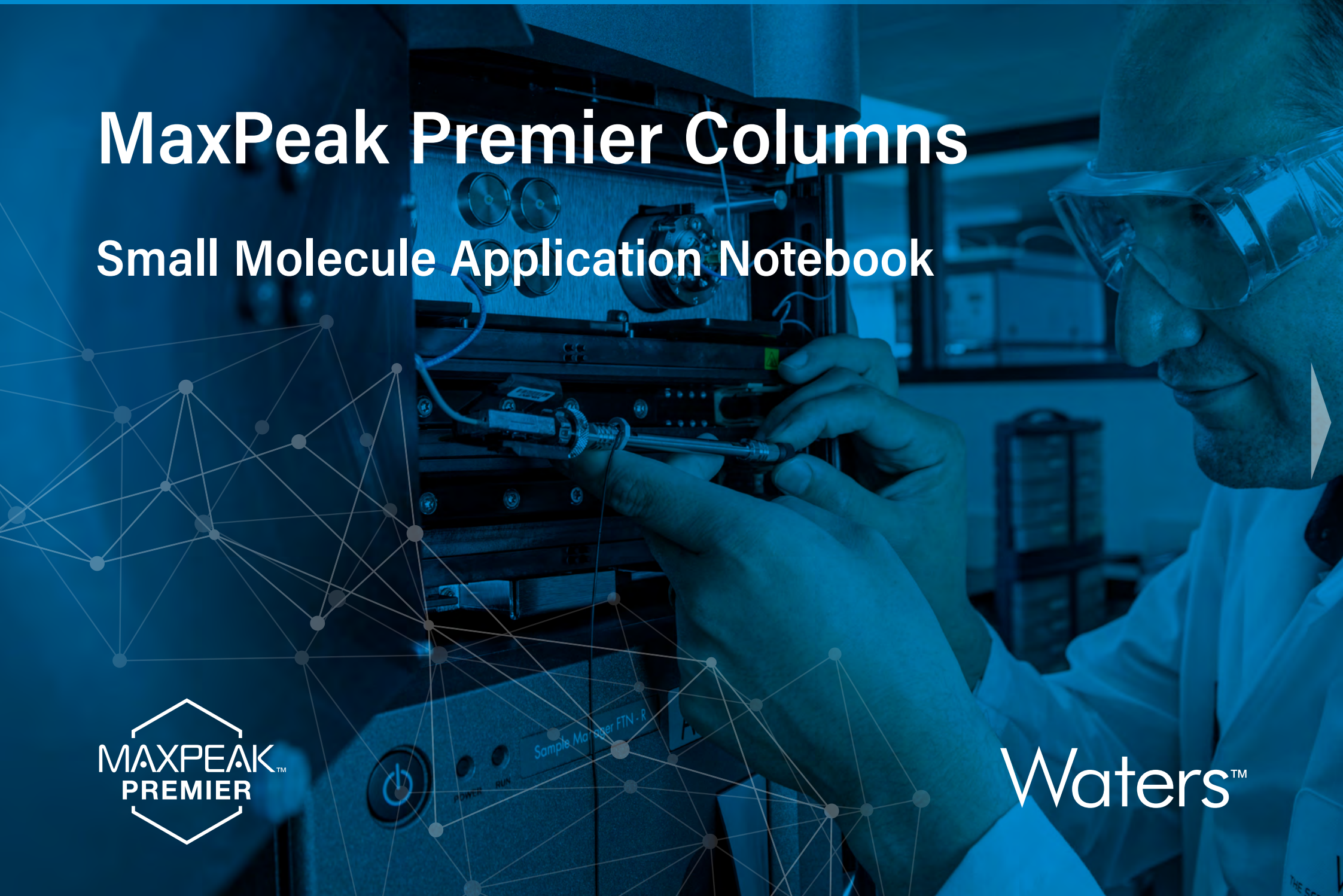
MaxPeak Premier Columns

Small Molecule Application Notebook



MAXPEAK™ PREMIER

Waters™

CONTENTS

Non-Specific Adsorption: Interactions of Analytes with Metal Surfaces

Certain analytes may interact with the surface oxide layer present on the surface of stainless steel and other metals present in the column and HPLC system flow path.

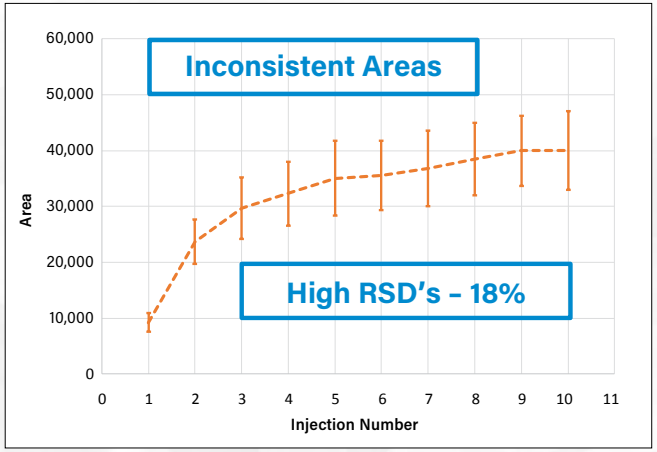
WHAT ARE THE CONSEQUENCES?

- High carryover
- On-column reactions leading to the formation of new peaks
- Broad peaks, tailing peaks, missing peaks
- Low area counts or reduced sensitivity with new columns that increases over time
- Conditioning is required with new columns
- Poor reproducibility from injection to injection
- Complex mobile phases required to suppress adsorption

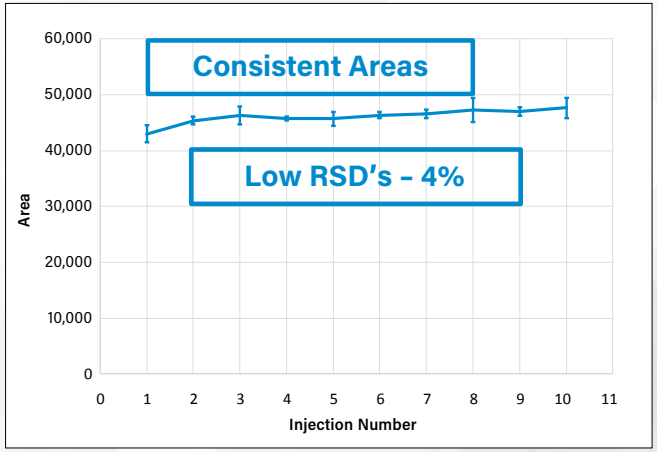
MITIGATION OF THESE INTERACTIONS CAN LEAD TO IMPROVEMENTS IN:

- Sensitivity
- Peak shape
- Reproducibility
- Detection
- Analysis time
- Carryover

What happens when you are getting this...



But you need this....



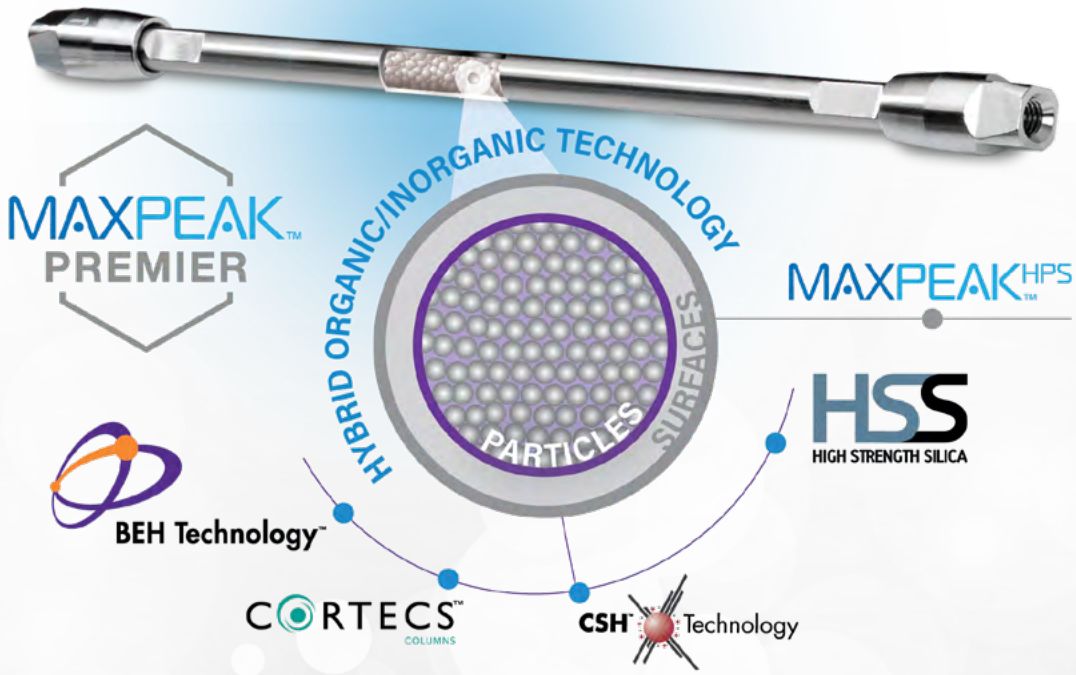
How Do Scientists Solve This Problem Today?

