Trends in Impurity Analysis

Determination of Extractables, Leachables, Residual Solvents, and Unknowns by Mass Spectrometry

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Although mass spectrometry has long been used for drug discovery and characterization, it has recently shown promise in the quality control laboratory as a tool for identifying and monitoring impurities.

The pharmaceutical industry is coming under increasing pressure to identify, quantify, and monitor impurities. Potential sources of these impurities include residual solvents or other chemicals used during the manufacturing process as well as degradants of the drug substance or excipients. FDA also is focusing on compounds extracted from packaging material and leachables from the container or closure system. The development, validation, and testing of these impurities must be conducted using International Conference on Harmonization (ICH) and United States Pharmacopeia (USP) guidelines in a laboratory that is compliant with current good manufacturing practices.

Many of the impurity methods found in a pharmaceutical quality control (QC) laboratory use high-performance liquid chromatography (HPLC) with UV detection (LC–UV). Some impurities are volatile molecules and are amenable to gas chromatography–flame ionization detection (GC–FID). With both of these modes of detection, peak heights or peak areas coupled with retention times are used to identify and calculate the concentration of the impurity. This procedure is usually sufficient because the drug products or raw materials are well characterized. However, in earlier stages of the drug development process or when investigative work requires identification of an unknown peak, more definitive

Table I: Elements addressed by *USP* ⟨467⟩.

Organic volatile impurity	Limit (μg/g)
Methylene chloride	600
Chloroform	60
1,4-Dioxane	380
Trichloroethylene	80

Table II: ICH Guidelines, *EP* 5.4, and *JP* XIV.

Class 2 solvents: potential organic volatile impurities	
Acetonitrile	Hexane
Chlorobenzene	Methanol
Chloroform	2-Methoxyethanol
Cyclohexane	Methylbutylketone
1,2-Dichloroethene	Methylcyclohexane
Dichloromethane	*N*-Methylpyrrolidone
1,2-Dimethoxyethane	Nitromethane
N,*N*-Dimethylacetamide	Pyridine
N,*N*-Dimethylformamide	Sulfolane
1,4-Dioxane	Tetralin
2-Ethoxyethanol	Toluene
Ethyleneglycol	1,1,2-Trichloroethane
Formamide	Xylene

Table III: Relative costs.

Instrument	Relative cost
LC–UV	1×
GC–MS	2×
LC–MS	4×
ICP–MS	4×
LC–MS–MS	8×

information may be required. Thus, the typical LC–UV or GC–FID approach may not yield sufficient information because UV and FID detectors provide very little structural information. However, advances in mass spectrometry (MS) have made MS a valuable tool for the QC laboratory (1).

Leachables and extractables

Leachables and extractables are chemicals that can be released or can migrate from containers, closure systems, and other packaging components and have the potential to contaminate the drug product. Sources of leachable and extractable contamination include plastic components, elastomers, coatings, accelerants, antioxidants, and vulcanizing agents. Plasticizers such as bis(2-ethylhexyl)phthalate (BEHP) are ubiquitous and may be carcinogens. This colorless, odorless liquid is added to plastics to make them more flexible and can be found throughout the manufacturing process.

Another potential source of leachable–extractable contamination is a class of carcinogenic compounds called *nitrosamines*, which are found in products made of rubber. Many drug products are distributed or administered in packages made of plastic and rubber components; therefore, phthalates and nitrosamines could come into contact with the drug product. Metered-dose inhalers (MDIs) and dry-powder inhalers (DPIs) are constructed of various plastic, rubber, and stainless steel components. An FDA draft guidance includes guidelines for MDIs and DPIs but is for the most part vague; however, the guidance clearly states that "applicable methods must be developed and validated" for MDIs and DPIs (2). Many of the extractables and leachables lend themselves to GC–MS analysis.

Residual solvents

Residual solvents are organic volatile impurities that remain in active substances, excipients, and other products after processing. The *USP* compendia address only four commonly used solvents (see Table I). Even with this short list of compounds, there is ambiguity that is inherent in the analysis by GC–FID with a single column. In several instances, MS has been used to confirm the absence of a false positive by FID.

