CASE STUDIES

Eliminating Mobile Phase Induced Peak Splitting on Charged Surface Columns:

A Decisive Milestone for On-time, On-budget Project Delivery

Introduction

Porton PharmaTech, Slovenia (PSI) is the European subsidiary of Porton Pharma Solutions Ltd. This case study demonstrates the analytical depth, agility, and problem-solving capability of the R&D Analytical Team at PSI. By combining advanced chromatographic expertise, rigorous experimental design, and cross-platform studies, PSI analytical scientists rapidly diagnosed and resolved a subtle but project-threatening chromatographic anomaly.

The work was completed entirely by PSI's Analytical R&D team while operating within permissible variations confirmed by the customer. To maintain alignment and customer specifications, the team was required to use the analytical method, including the mandatory use of trifluoroacetic acid (TFA). This constraint eliminated conventional method redevelopment options and demanded a solution that was both scientifically sound and operationally fast.

Subtle analytical problems can quietly derail development programs. This one nearly did. Until PSI analytical team traced the root cause, proved the mechanism, and implemented a simple, durable control strategy. Addressing mobile-phase-induced peak splitting on charged surface columns became a decisive turning point; without this investigation, the project would not have met its approved timeline.

Executive Summary

Charged surface columns contain stationary phases modified with a fixed, low-level positive charge (quaternary ammonium–type groups). When used with TFA-modified mobile phases, trifluoroacetate anions gradually adsorb to these charged sites. This slow, cumulative process can introduce dual retention behaviour that manifests as peak doublets and retention variability over time, particularly in gradient methods and long injection sequences.

PSI Analytical R&D confirmed that:.

- Peak splitting intensified with increasing sequence length and elevated column temperature.
- The phenomenon was clearly visible on high sampling-rate detectors and largely masked at lower acquisition rates.
- A robust, periodic strong-wash protocol fully reversed the effect, harmonized chromatographic profiles across LC systems from different vendors, and eliminated an artefact peak coeluting with one impurity, without impacting critical impurity identification or quantification.
- Optimization of mobile phase composition to 0.15% v/v TFA eliminated peak splitting, stabilized retention, and maximized reproducibility across platforms.

By combining an optimized mobile phase with a disciplined column-wash strategy, PSI restored consistent chromatography across different LC systems, safeguarded data integrity, and preserved both project budget and schedule.

Context and Hypothesis

Charged surface stationary phases are designed to provide complementary selectivity through a mild, fixed positive surface charge. In mobile phases containing TFA, trifluoroacetate anions are electrostatically attracted to these sites and accumulate gradually with repeated injections.

Over time, the column surface transitions from its native state to a partially TFA-modified state, creating spatially and temporally heterogeneous retention behaviour. In practice, this might appear as:

- Peak doublets or shoulders (apparent "dual" analyte populations)
- Retention time drift or variability
- Occasional late-eluting, low-intensity artefact peaks caused by unpredictable TFA desorption

Because the effect develops cumulatively, it is often misattributed to sample instability, instrument variability, or operator technique. PSI's working hypothesis centered on TFA–charged surface interactions as the primary driver, with the effect amplified by temperature, sequence length, and detector acquisition rate.

Diagnostic Investigation

The investigation was deliberately structured to distinguish true chemistry-driven behaviour from instrument or perception-related artefacts:

- Column conditioning challenge
  Comparison of routine sequences versus runs incorporating prolonged, high-strength wash steps
- Temperature sensitivity
  Systematic elevation of column temperature to assess kinetic amplification
- Detector sampling-rate sensitivity
  Cross-platform comparison to determine whether acquisition settings influenced visibility
- Cross-system reproducibility
  Side-by-side evaluation on different LC systems from different vendors

This approach allowed PSI to decouple surface-chemistry effects from detection artefacts and to determine whether surface modification was reversible through controlled cleaning.