SOLUTION	HOW DOES IT WORK?	WHAT IS THE CONSEQUENCE?
Passivation of surfaces with acid	Removes free iron from steel surface	■ Time consuming ■ Requires strong acids ■ Not stable, needs to be repeated
Conditioning of surfaces with sample or matrix	Analyte or matrix coats reactive surfaces	■ Time consuming ■ Not stable, needs to be repeated
PEEK or PEEK lined steel columns	Replaces metal surface with an organic polymer surface	■ PEEK alone is not high pressure tolerant PEEK materials have: – Higher dimensional variability – Lower frit permeability – Incompatibility with some solvents
Titanium in columns or instrument parts	Titanium is less susceptible to oxidation than stainless steel. However, the titanium oxide surface still causes non-specific adsorption	■ Low sensitivity ■ Poor peak shape ■ Poor reproducibility ■ Possible analyte loss
Industrial coatings	Covers the metals with an inert material, e.g silica/other materials	■ MS bleed, and other unexpected problems ■ These were never designed for LC and LC-MS applications
Additives in mobile phases	Bind to metal surface to prevent analyte adsorption	■ Ion suppression and other unknown effects ■ Continued use necessary ■ Possible solubility issues

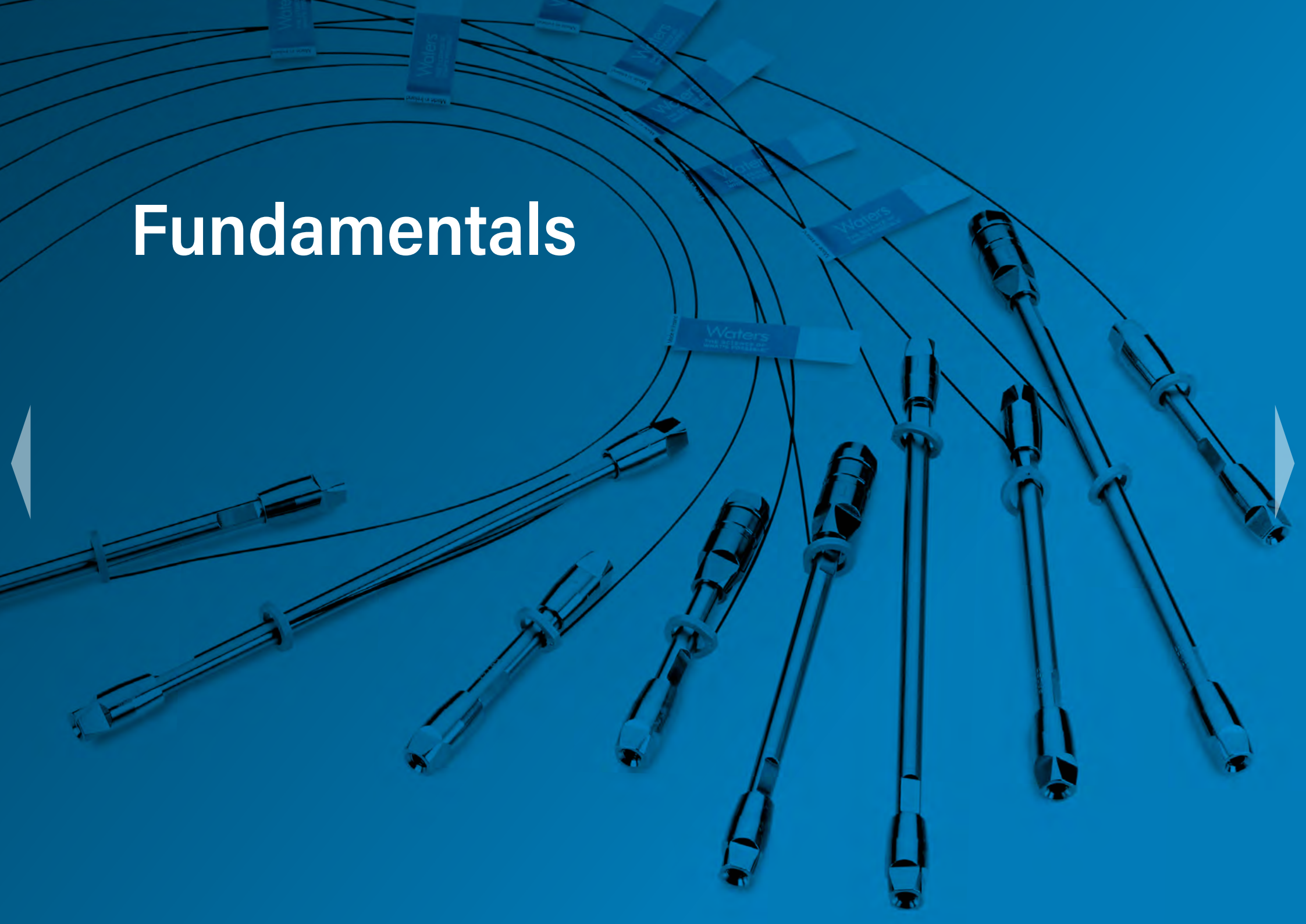
Precision Chemistry for Particles and Surfaces

MaxPeak Premier Columns, constructed with MaxPeak High Performance Surfaces (HPS) Technology, were created to address the concern of non-specific adsorption by mitigating interactions of analytes with the metal surfaces.

These new and innovative technologies are designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte-surface interactions that can lead to sample loss.



Fundamentals



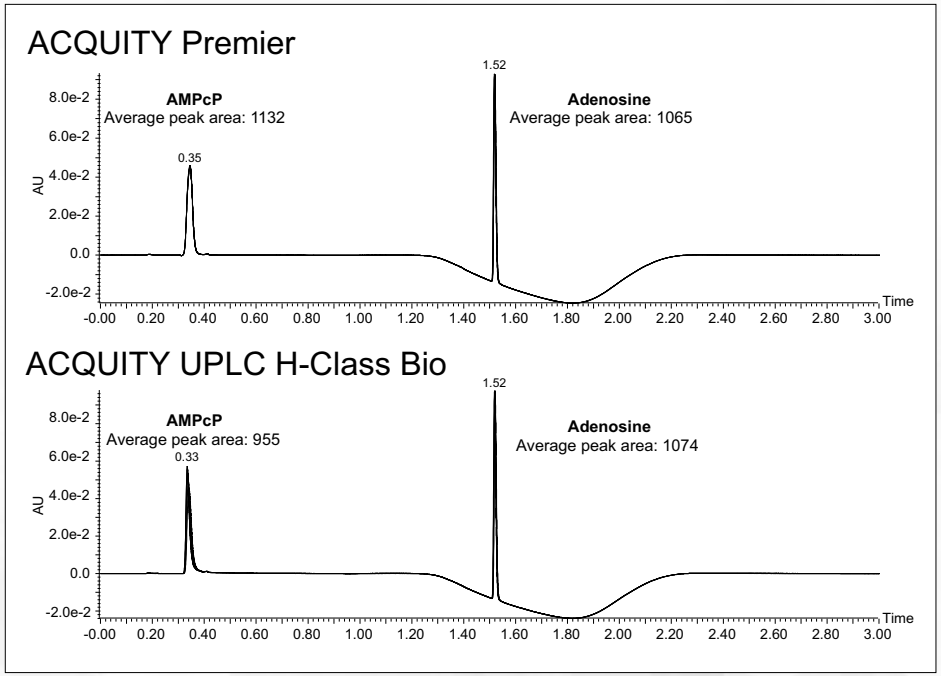
Waters Premier Standards to Investigate the Inertness of Chromatographic Surfaces

Ensuring that instruments are in proper working order is a critical aspect of making any analytical measurement. Failure to confirm system suitability can lead to uncertainty in results and incorrectly formed conclusions. Evaluating system suitability for analytes that are challenging to handle, separate, and measure requires special considerations. Analytes with a propensity to chelate to metals are one such class of molecules. To better facilitate separations of these types of molecules, Waters has designed the ACQUITY Premier System and MaxPeak Premier Columns to have chromatographic surfaces based on hybrid silica instead of metal or metal alloy surfaces. When adopting MaxPeak Premier Instruments, or attempting to make do with workarounds, analysts should consider test approaches that would report on their system's inertness to metal sensitive compounds. To this end, two test standards have been developed. Chemically, a nucleotide would be a useful test probe, however, they are subject to hydrolysis. As an alternative, we have made use of a non-hydrolyzable analog of adenosine diphosphate (ADP) to improve shelf life and solution stability of the standard. This molecule is adenosine 5′-(α,β-methylene)diphosphate (AMPcP) and is formulated as an AMPcP-only standard, as well as an equimolar mixture of AMPcP and adenosine.

Read the Full Application Note

BENEFITS

- MaxPeak High Performance Surfaces (HPS) Technology improve recovery and peak shape of metal sensitive analytes
- Waters Premier Standards used as quality control reference materials (QCRMs) with potential system suitability techniques for inert LC instruments
- Improved certainty in results for historically problematic metal sensitive analytes



Overlay of five replicate injections of AMPcP and Adenosine Standard on an ACQUITY Premier System (top) and five replicate injections on an ACQUITY UPLC H-Class PLUS Bio System (bottom).

