

LC analyte recovery

Analyte adhesion to LC vials: impact of surface chemistry on recovery at low concentrations

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Keywords

GOLD-grade polypropylene vial, analyte adhesion, adsorption, low concentration, HPLC vials, nonspecific binding, low bind vials, peptides, proteins, mAbs

Goal

To understand the fundamental mechanisms of analyte adhesion to LC vial surfaces and demonstrate how optimized vial surface chemistry and conditions improve analyte recovery, especially at low concentrations.

Introduction

The use of advanced high-throughput liquid chromatography-mass spectrometry methods is essential in the evolving landscape of drug discovery and the development of complex biotherapeutics. As the industry strives to improve drug efficacy and analyte concentrations decrease, analytical methods must deliver higher selectivity and sensitivity to meet increasingly stringent validation requirements. A critical challenge in this context is the phenomenon of nonspecific binding (NSB).¹

NSB is often overlooked and can lead to poor, nonlinear, or non-reproducible analyte recoveries, negatively impacting overall method robustness, particularly when working with trace-level concentrations. Analytes are prone to adsorption onto multiple surfaces throughout sample preparation, injection, separation, and detection.¹ These surfaces include sample handling equipment, liquid chromatography (LC) system components, and analytical columns. While pharmaceutical guidelines emphasize consistent recovery, stability, accuracy, and precision, they typically do not extend the investigation of NSB to sample handling materials. This gap can critically influence method performance, particularly sensitivity, precision, and accuracy. Furthermore, limited cross-validation between different materials may result in inaccuracies and inter-laboratory variability due to insufficient understanding of analyte adsorption behavior.

Analyte loss can arise from complex interactions such as electrostatic forces, hydrogen bonding, and hydrophobic or hydrophilic interactions between biomolecules and contact surfaces, including sample containers, pipette tips, and vials.¹ Differences in sample properties and vial materials, such as glass or plastic (polypropylene), can therefore lead to significant analyte loss due to NSB. Because samples are commonly stored and analyzed in vials, this study focuses on the impact of vial material, sample environment, and instrumental conditions. By investigating biotherapeutics with different adhesion mechanisms, a clearer understanding of how to mitigate analyte loss can be achieved.

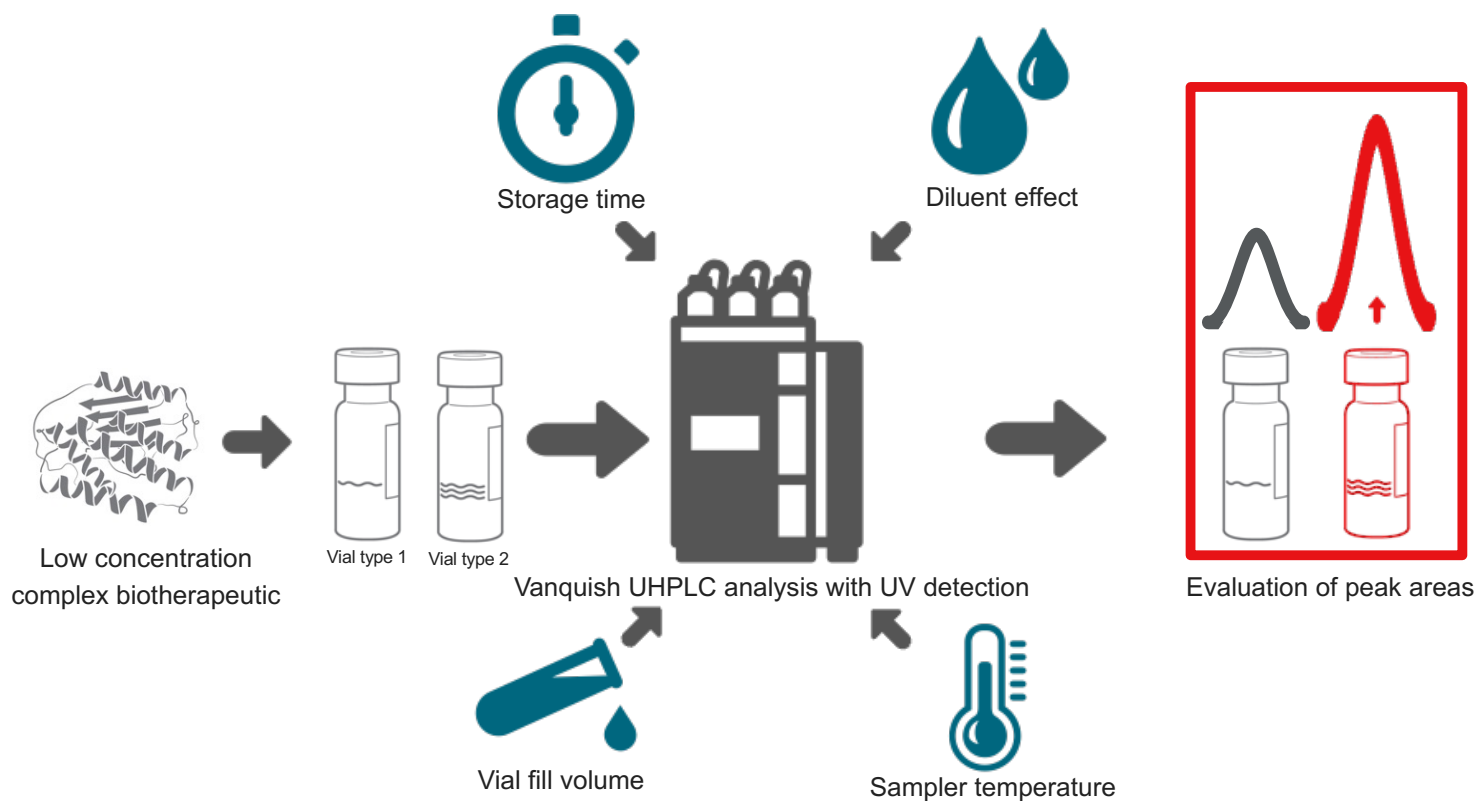


Figure 1. Simplified scheme of analysis workflow for the determination of peak recoveries in vials.

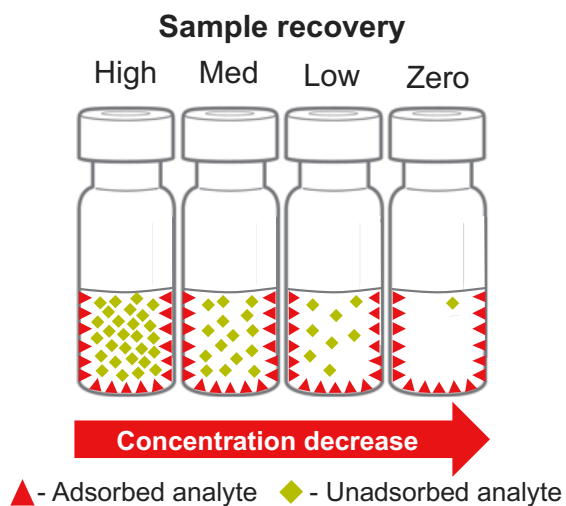


Figure 2. Representation of adsorption phenomenon in the vials with varying sample concentrations.