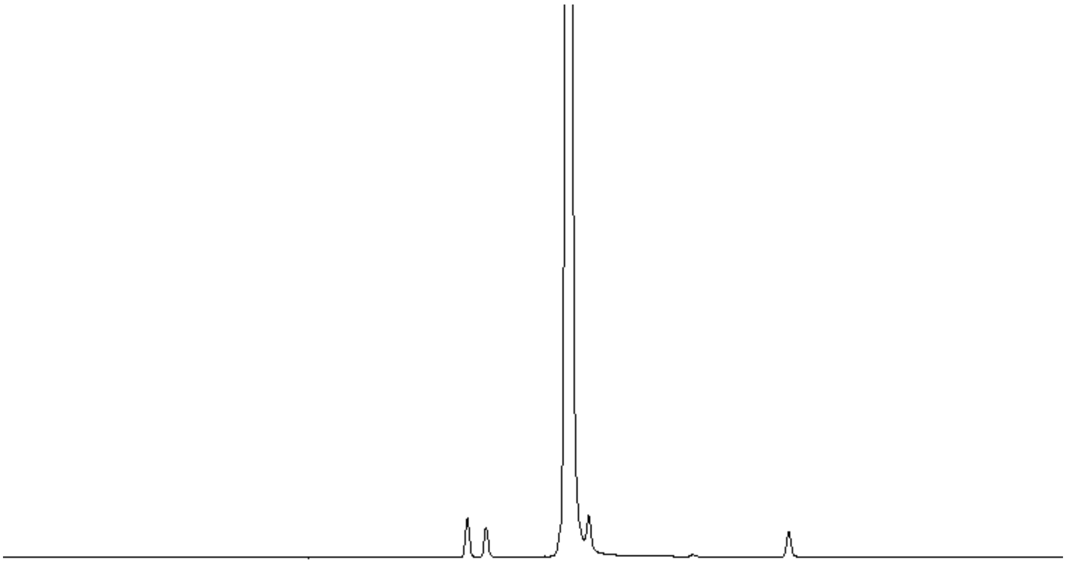

Figure 1. Chromatogram of standard marker solution

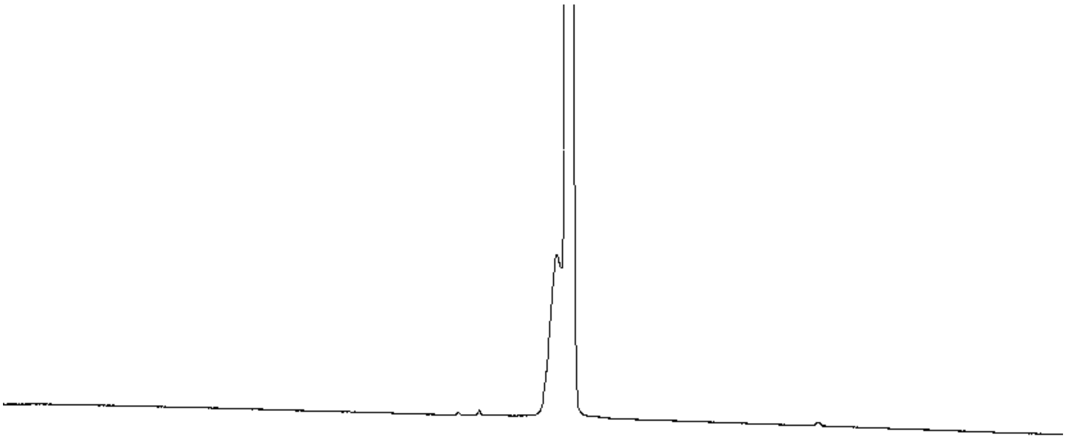

Figure 2. Chromatogram of standard marker solution after repeated injections within the same sequence

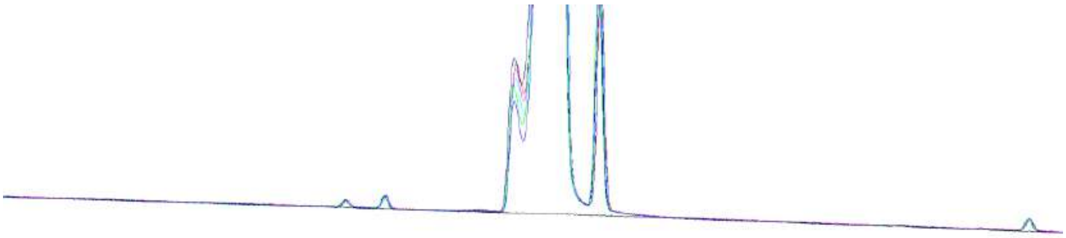

Figure 3. Overlay of standard marker solution repeated injections within the same sequence