ICH has proposed a set of guidelines that has been adopted by the European and Japanese pharmacopoeias (EP and JP) (see Table II) (3). This set of guidelines is a much more substantial list of volatile compounds, which lend themselves to MS because of their complexity. Many of the residual solvents such as toluene, dioxane, xylene, and pyridine are volatile organic compounds routinely monitored by the chemical industry.

Unknowns

Unknowns mean exactly that—not known. Even though every degradation pathway is considered, and every attempt is made to minimize impurities, pharmaceutical chemistry can still be unpredictable. The extra, unexpected peak in a chromatograph or the mysterious black dust forming on a medical device could be an extractable–leachable, a residual solvent, or an unexpected degradant and must be investigated and identified, which can be achieved using MS.

Mass spectrometry

MS is a powerful analytical tool because it can provide valuable structural information with a high degree of specificity. MS is widely recognized as the technique that obtains the most-defensible data. The distinctive mass spectrum or fragmentation pattern acquired for each molecule makes it a definitive and effective tool for identifying unknown impurities or degradation products. Comparing the MS "fingerprint" with large mass spectral databases further facilitates identification. FDA and many research laboratories have realized the need for MS; therefore, the use of MS is not only commonplace, but also necessary in the pharmaceutical laboratory.

Coupling GC to MS creates an instrument that is much more powerful than the sum of the two individual instruments—a result that led researchers to other important discoveries. At one time, coupling LC to MS was a difficult undertaking and limited to the R&D laboratory. The main obstacle was the interface of the two methods because a great difference existed between the mass flows involved in HPLC and those accommodated by conventional MS vacuum systems. The advent of thermospray and electrospray interfaces allowed LC–MS to become more mainstream and cost-effective and to be used in the QC laboratory. LC–MS allows one to obtain molecular weight and structural information that can facilitate the identification of an impurity. LC–MS can be used to identify impurities and degradation products where peak purity measurements with photodiode array (PDA) detection fall short.

Even tandem instruments such as LC–MS–MS, also known as a *triple-quad LC–MS*, have become standard in the pharmaceutical analytical laboratory. For the quantitative analysis of many analytes, LC–MS–MS is a fast, universal, selective, and sensitive tool. The quantitation is typ-

Table IV: Common isolation and preparation techniques.

Class of Compounds	Techniques
Organic	Solvent extraction: a suitable solvent is used to separate impurities from the matrix (drug products, excipients, etc.) Cleanup: a solvent extract is treated to remove interferences Solid-phase extraction (SPE): uses both a solid and a liquid phase to isolate one or one type of impurity from a matrix; can also be used for cleanup Fractionation: similar to cleanup; typically used to separate compounds based on functionality and molecular weight
Inorganic	Acid digestion: acid is used to dissolve (isolate) the metals Wet oxidation: very strong acids (oxidizing agents) are used Dry ashing: a sample is heated to oxidize (remove) the organic material and leave (isolate) the inorganic impurities Ion exchange: similar to SPE

Table V: Suitable techniques.

Class	Properties	Technique
Inorganic	Elemental	ICP–MS
Organic	Polar, thermally unstable, low volatility	LC–MS–MS
	Nonpolar, volatile–semivolatile, thermally stable	GC–MS

Table VI: Applicable EPA Methods (4).

Method	Technique	Application	Isolation
SW846 Method 8260	GC–MS	Volatile organics	Purge-and-trap
SW846 Method 8270	GC–MS	Semivolatile organics	Solvent extraction
SW846 Method 8321	LC–MS	Nonvolatile organics	Solvent extraction
SW846 Method 6020	ICP–MS	Elemental	Digestion

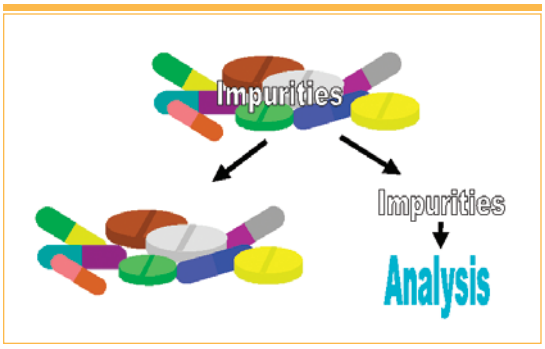

Figure 1: Impurities must be isolated from drug products.

ically performed with high selectivity, which practically eliminates matrix components. With this more-streamlined sample preparation, extracts of complex sample matrices can be analyzed with limited or no cleanup. LC–MS–MS is also a very important technique in the bioanalytical arena for characterizing large molecules such as proteins and peptides, which are typically present in challenging matrices.

Inductively coupled plasma (ICP)–MS provides elemental and isotopic information for a wide variety of applications in pharmaceutical chemistry. This technique, which uses plasma as the ionization source and a mass spectrometer analyzer to detect the ions produced, offers better speed, sensitivity, and performance than graphite furnace atomic absorption spectrometry. ICP–MS can simultaneously determine many elements and measure their concentration at the parts-per-billion or even parts-per-trillion level.

Although the instrumentation has come down in price in recent years, it is still more expensive than HPLC. Nevertheless, the benefits far outweigh the cost. The relative costs of various types of equipment are shown in Table III.

Isolation

Isolation is perhaps the greatest challenge in accurately quantitating and identifying impurities. The impurity of interest must be isolated from the drug product, excipients, or device (see Figure 1).

Isolation is accomplished through various sample preparation procedures followed by LC–MS. Although sample preparation is not as alluring to a methods development chemist as the instrumentation, it is just as important to the overall process. Sample preparation is the area in which an experienced chemist's practical knowledge of traditional chemical procedures is very valuable. Table IV lists some common isolation techniques. Once the impurities are isolated, they are subjected to the appropriate determinative chromatographic technique (see Table V); however, the properties of the impurity are not always known.

The Environmental Protection Agency (EPA) recognized the potential of MS when coupled with GC and promulgated GC–MS methods to determine various analytes in environmental and process samples (see Table VI). After extracting the compounds from the matrix and performing a cleanup of the extract, the GC–LC separates the various components for MS to identify.

Strategies

Many extractables–leachables and residual solvents have been monitored by the chemical industry and are addressed in the methods listed in Table VI. These methods can provide a starting point for developing a suitable impurities method.

When unexpected impurities or degradants appear in a stability-indicating assay or dissolution, a good approach is to mimic the LC–UV analysis with an LC–MS instrument that is equipped with a PDA detector. This procedure allows one to find the specific peak (with the PDA functioning similar to the UV detector) and then use MS to obtain molecular weight information.

If an unknown is determined to be inorganic, then the sample is digested and analyzed by ICP–MS to provide information about the elemental makeup. If an unknown is determined to be organic, then LC–MS and GC–MS can provide the impurity's structure and sometimes identify it. In some cases brute force is required, and LC–MS, GC–MS, and ICP–MS are all performed.

In all cases, laboratory personnel and the client must work closely together. The client most likely has the critical infor-

mation that, when joined with the MS data, can solve the problem.

Applications of MS

Many applications of MS exist in the laboratory today. The following scenarios are a few examples in which an MS method was used:

- A GC–MS method was developed to accurately quantify BEHP in tablets at a concentration of 6 ppb when a manufacturer had to monitor its processes and eliminate phthalate esters, potential carcinogens, and endocrine disrupters.
- A GC–MS method was developed to accurately quantitate the polynuclear aromatic hydrocarbons (PAHs) in a product when a consumer product had to meet a maximum concentration standard of PAHs to comply with state environmental regulations.
- When a film formed on the surface of a drug product in an identification test, GC–MS confirmed that the film was benzaldehyde.
- LC–MS identified a mystery peak that appeared in kinetic drug release data as a process impurity and not a degradant of the active ingredient.
- Impurity profiles sometimes act as fingerprints of a patented drug synthesis. LC–MS was used to confirm the presence of a specific impurity for a patent infringement lawsuit.
- When a drug product dispensed in a new syringe lost activity, GC–MS was used to show that the drug product lost its activity when it came in contact with the extractable components found in the plastic and rubber components of the syringe.
- ICP methods were developed to monitor leachable metals to prevent metals from rubber components in vials and stainless steel reactors from leaching into a variety of drug products during the manufacturing process.

Conclusion

An increasing need exists for methods that quantitate and identify impurities such as extractables and leachables, residual solvents, and unknowns. Mass spectrometry is an exceedingly powerful tool for accomplishing this task. The MS methods that have been developed and the data generated from these methods assist the end user in complying with FDA, EPA, and state regulations; filings for new drug applications; and raw materials and release testing.

References

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