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ANALYSIS OF EXTRACTABLES AND LEACHABLES

OVERVIEW, TECHNOLOGIES, BEST PRACTICES

Manufacturers of pharmaceuticals, drug delivery systems, and biomedical devices have come under growing pressure to perform sensitive and accurate analytical studies to detect, identify, and quantify extractable and leachable compounds (E&Ls). E&Ls may be inherently toxic or may contaminate or interact with drug products, posing a potential danger to patients. Even as regulatory guidance related to the application, performance, and reporting of E&L studies increases and examples and data accumulate, E&L analysis is still an evolving area of investigation. This whitepaper provides an overview of the current best practices in the analysis of E&Ls, including the basic principles of E&L analysis, how to design an E&L study, and a detailed look at workflows, focusing on analytical techniques and instrumentation, sample selection, and extraction conditions. The discussion also highlights the advantages and limitations of available and emerging separation, detection, and identification technologies, software tools, and quantitative methods development.

THE IMPORTANCE OF E&L ANALYSIS

Extractables are substances that can be extracted from pharmaceutical packaging materials, a biomedical device, or a drug delivery system using extraction solvents (polar or non-polar) and various extraction conditions (temperature, exposure time) that are at least as aggressive as the expected conditions of use. Whereas leachables are trace amounts of chemicals that originate from packaging materials, medical devices, drug delivery systems, or processing equipment and can leach into and contaminate a product. Whether an extractable will also be a leachable will depend on the solubility of the extractable compound and the use conditions of the device or packaging.

The purpose of extractables analysis is to determine the worst-case leachables scenario and to measure the accumulation levels of detected substances over the shelf-life of a product. This is also why leachables analysis should mimic the most stringent use conditions of a product. Ultimately, the identification and quantitation of leachables makes it possible to perform a toxicological evaluation

and determine product safety. A typical E&L analysis might reveal common plastics additives such as antioxidants, surfactants, slip agents, plasticizers, acid scavengers, and cross-linking agents, as well as residual monomers and oligomers, polymerization side products, and process impurities.

"THE COMPLEXITIES IN THE ANALYSIS OF E&L IS A DIRECT RESULT OF ALL THE VARIOUS ROUTES OF DRUG ADMINISTRATION THAT THEN REQUIRE THE USE OF A LARGE VARIETY OF DRUG DELIVERY TECHNOLOGIES."
— SMRITI KHERA

Why is E&L analysis so important? Chemicals that can leach from medical devices and packaging materials represent an enormous risk to modern drug delivery. "The complexities in the analysis of E&L is a direct result of all the various routes of drug administration that then require the use of a large variety of drug delivery technologies," says Smriti Khera, Ph.D., Global Pharmaceutical Segment Manager at Agilent Technologies. "The risk increases as more components come in contact with the drug product," she adds, with risk rising as drug formulations become more complex, going from oral to topical, transdermal, injectable, and inhaled. The dose volume and frequency and duration of contact between the drug and packaging also contribute to overall risk.

Drug recalls have been on the rise in recent years and leached impurities have been a key factor responsible for many of these. In 2014 FDA recalls surged to 836. Recalls affect patients, denying them access to approved medications, as well as manufacturers of drugs, delivery systems, devices, and components, often imposing a significant financial burden.

Drug recalls have been on the rise in recent years and leached impurities have been a key factor responsible for many of these. In 2014 FDA recalls surged to 836. The U.S. Pharmacopeial Convention (USP) has issued regulations for the characterization of materials-of-construction, packaging systems, and pharmaceutical products that include E&L analysis.

"The results of studies designed to detect extractables can support the development of safe and effective manufacturing and packaging of drugs and devices," says Mark Jordi, Ph.D., President, Jordi Labs. "This information can also facilitate investigations into the origin(s) of identified leachables whose presence causes out-of-specification results for a marketed product."