Experimental details

Chemicals

Chemical name	Part No.
Thermo Scientific™ Barnstead™ GenPure™ Pro UV TOC Water Purification System, deionized water, 18.2 MΩ.cm	50131948
Fisher Chemical™ Optima™ Water with 0.1% Formic Acid (FA) (v/v), LC/MS grade	LS118
Fisher Scientific™ Optima™ Acetonitrile with 0.1% Formic Acid (v/v), LC/MS grade	LS120
Fisher Chemical™ Optima™ Acetonitrile, LC/MS grade	A955
Fisher Chemical™ Optima™ Methanol, LC/MS grade	A456
Thermo Scientific Chemicals Trifluoroacetic acid, 99%	A12198.22

Sample handling

Item name	Part No.
Thermo Scientific™ Finnpiptette™ F1 Variable Volume Single-Channel Pipettes, 100–1,000 µL	4641100N
Thermo Scientific™ Finnpiptette™ F1 Variable Volume Single-Channel Pipettes, 10–100 µL	4641070N
Fisherbrand™ Mini Vortex Mixer	14-955-152
Thermo Scientific™ SureSTART™ 0.4 mL Screw Top Clear Polypropylene Plastic Microvials, Level 1	6ESV9-04PP
Thermo Scientific™ SureSTART™ 0.25 mL GOLD-Grade Borosilicate Glass Inserts, Level 3	6PME03C1SPG
Thermo Scientific™ SureSTART™ 0.25 mL Clear Glass Silanized Inserts, Level 3	6PME03C1SSP
Thermo Scientific™ SureSTART™ 2 mL Glass Screw Top Vials, Level 2	6ASV9-1P
Thermo Scientific™ SureSTART™ 0.4 mL Screw Thread GOLD-Grade Polypropylene Microvials, Level 3	6PSV9-04GPP
Thermo Scientific™ SureSTART™ 9 mm Blue Polypropylene AVCS™ Screw Caps with Soft Blue Silicone/Clear PTFE Septa, 100/pack, Level 3	6PSC9ST101
Vendor W low adsorption vials	

Instrumentation

Module	Part No.
Thermo Scientific™ Vanquish™ Flex UHPLC System consisting of:	
Thermo Scientific™ System Base Vanquish™ Duo UHPLC System	VF-S02-A-02
Thermo Scientific™ Vanquish™ Dual Pump F	VF-P32-A-01
Thermo Scientific™ Vanquish™ Split Sampler FT	VF-A10-A-03
Thermo Scientific™ Vanquish™ Column Compartment H	VH-C10-A-03
Thermo Scientific™ Vanquish™ Diode Array Detector CG	VC-D11-A-01
Thermo Scientific™ Vanquish™ Semi-Micro Biocompatible Flow Cell, 2.5 µL, path length 7 mm, MP35N	6083.0550

Sample preparation

Biotherapeutic samples (Table 1) were prepared from stock solutions by diluting them into two different concentrations with varying diluents. One is a high concentration reference for recovery determination at 100 µg/mL for all biotherapeutics. Second is a trace concentration for all tested samples. These values were based on recovery versus concentration curves—high enough concentration for reference to consider adsorption effects negligible, and trace concentrations where a significant recovery drop is observed. For example, the insulin recovery versus concentration linearity curve was generated by diluting 4 mg/mL of stock solution to 200, 100, 50, 20, 10, 4, 2, and 1 µg/mL concentrations (Figure 3). Recoveries were based on considering 200 µg/mL as a 100% point.

Table 1. A list of all tested samples, diluents, and concentrations.

Test sample	Diluent	High concentration reference	Tested trace concentration
Insulin	0.1% FA in water	100 µg/mL	4 µg/mL
Glucagon	0.1% FA in water	100 µg/mL	4 µg/mL
Semaglutide	0.1% FA in water	100 µg/mL	8 µg/mL
NISTmAb	0.1% FA in water	100 µg/mL	2 µg/mL
Trastuzumab	0.1% TFA in water	100 µg/mL	4 µg/mL
Tryptic cytochrome c digest	0.1% FA in water	100 µg/mL	10 µg/mL
Magainin 2	Varied, between 0.1% TFA in water and Type 1 water	100 µg/mL	4 µg/mL
Mastoparan X	Varied, between 0.1% TFA in water and Type 1 water	100 µg/mL	4 µg/mL

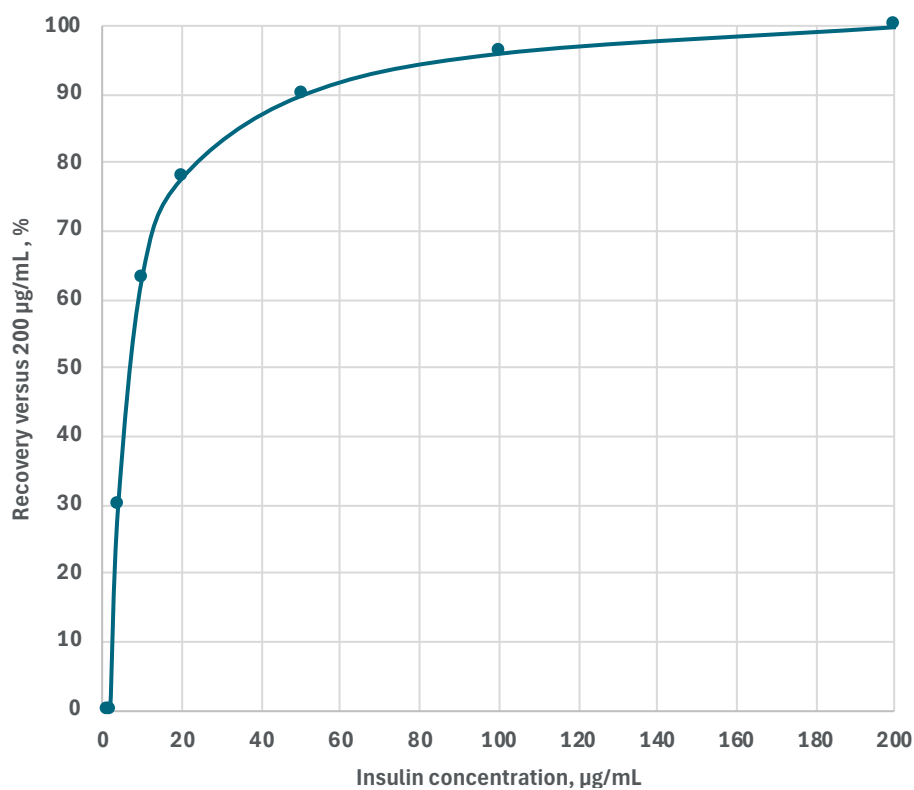


Figure 3. Insulin recovery versus concentration curve in polypropylene (diluent 0.1% FA in water, autosampler temperature 5 °C, vial fill volume 1/3 of total volume).

Chromatography data system

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) was used for data acquisition and processing. Errors were calculated by measuring the standard deviation of 3 consecutive injections.

Results and discussion

Proteins and peptides, as representative examples of complex biotherapeutics, often exhibit recovery losses in LC workflows that are strongly influenced by the surface chemistry of sample containers and by experimental conditions affecting surface–analyte interactions. At high concentrations, adsorption effects may be masked because surface sites become saturated. However, as concentration decreases, the surface-to-analyte ratio increases, and even minor surface interactions can significantly reduce recovery. All calculated recoveries in the following experiments were determined by evaluating target peak areas at trace concentrations versus a high concentration reference, where adsorption effects are negligible.

