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# Strategies of Method Development of Leachables Impurities Analysis Using Liquid Chromatography–Mass Spectrometry

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## Introduction

The term “leachables” refers to impurities in pharmaceutical products with an origin of the pharmaceutical container closure system in either direct or indirect contact with the formulation [1]. Pharmaceutical regulatory authorities, such as European Medicinal Agency (EMA) and Food and Drug Administration (FDA), periodically issue guidelines on leachables quantitation in pharmaceutical manufacturing and finished drug product. These guidelines make it critical to be able to develop an accurate and generalized method at ultra-low levels for the leachable impurities. High-performance liquid chromatography-mass spectrometry (LC/MS) has had a significant impact on drug development over the past decade because LC/MS meets many of the demands of drug development, such as sensitivity, selectivity, speed, and cost effectiveness [2]. The combination of HPLC with mass spectrometry allows LC/MS to accomplish structural analysis (i.e., molecular structure elucidation), qualitative analysis (i.e., high sensitivity confirmation of the presence or absence of a target analyte), and high sensitivity quantitative analysis for leachables impurity analysis [3]. However, there are still specific challenges to be addressed for the leachables impurity method development using LC/MS. This white paper provides a summary of the best strategies for method development of leachables analysis at Boston Analytical Inc (BA).

## Instrumental Condition

LC/MS method development for leachables analysis is more comprehensive than assay analysis. Very often, the target leachable compounds include more than one compound. This challenges the developer to combine several compounds within one method under the same instrumental conditions. Figure 1 shows the percentage of analytes in a typical leachable method based on 25 methods recently developed at BA using LC/MS. Note that 16% of the leachables methods developed included more than 16 analytes. BA successfully developed these leachable methods using an Agilent 6500 series high resolution Q-TOF LC/MS system. The high resolution Q-TOF LC/MS not only quantitates the leachables at a relative low concentration levels (µg/L), it also provides molecular structure information (fragment profiles), that are essential for later unknown identification.

Typical LC/MS instrumental conditions for leachable development are summarized in Table 1. The mobile phases 0.01% ammonium acetate in water and 0.01% ammonium acetate in methanol provide a better generalized separation and ionization of most common leachable analytes. The gradients listed in Table 1 cover a broad polarity of leachable analytes.

## Sample Preparation

For leachable method development using LC/MS, the major bottleneck within the laboratory is sample preparation. The sample preparation used depends on the drug product categories, leachable analyte structural diversity, matrix complexity, and the analytical evaluation threshold (AET) level of the drug product.

Drug matrix characteristics are critical to determining what sample preparation is used. Figure 2 shows the percentage of the drug product categories that BA has worked with for leachables method development. The majority (44%) of drug products are injectable drugs. The matrix of injectable drug products is simple, compared to other drug products. For the injectable drug, directly spiking the target analytes in the sample matrix at the AET level is possible, assuming the LC/MS can reach the AET level for each analyte. If the AET cannot be reached by directly spiking, a method concentrating the sample is needed after liquid-liquid extraction (LLE).

For more complex matrices, such as antibiotics, a more complicated preparation is required. Precipitation is a suitable option to reduce the matrix complexity. Normally, methanol, acetonitrile, and isopropanol are suitable solvents to utilize in the process.

Topical cream and ointment drug products present the most challenging matrices. Solid phase extraction (SPE) sample preparation is often used for the method development on these drug products. A polar, non-polar, ion exchange, or mixed mode SPE cartridge can be used based on the drug formulation and target analytes.