Regulations and standards governing E&L analysis have proliferated in recent years, in particular related to pharmaceutical packaging and biomedical devices. The regulations often provide helpful guidance on a range of topics including the characterization of packaging materials and systems, toxicological assessment, and E&L testing specifications and experimental design. The primary FDA guidance document, U.S. FDA 21 CFR 211.94(a), states that, "Drug product containers and closures shall not be reactive, additive or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug beyond the official or established requirements.... Standards or specifications, methods of testing, and, where indicated, methods of cleaning, sterilizing, and processing to remove pyrogenic properties shall be written and followed for drug product containers and closures."

E&L STUDY DESIGN

A well-designed E&L study is of utmost importance in evaluating the risk from potential leachables. Due to the lack of universally applicable rules for E&L study design, any analytical methodology requires a great deal of thought so as not to omit any possible hazardous compounds that could be traced back to the drug packaging components. In general, a well-designed E&L study has four main steps:

1 Gather background information on the components and composition of the device, delivery system, and packaging materials, and on the use conditions

2 Develop a study protocol for profiling of extractables that specifies:
   a Sample selection
   b Extraction protocol
   c Analytical evaluation
3 Risk assessment: evaluate safety risk of extractables and leachables based on available toxicological data for known compounds or using structure-activity relationship (SAR) data for new compounds
4 Leachables detection, identification, quantitation, and determination of toxicologic thresholds

GATHERING BACKGROUND INFORMATION

Important background information includes facts about the materials of construction (e.g., additives, multilayer films, printing inks) and whether any processing aids were used in manufacturing. Also crucial is the make-up of the finished packaging in relation to the product: which surface does the product contact, and is it possible for non-product contact components to migrate?

"This information helps you understand what components have the potential to interact with the product," says Kevin Rowland, Laboratory Manager, Jordi Labs. How a system will be used should be the basis for determining how it should be extracted, with variables such as temperature, solvent strength, and time used to simulate the use conditions.

SAMPLE SELECTION FOR PROFILING EXTRACTABLES

The sample to be tested should be representative of the final product, mimicking its intended use by a patient. In some cases, the extraction sample may be an entire device or packaging. When that is not practical or necessary, however, alternative strategies may include cut and cover, full fill, one-sided, flow through, or large volume dynamic headspace extraction.

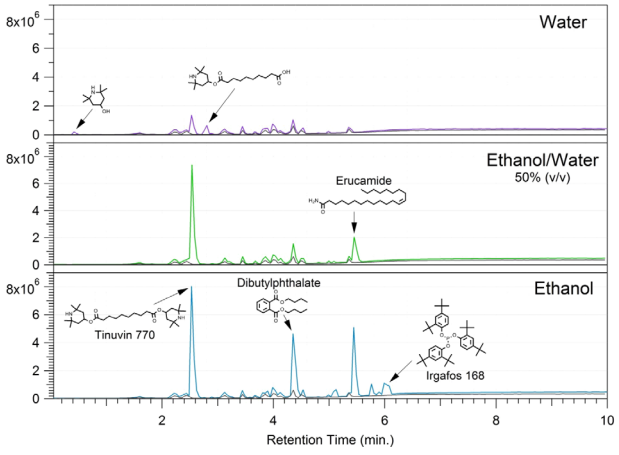
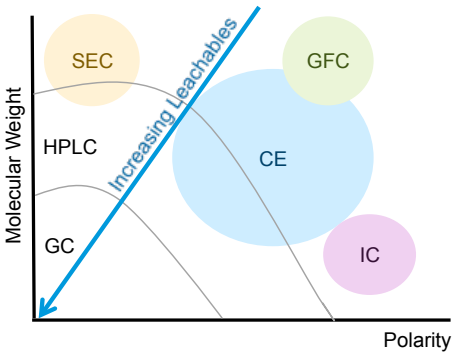


Figure 1. Solvent polarity affects which compounds will be extracted and which degradants of those compounds may be observed, as shown in these spectra comparing extraction with water, ethanol/water, or ethanol. Analysis was performed using an Agilent 1290 Infinity UHPLC; Agilent 6520 QTOF-MS; Agilent Zorbax Eclipse Plus C8 column; elecrospray ionization, polarity: positive.



CE: Capillary electrophoresis
GFC: Gel-filtration chromatography
SEC: Size-exclusion chromatography
IC: Ion chromatography

Figure 2. Graph showing the types of analytes detectable with various analytical technologies.