Influences of common vial materials and surface chemistry

The most frequently used LC sample containers differ significantly in surface chemistry and interaction potential. Unmodified borosilicate glass contains active silanol groups that can participate in hydrogen bonding and electrostatic interactions, which may promote NSB depending on analyte charge and pH. Silanized (deactivated) glass is chemically treated to reduce silanol activity and minimize polar interactions, although residual surface interactions may still occur. Polypropylene, an alkali-free polymer, eliminates silanol-related and ion exchange mechanisms but presents a hydrophobic surface that can interact with hydrophobic regions of proteins and peptides. Understanding the role of vial surface chemistry when working with adsorption-sensitive biomolecules is essential for correctly interpreting experimental data and evaluating the influence of molecule-specific structural properties.

Factors influencing adsorption

To systematically evaluate adsorption behavior, a structured design of experiment (DoE) was implemented. The experimental matrix included five variables suspected to significantly influence adsorption: autosampler temperature, organic modifier in the diluent, storage time in the autosampler, vial fill volume, and the aforementioned vial materials. These parameters were selected because they can be readily adjusted within routine analytical workflows without requiring major hardware modifications, enabling a controlled assessment of how method conditions affect the recovery of different proteins at low concentrations.

The highlighted results with selected proteins/peptides demonstrate that, beyond vial material selection, several analytical parameters can substantially influence NSB.

Table 2. Design of experiment.

Factor	Variable A	Variable B	Variable C
Vial fill volume	1/3 of total vial volume	2/3 of total vial volume	-
Vial storage time in autosampler	6 h	12 h	24 h
Autosampler temperature	5 °C	25 °C	-
Organic diluent content	0% ACN	20% ACN	-
Vial material	Unmodified glass	Silanized glass	Polypropylene

Autosampler temperature

Temperature is known to affect adsorption equilibrium and desorption kinetics. In general, increasing temperature should promote desorption of peptides and proteins from vial surfaces; however, the observed effects were analyte dependent. While some proteins showed improved recovery at elevated temperatures, others exhibited minimal change or even reduced recoveries (e.g., insulin in silanized glass). Therefore, temperature optimization must balance potential recovery gains against the risk of analyte degradation.

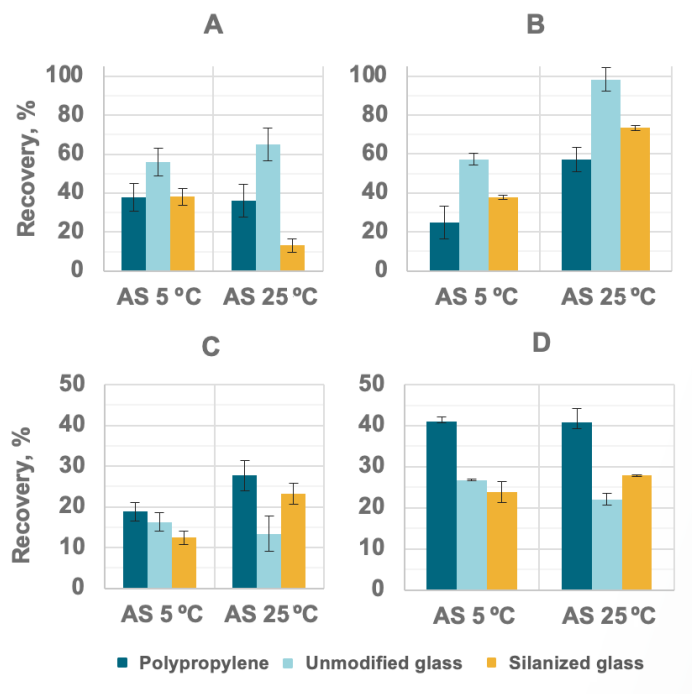


Figure 4. Autosampler temperature influence on recovery. (A) insulin (B) glucagon (C) semaglutide (D) NISTmAb.

Organic modifier in diluent

Among the tested variables, the presence of organic solvent had the most pronounced influence on recovery for majority of proteins. The addition of acetonitrile reduced hydrophobic interactions between biomolecules and vial surfaces, in some cases restoring recovery close to reference levels. However, solvent composition must be carefully optimized to maintain analyte stability and solubility.

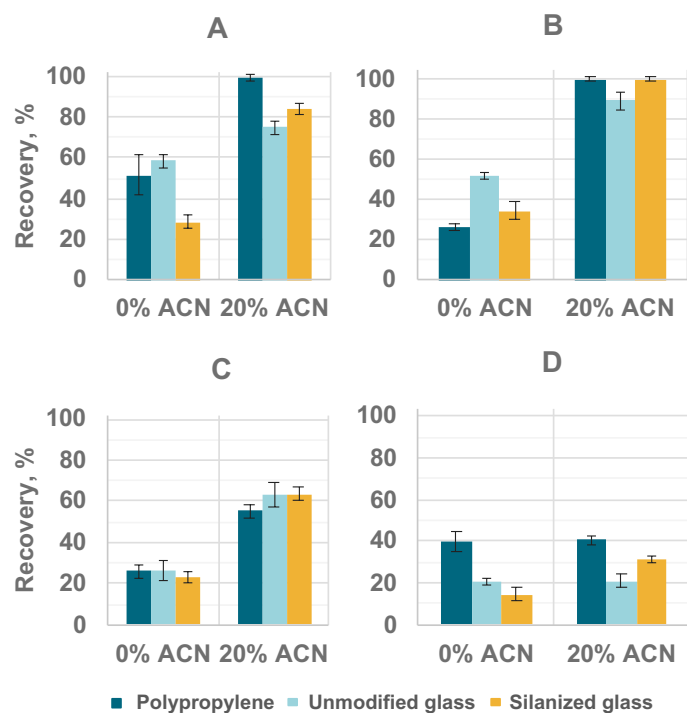


Figure 5. Organic diluent content influence on recovery. (A) insulin (B) glucagon (C) semaglutide (D) NISTmAb.

Storage time in autosampler

Extended residence time in the vial may affect recovery depending on the analyte. While some proteins exhibited minimal time-dependent effects, others showed measurable recovery decreases during prolonged storage. These findings highlight the importance of injection sequence planning and minimizing unnecessary sample storage time.

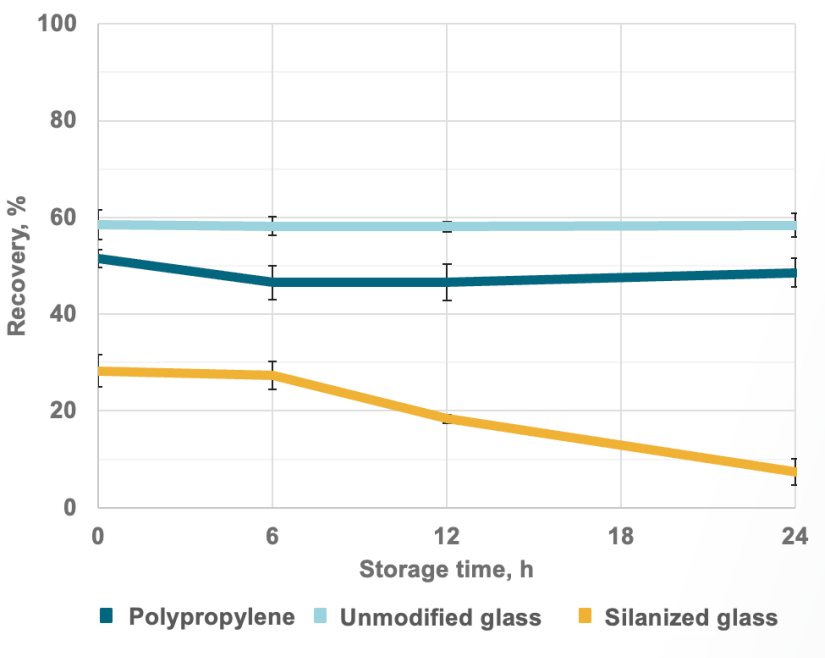


Figure 6. Insulin vial storage time in autosampler influence on recovery.

