IMPROVING CHROMATOGRAPHIC PERFORMANCE FOR METAL SENSITIVE ANALYTES USING HYBRID SURFACE BARRIER TECHNOLOGY

Nikunj Tanna, Robert S. Plumb, Kim Mulder
Waters Corporation, 34 Maple Street, Milford, MA , USA

INTRODUCTION

Analytes with electron rich moieties such as phosphate and carboxylate groups are susceptible to chelation with metal surfaces in chromatographic systems and columns. This often results in poor peak shape, reduced sensitivity, and poor reproducibility, resulting in less than desired quantitative performance.

Conditioning or passivation of LC systems and columns, using high concentrations of the analyte, to block sites of adsorption is often used to mitigate this issue. While effective, this passivation is not permanent. As an alternative, use of chelating reagents in mobile phases, such as EDTA, is often used. While also effective, use of chelating additives often negatively impacts LC-MS assays, suppressing MS signal and limiting sensitivity.

The work described herein, demonstrates improved chromatographic performance for metal-sensitive analytes, using a low-dispersion UHPLC system and sub 2-micron particle column, both of which incorporate a novel hybrid organic-inorganic surface technology (HST) specifically designed to mitigate issues resulting from the adsorption of analytes to metal surfaces. Both hydrocortisone and dexamethasone phosphates extracted from human plasma, showed improved chromatographic performance and sensitivity using the MaxPeak HST technology incorporated in the system and column as compared to the conventional UHPLC system and column.

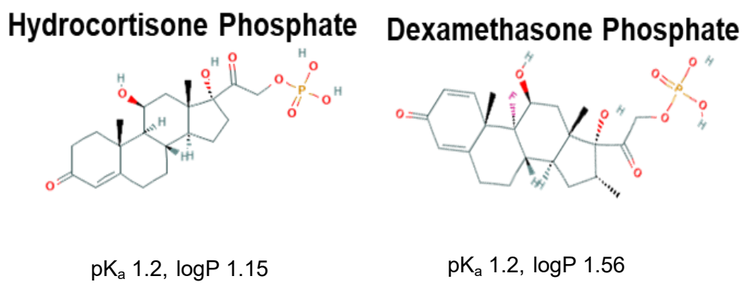

Figure 1. *Chemical structure and physiochemical properties for the metal sensitive analytes, hydrocortisone phosphate and dexamethasone phosphate.*

METHODS

Sample Preparation

Working solutions of 100 µg/mL and 10 µg/mL hydrocortisone phosphate and dexamethasone phosphate were spiked into human plasma to generate a calibration curve from 5–1000 ng/mL for hydrocortisone phosphate and 1–1000 ng/mL for dexamethasone phosphate. 100 µL of each sample was transferred to a micro-centrifuge tube and extracted using 300 µL of methanol. Samples were vortexed and centrifuged at 13,000 rcf for 10 min. Aliquots of the supernatant (200 µL) were transferred to LC-MS vials for analysis.

LC-MS/MS Conditions

Quantification of analytes was performed using a Waters Xevo™ TQ-XS tandem quadrupole MS (ESI-). Chromatographic performance assessment of metal sensitive analytes was performed using the ACQUITY™ Premier System or a traditional ACQUITY UPLC I-Class PLUS System with an ACQUITY Premier Column or an ACQUITY UPLC Column (HSS T3 , 1.8 µm, 2.1x50 mm). The ACQUITY Premier technology incorporates MaxPeak High Performance Surface (HPS) technology to mitigate non-specific adsorption due to ionic interactions. Mobile phases A and B consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. A gradient from 5-75% B over 2.50 minutes was used at a flow rate of 0.6 ml/min. Column temperature was 60 °C. Samples were kept at 5 °C. Sample injection volume was 10 µL.