EXTRACTION PROTOCOL FOR PROFILING EXTRACTABLES

The background information and probable extractables will guide solvent selection. It is generally recommended to utilize three solvents spanning a wide range of solvent polarity from polar to non-polar. "Solvent polarity choice determines which compounds you will extract and which degradants of those compounds (e.g., as a result of hydrolysis) you may observe," says Kevin Rowland.

ANALYTICAL EVALUATION OF EXTRACTABLES AND LEACHABLES

The E&L analytical workflow and the method of detection employed will depend on the physicochemical characteristics of the analytes. No universal analytical technique exists to detect the range of known and unknown organic and inorganic compounds that may be present at risk assessment threshold levels in an E&L study. Typically, "the volatility of residues drives the selection of analytical technique employed," says David Weil, Ph.D., Senior Application Scientist, Agilent Technologies. Thus, based on volatility of the fraction to be analyzed, the most common analytical techniques employed for extractables profiling are:

- Non-volatile residues: LC/MS, using a targeted triple quad approach (LC/QQQ) or an untargeted (LC/QTOF) mode or other high resolution system
- Volatile or semi-volatile residues: GC/MS (headspace GC/MS, GC/QTOF)
- Heavy metals: ICP/MS, ICP/OES, AA

THE ACCURACY AND RELIABILITY OF AN E&L STUDY SHOULD BE CONFIRMED USING RIGOROUS QUALITY CONTROL MEASURES THAT INCLUDE ANALYSIS BLANKS, NEGATIVE AND POSITIVE CONTROLS, AND SPIKING STUDIES.

— MARK JORDI

Analytical innovations make it possible to identify more chemicals. LC/MS and GC/MS are currently the workhorse techniques for E&L analysis, but several emerging technologies offer the potential to improve analytical efficiency, resolve complex mixtures, and enhance the sensitivity and expand the detection limits of conventional methods. New analytical technologies that offer better performance for analyzing non-volatile E&Ls include evaporative light scattering detection (ELSD), supercritical fluid chromatography (SFC), two-dimensional LC, and ion mobility MS (IMS).

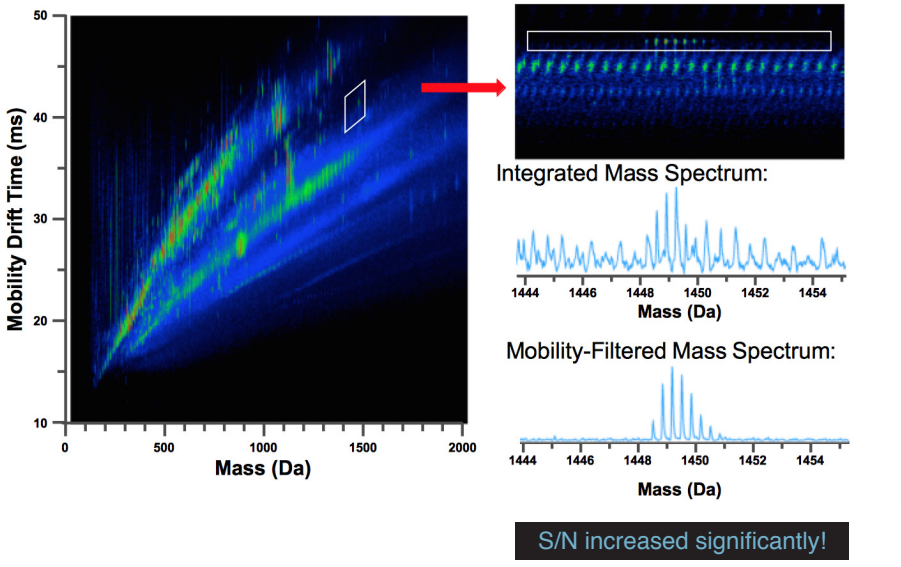


Figure 3. Ion mobility provides greater selectivity, thereby lowering detection limits.