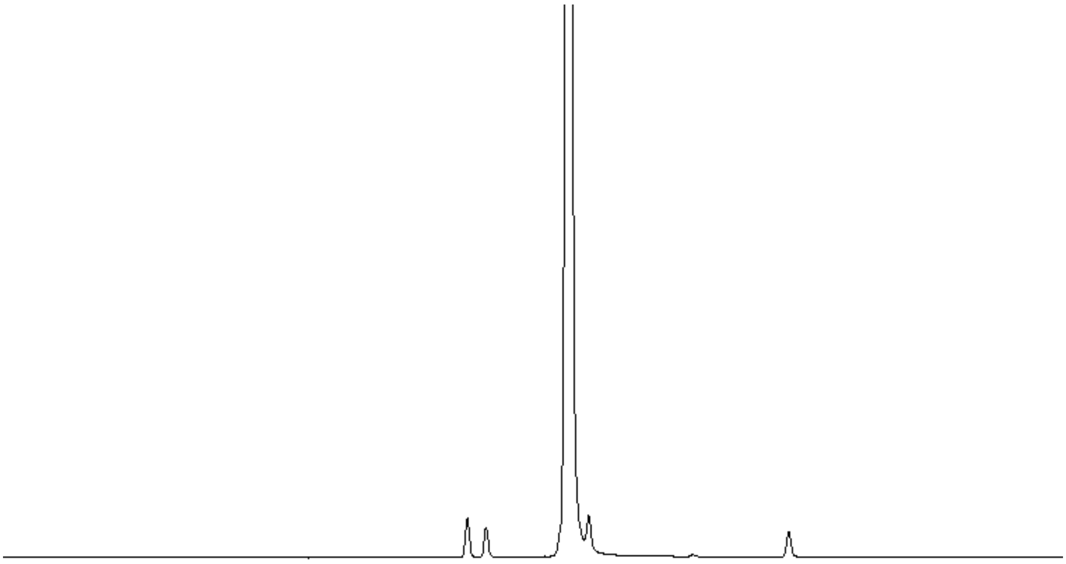

Figure 4. Chromatogram of standard marker solution after column wash

Key Findings

- Mechanism confirmed
  Progressive accumulation of TFA on charged surface stationary phases drives dual retention and peak splitting. Strong pre- and post-sequence washes collapsed doublets into single, symmetrical peaks, directly linking the phenomenon to a reversible surface modification.
- Acquisition rate governs visibility, not existence, of the effect
  High-frequency acquisition reveals the artefact peak presence. The underlying chemistry is present regardless of sampling rate.
- Artefact peak is removable
  After comprehensive column cleaning, the artefact peak disappeared.
- Temperature amplifies the phenomenon
  Elevated column temperatures increased peak splitting, consistent with faster adsorption–desorption kinetics on a partially modified surface.

While the wash protocol initially restored performance, chromatographic anomalies re-emerged during extended use, indicating the need for further refinement.

Mobile Phase Optimization: Establishing the Effective TFA Threshold

Subsequent investigation revealed that absolute TFA concentration in the mobile phase was decisive in stabilizing ion-pairing interactions. Small changes in additive level produced disproportionately large effects on chromatographic behaviour.

Experimental Progression

- **0.05% v/v TFA**
  Insufficient stabilization; peak splitting persisted and retention drift remained significant.
- **0.10% v/v TFA**
  Substantial improvement in peak symmetry and reproducibility, though minor variability appeared in long sequences.
- **0.15% v/v TFA**
  Optimal condition; peak splitting eliminated, retention stabilized, and reproducibility maximized across different platforms.

