standard storage or usage conditions, potentially compromising its safety, efficacy, and stability.

In lyophilized drug products, E&L interactions predominantly occur through outgassing, where volatile and semi-volatile organic compounds from packaging materials, particularly rubber stoppers, migrate into the vial's headspace and adsorb onto the dry lyo cake. The high surface area and extreme dryness of the lyo cake exacerbate this absorption. Additionally, reactive leachables may interact with APIs or excipients, creating byproducts that can compromise drug quality and pose safety risks to patients.

Regulatory frameworks for E&L evaluation

Both FDA and *United States Pharmacopeia* (*USP*) <1664> categorize prod-

E&L Testing Trends: Current Greatest Areas of Need

Dujuan Lu, PhD, manager and global lead of the extractables and leachables (E&L) team at SGS Health Science, spoke with *Pharmaceutical Technology*® on a variety of E&L-related topics, including: testing needs for single-use products, regulatory differences for combination products versus single or simplified drug products, the financial outlook for the market in 2025 and beyond, and the research that Lu and her team have been conducting and publishing on the challenges that advancements in the field have presented.

Lu previously sat down with *Pharmaceutical Technology*® at AAPS PharmSci 360 in 2023, for a series of videos that expanded upon her talk at the conference, "Regulatory Expectations in Extractables and Leachables Testing of Combination Products" (1–3). One of the topics covered in those interviews was the common ground in analytical testing under both *United States Pharmacopeia* (*USP*) and International Organization for Standardization (ISO) standards, despite different extractables that may be tested.

"There is GC–MS [gas chromatography with mass spectrometry] for volatiles; there is direct injection GC–MS for semi volatiles, LC–MS [liquid chromatography with mass spectrometry] for the nonvolatiles, and the ICP–MS [inductively coupled plasma mass spectrometry] for elemental impurities," Lu said in her comparison (2).

Lu again addresses the distinction between *USP* and ISO, as well as other E&L considerations, in the questions below.

PharmTech: How has the need for testing in single-use products evolved in recent years? Can you explain how the problem differs in single-use versus reusable products?

Dujuan Lu (SGS): The single-use product typically has a lower risk in terms of E&L, compared to reusable products, as the patients are exposed to the single-use packaging or devices for a limited time. The toxicological risk could be considered lower based on the duration and frequency of the treatment.

PharmTech: What are the differences with combination products versus single/simplified drug products? How are regulations adapting to this?

Lu (SGS): Per the definition from FDA, a combination product is a product composed of any combination of a drug and a device, a biological product and a device, or a drug and a biological product, or a combination of a drug, device, and a biological product (4). All aspects of the regulations will be considered when evaluating the regulation submission of the combination products. The product's primary mode of action (PMOA) will be crucial and could lead to a suitable regulatory submission pathway. If the PMOA of a drug-device combination product is 'drug,' most of the pharmaceutical regulatory testing will apply. When a PMOA of a drug-device combination product is 'device,' the combination product will be considered as a medical device. There are different E&L regulatory guidances in pharmaceutical testing versus medical device testing. The extractables and leachables study designs of pharmaceutical and biological drug products associated with pharmaceutical packaging/delivery systems typically follow *USP* <1663> for extractables testing and *USP* <1664> for leachable testing (5,6). The Product Quality Research Institute (PQRI) guidance also provides good recommendations to design extractables and leachables studies for parenteral and ophthalmic drug products (PODP) and orally inhaled and nasal drug products (OINDP) (7). As for testing of extractables and leachables of devices, it is usually considered as a chemical characterization of medical device materials per ISO 10993-18 (8).

PharmTech: What do testing teams miss most often in their attempts to detect and quantify extractables and leachables, or, what are their biggest challenges?

Lu (SGS): There are a lot of analytical challenges regarding extractable and leachable testing. The major challenges are associated with analytical evaluation threshold (AET), confident identification, and semi-quantitation. They are outlined in one of our [SGS'] recent review articles (9).

PharmTech: Financially, what is the state of the E&L testing market early in 2025, and how is the market positioned to grow through the second half of the 2020s? What factors are driving growth, and what could hold that growth back?

Lu (SGS): The current E&L testing market is still growing, given more stringent regulatory requirements. There have been numerous E&L industry guidances published in recent years or expected in upcoming years, including *USP* <665>/<1665> for pharmaceutical/biopharmaceutical manufacturing materials, ISO 10993-18:2020, FDA guidance on chemical testing of medical devices, and the upcoming ICH (International Council for Harmonisation) Q3E. Most of the testings are carried out at third-party contract research organizations, as they have dedicated instrumentation and technical expertise built on many years in the industry. Some big pharmaceutical and medical device companies are also performing E&L testing in-house, due to confidentiality of the products in development or other historical reasons.