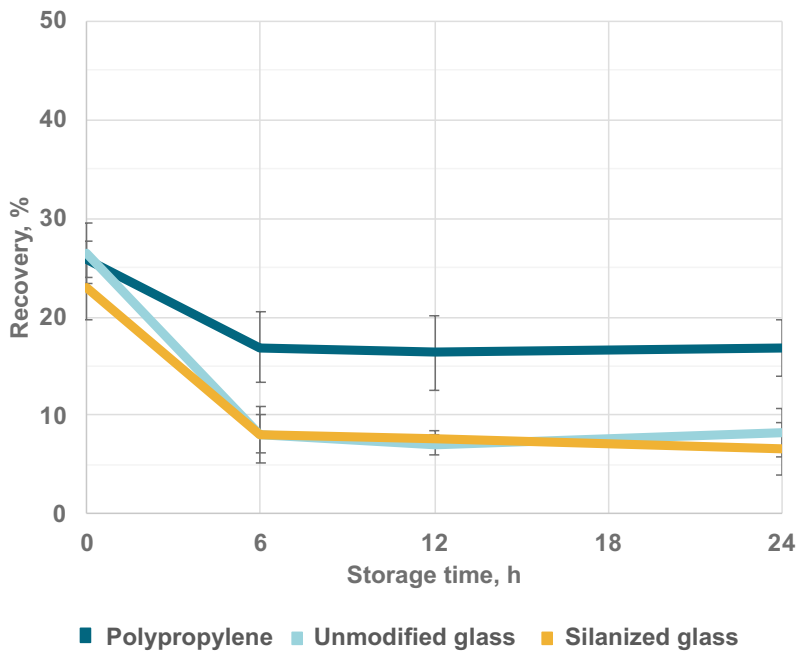


Figure 7. Semaglutide vial storage time in autosampler influence on recovery.

Vial fill volume

Although reduced fill volume generally increases the surface-to-volume ratio, no significant differences in recovery were observed between the tested volume conditions due to the V-shaped bottoms, and the results were within experimental error range. Under the studied conditions, fill volume was not a dominant factor influencing adsorption.

Importance of low-adsorption vial technologies

The results demonstrated that both vial material and controllable analytical parameters influence protein and peptide recovery. However, while method optimization can mitigate NSB to some extent, adsorption effects cannot always be fully eliminated through instrumental adjustments alone.

This is where specifically designed surfaces are introduced to address surface-analyte interactions at the material level. Rather than relying on method modifications (e.g., temperature or solvent adjustments), these “low-adsorption” vial technologies/surfaces offer a way to reduce NSB by minimizing electrostatic interactions, reducing hydrogen bonding potential, limiting hydrophobic surface affinity, preventing ion exchange mechanisms, and providing chemically inert and stable contact surfaces. As such, these technologies seek to create a more predictable and reproducible recovery profile across different biomolecules.^{2,3}

Engineered low-adsorption surface: GOLD-Grade polypropylene

To address adsorption at the material level, Thermo Fisher Scientific has developed a surface-engineered vial designed to reduce NSB mechanisms associated with both glass and conventional polypropylene materials.

GOLD-Grade polypropylene vials combine both the advantages of polypropylene and glass with a modified surface architecture composed of 100% silicon dioxide, intended to minimize surface-analyte interactions. Unlike unmodified glass, this design significantly reduces silanol-related electrostatic and hydrogen bonding interactions. Compared to standard polypropylene, the engineered surface further lowers the hydrophobic interaction potential and overall surface activity. In addition, the alkali-free composition helps prevent ion-exchange effects and reduces the risk of MS adduct formation. The modified surface is also chemically resistant and supports stable performance across varying solvent compositions.

By targeting multiple adsorption mechanisms simultaneously, this surface engineering approach provides more consistent recovery for complex biomolecules such as proteins and peptides, particularly at trace concentrations where surface effects are amplified.

Effects on small protein samples

To evaluate the practical impact of surface effects, selected proteins were analyzed at low concentrations using a wide array of different vial materials, including unmodified glass, silanized glass, standard polypropylene, low adsorption vial from Vendor W, and GOLD-Grade polypropylene. All subsequent results were generated at 5 °C autosampler temperature using the diluents listed in Table 1. Chromatographic overlays, recovery comparisons, and stability assessments were performed to illustrate differences in real analytical workflows.

A panel of representative small proteins was selected, including insulin, glucagon, and semaglutide from the influencing-factor experiments, as well as cationic membrane-active proteins such as Magainin 2 and Mastoparan X. These analytes differ in molecular weight, charge distribution, hydrophobicity, and structural features, making them suitable candidates for investigating distinct adsorption mechanisms. Insulin and glucagon are therapeutically relevant proteins with varying polarity and conformational properties, while semaglutide contains a C18 fatty diacid side chain attached to a lysine residue, increasing its hydrophobic character. In contrast, Magainin 2 and Mastoparan X are strongly cationic proteins known for high surface affinity driven primarily by electrostatic interactions. This diverse selection enables assessment of multiple interaction pathways commonly encountered in protein bioanalysis.

