Control Method-Related Data Quality Risks During Chromatographic Analysis with Fit-for-Purpose, High Quality Vials

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INTRODUCTION

Controlling risks that compromise analytical method performance is an important aspect of Method Lifecycle Management (MLCM). MLCM is an approach to method management that ensures methods are fit-for-purpose throughout their lifetime. Assessing the risks and implementing control strategies are part of applying Analytical Quality by Design (AQbD), a risk-based approach to method development[1] and is an integral part of MLCM Stage 1: Method Design and Development.

Typical method-related risks associated with vial quality include adsorption of analyte, appearances of unexpected or "ghost" peaks, ionic effects such as ion suppression or pH changes, mechanical effects (*e.g.*, septa blocking the autosampler needle), and evaporation and chemical incompatibility caused by interactions between the septa/caps and sample.

Understanding the method performance objectives as outlined in the Analytical Target Profile (ATP) and determining the correct vial for the intended method in MLCM Stage 1 can significantly improve the quality, reliability, and consistency of data generated by the analytical method. Controlling the vials used during sample handling and analysis also reduces aberrant data investigations and helps maintain good method performance.[1]

The case studies in this paper illustrate the types of issues that occur when using inadequate quality vials. These case studies prove that the root cause for poor data quality is poor quality vials. Methods for choosing the appropriate vial type to control the risks of these issues from occurring are also discussed.

This paper focuses on the use of glass vials for LC and LC-MS small molecule applications. To address challenges of data quality involving similar applications for large molecules such as peptides and proteins, please reference Waters™ white paper "Achieving Maximum Protein and Peptide Recovery, Sensitivity, and Reproducibility using QuanRecovery™ Vials and Plates" (p/n: 720006571EN).

UNEXPECTED CONTAMINANT OR GHOST PEAKS

One of the potential risks of HPLC testing is the presence of unexpected ghost peaks, resulting from the sample vials. Such peaks may be introduced by contaminants from the vial, septa, or the unpredictable degradation of the analytes caused by impurities present in the glass.

- **Contaminants** can be introduced during vial manufacturing, such as oils used in the vial manufacturing equipment. These VOCs (volatile organic compounds) can remain on the glass surface, if the vial is not properly treated prior to packaging.
- The **septum** is a more common source of contamination. The silicone material can be improperly cured. This can lead to catalysts and other additives to not being integrated fully into the material. These impurities can cause contaminant peaks, as shown below.
- The **surface properties of the sample vial** are a third aspect to consider. Although most sample vials are made from USP Type 1 borosilicate glass, certain steps during manufacturing impact the surface properties greatly. Free ions, such as sodium, can bloom to the surface and impact the pH of the diluent, which can trigger a degradation reaction in the vessel, leading to ghost peaks.

CASE STUDIES

IMPACT OF GLASS CLEANLINESS AND PURITY[2]

Sensitivity is important when analyzing samples via LC-MS and when the presence of background noise prevents the ability to accurately detect and quantify peaks. Background noise can come from many factors such as instrument, sample, and vial cleanliness. Variability in the levels of vial cleanliness from one manufacturer to another can be seen in the MS scans of a solvent sample stored in a competitor's vial and in a Waters LCMS Certified Vial (Figure 1). The solvent samples were stored in the vials for a fixed amount of time, removed, and analyzed via LC-MS. The competitor's vial scan is typical of vials commonly purchased around the world. The reference scan is from a solvent sample that was not stored in a vial. To mitigate the presence of background noise during LC-MS analysis, Waters LCMS Certified Vials may be used as a control strategy to ensure peak detection and quantitation are accurate to further enhance data quality.