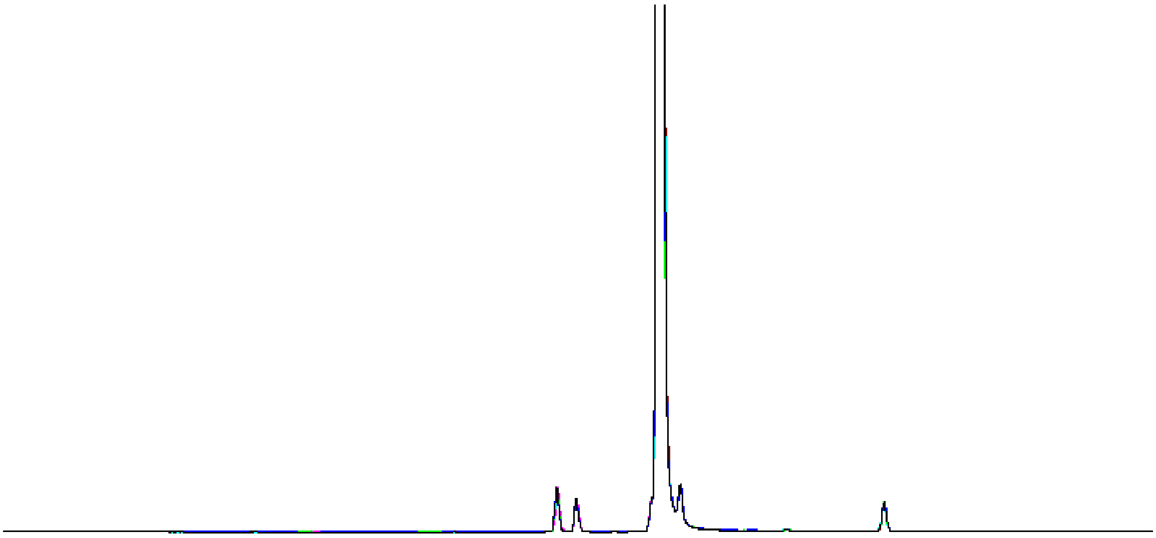

Figure 5. Chromatogram of marker standard solution on 0.10% TFA in mobile phase

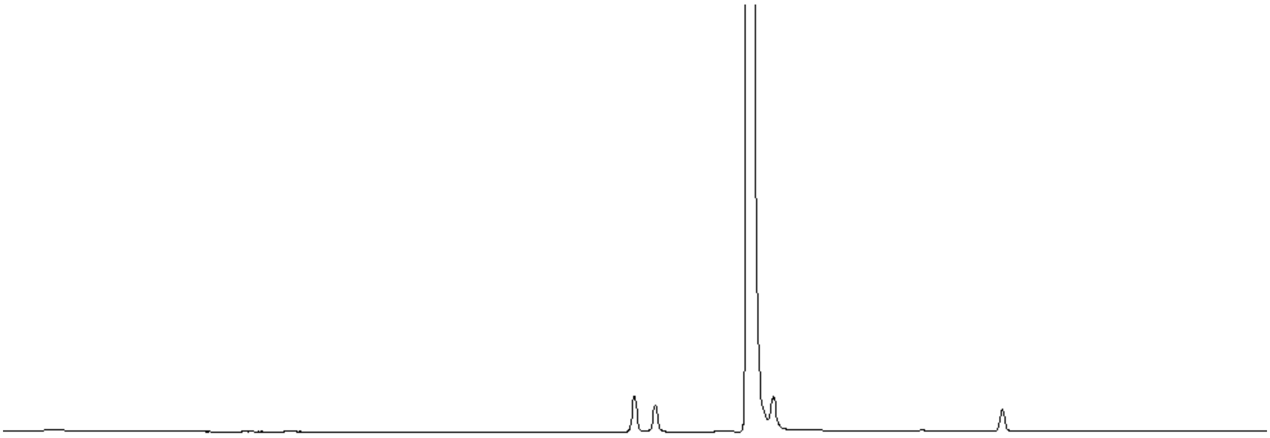

Figure 6. Chromatogram of marker standard solution on 0.15% TFA in mobile phase

Implications

- Below 0.1% v/v, TFA levels were insufficient to saturate ion-pairing sites, leaving the charged surface vulnerable to progressive modification.
- At 0.1–0.15% v/v, ion-pairing equilibria were stabilized, minimizing adsorption–desorption dynamics.
- 0.15% v/v TFA represented the most robust and reproducible operating point.