PharmTech: Are there any emerging pharmaceutical areas where E&L testing is currently being emphasized?

Lu (SGS): There are several emerging areas with more E&L focus these days. One of the areas is related to pharmaceutical/biopharmaceutical manufacturing materials. During the manufacturing process of the pharmaceuticals or biologics, more and more plastic single-use systems have been utilized these days, instead of the traditional stainless-steel materials in the old days. However, those plastic additives can easily leach out into the drug substance and drug product during the manufacturing process. With the new *USP* <665> scheduled to become official in 2026, all drug/biological manufacturers will need to perform risk assessment and testing based on the risk level.

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uct interaction risk (1) or specifically leachables risk (2) based on the dosage form and administration route. For lyophilized drugs (powders for injection/reconstitution), while the leachables risk of direct interaction during solid-state storage is considered low, the reconstitution phase significantly elevates these risks. Furthermore, worst-case scenario testing can be utilized to ensure leachables remain within acceptable safety limits under real-world conditions.

The European Medicines Agency (EMA)'s guidelines includes a decision-tree approach for assessing packaging interactions (3). For drugs intended for parenteral administration, the International Council for Harmonisation (ICH) also recommends long-term stability studies and accelerated testing to identify cumulative leachables over the product's lifecycle (4). Real-world simulations, including variations in temperature and humidity, are emphasized to ensure comprehensive compliance.

Challenges in E&L testing for lyophilized products

The following are some challenges in E&L testing for lyophilized products.

Outgassing and progressive accumulation. Rubber stoppers release volatile and semi-volatile organic compounds, which continuously accumulate on the lyo cake during storage. This progressive outgassing, coupled with the lack of equilibrium in the vial's headspace, leads to increasing contamination levels over time.

Reconstitution-induced risks. Reconstitution introduces additional complexities as volatile and semi-volatile compounds solubilize in the diluent. This step also allows short-term interactions between the liquid drug product and the primary packaging, potentially introducing new contaminants, including metals and ions. Such multifaceted interactions demand extensive testing to ensure product safety.

Analytical complexities and the absence of true blank solutions. A major limitation in E&L testing for lyophilized products is the challenge of establishing a true blank solution. Because the lyophilization process inherently involves packaging contact, alternative approaches, such as time-point zero baselines, must be employed. However, these methods are imperfect and introduce uncertainties in distinguishing between inherent impurities and leachables. Advanced analytical techniques, including high-sensitivity liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–MS (GC–MS), are indispensable but require significant expertise and resources to implement effectively.

Strategies for mitigating E&L risks

The choice of packaging materials plays a pivotal role in minimizing E&L risks. Utilizing low-alkali glass and high-quality, low-residual rubber stoppers can significantly reduce contamination. Proactive collaboration with suppliers during material selection and validation helps demonstrate compatibility with the lyophilized drug product.

E&L studies must encompass both extractables and leachables testing under simulated real-world and worst-case scenarios. This includes rigorous assessments during storage, reconstitution, and administration. Predictive modeling tools and accelerated testing techniques can enhance study efficiency and provide robust data.

Innovative packaging designs, such as non-contact systems and reduced headspace configurations, minimize the accumulation of leachables. Coated or laminated materials further reduce the migration of harmful compounds, ensuring better product safety.

Developing standardized methodologies across the pharmaceutical industry ensures consistency, improves compliance, and facilitates data sharing. Such protocols should address the entire lifecycle of lyophilized products, from manufacturing to patient administration.

Post-market surveillance programs and ongoing reviews of packaging materials and processes are essential for adapting to emerging risks and regulatory changes. Pharmaceutical companies can leverage feedback from real-world usage to help collaborate with container manufacturers and analytical laboratories to identify and address vulnerabilities proactively.

Conclusion

The unique characteristics of lyophilized drug products amplify the unique E&L risks associated with them. These various challenges arise from complex interactions between the drug product and its packaging during storage, reconstitution, and administration. Addressing these risks requires a multidisciplinary approach that involves, among other factors, advanced material science, rigorous testing methodologies, and compliance with global regulatory standards. By adopting innovative packaging technologies, implementing comprehensive E&L studies, and fostering continuous improvement, the pharmaceutical industry will be able to ensure the safety, efficacy, and quality of lyophilized medications. As advancements in both the areas of analytical tools and packaging materials continue to be made, continuous investment in E&L practices will further enhance the reliability of life-saving therapeutics, safeguard patient health and maintain global trust in pharmaceutical innovations.

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