RESULTS

IMPROVED CHROMATOGRAPHIC PERFORMANCE USING ACQUITY PREMIER WITH MAX PEAK HPS TECHNOLOGY

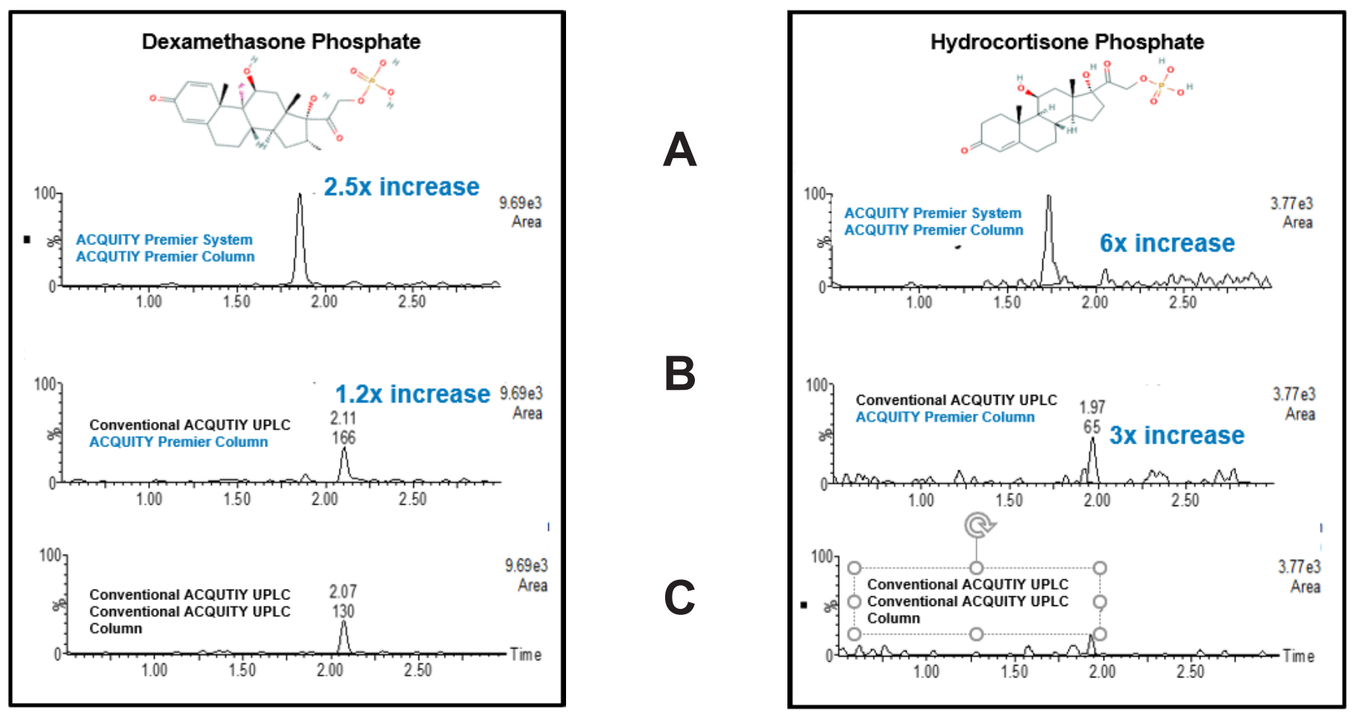

Figure 2. *Representative chromatograms comparing **dexamethasone phosphate** (2.5 ng/mL) chromatographic performance extracted from human plasma using **A)** ACQUITY Premier System/ACQUITY Premier Column, **B)** Conventional ACQUITY UPLC/ ACQUITY Premier Column, and **C)** a conventional ACQUITY UPLC/Conventional ACQUITY UPLC Column.*

Figure 3. *Representative chromatograms comparing **hydrocortisone phosphate** (2.5 ng/mL) chromatographic performance extracted from human plasma using **A)** ACQUITY Premier System/ACQUITY Premier Column, **B)** Conventional ACQUITY UPLC/ ACQUITY Premier Column, and **C)** a conventional ACQUITY UPLC/Conventional ACQUITY UPLC Column.*