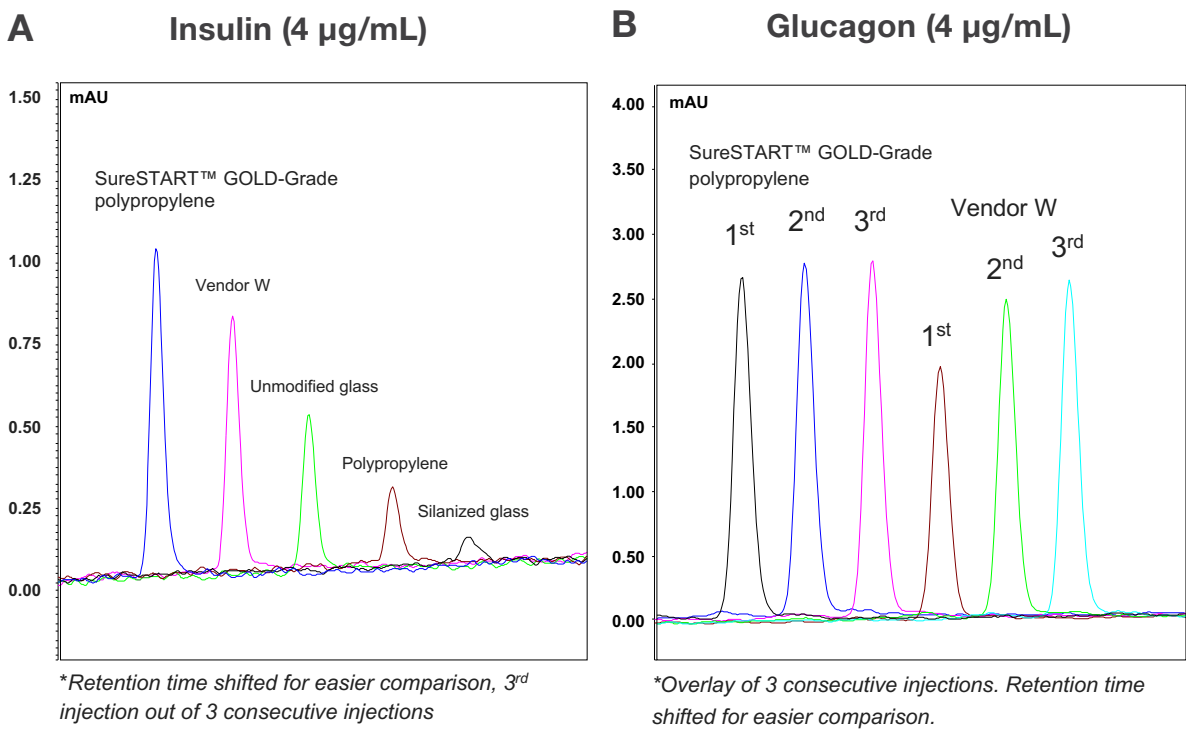


Figure 8. Overlay chromatograms of (A) insulin and (B) glucagon at low concentration in different vial materials.

Initial evaluations were performed using the therapeutically relevant proteins insulin and glucagon (Figure 8). From the beginning, clear performance differences were observed between vial materials under low-concentration conditions. For insulin (Figure 8A), recovery comparisons across a concentration range and during extended autosampler storage demonstrated consistent material-dependent behavior.

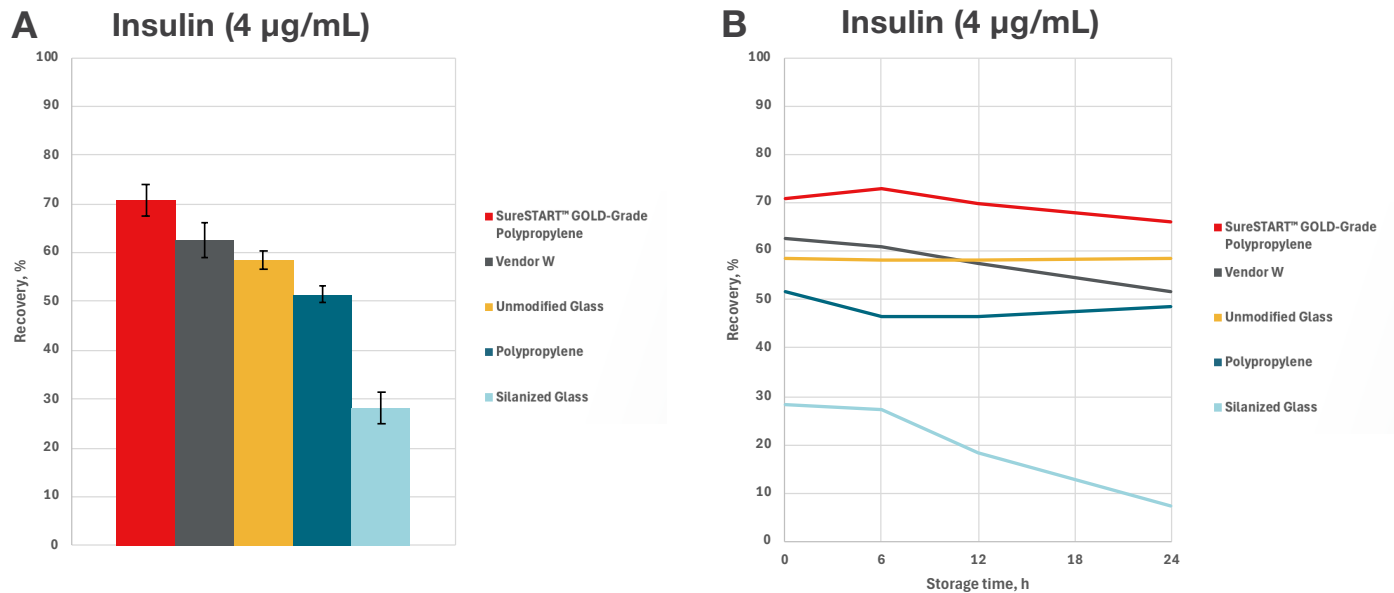
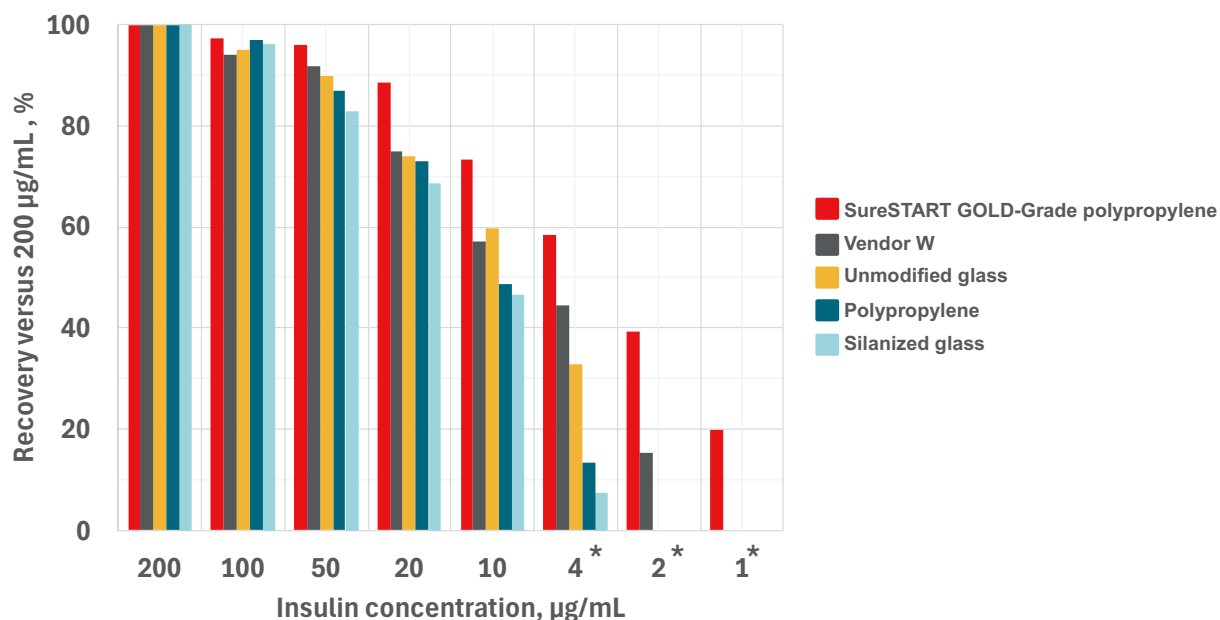


Figure 9. (A) Insulin recovery at low concentration and (B) effect of autosampler storage time across different vial materials.