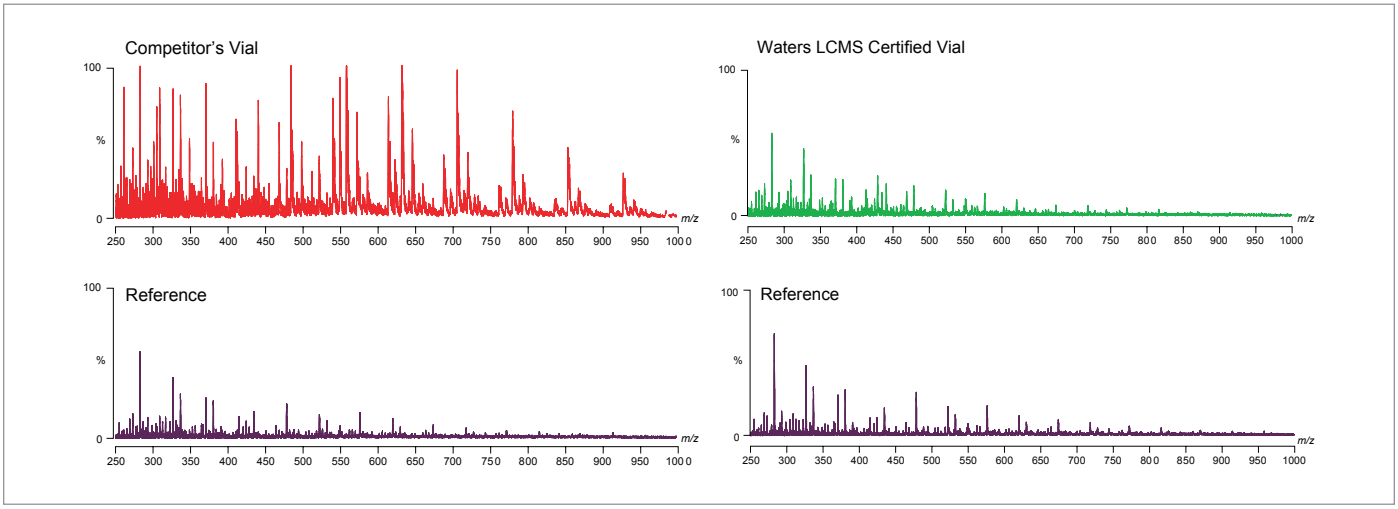

Figure 1. Comparing typical MS scans for Waters LCMS Certified Vials, vials from other sources, and clean solvents.[2]