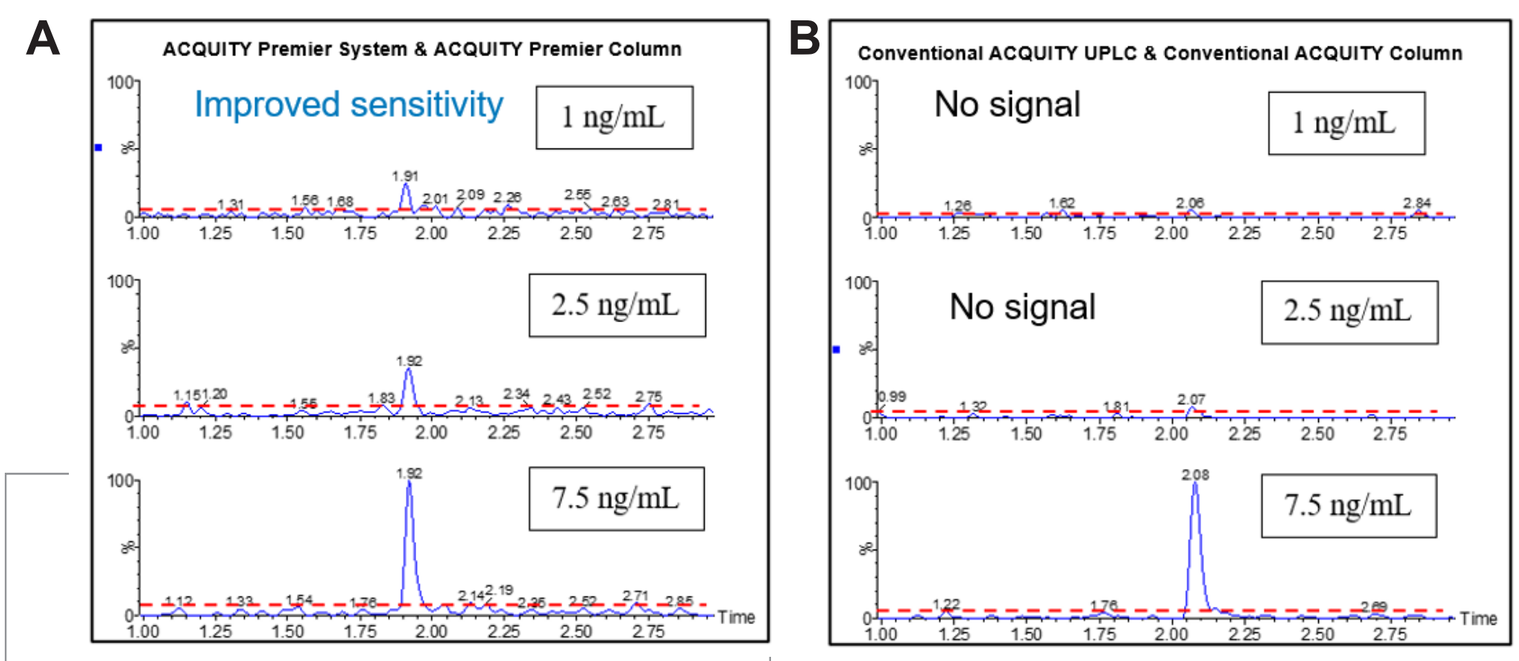

Figure 4. *Representative chromatograms for **dexamethasone phosphate** illustrating improved sensitivity at 1, AND 2.5 ng/mL on the ACQUITY Premier System/ACQUITY Premier Column (A) versus the Conventional ACQUITY UPLC System/Conventional ACQUITY UPLC Column. (B).*

ACCURATE AND REPRODUCIBLE QUANTIFICATION

Calibration Curve Performance

	Linear Range (ng/mL)	Weighting	r^2	Slope	Intercept
Dexamethasone phosphate	1-1000	1/x	0.992	0.119	0.129

Table 1. *Representative standard curve performance for **dexamethasone phosphate** extracted from human plasma using the ACQUITY Premier System & ACQUITY Premier Column.*

Quality Control Performance

	Expected Concentration (ng/mL)	Mean Observed Concentration (ng/mL)	Precision (%)	Accuracy (%)
LLOQ	1	0.97	5.97	96.67
LQC	10	11.10	4.68	111.00
MQC	75	80.17	4.39	106.89
HQC	750	694.73	7.49	92.63

Table 2. *Representative quality control performance for **dexamethasone phosphate** extracted from human plasma using the ACQUITY Premier System & ACQUITY Premier Column.*

STUDY HIGHLIGHTS

- *Use of the low adsorption ACQUITY Premier System and ACQUITY Premier Column, with MaxPeak HPS Technology improved chromatographic performance and sensitivity for metal sensitive analytes.*
- *Compared to a conventional ACQUITY UPLC System & Column, the ACQUITY Premier System and column produced improved analyte recovery, narrower peak widths with improved peak shape, which collectively improve overall robustness and repeatability of this analytical method.*
- *Excellent quantitative performance with LODs of 1 to 2.5 ng/mL, excellent linearity ($r^2 \geq 0.99$), and accuracies ≤15%. Assay precision for hydrocortisone phosphate and dexamethasone phosphate across the QC levels was <11%.*

CONCLUSION

The use of the ACQUITY Premier System with an ACQUITY Premier Column enabled the development of a sensitive quantitative MRM method for the metal sensitive analytes dexamethasone phosphate and hydrocortisone phosphate, achieving LLOQs of 1 ng/mL in extracted plasma. The ACQUITY Premier with MaxPeak HPS Technology greatly improved quantification performance, with improved sensitivity, linearity, accuracy, and reproducibility of the developed analytical method. The proof of concept method shows great promise for accurate quantification of steroid phosphate therapeutics. In support of drug discovery and research.

References

1. Hassn M. E. Dexamethasone in Severe COVID-19 Infection: A Case Series. *Respiratory Medicine Case Reports*. 2020, 31, 101205.
2. Cevc, G.; Blume, G. Hydrocortisone and Dexamethasone in ery Deformable Drug Carriers Have Increased he Biological Potency, Prolonged Effect, and Reduced Therapeutic Dosage. Biochimica et Biophysica Acta (BBA) – *Biomembranes*. 2004, 663 (1–2), 61–73.
3, Lauber, M.; *et al*. Low Adsorption HPLC Columns Based on MaxPeak High Performance Surfaces. Waters White Paper, 720006930EN <https://www.waters.com/waters/library.htm?cid=511436&lid=135074404>, 2020.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS