Technical Guide Series
Trends in Impurity Analysis

By J. Menoutis, Ph.D., F.A.I.C., C.P.C., C.Ch.E. and A. I. Paris

Drug manufacturers are coming under increased regulatory scrutiny to identify, quantify, and monitor impurities. Sources of potential impurities include compounds used in the synthesis of active pharmaceutical ingredients (API), reaction by-products formed during synthesis, residual solvents, API degradants or excipients. There is also growing regulatory efforts focused in identifying compounds extracted from packaging material and leachables from the container or closure system. Most methods used in the pharmaceutical quality control (QC) lab for determining impurities use HPLC with either UV or PDA detectors. For more volatile impurities, such as residual solvents, GC using either liquid injection or static headspace is used. In either instrumental method (HPLC or GC), identification is made using retention times.

These approaches are generally sufficient with established and well characterized drug products or raw materials. However, when issues arise in the identification of an unknown peak whether in an established drug product or in earlier phases of drug development, more substantive information may be needed. HPLC and GC, while providing basic identification using retention time, provide no structural information. Mass Spectrometry (MS), on the other hand, as a hyphenate technique such as LC-MS or GC-MS, can provide not only retention time, but structural information as well.

Furthermore, 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.

Mass spectrometry

Mass spectrometry is widely recognized as yielding substantive information. Its power as an analytical tool rests with its ability to provide valuable structural information with a high degree of specificity. The distinctive mass spectrum or fragmentation pattern acquired for each molecule makes it an ideal tool to aid in identifying unknown impurities or degradants.

Hyphenated GC-MS (coupling GC to MS) has resulted in an instrument that is much more powerful than the sum of its individual parts, GC and MS. The technique provides identification by both retention time and the distinctive mass spectrum or fragmentation pattern obtained for a molecule. With the advent of well established mass spectral databases, such as the NIST MS database, the identification of an unknown by its mass spectrum is greatly facilitated.

The development of electrospray (ESI) and atmospheric pressure chemical ionization (APCI) interfaces has allowed hyphenated LC–MS (coupling HPLC to MS) to become both a cost-effective and a more routine technique. LC–MS allows for obtaining molecular weight and structural information that can facilitate the identification of an impurity. Further recent advancements have also made tandem LC-MS such as triple quadrapole LC-MS (LC-MS-MS) available as a routine technique. For the quantitative analysis of many analytes, LC–MS–MS is a fast, selective, and sensitive tool. The quantitation is typically performed with a high degree of selectivity which eliminates most if not all matrix effects. With this technique, extracts of complex sample matrices can be analyzed with minimal or no sample cleanup. LC–MS–MS is also a significant technique in bioanalytical analysis involving the characterization of large molecules such as proteins and peptides, which are typically present in very complex biological matrices.

"Mass spectrometry long the province of drug discovery as a powerful characterization tool, now serves as a powerful analytical tool for identifying and measuring degradants, impurities and unknowns in APIs, excipients, finished drug products and packaging."

Aiding in the identification of inorganic impurities, inductively coupled plasma-mass spectrometry (ICP-MS) provides elemental and isotopic information for a wide variety of applications. The technique, employs an argon plasma as the ionization source and a mass spectrometer to detect the ions produced. In comparison to graphite furnace atomic absorption spectrometry, it offers better speed, sensitivity, and performance. It can simultaneously determine many elements and measure their concentrations at the parts-per-billion or even parts-per-trillion level.

Residual Solvents – The power of GC-MS vs. GC

Residual solvents are trace level chemical residues that remain in active pharmaceutical ingredients (APIs), excipients and drug products after the manufacturing process. They can also form during packaging or storage. USP General Chapter <467> Organic Volatile Impurities has been the generally used compendial method for identifying and quantifying residual solvents when no information was available on what solvents were likely to be present.

To better mirror the ICH guidelines, On July 1, 2008, the USP implemented a new test requirement for the control of residual solvents in drug products. The new test requirement, USP General Chapter <467> Residual Solvents, replaces the previous USP General Chapter

This new method involves a much more extensive analysis, using a preliminary screening GC analysis, followed by a confirmatory GC analysis using dissimilar columns. If any compound is detected using both systems, a third analysis is necessary to quantify the impurity. Even with this approach, there can be some ambiguity. Coelution on one or both columns can present problems. Using mass spectrometry as the detector can solve this ambiguity.

Whether the issues relate to coelution or possible sample matrix interferences, mass spectrometry has the ability to overcome them. Co-elution is usually not a problem with mass spectrometric detection since in most cases the co-eluting compounds each have unique molecular ions and differing ion ratios. Matrix interferences can in most cases be overcome by adjusting the mass scan range or by the use of Selective Ion Monitoring (SIM). A benefit of SIM is that it provides the advantage of routine ultra-sensitive detection. Also, to obtain more information for confirmation and unambiguous identification of unknowns, e.g., during an out-of-spec investigation, mass spectrometry is the method of choice.

Impurities & Degradants - LC-MS vs. LC

The isolation of impurities is critically important to the successful development of processes for the synthesis of pharmaceutical candidates. Reversed-phase HPLC (RP-LC) is the method of choice for general purity analysis in pharmaceutical process research. Project chemists routinely use reversed-phase HPLC to monitor reactions and to establish the purity of starting materials and final products. Using the information obtained from RP-LC, degradation pathways are evaluated and impurities formation from the synthesis process are minimized.

While HPLC (LC) is a valuable tool in monitoring purity, identification of impurities and degradants can pose a challenge using just LC. The extra, unexpected peak in a chromatogram, an unexpected degradant or an unexpected impurity or degradant in a stability-indicating assay or dissolution must be investigated and identified. Mass spectrometry is an ideal analytical tool for accomplishing this. Using the RP-LC method which identified the presence of the unexpected peak, impurity or degradant is a good starting point for developing a suitable LC-MS method which identifies its structure. If the method uses mobile phase buffers from salts, a problem for LC-MS, then a method will have to be developed using organic acids, such as formic, to provide comparable separation. Aiding in the development process is the use of a photodiode array detector (PDA). By equipping the LC-MS with a PDA in between the LC and MS allows tagging the specific peak(s) of interest with the PDA functioning similarly to a UV detector used in the original method. This allows the identification of the peak(s) of interest as it leaves the PDA and enters the MS. Then the use of the MS yields the needed mass spectral information to obtain molecular weight and other information to provide identification of the unknown, impurity or degradant.

Genotoxic Impurities - GC-MS, LC-MS and ICP-MS

Reactants and catalysts that are employed in the synthesis of APIs or reaction by-products formed during synthesis, have the potential to remain as impurities in finished APIs. Some of these compounds, if present, may have genotoxic properties, and as a result have the potential of being carcinogenic, mutagenic or teratogenic agents. Consequently, the toxicity of these compounds make it necessary that they not be present or be limited to extremely low levels in an API. Recently, in response to such concerns, the European Medicines Agency (EMEA) released industry guidance on acceptable limits of potential genotoxic impurities in APIs.

Hyphenated analytical techniques such as GC-MS, LC-MS and ICP-MS provide the sensitivity, selectivity and specificity to identify and measure potential genotoxic impurities. Many of these compounds tend to be volatile or semivolatile small molecules which lend themselves to GC-MS. Those which are non-volatile or thermally liable can be readily analyzed by LC-MS, while, those that are inorganic elemental impurities can be analyzed by ICP-MS.

QUANTEX LABORATORIES

AN EXTENSION OF YOUR TEAM℠

For more information on our capabilities please contact our Business Development group at (732) 248-3335, extension 404.