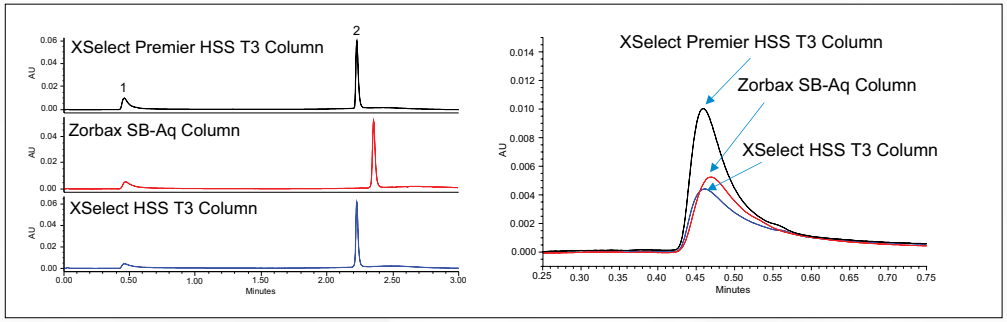
Improving Metal-Sensitive Analyte Recovery on Various LC Systems Using MaxPeak Premier Columns

Metal adsorption can be a significant problem in LC analysis, particularly for highly acidic probes like phosphorylated peptides, or small molecules such as nucleotides. These probes can interact with chromatographic metal surfaces, leading to poor peak shape, reduced peak area, or even complete loss of recovery.[1] This issue can be mitigated by implementing MaxPeak High Performance Surfaces (HPS) Technology, which has been applied to Waters LC columns and LC systems. However, competitive LC systems do not employ this technology. Here, we demonstrate the use of MaxPeak Premier Columns on three different, non-Waters LC systems for the analysis of a metal-sensitive analyte, adenosine 5′-(α,β-methylene) diphosphate (AMPcP). This probe has shown strong metal-sensitivity and is more stable than compounds such as adenosine triphosphate or adenosine diphosphate.[2] Our results show that regardless of the LC system used, a MaxPeak Premier Column yielded higher peak areas and thus analyte recovery for AMPcP compared to stainless steel columns. This indicates that the advantages of the MaxPeak Premier Columns are system-agnostic and would prove beneficial to almost all system configurations.

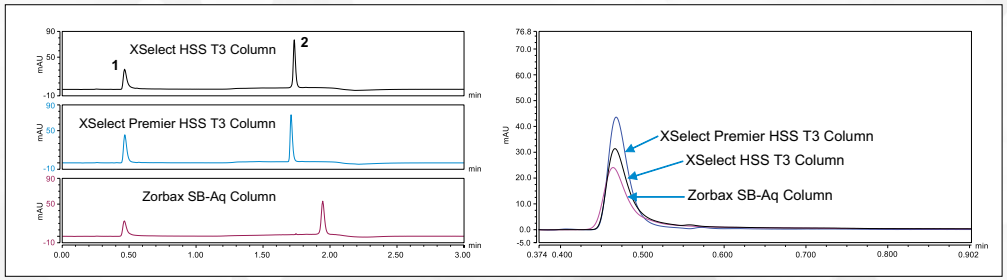
Read the Full Application Note

BENEFITS

- Increased peak area, or recovery, for AMPcP using MaxPeak Premier Columns
- Characterization of the extent of metal interaction using an AMPcP/Adenosine standard on various LC systems
- System-agnostic recovery advantages for metal-sensitive analytes



Chromatograms of the three columns tested on a Shimadzu Nexera-I 2040 with UV detection at 260 nm. Elution Order: 1) AMPcP and 2) Adenosine. Insert is a zoomed in portion of the X-axis with overlaid chromatograms to show the peak height and peak shape differences for AMPcP on the three columns.



Chromatograms of the three columns tested on a Thermo Vanquish with UV detection at 260 nm. Elution Order: 1) AMPcP and 2) Adenosine. Insert is a zoomed in portion of the X-axis with overlaid chromatograms to show the peak height and peak shape differences for AMPcP for the three columns.

1. Jung M, Lauber M. Demonstrating Improved Sensitivity and Dynamic Range with MaxPeak High Performance Surface (HPS) Technology: A Case Study in the Detection of Nucleotides. Waters Application Note. 720007053EN.

2. Lauber, M.; et al. Low Adsorption HPLC Columns Based on MaxPeak High Performance Surfaces. Waters White Paper, 720006930EN, 2020.

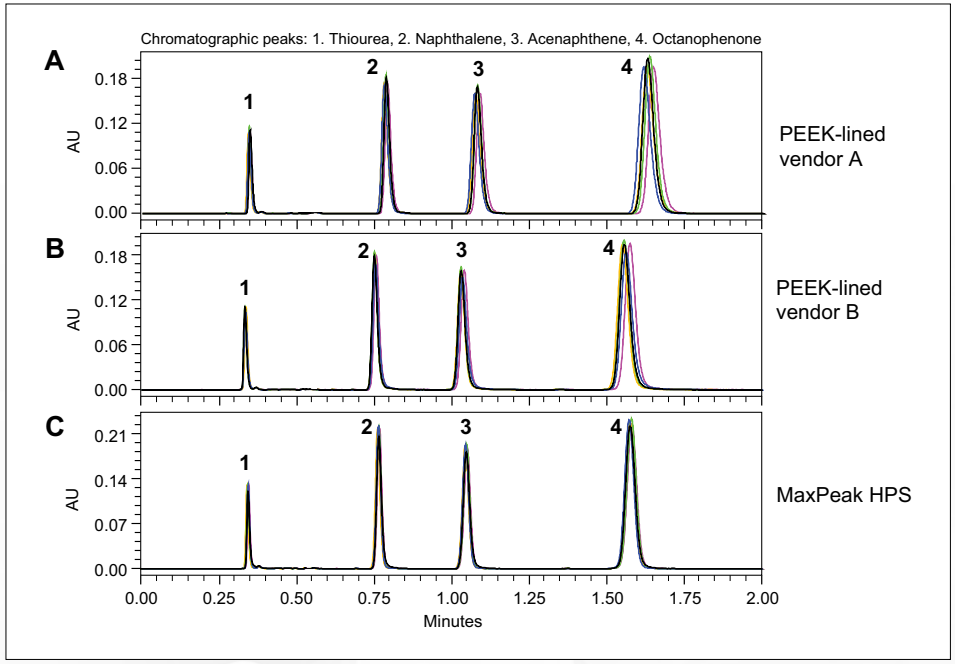
A Comparison of MaxPeak Premier Columns with MaxPeak HPS Technology Versus PEEK-lined Column hardware

Anionic or electron-rich analytes often have poor peak shape and low signal intensity in liquid chromatography (LC) analysis due to analyte loss via adsorption on electron-deficient metal surfaces, such as stainless steel.[1] Alternative column hardware made entirely of PEEK (Polyether Ether Ketone), or stainless-steel columns with a PEEK lining, have recently been utilized for these applications to eliminate analyte loss. However, columns that utilize PEEK materials can exhibit other undesirable problems such as lower plate efficiency and more column-to-column variability than traditional stainless-steel hardware.

The MaxPeak Premier Columns feature MaxPeak High Performance Surfaces (HPS) Technology, making it possible to more easily study metal-sensitive analytes. The MaxPeak HPS Technology provides an effective barrier to metal-analyte interactions and any related loss of sample due to non-specific adsorption.

BENEFITS

- Better column efficiency
- Better peak shape
- Lower column backpressure
- More consistent column-to-column reproducibility
- Excellent analyte recovery



Chromatograms for N=5 columns packed in (A) PEEK-lined Vendor A hardware, (B) PEEK-lined Vendor B hardware, and (C) MaxPeak HPS hardware. Analyses were performed with an ACQUITY UPLC chromatograph and 2.1 x 50 mm columns packed with C_{18}, 2.5 µm stationary phase. Isocratic separation conditions included a flow rate of 0.30 mL/min, column temperature of 30 °C, 75% acetonitrile, 254 nm UV detection, and 1 µL injection volumes.

Read the Full Application Note

1. Lauber, M.; et al. Low Adsorption HPLC Columns Based on MaxPeak High Performance Surfaces. Waters White Paper, 720006930EN, 2020.

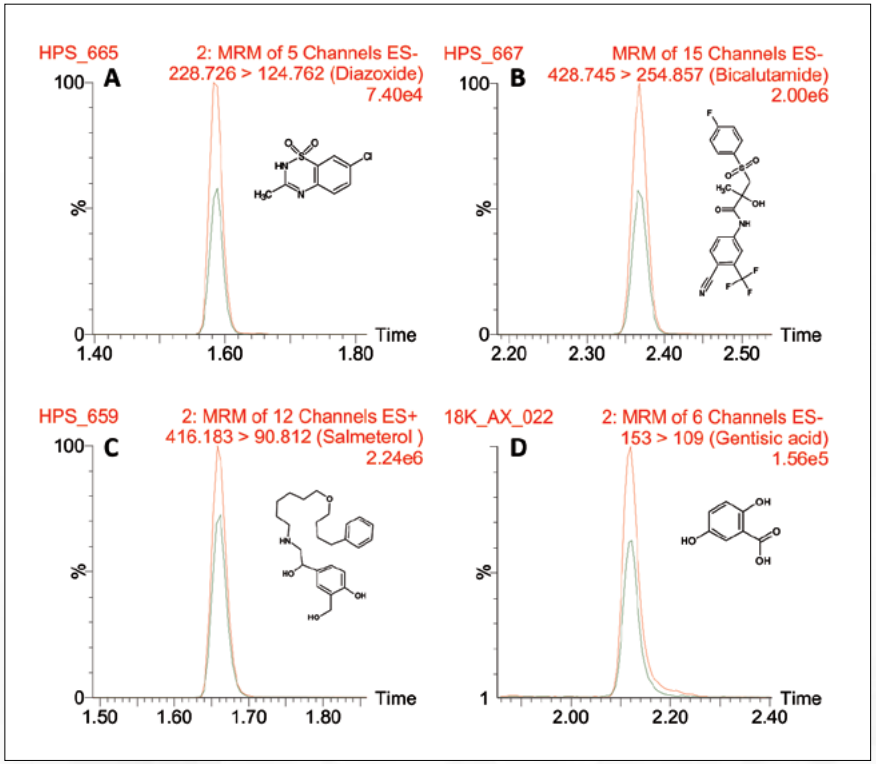
Utilization of MaxPeak High Performance Surfaces and Atlantis Premier BEH C_{18} AX Column to Increase Sensitivity of LC-MS Analysis

This application brief shows the benefit of MaxPeak HPS Technology in the mixed-mode Waters Atlantis Premier BEH C_{18} AX Column.