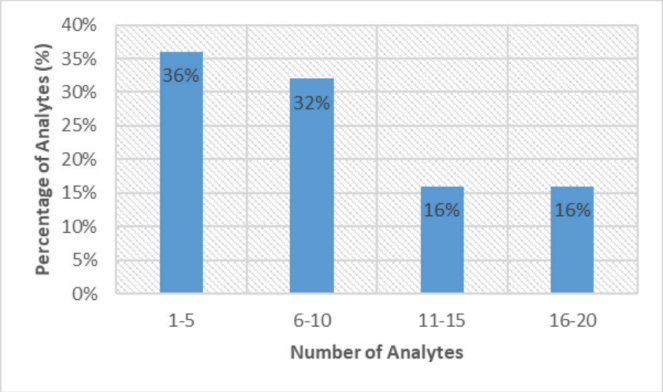


**Figure 1** **Percentage of analytes in a typical leachable method based on 25 recent methods developed by BA using LC/MS.**

**Table 1** **Optimized Instrument Conditions**

| PARAMETER | VALUE |
|---|---|
| **Instrument** | Agilent 1290 HPLC with Agilent 6500 QTOF LC/MS |
| **Column** | Zorbax Eclipse Plus C8 2.1 x 50mm x 1.8µm |
| **Mobile phase** | A: 0.01% ammonium acetate in water<br>B: 0.01% ammonium acetate in methanol |
| **Gradient** | **Time (min) %A %B**<br>0.0 70 30<br>2.5 20 80<br>9.5 0 100<br>14.0 0 100<br>14.1 70 30<br>**(2-min post-time)** |
| **Flow rate** | 0.4 mL/min |
| **Injection volume** | 1 µL |
| **Column temperature** | 40 °C |
| **Ion-mode** | Dual AJS-ESI |
| **Polarity** | Positive and Negative Modes |
| **Drying gas** | Nitrogen, 15 L/min at 150 °C |
| **Nebulizer pressure** | 25 psi |
| **Sheath gas** | Nitrogen, 11 L/min at 250 °C |
| **Capillary voltage** | 3500 V |
| **Nozzle voltage** | 0 V |
| **Fragmentor** | 275 V |
| **Scan range** | 50–1700 m/z |
| **Scan rate** | 3 spectra/s |
| **Reference ions** | ESI+: 121.0509 and 922.0098<br>ESI-: 119.0363 and 980.0164 |

Leachables are compounds that leach into the drug product formulation from the container closure system as a result of direct contact with the formulation. Leachable analytes can co-elute with the active pharmaceutical ingredient (API) peak of the drug product as shown in Figure 3. In such a case, the ion suppression from the API peak can lead to ultra-low recovery of the leachable analytes. Three strategies can overcome this issue. One strategy is to adjust the mobile phase gradients to separate the API and leachable analytes. Second, consider the LLE sample preparation since it could reduce or eliminate the API peak, removing the interference. Third, a suitable internal standard can be spiked along with the leachable analytes before sample preparation. Recovery of the leachable analytes can be determined using the internal standard response.

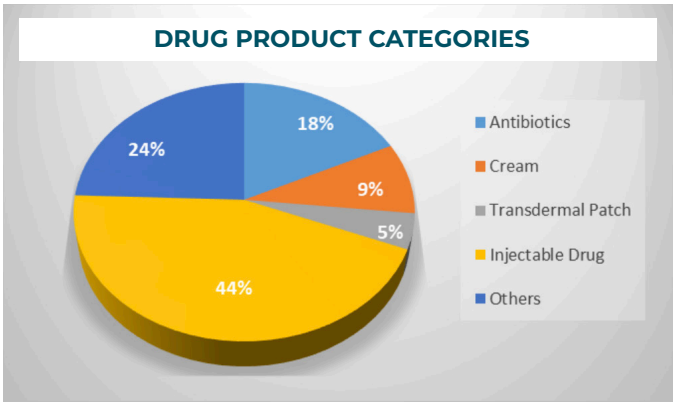


**Figure 2** **Percentage, by category, of drug products based on 45 drug products for which BA developed leachable methods.**