Corrective Action and Control Strategy

PSI translated these findings into a streamlined, durable control strategy addressing both root cause and data presentation:

Mobile Phase Adjustment (Root-Cause Control)

- Target concentration: 0.15% v/v TFA
- Effect: Stabilizes ion-pairing interactions and prevents progressive surface modification
- Outcome: Harmonized retention and reproducibility across platforms, even in extended sequences

Column Wash Protocol (Root-Cause Control)

- **Schedule**: Strong column wash performed after usage rather than after every 10 injections
- Effect: Restores the stationary phase to a consistent surface state
- Outcome: Equivalent chromatographic profiles across different LC system brands

Acquisition Tuning (Perception Control)

- Sampling rate: ≤5 Hz for routine analyses
- Rationale: Reduces apparent microstructure once chemical stability is ensured, without compromising quantitation

Operational Safeguards

- Embed scheduled washes in long sequences rather than deferring recovery to end-of-day
- Maintain platform parity across instruments using the same control strategy

Project Impact and Lessons Learned

This investigation marked an inflection point for the program. By isolating the TFA–charged surface interaction and institutionalizing a control strategy, PSI avoided the delays typically associated with elusive chromatographic variability.

Budget and Timeline Protection

- Eliminated emergency method redevelopment and unnecessary column replacement
- Preserved batch throughput and downstream manufacturing schedules
- Enabled cross-platform continuity, minimizing bottlenecks and overtime

Data Integrity Enhancement

- Stable peak shape and retention strengthened decision-making and trending
- Confirmation and elimination of a non-interfering artefact peak ensured fitness for routine release and stability testing

Operational Insights

- Surface chemistry matters: Even weak ionic interactions accumulate over long sequences
- Perception: High sampling rates reveal, but do not cause the problem
- Preventive discipline: Proactive washes are more efficient than reactive troubleshooting

Conclusion

This case study demonstrates how rigorous analytical logic, disciplined execution, and targeted experimentation can resolve complex chromatographic challenges. PSI's Analytical R&D team delivered a robust solution that safeguarded data integrity, protected project timelines, and preserved budget - reflecting a clear customers-first mindset supported by efficient execution and strong teamwork.

Importantly, the solution has broad industry applicability. Charged surface columns and TFA-containing mobile phases are widely employed for the analysis of complex modalities such as peptides and highly polar small molecules. The control strategy established here - optimized TFA concentration, disciplined wash protocols, and fit-for-purpose acquisition settings - provides a transferable framework for stabilizing ion-pairing interactions across analytical platforms. This approach exemplifies PSI's commitment to the pursuit of excellence and willingness to embrace change through thoughtful, data-driven optimization.

For Porton Pharma Solutions, this work reinforces our mission of **enabling earlier access to good medicines** by supporting global pharmaceutical partners with scientifically rigorous, aligned, and operationally efficient analytical solutions. By transforming a subtle chromatographic anomaly into a controlled and well-understood variable, PSI ensured both scientific integrity and program delivery, demonstrating how precision at the molecular level, guided by clear values, can ultimately accelerate and determine project success.

Verica Zlatevska

AR&D Senior Analyst

Porton Slovenia

Luka Benić

AR&D Manager

Porton Slovenia

About Porton Slovenia (PSI)

Founded in 2022, Porton PharmaTech, Slovenia is the European subsidiary of Porton Pharma Solutions Ltd. It is the first CDMO in the pharmaceutical industry in Slovenia, dedicated to providing comprehensive services to Customers, encompassing synthesis route development, process familiarization, development & characterization, analytical methods development & validation, followed by right-first-time process scale up in production facilities.

PSI's R&D Analytical Team integrates advanced chromatographic science and expertise to solve complex analytical challenges. With scientists averaging approximately 5-10 years of industry experience, the team globally supports pharmaceutical companies. Equipped with state-of-the-art instrumentation and analytical algorithms, PSI ensures data integrity and delivers projects on time and within budget.

Porton Pharma Solutions Ltd.
www. portonpharma.com
business@portonpharma.com