Across the tested conditions, more hydrophobic surfaces, such as standard polypropylene and silanized glass, generally exhibited lower recovery compared to engineered low-adsorption surfaces. GOLD-Grade polypropylene provided the highest relative recovery throughout this comparison. Although minor adsorption effects were observed over 24 hours for most vial types (Figure 9B), the relative performance ranking remained largely unchanged compared to time-zero measurements. This indicates that concentration-dependent surface interactions were the dominant factor, rather than progressive time-dependent adsorption.



*Only peaks >LOD (S/N > 3) are evaluated. Some peaks are <LOQ (S/N < 10). Results are average of 3 consecutive injections.

Figure 10. Insulin recovery versus concentration across different vial materials.

Evaluation across a broader concentration range further confirmed this trend (Figure 10). Recovery differences between materials remained consistent, and GOLD-Grade polypropylene enabled reliable detection at the lowest tested concentrations (S/N > 3), where other materials approached reduced or non-detectable signal levels.

Glucagon: injection-to-injection reproducibility

For glucagon (Figure 8B), average recoveries across three consecutive injections were more comparable between vial materials. However, differences in injection-to-injection reproducibility were observed in certain vial types. In contrast, GOLD-Grade polypropylene demonstrated consistent peak area response from the first injection onward, indicating improved precision under low-concentration conditions.

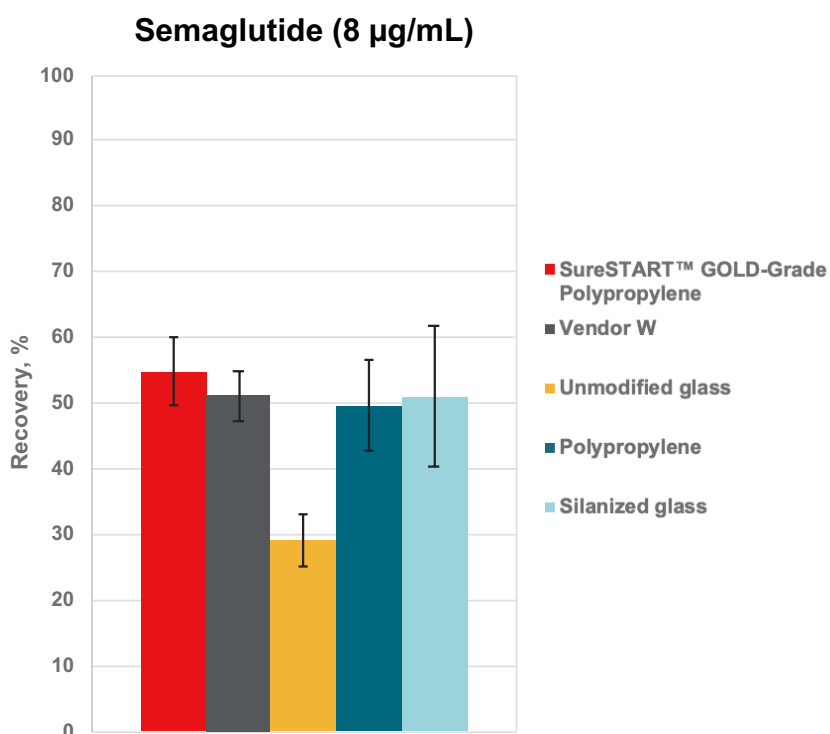


Figure 11. Semaglutide recovery at low concentration across different vial materials.

To further evaluate structural influences, semaglutide was investigated (Figure 11). Due to increased adsorption observed at trace levels across all tested vial materials, the sample was evaluated at a slightly higher concentration. As semaglutide contains a C18 fatty diacid side chain, increased hydrophobic interactions were anticipated. However, under these conditions, more hydrophobic surfaces, such as silanized glass and standard polypropylene, showed relatively improved recovery compared to their performance with insulin. In some replicate injections, recoveries were comparable to low-adsorption vials; however, repeatability remained inferior. GOLD-Grade polypropylene and Vendor W low-adsorption vials demonstrated more consistent injection-to-injection precision, supporting their suitability for structurally modified proteins.

Cationic membrane-active proteins: electrostatic interactions

To further investigate electrostatic effects, cationic membrane-active proteins were evaluated under different diluent conditions. Type I borosilicate glass contains active silanol groups capable of participating in electrostatic interactions. Because silanol groups can adopt negative charge states under neutral conditions while basic proteins remain positively charged, these analytes serve as effective models for assessing electrostatic adsorption.⁴

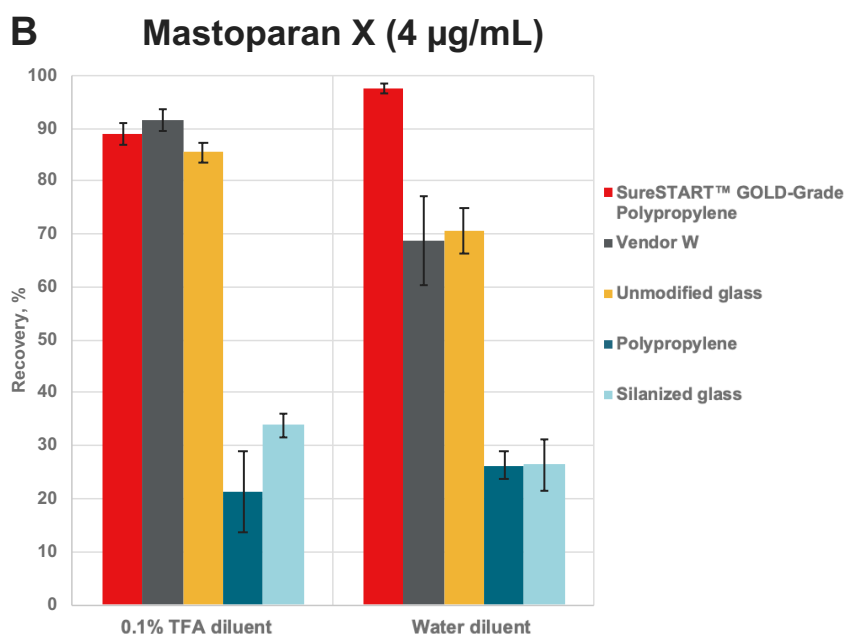
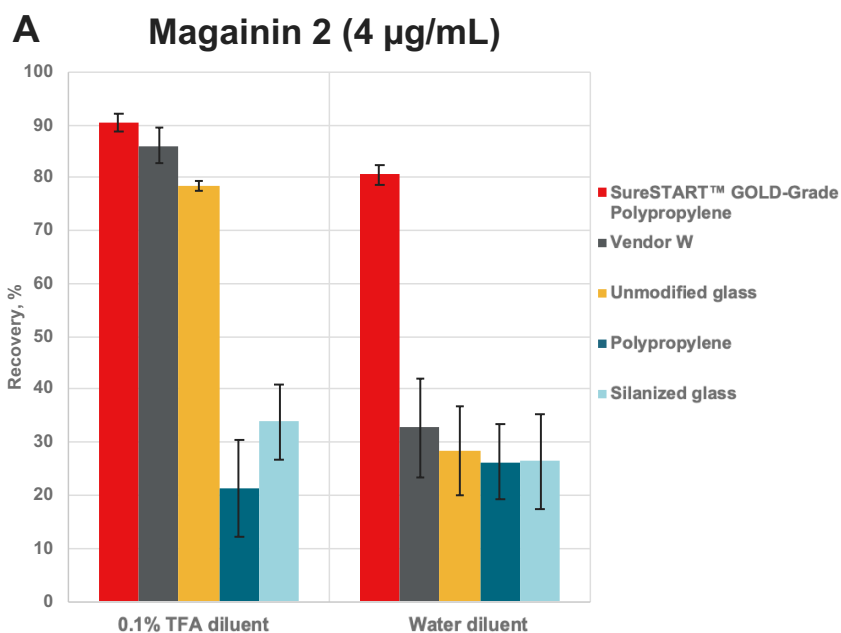


Figure 12. Recovery of (A) Magainin 2 and (B) Mastoparan X at low concentration in different vial materials under varying diluent conditions.