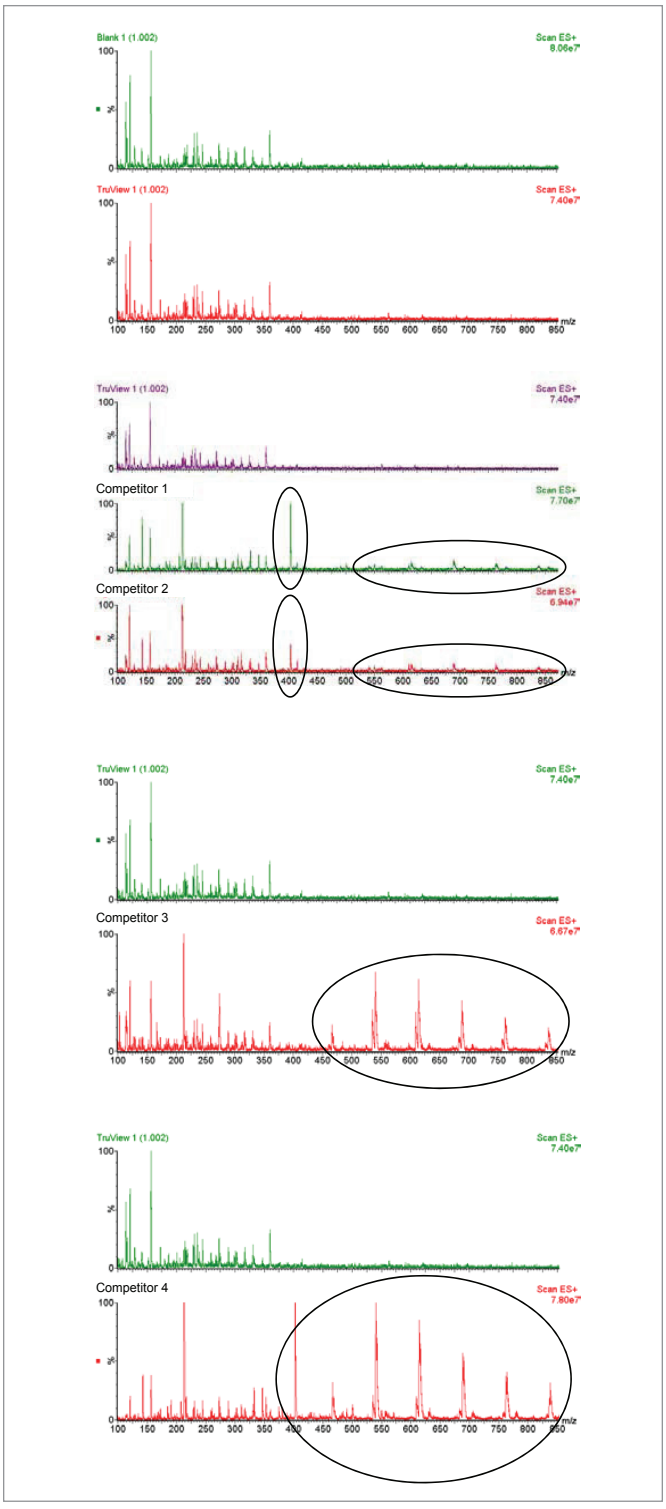

Figure 2. Comparisons of extractables from septa in different manufactured vials and TruView pH Control LCMS Certified Vials.

EXTRACTABLES FROM CAPS AND SEPTA

In addition, the glass, caps, septa, and even the packaging components of the vial can affect the chromatograms and data quality. This is especially true when working with low concentrations of analytes and using mass spectrometry. To demonstrate this, 1 mL of 95% methanol in water was added to vials from different sources. The caps were attached, and the vials were incubated at room temperature for one hour. Septa were punctured three times with an autosampler, and the solutions were analyzed by LC-MS. The results, shown in Figure 2, show that the TruView™ pH Control LCMS Certified Vials showed mass spectra like that of the solvent blank, but numerous peaks, at various masses, were observed with vials from other sources.

IMPACT OF pH

The change of the pH of the sample solution can have a significant impact on the results as the glass composition can also affect detection sensitivity. In the following case study, it was found that the analyte sofosbuvir showed a measurable level of degradation while sitting in the sample vial (Figure 3). This degradation presented itself as a second peak eluting just before the main component. This initiated a study that demonstrated the glass composition and the sample reacting with the glass components were the root causes of degradation. It was clearly shown that this effect varied greatly depending on the source of the glass (Figure 4).[2, 3]

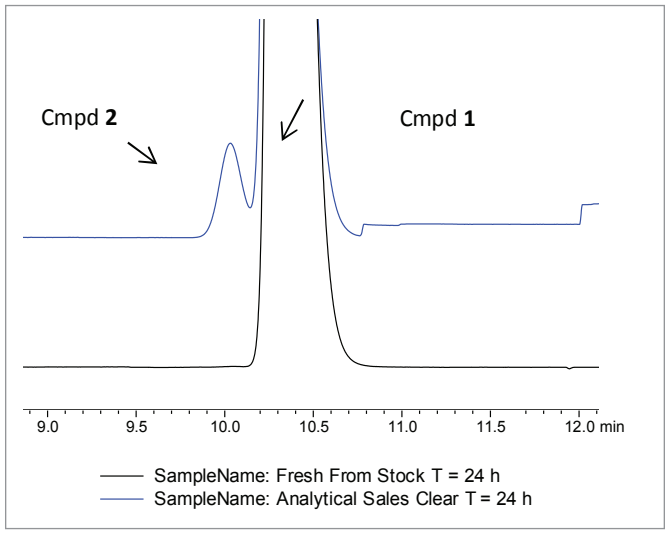

Figure 3. Overlay of 24-hour sample chromatogram of sofosbuvir showing degradation peak caused by sample's interaction with the glass vial.[3]