ELSD, a technique that complements LC/MS, has a sensitivity in the low nanogram range and provides a more uniform response. With sub-ambient evaporation at 10°C, ELSD can enhance detection of semi-volatile compounds. The key advantages of an SFC separation are its speed, with separation times 2-5 times faster than LC orthogonality, and the flexibility to switch between SFC and UHPLC using the 1260 SFC/UHPLC Hybrid system, which reduces the barrier to entry into the new technology. Other benefits of SFC are the ability to switch elution order compared with reverse-phase HPLC separations, and the ability to couple a "normal phase" separation mass spectrometry, unlike a traditional normal phase LC separation.

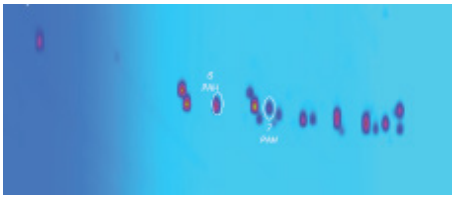
ION MOBILITY ADDS VALUE AND ANOTHER DIMENSION TO LC — A WAY TO REDUCE CHEMICAL BACKGROUND NOISE AND PROVIDE GREATER SELECTIVITY, LOWERING DETECTION LIMITS.

— DAVID WEIL

Additionally, "2D LC with MS detection is gaining in importance in E&L analysis," says David Weil. Two-dimensional chromatography separates compounds serially using two of the same or complementary columns with different solvent gradients, thereby exponentially increasing peak capacity. Also attracting attention is ion mobility-MS, which separates and identifies ionized molecules in the gas phase based on their mobility in a carrier buffer gas. Whereas MS separates molecules based on their mass alone, in IMS a molecule's gas phase mobility (how fast it moves through the drift tube) is a function of its size and conformation/shape.

Instrument detection limits. The techniques and instruments used for E&L analysis have different limits of detection. "To reach the detection limits using the instruments we have today, it is often necessary to concentrate extracts," says Kevin Rowland.

Separation of 25 PAHs



Separation of 17 Phthalate Esters

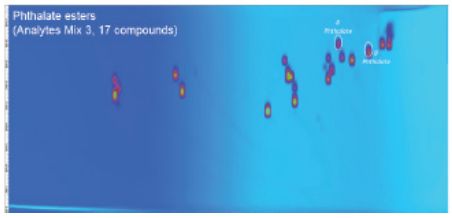


Figure 4. 2D LC and MS used to separate and detect polycyclic aromatic hydrocarbons (PAHs) and phthalate esters.

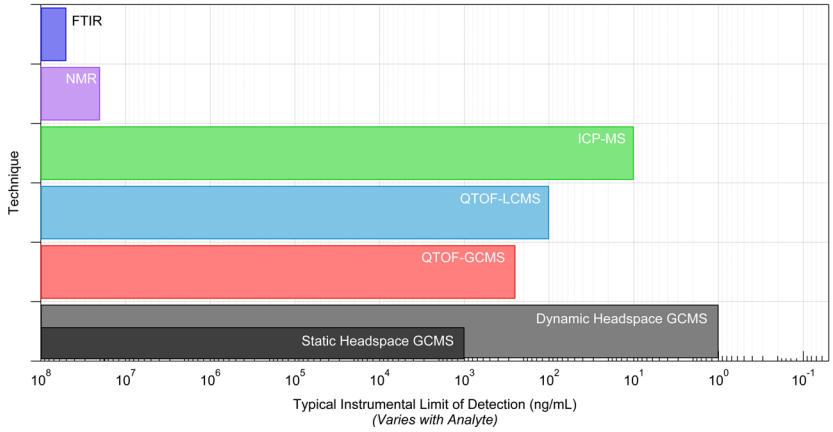


Figure 5. Range of techniques for detecting unknowns in qualitative analysis and their typical limits of detection.

But, “extractables and leachables can be lost during the sample concentration process. Therefore, it is critical to minimize losses and to know what might be lost to avoid false negative results. Best practice for concentration is the use of relatively mild conditions and to validate the methods used and know their capabilities.”