BENEFIT

- MaxPeak High Performance Surfaces (HPS) Technology are new and innovative technologies designed to increase analyte recovery, reproducibility, and sensitivity by minimizing negative analyte/surface interactions that can lead to sample losses

Read the Full Application Note



Peak area recovery of diazoxide (A), bicalutamide (B), salmeterol (C), and gentisic acid (D) on the Atlantis BEH C_{18} AX sorbent with MaxPeak HPS Column hardware (red line) and standard column hardware (green line).

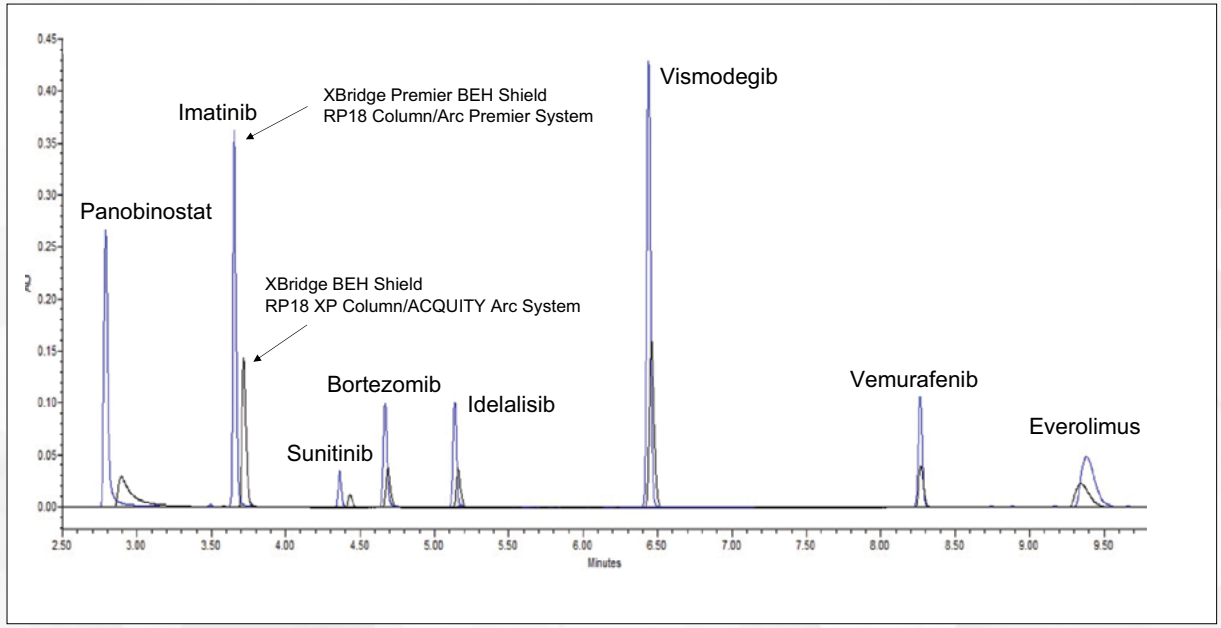
Pharmaceutical Applications

Advantages of Using MaxPeak HPS Technology for the Analysis of Targeted Cancer Growth Inhibitor Therapies

Non-specific binding of analytes to material surfaces is an inherent characteristic of conventional chromatographic columns and systems. This phenomenon leads to reduced sensitivity and sometimes poor peak shape. The MaxPeak HPS Technology provides a solution without the need for strong mobile phase additives, chelators, or lengthy passivation protocols. A panel of targeted cancer growth inhibitor therapies was utilized to demonstrate the chromatographic benefits provided when using columns and systems equipped with MaxPeak HPS Technology.

BENEFITS

- MaxPeak HPS Technology provides improved chromatographic peak performance for targeted cancer growth inhibitor therapies without the need for strong mobile phase additives, chelators, or lengthy passivation protocols
- The chromatographic sensitivity of cancer growth inhibitors is improved when using materials equipped with MaxPeak HPS Technology



Chromatographic overlay of the panel of targeted cancer growth inhibitor therapies analyzed using the (black) XBridge BEH Shield RP18 XP Column/ACQUITY Arc System and the (blue) XBridge Premier BEH Shield RP18 Column/Arc Premier System.

Read the Full Application Note

Improving Drug Metabolite Identification in Biofluids with the ACQUITY Premier and Hybrid Surface Technology: Increased Sensitivity and Reproducibility

Liquid chromatography coupled with electrospray tandem mass spectrometry is the primary platform for drug metabolite identification both in vivo and in vitro. Key to successful metabolite identification is the chromatographic resolution of the drug related analytes, both from each other and from endogenous components present in the matrix. TRANSITion metals present in chromatography systems and columns can act as Lewis Acids interacting with analytes containing phospho- groups, uncharged amines, hydroxyls, and deprotonated carboxylic acids resulting in poor chromatographic peak shape or even severe analyte loss. The ACQUITY Premier Chromatography System and columns employs a hybrid organic surface technology to eliminate this type of non-specific binding. The analysis of the in vivo metabolites of gefitinib using the ACQUITY Premier Chromatography System and columns showed improved peak shape, increased signal response and cleaner MS/MS spectra.

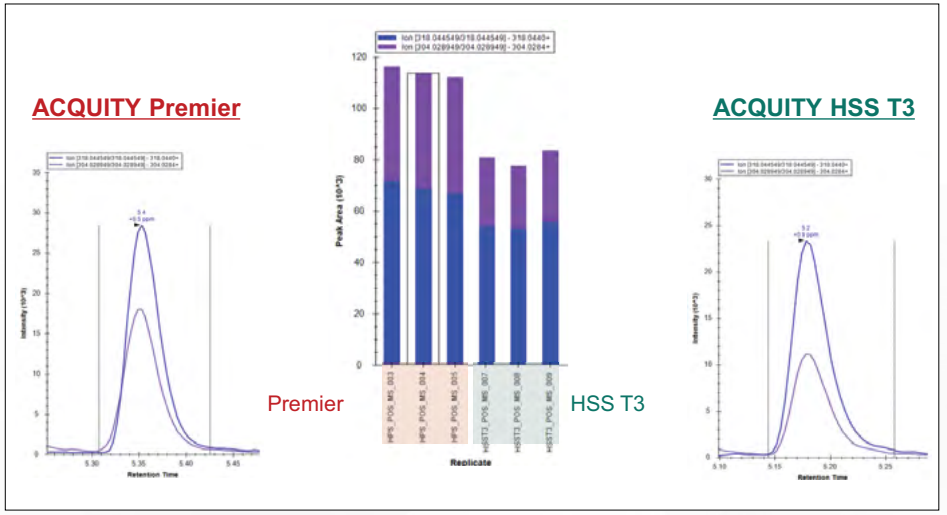


Figure 5. Improved fragment ion intensities demonstrated for gefitinib metabolite M7 (MQZP, m/z 378.1021). Increased ion intensities relating to transitions m/z 318.0440 and 304.0284 are shown to increase for the ACQUITY Premier HSS T3 Column.

BENEFITS

- Improved chromatographic resolution of drug metabolites
- Increased peak response
- Improved reproducibility

Read the Full Application Note

MaxPeak High Performance Surfaces Mitigate On-Column Oxidation

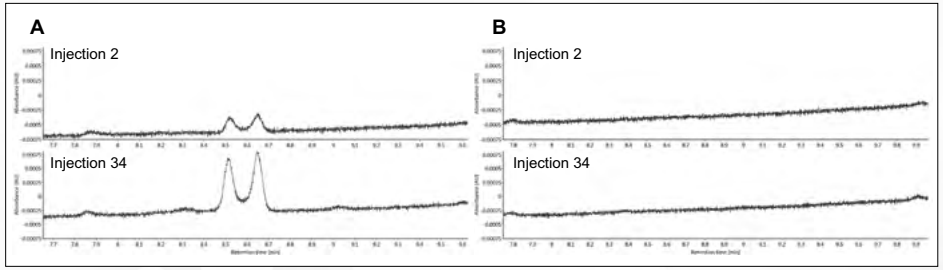
The advent of more robust chromatographic materials has facilitated the use of a wider range of method conditions. However, depending on the chemical properties of the analyte, sample degradation may occur during the chromatographic separation. This may be caused by various factors including the use of high temperature, stability in certain mobile phases, pH conditions, or even interactions with the stationary phase and columns.