Results shown in Figure 12 confirm the presence of electrostatic effects. For Magainin 2, several vial types (GOLD-Grade polypropylene, Vendor W, and unmodified glass) demonstrated excellent recovery when diluted in 0.1% TFA in water. However, upon removal of the acidic modifier, recovery decreased sharply in all vial materials except GOLD-Grade polypropylene, indicating pronounced electrostatic interactions under neutral conditions. GOLD-Grade polypropylene maintained stable recovery, suggesting reduced sensitivity to pH-dependent surface charge effects and providing a more universal solution for adsorption-sensitive basic peptides.

Effects on protein digest samples

An additional area of investigation involved peptide mapping analysis using cytochrome c tryptic digest as an example. Due to its relatively low sequence complexity and well-characterized peptide profile, cytochrome c digest provides a practical framework for evaluating adsorption behavior at the individual peptide level. This approach allows assessment of whether specific peptide fragments exhibit increased affinity toward particular vial surfaces.

Cytochrome C tryptic digest (10 µg/mL)

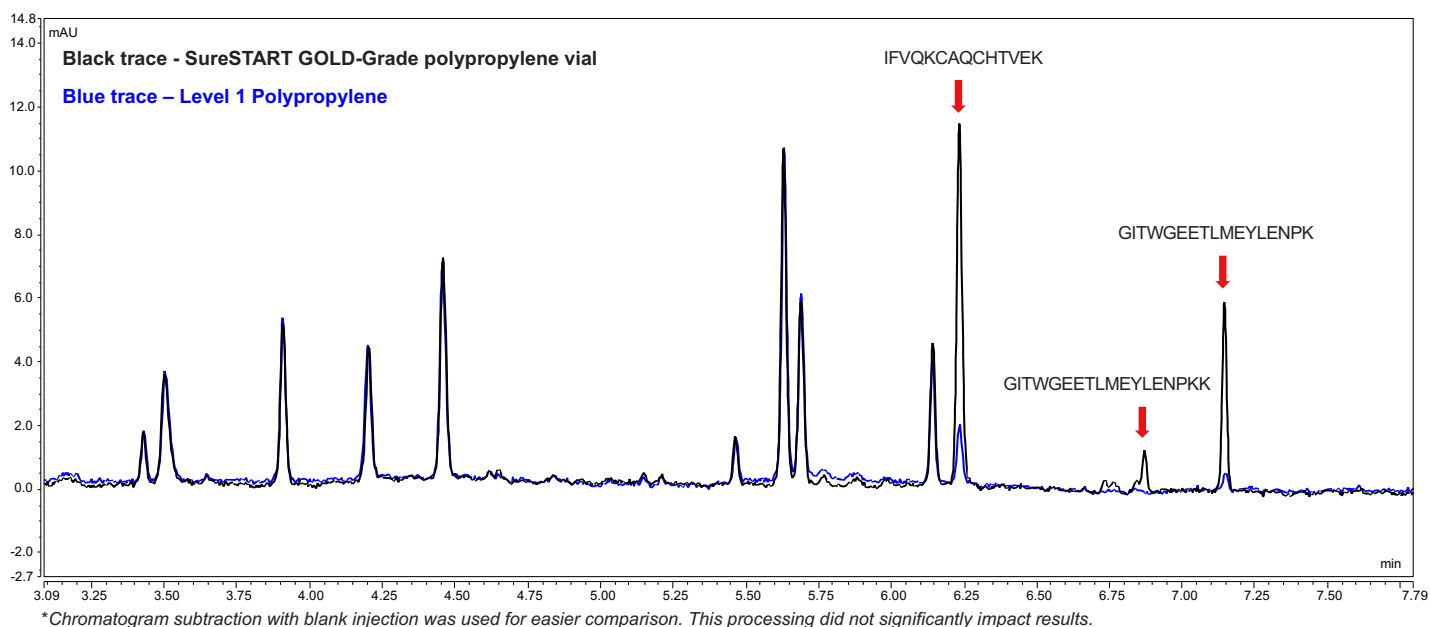


Figure 13. Overlaid chromatograms of cytochrome c tryptic digest at low concentration in GOLD-Grade and Level 1 polypropylene vials.

Figure 13 presents overlay chromatograms comparing GOLD-Grade polypropylene and Level 1 polypropylene vials under low-concentration conditions. For the majority of more hydrophilic peptide fragments, no substantial differences in peak response were observed between the two materials. However, for several later-eluting peptides with greater hydrophobic character, Level 1 polypropylene exhibited significantly reduced signal intensity, with some peaks approaching or falling below the detection threshold. In contrast, these peptides remained clearly detectable in GOLD-Grade polypropylene vials, indicating reduced adsorption effects. The high signal intensity for these hydrophobic fragments suggests that surface engineering may be particularly beneficial when analyzing peptide sequences with limited intrinsic detectability or increased surface affinity.

Cytochrome C tryptic digest (10µg/mL)

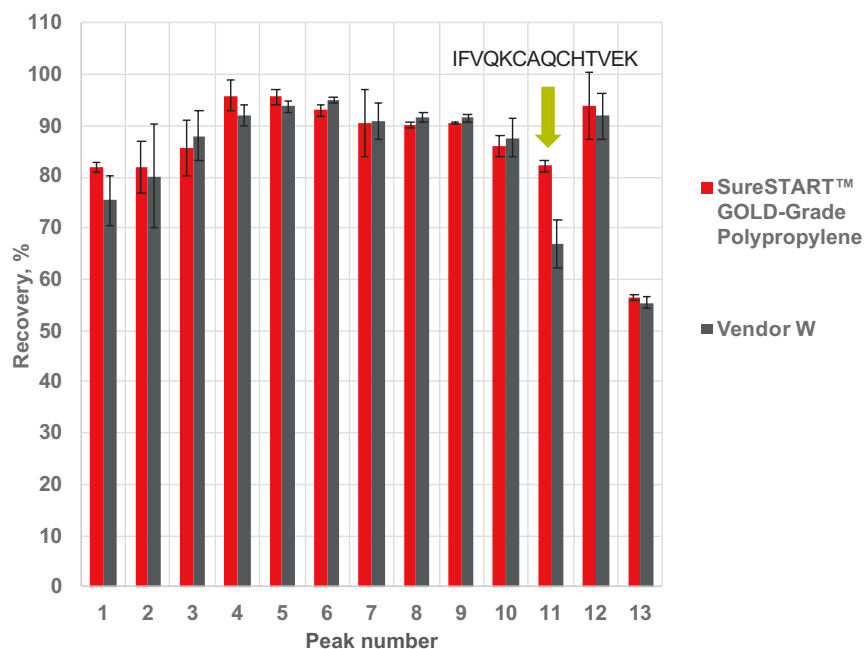


Figure 14. Recovery comparison of cytochrome c tryptic digest in GOLD-Grade polypropylene and Vendor W low-adsorption vials for each major individual peak.

