Extractable and Leachable Database Building for High Confidence Identification by Liquid Chromatography-Mass Spectrometry

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INTRODUCTION

Liquid chromatography-mass spectrometry (LC-MS) is a powerful technique for extractables and leachables (E&L) analysis, capable of identifying and quantifying E&L compounds during medical device testing. These analyses are frequently challenging because of high sample complexity, with a widely diverse set of possible extractables. E&L testing laboratories must be able to identify a range of compounds with high certainty. While the value of subject matter expert data interpretation cannot be understated, a comprehensive mass spectral database that allows for retention time, accurate mass spectrum, and tandem mass spectrum matching is the gold standard for compound identification. It is crucial to add a range of compounds to the database from each compound class frequently observed in E&L studies. The process is iterative, producing an increasingly comprehensive library as more additions are made. Moreover, regularly observed compounds should be procured and added to the library as they are identified.

When preparing a medical device chemical characterization report, Eurofins EAG Laboratories provides confidence levels alongside identifications to give more context to the toxicologist providing the risk assessment (see inset). Higher identification confidence yields better outcomes in toxicological risk assessments (TRAs). For compounds with limited data, "tentative" identifications provide class-level information without sufficient data to support a single structure. Both "confirmed" and "confident" identifications solve the data for a unique compound, but confirmed IDs provide extra assurance through matching retention time, accurate mass, and tandem mass spectra to EAG's internal databases. Through database building, EAG provides higher-quality chemical characterization reports with reduced turnaround times to support medical device biocompatibility programs.

EUROFINS EAG LABORATORIES CONFIDENCE LEVELS (BASED ON GUIDELINES FROM USP 1663)
Confirmed: mass spectral and retention time match with database reference compound.
Confident: sufficient confirmatory information to preclude all but the most closely related structures.
Tentative: data consistent with a class of molecule only.

GROWING THE DATABASE

To make additions to the database, reference standards for the selected compounds are obtained from reputable chemical suppliers. Prior to sample handling, the safety data sheet for each compound is reviewed to account for potential hazards. Solutions of each standard are prepared at concentrations applicable to the instrumental methodology. Each prepared standard is analyzed using the same validated E&L LC-MS method used for medical device chemical characterization, ensuring that the data will be representative of how that compound might be detected in a device extract. The collected data are evaluated for quality and processed using ThermoFisher mzVaultTM to collect representative tandem mass spectrometry data from all species (adducts, in-source fragments, etc.). After adding the data as a new entry in the database (including retention time, formula, identifying information, etc.), a second subject matter expert reviews the entry for accuracy.

Compounds selected for inclusion in EAG's E&L mass spectrometry database represent a diverse profile of E&L classes. Tailoring growth to the needs of Study Sponsors, data analysts request compounds to be added to the database from studies they complete. Thus, the challenging chemical identification process is translated into institutional memory when compounds first identified from device extracts are procured, analyzed, and added to the database. EAG welcomes requests from Study Sponsors, so every compound singled out for targeted analysis is also added to the E&L database. Examples of the included compound classes are shown in the table on the next page.

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CLASS	DESCRIPTION	EXAMPLE(S)
Polymers	Common materials of construction	Polyethylene glycol, nylon
Surfactants	Cleaning, wetting, dispersing agents and lubricants	Sodium dodecyl sulfate
Antioxidants	Additives for plastics or rubber to prevent oxidation or degradation	Butylated hydroxytoluene, Irganox 1076
Plasticizers	Additives for plastics to increase flexibility, durability, or transparency	Bisphenol A, dioctyl phthalate
Dyes	Additives which impart color to a medical device	Disperse Blue 106, HC Red 3
Sensitizers	Additives which may cause irritation to skin, eyes, or lungs after exposure	Activated triazines, organophosphorus, isocyanate compounds
Per-and-polyfluoro alkyl substances	Coatings, lubricants, and processing aids	Perfluorooctanoic acid, perfluoro octane sulfonic acid
Cohorts of concern	Intentional or unintentional additives with acute toxicity and/or mutagenic potential	Aflatoxin-like, N-nitroso, alkyl-azoxy, organophosphorous compounds

As the database grows, the frequency of "confirmed" identifications grows, which provides the most valuable information to Study Sponsors and their toxicology teams. Moreover, database matches are much quicker than solving structures independently, and these time savings help EAG offer industry-leading turnaround times. Tandem mass spectra from known compounds in the library also aid in interpreting other structurally similar compounds.

WHERE WE ARE NOW

EAG continues to maintain its dedication to its ever-growing E&L database. This year, an average of more than 80 new compounds have been added each month. The E&L database is an invaluable tool for our LC-MS subject matter experts, complementing EAG's fleet of state-of-the-art ThermoFisher Orbitrap™ mass spectrometers.

REFERENCES

1. ISO 10993-18:2020: Biological evaluation of medical devices — Part 18: Chemical characterization of medical device materials within a risk management process. *International Organization for Standardization*, 2020.

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3. General Chapter, (1663) Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems. *United States Pharmacopeia*, 2022.