SPOTLIGHT feature

Sample Preparation & Processing

How Glass Vial Quality Affects Data Accuracy

Antonietta Wallace, Global Marketing Manager, Thermo Fisher Scientific

When testing for specific analytes, researchers prioritise data accuracy and reproducibility, especially in clinical research. The sample handling and preparation stages are particularly important when either the sample volume is limited or when detecting and quantifying analytes present at low concentrations. The quality of glass vials that these samples are prepared and stored in, a commonly overlooked factor, can have a major impact on the results. Here, we discuss how the surface and composition of the glass vials used in laboratories can affect data output and accuracy, ultimately impacting research findings and patient care. We also provide key considerations to choose the best glass vial quality for analytical applications.

Glass Quality Affects Data Accuracy

Glass vials often carry sub-micron particles on their surface as a by-product of the manufacturing process. Poor glass quality can affect analytical applications in two broad ways: (i) analytes in the sample react with inorganic particles on the glass surface and are retained in the vial, resulting in skewed quantitation numbers, (ii) contaminant particles enter the sample stream and accumulate on the column, resulting in distorted peaks and directly affecting data integration and interpretation.

Gas chromatography (GC) methods typically employ injection port liners as a way to protect the column from particulate damage, while high-performance liquid chromatography (HPLC) methods use guard columns. However, as more and more analytical methods use direct on-column injections or the use of capillary columns for finer separation, using vials with minimal background particulates can maintain data consistency and prolong column life.

Common constraints in translational and clinical research, such as the use of rare samples, limited sample volumes and the need to quantify trace levels of analytes, make it even more important for researchers to minimise background interferences. To detect low-abundance analytes, researchers often use high-sensitivity instrument settings. Even under these settings, using unprocessed glass vials can result in higher background noise. In fact, vials that have not been processed for analytical application can exhibit particle counts exceeding 5000 particles per mL, with the highest counts occurring below 0.5µm [1].

Why is High Quality Glass Important?

Contaminant particles on the glass surface stem from the vial manufacturing process which leaves behind free silanol groups along the glass walls, that can then react with and bind to analytes. The percentage of silanol groups on the glass wall, often referred to as the expansion coefficient, describes the 'activity' of the glass surface and, therefore, determines the glass quality. For example, a 70-type glass contains 70% free silanol groups on its surface, while a 33-type glass only has 33% of free silanol. The larger the expansion coefficient number, the greater the adsorption of analytes. Additional surface imperfections on the glass surface, such as scratches or holes, can increase surface adsorption. The time that that sample is present in the vial will also contribute to the degree of analyte adsorption.

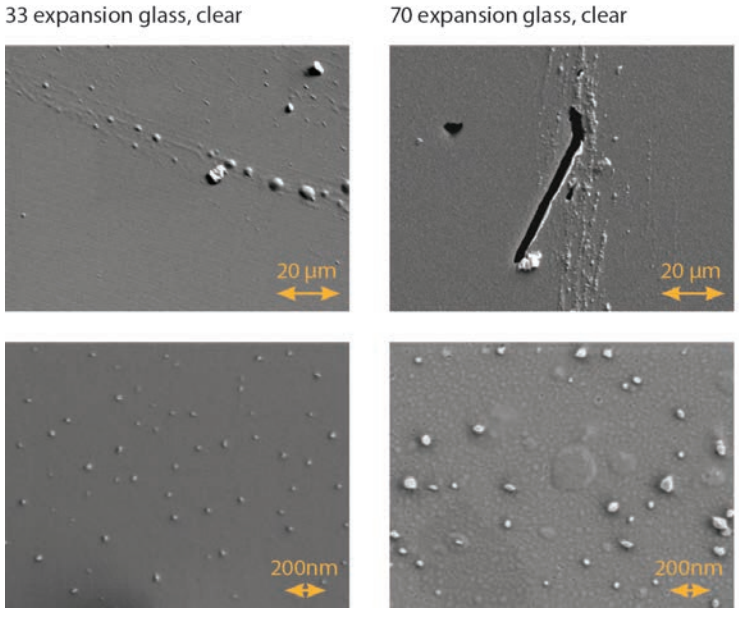

Figure 1. Glass quality comparison showing 1st hydrolytic class glass, types 33 vs 70.

To study the effect of glass quality on analyte adsorption, a typical routine analysis was replicated with glass vials of varying qualities. Tricyclic antidepressants (TCAs) were prepared in serum using five different glass vials with the goal of finding a vial type with least adsorption and good vial-to-vial reproducibility. LC-mass spectrometry (MS) results were obtained 0, 2, 4, 12 and 24 hours after filling up the vial. The most drastic adsorption effect was observed with TCA doxepin as it contains trisubstituted N-atoms that strongly react with silanol groups on the glass surface, suggesting that analyte chemistry can influence adsorption. Despite having the same expansion coefficient, the 51 type glass vials hailing from different countries exhibited varied performance, demonstrating that the underlying manufacturing process in the country of origin affects data output [2]. The Thermo Scientific Chromacoal GOLD-grade vials showed least vial-to-vial variability in adsorption, even over prolonged incubation periods.