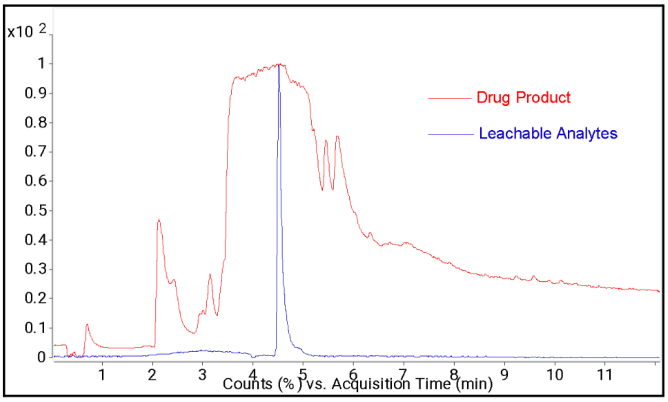


**Figure 3** **LC/MS chromatograms showing co-elution of drug product and leachable analytes.**

## Typical Parameters Recommended

Leachables methods development should be accomplished according to accepted practices, criteria, and guidelines [1]. Development parameters may include: accuracy, precision (repeatability), specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity, and range. System suitability tests and criteria should also be developed for each leachable analyte in a typical method. The specific method development parameters also depend on the goals of the leachables analysis and intended purpose of the developed method. The following considerations for the individual parameters are for a leachable impurities method:

**Accuracy:** Accuracy can be evaluated by spiking the drug product with the target leachable analytes at the AET level. Typically, three total spiking levels are evaluated. The spiked samples are analyzed using the draft method, and the analyte levels calculated from a linearity curve are compared to the spike levels.

**Precision (Repeatability):** Repeatability can be determined through preparation of multiple (six) replicates of spiked samples (often in conjunction with accuracy) followed by statistical analysis of the results.

**Specificity:** Specificity can be evaluated by extracted ion chromatogram (EIC) of the spiked and non-spiked drug product samples. Signal carry-over is common with LC/MS analysis for some analytes, such as slip agents, fatty acids, and amides. In these cases, it is important to discern signal carry-over from actual interference from the drug product matrix.

**Linearity and Range:** The method linear range should include the AET level (LOQ) up to the maximum accumulation levels of each target analyte, since the potential leachables are present at widely varying levels.

**LOD/LOQ:** The LOQ must be at or below the designated AET level. Figure 4 shows the distribution of LOQs by concentration in LC/MS leachable methods based on 202 target leachable analytes at BA. Note that 34% of LOQs are between 11 and 50 µg/L. These will likely require LLE sample preparation followed by concentrating several times to reach the AET level.

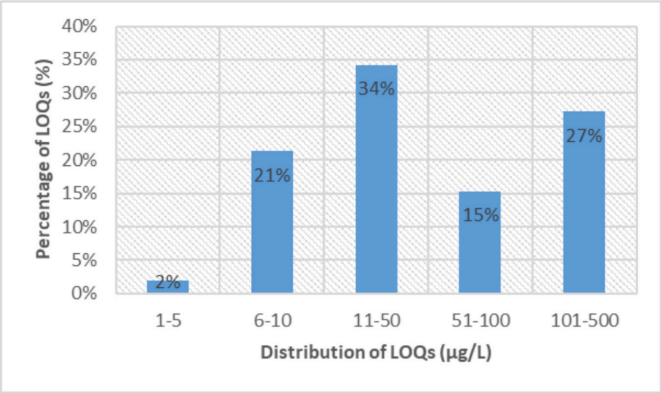


**Figure 4 LOQs of leachable analytes by concentration in leachables methods based on 202 analytes.**

## Future Perspectives

BA has considerable experience in development of methods for leachables impurity analysis using high resolution Q-TOF LC/MS. LC/MS applications in leachables impurity analysis are expected to remain an active area of development. To successfully develop leachables impurities methods, current LC/MS sensitivity and dynamic range and sample preparations need to be continually improved.

## REFERENCE

1. USP <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems.

2. Mike S. Lee, Edward H. Kerns, LC/MS Applications in Drug Development, Volume 18, Issue 3-4, 1999, Pages 187-279.

3. Daniel L. Norwood et al., HPLC and LC/MS Analysis of Pharmaceutical Container Closure System Leachables and Extractables, J. Liq. Chromatogr. Relat. Technol., 32:11-12, 2009, 1768-1827.