

Utilization of MaxPeak High Performance Surfaces and the Atlantis PREMIER BEH C₁₈ AX Column to Increase Sensitivity of LC-MS Analysis

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GOAL

To show the benefit of MaxPeak™ HPS in the mixed-mode Waters™ Atlantis™ PREMIER BEH C₁₈ AX Column.

BACKGROUND

Variability in the separation and detection of compounds can be caused by many factors. One factor is analyte/surface interactions of compounds with the analytical column.¹ Such interactions can be problematic, especially at very low concentrations of analytes. To address this, Waters has developed a novel column hardware using MaxPeak High Performance Surfaces (HPS). MaxPeak HPS are new and innovative technologies designed to increase analyte recovery, sensitivity, and reproducibility by minimizing the analyte/surface interactions that can lead to sample losses. The Atlantis PREMIER BEH C₁₈ AX Column, the first chromatographic column to incorporate MaxPeak HPS technology, is a mixed-mode anion exchange (AX) column that has been designed to improve the retention of polar acidic analytes under reversed phase chromatographic conditions, while also minimizing negative analyte/surface interactions for compounds.²

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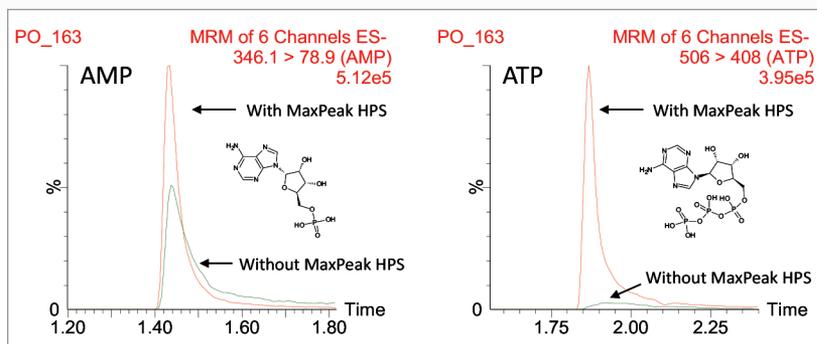


Figure 1. Separation of AMP (left) and ATP (right) on the Atlantis BEH C₁₈ AX sorbent with MaxPeak HPS column hardware (red line) and standard column hardware (green line).

In this work, metal sensitive compounds, pharmaceutical drugs, and biological metabolites were tested using the Atlantis BEH C₁₈ AX material with and without MaxPeak HPS column hardware.

THE SOLUTION

It is known that phosphorylated compounds can interact with stainless steel to reduce analyte recovery and that this interaction increases with the number of phosphorylated moieties present.³ To illustrate the benefit of MaxPeak HPS column hardware, standard solutions of adenosine 5'-monophosphate (AMP) and adenosine 5'-triphosphate (ATP) were chromatographically separated by the Atlantis PREMIER BEH C₁₈ AX material with and without MaxPeak HPS column hardware. Figure 1 shows the results of the analysis of a standard solution separated using a linear gradient of 10 mM ammonium acetate solution to 100% 10 mM ammonium acetate, pH 9.2, in 90:10 ACN/H₂O over three minutes. While the peak area of AMP increased 1.5x with the MaxPeak HPS hardware, the peak area of ATP increased 19x. These results follow the trend seen in the analysis of multi-phosphorylated compounds.

To further investigate the benefits of new column hardware, additional compounds, including pharmaceutical drugs and biological metabolites, were tested using a water and ACN gradient each containing 10 mM ammonium formate, pH 3.0. The compounds spanned a range of molecular properties and sizes. Four examples of compounds tested are in Figure 2. Diazoxide (anti-hypertensive medication), bicalutamide (anti-androgenic drug), salmeterol (asthma medication), and gentisic acid (potential cancer biomarker) showed peak area increases of 1.6x, 1.6x, 1.3x, and 1.5x respectively when analyzed using the MaxPeak HPS column hardware. Diazoxide and bicalutamide each contain sulfur dioxide, a constituent that can interact with metals.^{4,5} In fact, one possible mechanism of action of diazoxide is binding to the Zn²⁺ center of carbonic anhydrase to act as an inhibitor.⁶ Gentisic acid contains a carboxylic acid and hydroxyl moieties where salmeterol contains hydroxyl and nitrogen moieties that, when in a particular spatial arrangement, can act as a Lewis base and form chelates with metals.⁵ These results suggest that even seemingly innocuous molecular constituents can impact the recovery, and hence the sensitivity, of compounds. MaxPeak HPS can mitigate these effects by creating an inert surface, thus preventing the compounds' interaction with metals.

SUMMARY

In this work, we show the benefits of the incorporation of MaxPeak HPS with the Atlantis PREMIER BEH C₁₈ AX Column. Increases in chromatographic peak area under LC-MS conditions were observed in the range of 1.3- to 19-fold higher when comparing columns with and without MaxPeak HPS. Many classes of

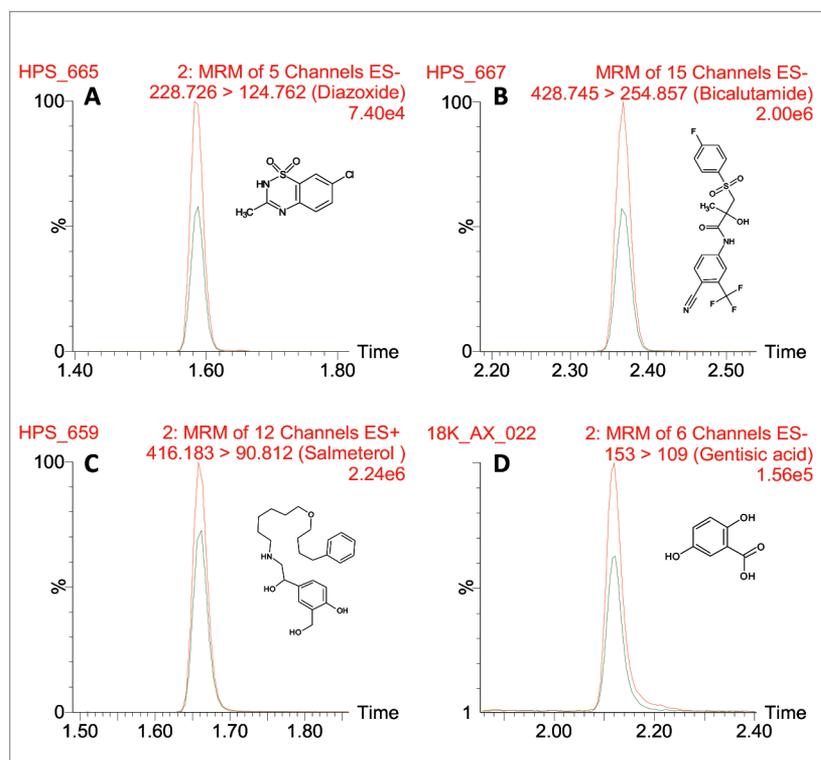


Figure 2. Peak area recovery of diazoxide (A), bicalutamide (B), salmeterol (C), and gentisic acid (D) on the Atlantis BEH C₁₈ AX sorbent with MaxPeak HPS column hardware (red line) and standard column hardware (green line).

compounds tested in this work showed benefits using this technology, such as phosphorylated compounds, pharmaceutical drugs, and biological metabolites.

References

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