For extractable and leachable compounds present at low concentrations, “better separations enhance detection” and “using the right column,” is critical, says David Weil. Thus, new and emerging separation techniques such as 2D LC or SFC offer advantages, as do orthogonal detection strategies such as a combination of ELSD or UV together with MS. Choosing an optimum ionization technique can also have a big impact on mass spectrometric detection. Compared to ESI, APCI, or PPI, a Jet Stream ion source, which uses superheated nitrogen sheath gas, yields a 10-fold increase in ions, lowering detection limits.

SOLVING A DIFFICULT EXTRACTION WITH 2D UHPLC

In the quantitation method development for bis-phenol A, Kevin Rowland describes the difficulty encountered in resolving the compound from background components on a UHPLC system (Figure [a]). He developed a 2D UHPLC methodology illustrated in Figure b, which included the use of a spiked extract and fluorescence detection.

Figure a: 1° chromatograms from 2D UHPLC workflow; analysis was performed using an Agilent 1290 Infinity UHPLC, Zorbax SB-C18 column, H2O - ACN gradient as mobile phase. Top, 230 nm (DAD) detection. Bottom, 230 nm (VWD) detection; collection mode: heart-cutting, 2.18-2.22 minutes.

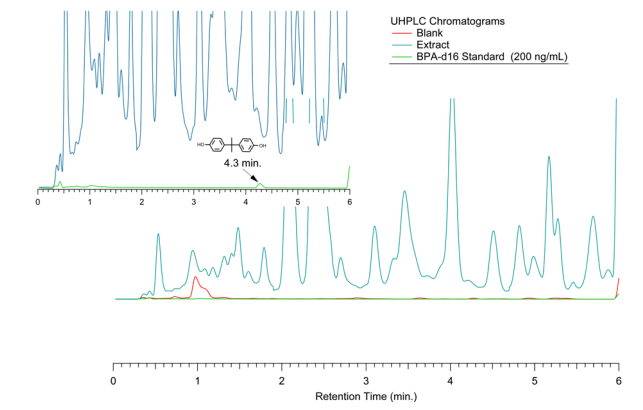
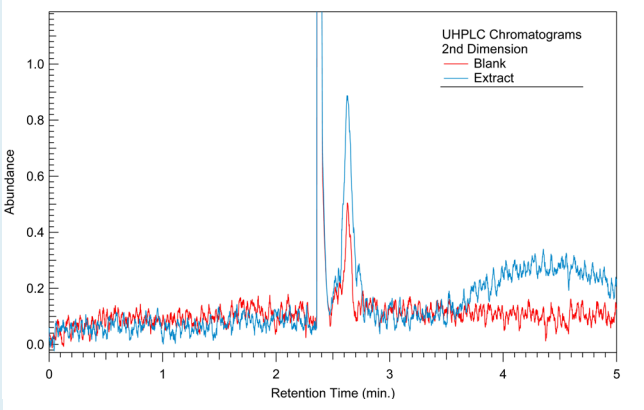


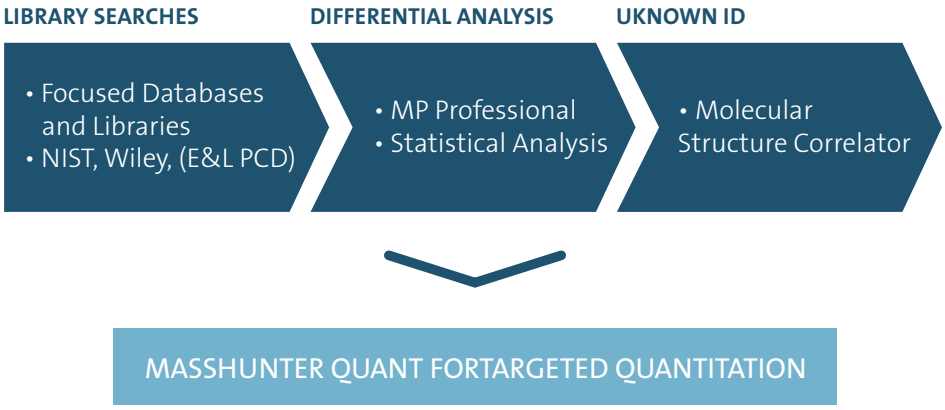
Figure b: 2° chromatogram in 2D HPLC workflow; analysis was performed using an Agilent Eclipse Plus Phenyl-Hexyl column and H2O - ACN isocratic mobile phase. Top, 230 nm (DAD) detection; Bottom, 225 nm excitation and 310 nm emission fluorescence detection.