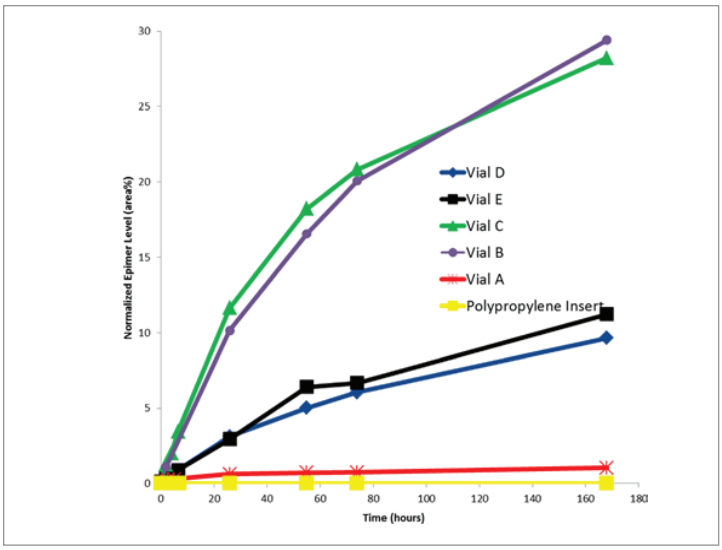

Figure 4. Graph of epimerization over time, showing the normalized area % of epimer. The monitored vials A (Waters), B, and D show different levels of degradation.[3]

In a different scenario, degradant peaks induced by the alkaline impurities from the glass vials were also detected in two ezetimibe solutions.[2] Each solution was stored in two different vials (Figure 5). To prevent the appearance of unexpected peaks during LCUV or LC-MS analysis, Waters TruView pH Control LCMS Certified Vials may be used as a control strategy.[4] The surface of TruView pH Control LCMS Certified Vials is modified to minimize blooming of free Na ions into solution. They have appropriate manufacturing and quality control procedures to ensure this property and are optimized for very low vial-to vial variability which can compromise data quality.

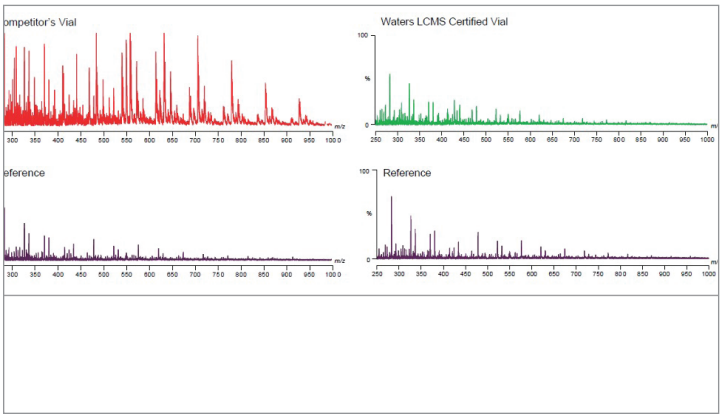

Figure 5. Top chromatogram shows normal behavior, and the bottom chromatogram shows ghost peaks.[4]

ADSORPTIVE LOSSES

In quantitative or low concentration qualitative LC-MS analysis, loss of analyte due to non-specific adsorption, non-specific binding on the surface of glass vials, and a high level of vial-to-vial variability are detrimental to data quality.[6,7] This is a significant problem in the lab because such interactions are often not recognized early enough and can happen immediately or over the course of a few hours while the sample is waiting for injection. The result is the cost of lost development and troubleshooting time. The extent of lost analyte has been shown to vary significantly among vials from different manufacturers (Figure 6). Waters TruView pH Control LCMS Certified Vials showed the lowest adsorptive losses and consistently performed better than competitor vials (Figure 7). Vial-to-vial adsorption variability demonstrates the variability between vials within a package.[4]

The impact of vial source variability on absorption is shown in Figure 7 where the nortriptyline peak is much smaller after four hours in vials from Vendors A and B. It is essentially unchanged in Waters TruView pH Control LCMS Certified Vials.

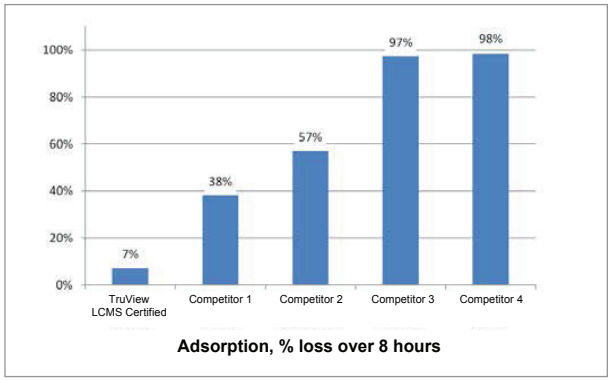

Figure 6. Comparison of adsorptive loss of chlorohexidine acetate in a variety of vials.

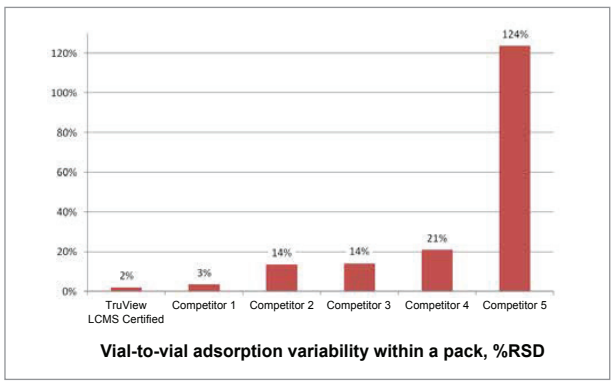

Figure 7. Comparing vial-to-vial variability (%RSD) for adsorptive loss of chlorohexidine acetate in vials from a variety of vendors.

The previous case studies demonstrate that selecting vials that are not fit-for-purpose with current method development can lead to costly and time-consuming investigations. To avoid unexpected delays, implement risk assessments as recommended in Stage 1 of MLCM in order to fully assess different vials and how they affect data quality. The following section describes the different types of vials and provides guidance in choosing the most suitable vial for your method's purpose.

VIALS FOR CHROMATOGRAPHIC USE

There are two main components to a sample vial – the glass vial and the cap with septa. Each of these components can have a potential impact on the analytes of interest and should be considered when choosing a vial for robust method development. A typical sample vial is made of USP Type 1 borosilicate glass which meets a certain level of USP requirements. It is important to remember that glass is an inorganic compound which exhibits an active surface and contains various levels of metals and free ions that can interact with the stored analytes. These metals and free ions are not considered by the USP guidelines. This means that the same USP glass type in sample vials from various manufacturers can cause variations in analytical results since the surface activity is not entirely specified.[5] During the typical residence time of a sample solution in a vial, it is possible for glass components such as sodium and other alkali metals to leach into the solution. This can significantly raise the sample pH and trigger changes such as unexpected degradation and lower analyte recovery. Other ions blooming to the glass surface can cause ion suppression effects in the ESI source of the MS and therefore alter the results. The polar binding of the analyte to the glass surface such as non-specific adsorption (NSA) or non-specific binding (NSB) can adversely affect detection or quantification. Analyte binding can be partially mitigated by surface alteration[4, 5, 6] during manufacturing. The second component is the caps and septa. In addition to the common considerations of chemical compatibility (Table 1), the cleanliness of the septa is important. During the manufacturing process, the septa material undergoes several finishing and conditioning processes that define the final purity of the septa. Impurities from the septa can be introduced into the sample vial by outgassing or contaminating at the point of sample injection.

Contaminants can originate from inside the glass vial, septa, and packaging. Polymers are commonly released by the septa. Contaminants from inside the sample vial are typically generated from lubricants and oils from the machines used during glass handling processes. Antistatic and other volatiles are known contaminants from packaging materials. Some level of these contaminants can be controlled by the manufacturing process, but it is important that the level of contamination does not exceed the sample and detection requirements of the analysis. Glass vials, caps, and septa quality should be considered when assessing method-related risks to achieve robust methods and good quality data. Choosing a manufacturer that can produce high quality vials with little-to-no lot-to-lot variation can serve as a control strategy to minimize such risks. The Waters certified glass product line discussed in this paper offer a variety of fit-for-purpose, high quality, and dependable glass vials. This helps ensure methods can generate good quality data, ultimately achieve good method performance, and support the QbD method development approach as suggested by MLCM.

Table 1. Overview of solvent compatibility and suitability of different septa materials

Material	Compatibility	Recommendations	Sealing
PTFE	All solvents	Suitable for one injection	No
PTFE/ Silicone	PTFE is resistant until puntured after first injection	Recommended for multiple injections	Very good
PTFE/ Silicone pre-slit	PTFE is resistant until punctured after first injection	Very good for multiple injections. Eliminates vacuum formation or pressure, enables accurate volume. Draw-in auto sampler, prevents coring with bottom draw needles	Good

LCGC CERTIFIED VIALS

High throughput laboratories rely on consistent chemical and mechanical product performance of sample vials. Waters LCGC Certified Vials are fit-for-purpose for LCUV analysis and tested for contaminants that can affect results at the parts per million (ppm) or µg/mL level.

The sample vials, septa and caps, as part of a finished Certified Vial kit, are tested and assembled to reflect the intended use. We use reference compounds across a wide range of polarities ensuring chromatographic integrity during the gradient RPLC test, measuring at a low wavelength of 195 nm to enhance detection sensitivity. Contaminants or extra tables of the highest level are quantified not to exceed 100 ppb.

Additionally, the septa material is tested with GC headspace to ensure the appropriate finish of the septa materials.

LCMS CERTIFIED VIALS

Waters LCMS Certified Vials are suitable for routine LC-MS applications, such as those that use the ACQUITY™ QDa™ or other single quad mass spectrometers and for analyte concentrations in the 10's to 100's ng/mL (parts per billion [ppb]). The LC-MS certification involves testing for contaminants typically found in lower end quality vials that can interfere with the electro spray ionization, such as surfactants, lubricants, antistatic agents, and silicone polymers from septa.

TruView pH CONTROL LCMS CERTIFIED VIALS

TruView pH Control LCMS Certified Vials are best suited for pH sensitive or unknown analyte compositions and when a high analyte recovery is required with analyte concentration levels of 1 pg/mL or below (1 ppb or below). These vials have very low vial-to-vial variability of blooming alkali and other ions causing pH changes, exhibiting very low adsorption, and making them ideal for handling solutions that have polar analytes at low level concentrations. The glass surface has been modified by a tightly controlled manufacturing process, achieving minimized pH changes and low adsorption properties. In addition to the MS-cleanliness tests, TruView pH Control LCMS Certified Vials are certified using UPLC™-MS/MS (MRM) for low analyte adsorption.

Every application requires different types of vials and therefore it is recommended to determine the best vial type that satisfies the application's needs and requirements.

CONCLUSIONS

It has been shown that vial quality does have an impact on data quality and should be considered when assessing method-related risks during Stage 1 of MLCM: Method Design and Development. Choosing the right vial, cap, and septa for sample analysis is important to ultimately achieve and maintain good method performance and is essential for method robustness. The sample vial coming from a single, specified source also reduces risks when the sample methods need to be deployed to different laboratories across the globe.

How to choose what type of vial is most appropriate for your application:

- If you require sensitivity, have pH sensitive compounds, or work with unknown analyte compositions in the low ppb range or below, TruView pH Control Vials will give you the best performance.
- If you are using MS for detection for a routine application with concentration in the low- to mid-ppb range, choose Waters LCMS Certified Vials.
- For high throughput routine analysis in a regulated QC environment, such as HPLC with UV detection or GC, and typical sample concentrations in the ppm range, Waters LCGC Certified Vials will meet your needs in the most cost-effective manner.

Waters sample vials offer the reliability and reproducibility required for consistent analytical method performance, and reduce aberrant data investigations.

Please visit waters.com/vials for further product information and literature. To see whether a vial is compatible with your autosampler, please visit our vial selector tool at find.waters.com/vials.

Table 2. Testing levels for Waters Certified Vials

	LCGC Certified	LCMS Certifed	TruView pH Control LCMS Certified	QuanRecovery with MaxPeak™ HPS
Dimensional Test	✓	✓	✓	✓
UPLC-UV Cleanliness Certified	✓	✓	✓	
MS Certified		✓	✓	
Low Adsorption Certified			✓	
pH Control Certified			✓	
Protein Revocery Certified				✓

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