On-column degradation is more commonly reported for biopharmaceuticals rather than for small molecules. These larger biomolecules can undergo conformational changes and on-column degradation with the stationary phase and LC conditions, respectively. While reports of on-column degradation of small molecules may be rarer, significant cases have been observed. Instances of on-column degradation of amino compounds have been encountered, and their degradation has been found to be exacerbated using high pH mobile phases.

This application note demonstrates that small molecule amines can indeed undergo on-column oxidation when separated with conventional stainless-steel columns. However, this degradation can be mitigated with the use of ACQUITY Premier Columns. ACQUITY Premier Columns feature MaxPeak High Performance Surfaces, which provide a barrier against metal-analyte interactions. This barrier, based on hybrid organic-inorganic silica, can also help protect metal LC surfaces from corrosion, which can further exacerbate analyte degradation. These results demonstrate that the MaxPeak Premier Technology can reduce on-column, metal catalyzed reactions like oxidation. ACQUITY Premier Columns should thereby be considered for use in LC work, purity measurements, and impurity profiling or wherever analytical artifacts might undermine the value of an assay.

BENEFITS

- Reduction in on-column degradation products versus conventional steel column technology
- Improved method robustness
- Higher fidelity data for impurity quantification

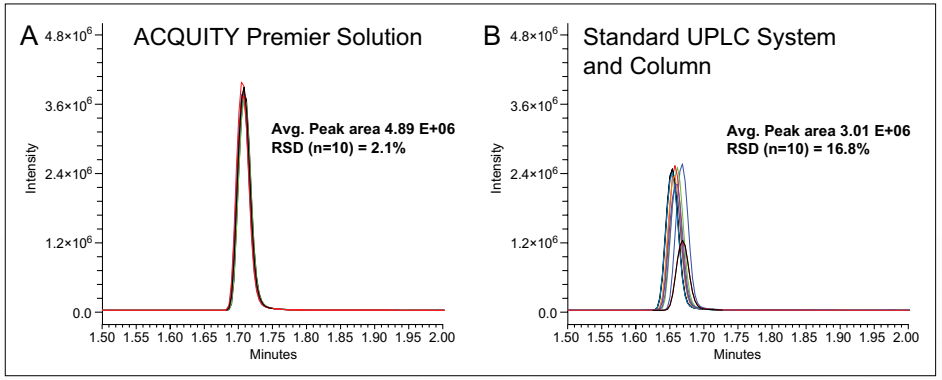


UV chromatograms at 290 nm detection from the 2nd and 34th separations of clozapine, showing its Z and E nitroso impurities, using mobile phases of ammonium hydroxide in water and acetonitrile, and an (A) ACQUITY BEH C_{18}, 1.7 µm, 2.1 x 50 mm Column and an (B) ACQUITY Premier BEH C_{18}, 1.7 µm, 2.1 x 50 mm Column. Separations were performed with an ACQUITY UPLC I-Class System, 0.31 mL/min flow rate, column temperature 30 °C, gradient of 25% to 80% acetonitrile in 10.31 min, and 0.15 µg mass loads.

Read the Full Application Note

ACQUITY Premier Solution Improves the UPLC-MS Analysis of Deferoxamine- an Iron Chelating Drugs

HPLC separations of metal-sensitive analytes are known to be impacted by interactions of the analytes with the metal surfaces in HPLC instruments and columns. This causes effects ranging from peak broadening and tailing to loss of peak area and high injection-to-injection variability. The ACQUITY Premier Solution mitigates these effects by employing the novel surface technology MaxPeak High Performance Surfaces. Here we compare UPLC separations for the iron chelating drug deferoxamine obtained using a standard UPLC system and column vs the ACQUITY Premier Solution. The results show that higher peak areas and improved injection-to-injection reproducibility are achieved using the ACQUITY Premier Solution.



(A) Overlay depicting 10 replicate injections of deferoxamine mesylate obtained using the ACQUITY Premier Solution. (B) Overlay depicting 10 replicate injections of the same analyte using a standard UPLC system and a standard column. ACQUITY UPLC HSS T3, 100 Å, 1.8 µm, 2.1 x 50 mm Columns were used in this study. The mass load of deferoxamine mesylate was 10 ng. Acetonitrile gradient separations were carried out with a 10 mM ammonium formate (pH 3.0) aqueous mobile phase, a flow rate of 0.5 mL/min, and a temperature of 30 °C. The deferoxamine peak was detected using an ACQUITY QDa Detector with SIR positive m/z 561.0.

BENEFITS

- The ACQUITY Premier Solution showed higher and more consistent peak areas for deferoxamine compared to a standard UPLC system and column
- No conditioning or unusual mobile phase additives were required to achieve reproducible separations using the ACQUITY Premier Solution

Read the Full Application Note

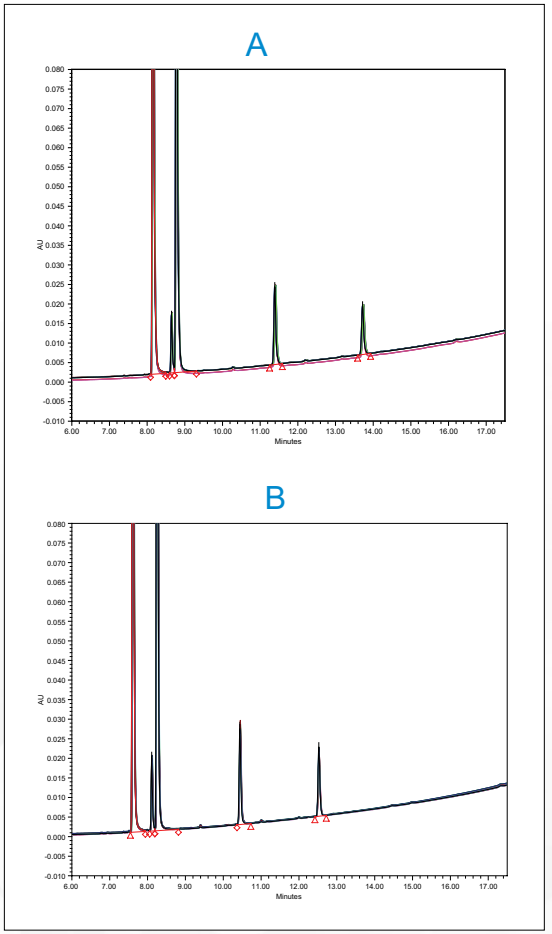
Batch-to-Batch Robustness of MaxPeak Premier Columns for the Analysis of Dexamethasone Phosphate and Related Compounds

An Ultra High Performance Liquid Chromatography (UHPLC) method that uses an Arc Premier System with MaxPeak Premier Columns was used to evaluate the batch-to-batch reproducibility of multiple columns. Different batches of both the construction material and the packing material of MaxPeak Premier XBridge™ BEH C_{18} and XSelect™ HSS T3 Columns were studied. Results showed that MaxPeak Premier Columns were very reproducible when used for the analysis of a mixture of metal chelating and non-metal chelating compounds. Various chromatographic parameters including relative retention time, critical pair resolution, and peak area were all investigated for reproducibility on the different columns. The columns showed excellent reproducibility for all the studied chromatographic parameters. For example, the %RSD for the peak areas for all peaks was always in the range of 0.1%–5.6% for all analytes. These findings indicate that the batch-to-batch reproducibility of MaxPeak Premier Columns is very high and these columns are very robust.

BENEFITS

- Batch-to-batch reproducibility of the construction material of MaxPeak Premier XBridge BEH C_{18} and XSelect HSS T3 Columns
- Batch-to-batch reproducibility of the packing material of MaxPeak Premier XBridge BEH C_{18} and XSelect HSS T3 Columns

Read the Full Application Note



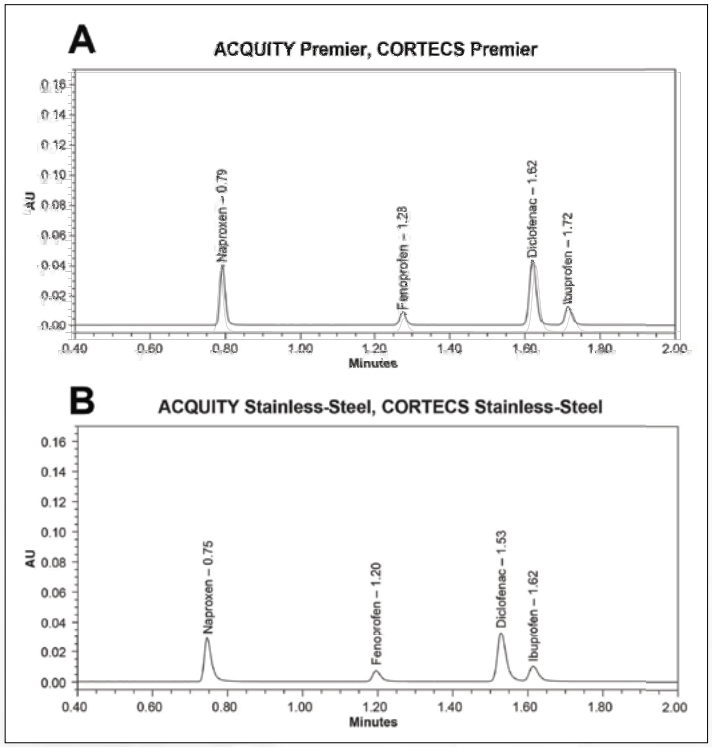
Overlay chromatogram of 18 injections on A: three different batches of the construction material for the MaxPeak Premier XSelect HSS T3 Column and B: three different batches of the construction material for the MaxPeak Premier XBridge BEH C_{18} Column. Peaks according to elution order are: hydrocortisone phosphate, betamethasone sodium phosphate, dexamethasone sodium phosphate, dexamethasone, and dexamethasone acetate.