CHEMICAL STRUCTURE IDENTIFICATION AND DIFFERENTIAL ANALYSIS

"Extracted components are identified using a combination of analytical methods including LC/MS, GC/MS, headspace MS, NMR, FTIR, and ICP/MS," says Mark Jordi. Mainly because of its sensitivity, selectivity, and low sample requirement, mass spectroscopy is the principle analytical method used for compound identification. For targeted identification to pinpoint known compounds, LC coupled with triple quadrupole MS with triggered multiple reaction monitoring (QQQ T-MRM) can be used. However, the best approach for non-targeted identification, when dealing with unknown compounds, is to couple GC (for volatiles) or LC (for non-volatiles) with TOF/QTOF and generate high resolution accurate mass MS and MS/MS spectra. These can then be matched with public or private databases using database searching to obtain a match with known reference standards. If an exact match cannot be found, then high resolution accurate mass QTOF data enable elemental formula generation, and software tools such as Molecular Structure Correlator can be employed to aid assignment of possible structures for the unknowns. Together with HR-MS and MS/MS experiments, spectral techniques such as NMR, and FTIR can also be employed for more confident structure determination.

"The qualitative analysis capabilities of MassHunter software can accelerate compound identification," says Smriti Khera. "The software is able to perform searches of large and diverse MS databases and libraries such as NIST 2014 LC MS/MS, NIST GC/MS SQ 2014, and Wiley GC/MS S 10th/NIST 2014. It is also easy to create a customized Personal Compound Database (PCD) for GC/MS or LC/MS data or a Personal Compound Library (PCDL) for MS/MS spectra in MassHunter. This speeds up identification based on the cumulative knowledge your laboratory has gathered from prior E&L sample analysis." The Molecular Structure Correlator (MSC) software tool can facilitate identification of an unknown compound by means of de novo structure prediction. The MSC workflow compares the LC or GC MS/MS spectra from E&L analyses to structure databases compiled from PCD/L, ChemSpider, PubChem, and other sources.



The addition of Mass Profiler software to MassHunter enables differential analysis through the comparison of sample sets and the identification of features that are either similar or differ between multiple groups of samples. Figure 6 shows an example of a differential sample analysis for extractables profiling that compares foil versus rubber. This is extremely beneficial in the case of E&L studies, since each analyzed sample may contain hundreds of peaks, and when

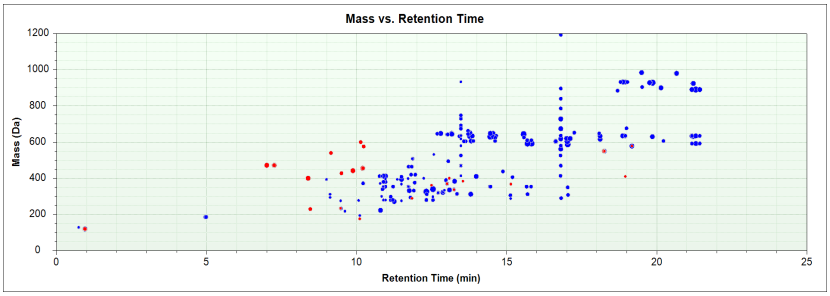


Figure 6: Differential analysis for extractables profiling comparing foil versus rubber, using MassHunter Mass Profiler.

making comparisons between sample and control, sample-to-sample or lot-to-lot differential analysis tools can bring a great deal of efficiency to the workflow and focus efforts on resolving the differences between sample sets.