Comparison with other low-adsorption vial vendor (Figure 14) showed that, for the majority of monitored peptide peaks, recoveries were comparable and remained within experimental variability. However, for selected fragments (e.g., peak 11), GOLD-Grade polypropylene demonstrated higher measurable recovery. This suggests that subtle differences in surface properties may influence adsorption behavior for specific peptide sequences, particularly those with increased surface affinity.

These findings emphasize the importance of evaluating vial surface effects not only at the intact protein or total digest level but also at the individual peptide level. In peptide mapping workflows, accurate representation of low-abundance or hydrophobic fragments is essential, as selective adsorption of specific peptides may alter sequence coverage and quantitative interpretation.

Effects on large protein samples

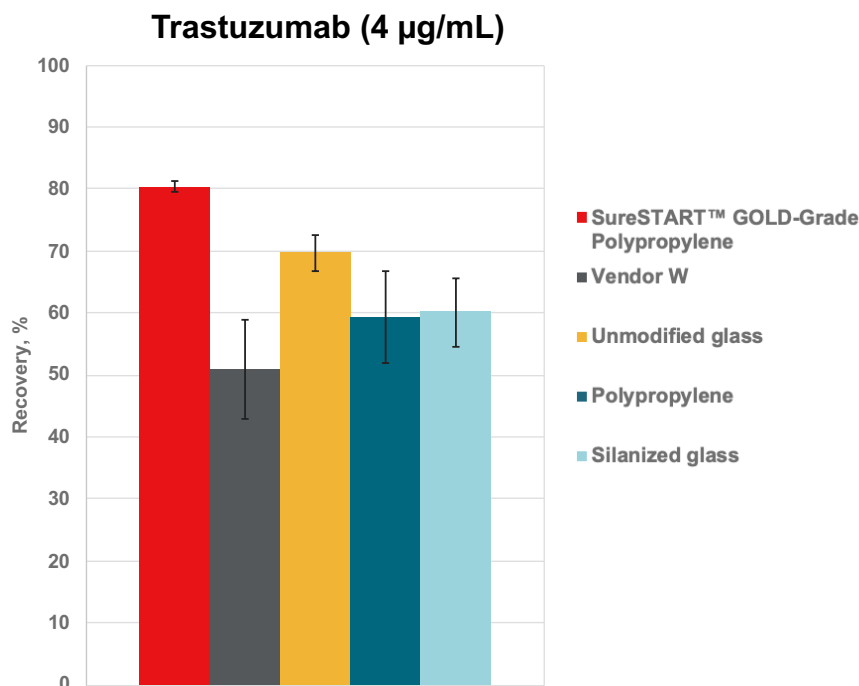


Figure 15. Trastuzumab recovery at low concentration across different vial materials

The complexity of larger biomolecules, such as NISTmAb, demonstrated that modifications of instrumental method parameters had a limited impact on recovery under the tested conditions. Consequently, vial surface properties become a key factor in determining analytical performance for monoclonal antibodies in low concentrations.

Such influences were observed for the therapeutically relevant monoclonal antibody trastuzumab (Figure 15). Across most tested vial materials, variability in peak response was detected, highlighting challenges in achieving consistent reproducibility at low concentrations. In contrast, GOLD-Grade polypropylene vials exhibited more stable peak area responses and improved injection-to-injection precision. Additionally, GOLD-Grade polypropylene provided the highest relative recovery among the evaluated materials, supporting its applicability for the analysis of larger protein therapeutics.

Conclusion

Complex biotherapeutics such as proteins and peptides exhibit recovery in LC workflows that are strongly influenced by vial surface chemistry, particularly at trace concentrations where adsorption effects can outweigh gains achieved through instrument optimization alone.

- Recovery and reproducibility are analyte-dependent and influenced by both controllable method parameters (temperature, diluent composition, storage time) and vial material selection.
- Hydrophobic peptides and cationic proteins, as well as large monoclonal antibodies, showed increased sensitivity to surface interactions, particularly under trace-level conditions.
- While glass and standard polypropylene remain appropriate for many applications, surface-engineered solutions such as GOLD-Grade polypropylene and GOLD-Grade glass demonstrated improved recovery consistency and precision under trace-level conditions, providing a practical and cost-effective way to enhance sensitivity without major hardware investment.
- There is no universal vial for every analysis. Selecting the appropriate vial based on analyte properties and method conditions rather than historical use is essential for reliable, high-quality data, however modified vials reduce the risk of analyte loss across multiple analyte classes.

Appendix:

Chromatographic conditions

Table 3. Default chromatographic conditions.

Column	Hypersil GOLD C18 Selectivity 100 x 2.1 mm; 1.9 µm (Part No. 25002-102130) / Hypersil GOLD Peptide 50 x 2.1 mm; 1.9 µm (Part No. 26002-052130) for insulin, glucagon, semaglutide, Magainin 2, Mastoparan X and tryptic cytochrome c digest MAbPac RP Phenyl (Part No. 088647) 100 x 2.1 mm, 4 µm for NISTmAb and trastuzumab
Solvent A	0.1% FA in water For Magainin 2, Mastoparan X and trastuzumab: 0.1% TFA in water
Solvent B	0.1% FA in ACN. For Magainin 2, Mastoparan X and trastuzumab: 0.1% TFA in ACN For cytochrome c digest: 0.085% FA, 60:40 ACN/water
Gradients	Gradient (insulin and glucagon): equilibrate for 4 min with 20% B, then ramp to 45% B in 4 min, clean with 95% B for 2 min. Gradient (semaglutide): equilibrate for 4 min with 35% B, then ramp to 65% B in 4 min, clean with 95% B for 2 min. Gradient (NISTmAb and trastuzumab): equilibrate for 3 min with 25% B, then ramp to 60% B in 6 min, clean with 60% B for 0.5 min. Gradient (cytochrome c digest): equilibrate for 10 min with 10% B, then ramp to 65% in 5 min, clean with 100% B for 0.5 min. Gradient (Magainin 2): equilibrate for 7 min with 25% B, then ramp to 60% in 2 min, clean with 95% B for 1 min. Gradient (Mastoparan X): equilibrate for 5 min with 30% B, then ramp to 95% in 1.8 min, clean with 95% for 1 min.
Flow rate	0.8 mL/min for insulin, glucagon and semaglutide 0.4 mL/min for cytochrome c digest 0.5 mL/min for trastuzumab and NISTmAb 0.6 mL/min for Magainin 2 and Mastoparan X
Column temperature	Active preheating enabled Insulin, glucagon, semaglutide: 50 °C, still air mode Trastuzumab and NISTmAb: 80 °C, still air mode Cytochrome c digest: 40 °C, forced air mode, fan speed: 5 Mastoparan X and Magainin 2: 50 °C, still air mode
Autosampler temperature	5 °C

Needle wash solution	10% methanol
Needle wash mode	After draw
Injection volume	10 µL
Detector settings	Insulin, glucagon, semaglutide, NISTmAb, trastuzumab: 280 nm. Cytochrome c digest, Mastoparan X, Magainin 2: 214 nm

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