Improved Chromatographic Analysis of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Using CORTECS™ Premier Columns That Feature MaxPeak™ High Performance Surfaces HPS Technology

NSAIDs are common pain-relieving anti-inflammatory medications used by millions of consumers worldwide, daily. To ensure the safety of consumers all around the globe, methods supporting the quality control sector of NSAIDs production are of great importance. In this application, we develop a rapid analysis for NSAIDs featuring CORTECS Premier Columns with MaxPeak HPS Technology. This method has proven to be reproducible and linear in its separation and detection of commonly used NSAIDs. Moreover, CORTECS Premier Columns that have MaxPeak HPS Technology showed improved chromatographic performance when compared to analysis on traditional CORTECS Columns packed in stainless-steel column hardware.

BENEFITS

- CORTECS Premier Columns with MaxPeak HPS Technology improved chromatographic performance when compared to traditional stainless-steel systems and columns
- This method could analyze multiple NSAID compounds in under two minutes
- CORTECS Premier Columns with MaxPeak HPS Technology delivers up to a 25% decrease in peak tailing and 39% increase to height signal when compared to traditional stainless-steel chromatographic systems



A: Chromatogram for injection five out of ten of the NSAIDs mix standard on the ACQUITY Premier System equipped with a CORTECS Premier C_{18} Column.

B:Chromatogram for injection five out of ten of the NSAIDs mix standard on the ACQUITY I-Class System equipped with a CORTECS C_{18} Column.

Read the Full Application Note

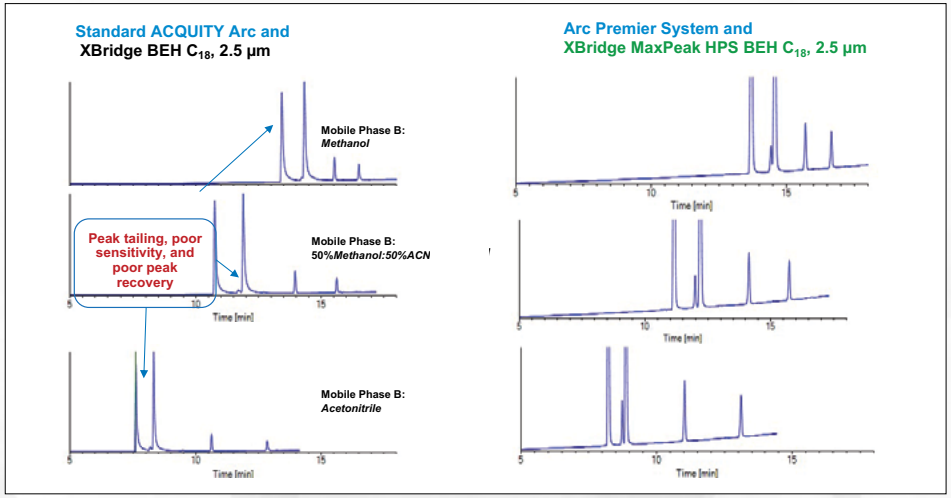
Analytical Quality By Design Based Method Development for the Analysis of Dexamethasone Phosphate and Related Compounds Using Arc Premier MaxPeak High Performance Surfaces Technology

An Ultra High Performance Liquid Chromatography method was developed for the analysis of a mixture of metal chelating and non-chelating compounds using the Analytical Quality by Design (AQbD) approach. DryLab, Empower,™ and Waters systems were used to automate the method development process. The performance of the Arc Premier System in combination with MaxPeak Premier Columns was compared to standard stainless-steel hardware. Results showed that MaxPeak Premier Columns on an Arc Premier System provides great performance for the separation of metal chelating compounds compared to stainless-steel hardware. The final method uses a MaxPeak Premier BEH C_{18} Column (10 cm × 4.6 × 2.5 µm), 0.1% formic acid in acetonitrile as an organic solvent, and 10 mM ammonium formate in water aqueous mobile phase. The method showed excellent separations between the peaks, ideal peak shapes, high recoveries, and good reproducibility. For example, the USP tailing was ≤1.1 for all peaks including the phosphorylated compounds. These findings indicate that using the MaxPeak High Performance Surfaces (HPS) Technology for the analysis of metal chelating compounds is greatly beneficial in mitigating undesired interactions with metal surfaces and achieving excellent separations.

Read the Full Application Note

BENEFITS

- Show the superior advantages for using the Arc Premier System's MaxPeak HPS Technology for the analysis of phosphorylated compounds
- Show the increased efficiency of method development using Arc Premier System in combination with Empower 3 Chromatographic Data System (Empower CDS) and DryLab4 Software
- Shows the seamless integration between DryLab and Empower to fully automate the method development process



Representative chromatograms from the 12 DOE runs. A: represents three experiments that were performed on a Standard ACQUITY Arc System under different scouting conditions and B represents three experiments that were performed on the Arc Premier System under the same conditions. Conditions in common between all chromatograms are: Mobile phase A: 10 mM Ammonium formate in water, flow rate 0.50 mL/min, temperature 30 °C, 0.0–15 min, and 10–90% B linear gradient. Variations in conditions are detailed in the figure.

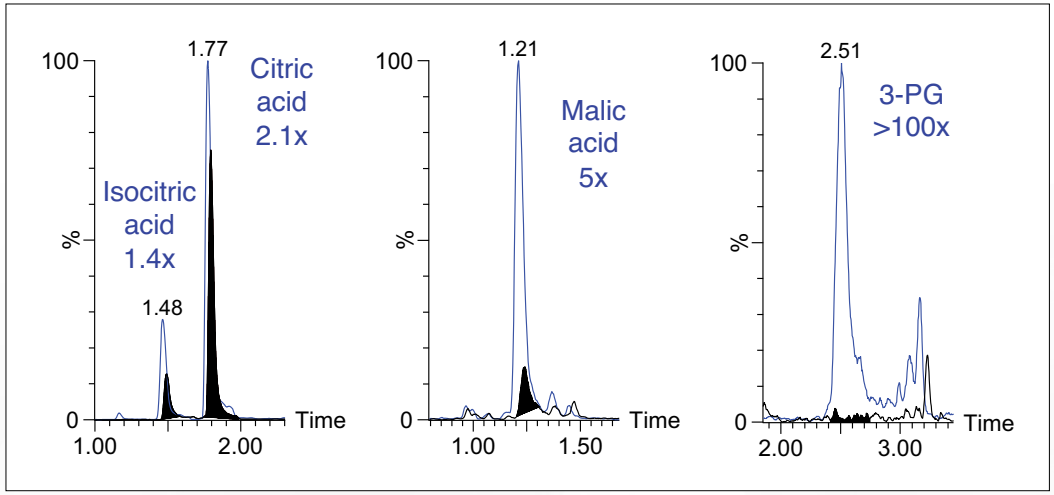
Metabolomic Applications

Utilization of MaxPeak High Performance Surfaces for Improved Separation and Recovery of Analytes Associated with the Tricarboxylic Acid Cycle

This application note presents a mixed-mode LC method that is MS compatible for the analysis of TCA cycle analytes as well as other related compounds without the use of sample derivatization or ion-pairing reagents.

BENEFITS

- The use of a hybrid organic-inorganic surface technology, MaxPeak High Performance Surfaces (HPS), within the ACQUITY Premier CSH Phenyl-Hexyl Column mitigates analyte interactions with metal surfaces
- A reproducible LC-MS method for the analysis of the TCA cycle analytes as well as other related compounds without the need for sample derivatization or ion-pairing reagents
- Data processing by Progenesis QI using a custom database that includes fragment and retention time match



Peak recoveries from a urine sample for isocitric acid and citric acid, malic acid, and 3-phosphoglyceric (3-PG) acid for ACQUITY Premier CSH Phenyl Hexyl Column and a standard CQUITY CSH Phenyl Hexyl Column (filled trace). Numbers show the improvement of peak area on the MaxPeak Premier Column.