QUANTITATIVE ANALYSIS OF E&LS

Quantitation is a very important part of the E&L workflow because it is essential to measure extractables and leachables down to the calculated Analytical Evaluation Threshold (AET). As Mark Jordi explains, "the AET is the threshold at or above which a chemist should identify a particular leachable and/or extractable and report it for potential toxicological assessment." The AET level for leachables will depend on the route of exposure, treatment duration, and daily exposure. Following E&L identification, the concentration of each component must be determined. Chromatography and mass spectrometry are the main techniques used in quantitative analysis. Currently, the most commonly applied quantitative methods are GC-MS, UHPLC-UV, UHPLC-CAD, and QTOF-GCMS.

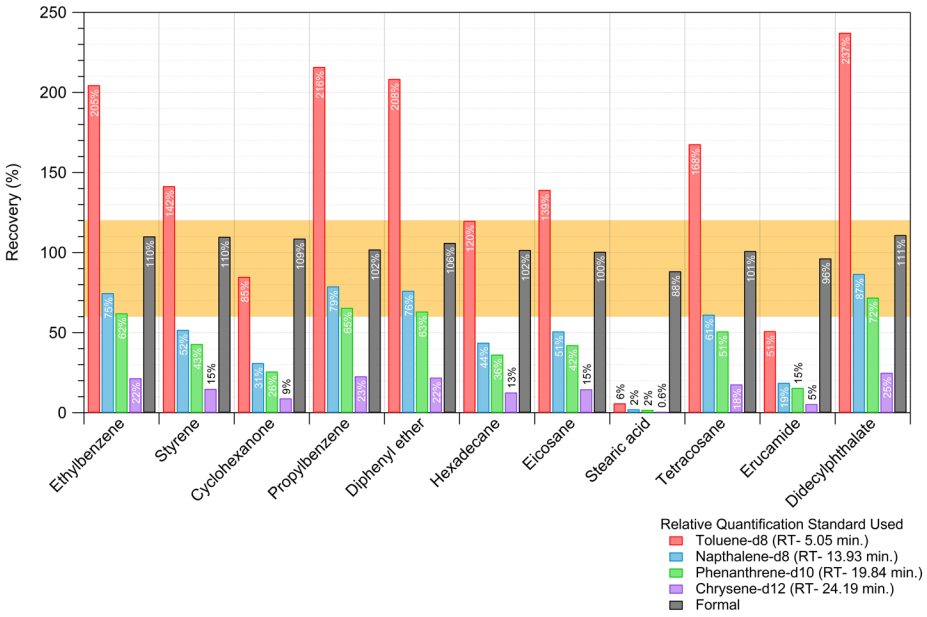


Figure 7: Comparison of recovery between Formal Quantitation method development and Relative Quantitation using various surrogate standards.

The two approaches to E&L quantitative analysis are Formal and Relative Quantitation. In Formal Quantitation, which is the preferred approach, compounds are quantified using high purity analytical standards (reference compounds) at a series of concentrations. However, when standards are not available, Relative Quantitation is employed. In the case of Relative Quantitation, the compounds in a sample are compared against surrogate standards; the accuracy of this method will depend on the surrogate standards used. The potential variability of the results obtained when using a Relative Quantitation strategy compared to Formal Quantitation is illustrated in Figure 7. Comparison of this must be kept in mind when using surrogates for quantitation.

CONCLUSIONS

The ability to perform accurate, sensitive qualitative and quantitative analysis of extractables and leachables is an essential part of pharmaceutical manufacturing and product testing. The potential toxicity of leachables on patient exposure and their ability to migrate from packaging materials, biomedical devices, drug delivery systems, or process equipment demands thorough evaluation of extractable compounds under regular use conditions, in the presence of environmental stressors, and in worst-case scenarios.

Product recalls due to contamination by unexpected leachables has a significant impact on patients and manufacturers. Robust E&L analytical studies to detect, identify, and quantify even low levels of a broad range of E&Ls can minimize or eliminate the risk of post-marketing recalls by enabling a data-driven toxicologic evaluation and determination of product safety. The application of well-defined E&L techniques, existing liquid and gas chromatography technology combined with mass spectrometry, and innovative new separation, ionization, detection, and MS approaches and software tools is improving the sensitivity and accuracy of E&L analysis, lowering limits of detection, and enabling advances in the identification and measurement of known and unknown compounds.

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