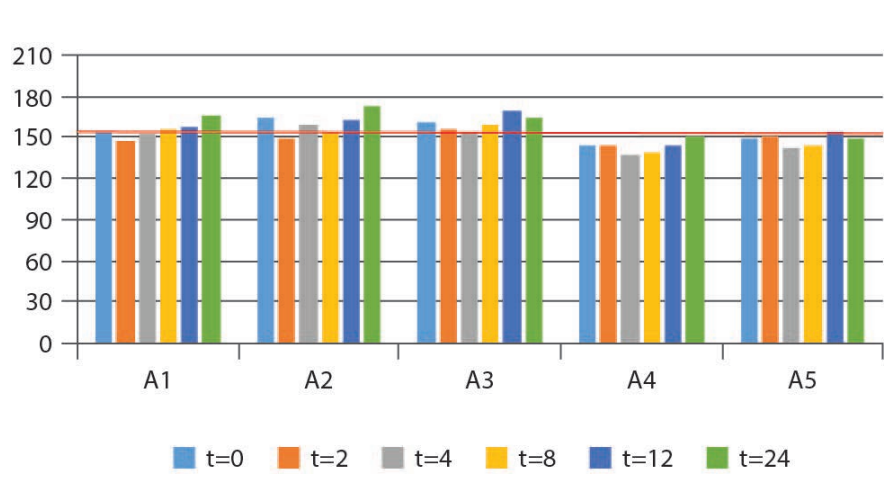

Figure 2. Results obtained for Vial A (Thermo Scientific Chromacoal GOLD-grade vial) shows excellent recovery and reproducibility from vial-to-vial and over a long period of 24 hours.

The study summarised the following glass-related factors that can directly affect sample integrity and data output [2]:

Vial-to-vial reproducibility: For protocols involving longer workflows or laboratories with higher throughput, using glass vials with lower adsorption coefficients can eliminate the need for interruptions during the routine workflow. It also sustains reproducibility between sample replicates or batch analysis.

Glass quality consistency: Even with the same glass quality configuration, glass types can vary depending on the manufacturing process across different countries, each influencing results in a different way. Choosing pre-processed vials that are most appropriate for the analytical application and staying consistent with that choice across experiments can eliminate glass-related variables.

Vial pre-processing: The detector can only measure the analytes entering the chromatography system. If analytes adsorb onto the glass vial surface, they never make it into the system to be quantified. To successfully quantify low-level analytes, choosing a high-quality, pre-processed glass vial becomes a crucial determinant, more important than the instrument itself.

The quality of glass vials, although seemingly minor, can have dire consequences on patient care through applications in clinical and translational laboratories. Inaccurate results obtained during clinical diagnostics, due to either analyte adsorption or abnormal peaks, can misguide subsequent decisions for medical intervention. Likewise, erroneous findings in population-level translational research can misrepresent biomarker data, causing serious delays in developing therapeutics.

Key Considerations for Choosing Glass Vials

For crucial applications in clinical laboratories and regulated research facilities, manufacturers now offer pre-cleaned or pre-processed vials with significant reduction in particle counts that minimise glass-related issues. With intentional steps during and after the manufacturing process dedicated towards eliminating glass particulates, these vials, specifically certified for analytical applications, are designed to meet high standards. In making a choice for the most suitable glass type, consider the following factors:

Physical uniformity: Are the vials manufactured in an automated facility with appropriate quality control to ensure structural uniformity between vials? Are the chosen raw materials chemically stable and inert? For example, the manufacturing process of Thermo Scientific MS Certified Vials includes a mandatory in-process step to verify 15 physical characteristics that are critical for reproducibility.

Removal of residual artifacts: Advances in analytical methods that facilitate highly sensitive detection can now catch even low concentration residual compounds. After the manufacturing process, are the vials subjected to additional processing to remove any particulate resides?

Standards of quality control and verification: What are the quality control tests performed by the manufacturer prior to packaging the vials? Each production lot needs to be tested for not only particulate counts, but also for any resulting background in LC or GC applications as a part of the quality control criteria. This ensures full transparency of the vial's performance especially for low-concentration analytes.

Packaging in cleanrooms: Once the vials are processed and cleaned, the steps involved in packaging and storing need to match the high standards of manufacturing. Gathering the manufactured vials on clean trays, encasing them into boxes and adding protective packaging are all steps that require the control of any additional particulate contamination. The MS Certified vials, for instance, are packed in a Class 10 cleanroom that's 1,000 times cleaner than those routinely used for glass or plastic packaging.

Vial caps: In addition to the vial glass surface, vial caps also need to remain clean. Ensure that the corresponding caps for the vial of choice have been selected based on both septa cleanliness and secure bonding. As with the glass vials, caps need to be packaged without contamination as well.

Questions to ask before choosing glass vials:

- Is it pre-cleaned? What is the particulate count?
- What is the percentage of silanol groups on the glass surface? The lower, the better.
- How does incubation time affect analyte adsorption?
- Is it manufactured exclusively for analytical applications?
- Has it been tested and certified for low background?

The careful consideration of glass quality and verification of pre-processing steps by the manufacturer upholds sample quality and analyte integrity by eliminating adsorption and helping ensure accurate numbers during analysis. By choosing consumables, such as glass vials manufactured in a good manufacturing practices (GMP)-compliant facility, laboratories can more easily comply with demanding regulations. Additionally, vials with non-adsorbing or minimally adsorbing surfaces also allow for sample incubation or backlogs during a normal laboratory workflow, without interrupting the routine or needing exceptions. Moreover, having a high vial-to-vial reproducibility boosts confidence in the results obtained, making troubleshooting and downstream analyses easier.

Conclusion

Even the slightest presence of particulate matter in glass vials can yield inaccurate results, especially if analytes are present in lower concentrations. Conclusive analysis in a clinical diagnostics laboratory or scarce sample analysis in bench-to-bedside research will depend on reliable data to inform subsequent choices in patient care. With numerous variables collectively influencing research data, the ability to fully control one by simply prioritising glass quality can help advance research efforts and maintain reproducibility in analytical applications.

References

1. L. Schaaf et al. Why is high glass quality so important when you want to detect low concentrations of analytes? Thermo Scientific.

2. MS Certified Vials: Pre-cleaned and certified vials for mass spectrometry. Thermo Scientific.