Read the Full Application Note

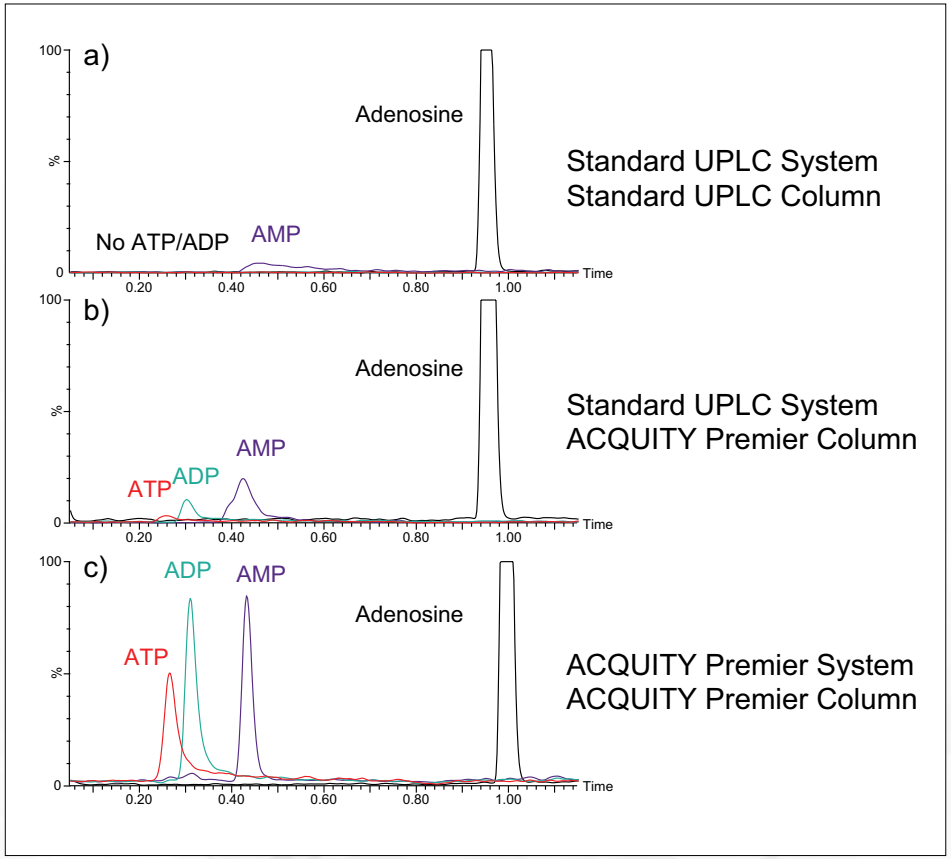
Demonstrating Improved Sensitivity and Dynamic Range with MaxPeak High Performance Surfaces (HPS) Technology: A Case Study on the Detection of Nucleotides

Acidic metal-sensitive analytes are challenging to robustly and sensitively assay by LC-MS. Numerous attempts have been made to mitigate analyte losses by modulating the chemical attraction to metal surfaces, but none have produced a universal solution that is compatible with highly sensitive UPLC-MS analyses. Using adenosine nucleotides as model analytes, we have demonstrated that the Waters ACQUITY Premier LC System with MaxPeak HPS Technology offers superior protection against metal-analyte interactions without compromising the benefits of high-efficiency UPLC separations with sensitive MS detection.

BENEFITS

- This case study shows when and how chromatographic surfaces can be of significant influence for analyte detection
- Waters ACQUITY Premier LC System and Columns with MaxPeak HPS Technology can dramatically improve the recovery and peak shape of metal sensitive analytes, like nucleotides and their analogs

Read the Full Application Note



Example chromatograms from 1 µL injections of mixture samples containing ATP, ADP, AMP, and adenosine (20 pg/µL each).

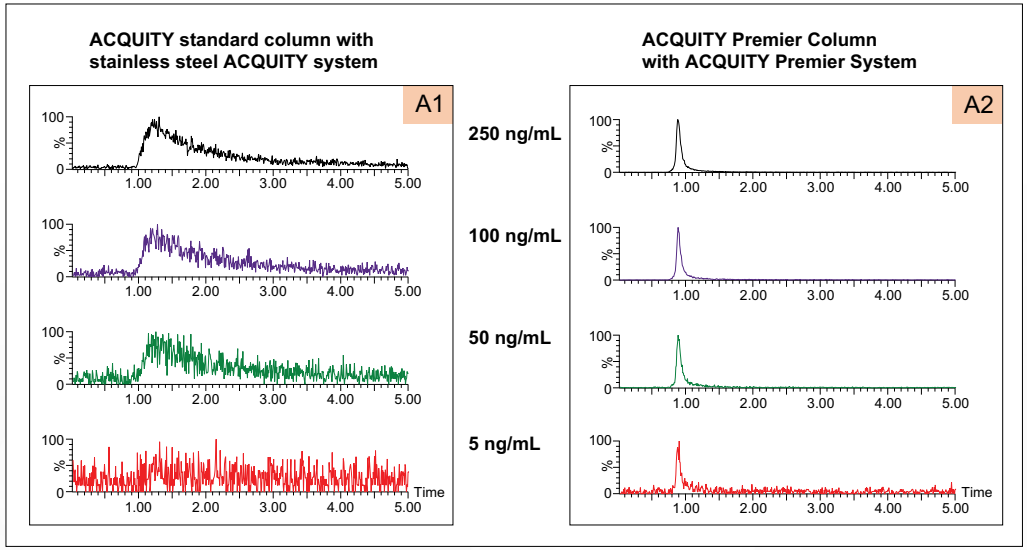
ACQUITY Premier LC Technology Significantly Improves Sensitivity, Peak Shape, and Recovery for Phosphorylated and Carboxylate Lipids

Phosphorylated and carboxylate lipid species are metal sensitive and can readily adsorb to stainless steel surfaces within the flow path of UPLC systems. This process can lead to poor peak shape, low recovery and reduction in sensitivity. Here we show that the ACQUITY Premier CSH C_{18} Column with ACQUITY Premier System can significantly improve sensitivity, peak shape, and recovery of phosphorylated and carboxylate lipids compared to ACQUITY standard column and stainless-steel ACQUITY UPLC I-Class System.

BENEFITS

The ACQUITY Premier technology provides:

- Increased sensitivity, recovery, and reproducibility for phosphorylated and carboxylate lipids
- Improved peak shape and reduced tailing by minimizing analyte-surface interaction
- Increased lipidomics coverage by simultaneous analysis of phosphorylated and carboxylate lipids with other lipid classes



Negative mode base peak extracted ion chromatogram of LPA (16:0/0:0) m/z 409.2355 at a concentration of 5, 50, 100, and 250 ng/mL. (A1) using ACQUITY standard CSH C_{18} Column with stainless steel surface ACQUITY System and (A2) using ACQUITY Premier CSH C_{18} Column with ACQUITY Premier System.

Read the Full Application Note

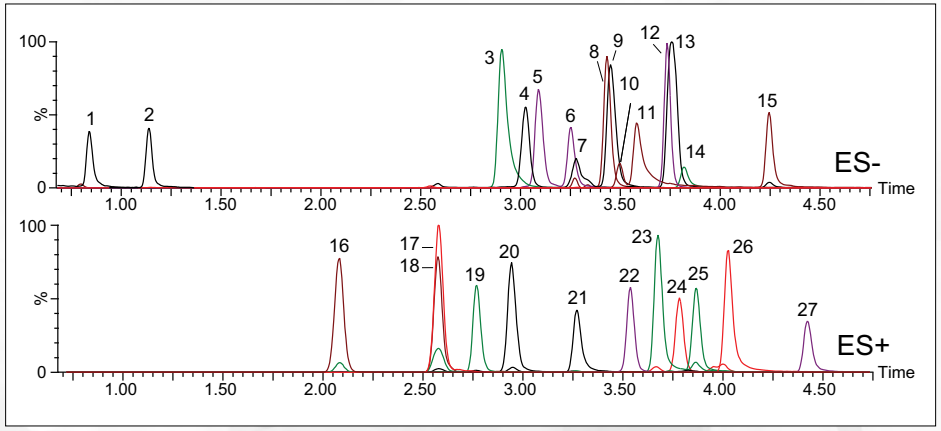
Separation of Pentose Phosphate Pathway, Glycolysis, and Energy Metabolites Using an ACQUITY Premier System with an Atlantis BEH Z-HILIC Column

Analyses of phosphorylated metabolites are challenging because of their interactions with metal surfaces in conventional LC systems and columns. Here, we describe a targeted UPLC-MS/MS method for 27 pentose phosphate, glycolysis, and energy metabolites in plasma and tissue extracts. The method leverages the benefits of MaxPeak High Performance Surfaces Technology used in the ACQUITY Premier System and the Atlantis Premier BEH Z-HILIC Column to mitigate interactions of the metabolites with metal surfaces. The stability of the column to high pH mobile phases is also key, as the best peak sharpness, peak symmetry, and sensitivity was achieved using a pH 9 ammonium bicarbonate buffer in the mobile phase. The results demonstrate that high efficiency, UPLC-pressure tolerant 1.7 µm Atlantis Premier BEH Z-HILIC Columns provide excellent separations for these challenging analytes.

Read the Full Application Note

BENEFITS

- A targeted UPLC-MS/MS method that provides sharp, symmetric peaks for 27 challenging metabolites
- The base-stable Atlantis Premier BEH Z-HILIC Column allows the use of an optimal pH 9 buffer
- MaxPeak High Performance Surfaces Technology in the UPLC system and column enables excellent peak sharpness, peak symmetry, and sensitivity
- of phosphorylated and carboxylate lipids with other lipid classes



Representative chromatograms of analyte standards in 50/50 ACN/water.

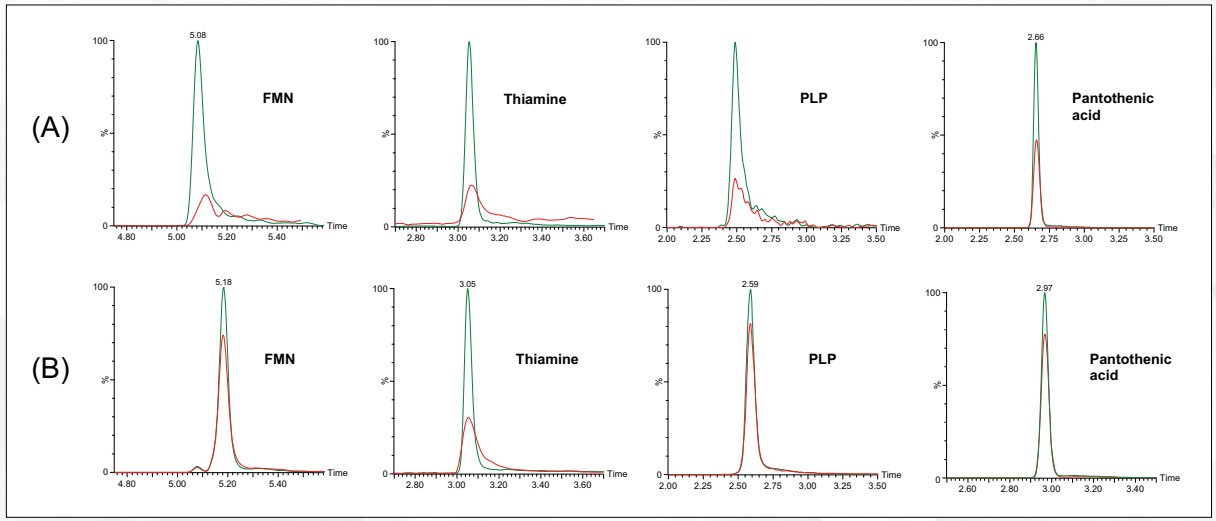
Food Applications

Enhancing the LC-MS/MS Analysis of B-group Vitamins with MaxPeak High Performance Surfaces Technology

Waters MaxPeak High Performance Surfaces (HPS) Technology provide an effective solution to mitigate interactions between analytes and metal surfaces in liquid chromatography. This application note investigates the effects of the MaxPeak HPS Technology on the LC-MS/MS analysis of B-group vitamins and demonstrates the key benefits of using the MaxPeak HPS Technology in the simultaneous analysis of B-group vitamins in energy drinks and vitamin B complex dietary supplements. The key benefits observed include high response, improved sensitivity, less peak tailing, better calibration linearity, and no carry-over compared to the stainless-steel surfaces in a conventional LC system setup. Greater sensitivity (3–10 times) was observed for riboflavin, thiamine, nicotinamide, flavin mononucleotide, pyridoxal 5′-phosphate, and 5-methyltetrahydrofolate using the Waters ACQUITY Premier Solution.

BENEFITS

- Waters ACQUITY Premier Solution improves LC-MS/MS analysis of B vitamins
- 3 to 10 times better sensitivity were observed for six B vitamins using the MaxPeak HPS Technology than the conventional stainless-steel surfaces
- Higher response, less peak tailing, and less carry-over are observed with Waters ACQUITY Premier Solution



Comparison of LC-MS chromatograms of FMN, Thiamine, PLP, and Pantothenic acid obtained on the HPS setup (green traces) and the SOP setup (red traces). (A) Observed during the initial injections of the same standard mix on fresh LC systems. (B) Observed in the B vitamin analysis of the same DS sample on LC systems that have been extensively used.

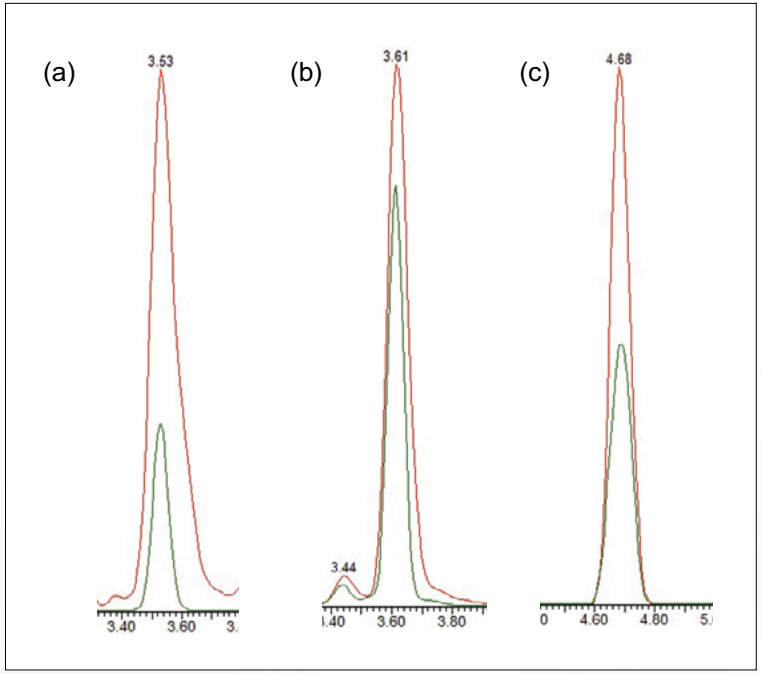
Read the Full Application Note

Enhanced Performance of the Analysis for Veterinary Drugs with Metal Affinity Using ACQUITY™ Premier and Xevo™ TQ-S Micro

The application of MaxPeak High Performance Surfaces (HPS) reduces interaction for metal sensitive compounds and so increases the response of these compounds in a liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Here we demonstrate the utility of the Waters ACQUITY Premier UPLC™ System-Xevo TQ-S micro for the analysis of a range of veterinary drugs representative of the major classes and show increased performance of MaxPeak High Performance Surfaces (HPS) for the known metal sensitive tetracyclines.

BENEFIT

- Sensitivity gains for metal sensitive veterinary drugs, extending detection limits of previous methods



Overlaid chromatograms showing the effect of HPS on known metal sensitive compounds. HPS – (red) and stainless steel (green) for (a) Tetracycline (ESI+, 445.45 > 410.00), (b) Chlortetracycline (ESI+, 479.30 > 444.18), and (c) Oxytetracycline (ESI+, 461.26 > 426.13).

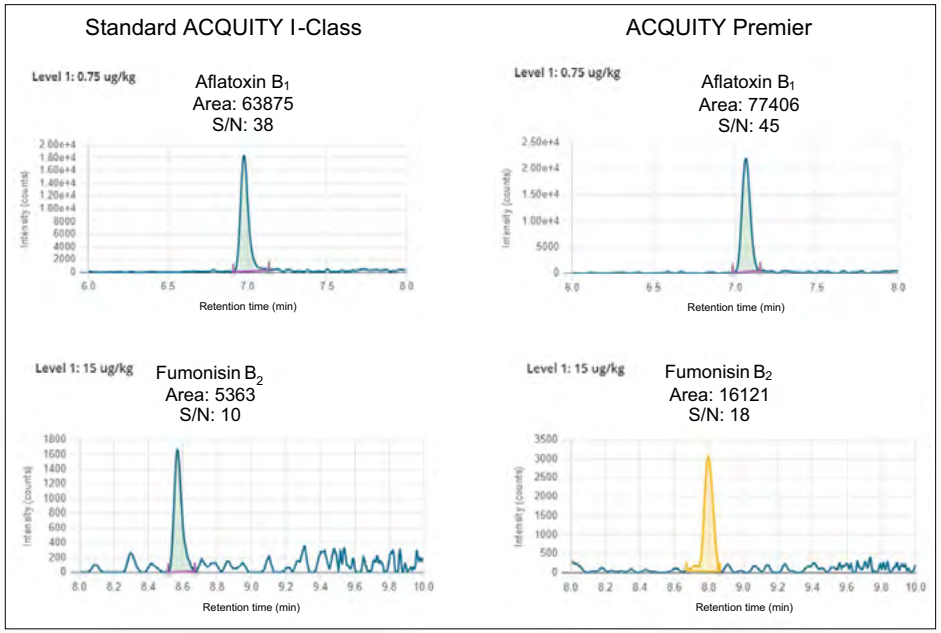
Read the Full Application Note

The Benefits of ACQUITY Premier UPLC for Multi-Mycotoxin Methods

Multi-mycotoxin methods typically present challenges, such as uneven response for different compounds, carryover in LC systems, and matrix effects. These factors can affect validation experiments and significantly impact the performance of the method. To address these challenges, Waters has developed the ACQUITY Premier System and analytical columns, which incorporate the MaxPeak™ High Performance Surfaces (HPS) Technology. In this work we successfully transferred a previously developed multi-mycotoxin LC-MS/MS method on the new ACQUITY Premier System, and we observed a significant reduction of carryover for fumonisins by almost 80% compared to a conventional UPLC. In addition, very good method performance was achieved, in terms of linearity, precision, peak shape, and retention time stability.

BENEFITS

- The ACQUITY Premier System and column effectively reduce carryover of fumonisins compared to conventional UHPLC systems
- On the ACQUITY Premier System, the addition of metal chelators in the mobile phase or washing solutions was not necessary
- Analytical throughput was improved on the ACQUITY Premier System as the number of washing cycles can be reduced
- Very good linearity, precision, peak shape, and retention time reproducibility were obtained, which allowed to meet SANTE/12089/2016 performance guidelines



Peak response and signal-to-noise ratio of aflatoxin B1 (0.75 µg/kg) and fumonisins B2 (15 µg/kg) on the ACQUITY I-Class (left) and on the ACQUITY Premier (right). Manually-modified integrations are characterized by yellow traces.

Read the Full Application Note

Links to Other Useful Materials

- MaxPeak Premier Columns
- To The Max: Eliminate Doubt with MaxPeak Premier Columns Brochure
- MaxPeak Premier Columns Ordering Guide
- Analytical Chemistry Article: Using Hybrid Organic-Inorganic Surface Technology to Mitigate Analyte Interactions with Metal Surfaces in UHPLC

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