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응용 자료

Utilization of the Arc Premier System and XSelect Premier Columns for Sensitive LC-MS Quantitation of the Steroid Phosphates, Betamethasone, Dexamethasone, and Hydrocortisone

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

LC-MS/MS quantification of steroid pharmaceuticals during pharmaceutical research, discovery, and manufacturing requires sensitive and robust analytical methods for their accurate quantification. This work highlights the increased chromatographic performance for the metal sensitive steroid phosphate analytes, dexamethasone sodium phosphate, betamethasone sodium phosphate, and hydrocortisone phosphate triethylamine using the Arc Premier System and XSelect Premier HSS T3 Column.

Benefits

- Use of the Arc Premier System and XSelect Premier HSS T3 Column yielded improved steroid phosphate recovery and peak shape, ultimately improving method detection limits and reproducibility
- Incorporation of Waters MaxPeak High Performance Surfaces (HPS) Technology to the Arc Premier System and MaxPeak Premier Columns, eliminated the need for lengthy system and column passivation and maximized system up-time
- Fast, accurate, and robust steroid quantitation with 1 ng/mL quantitation and 6.5 minute analysis time

Introduction

Amongst the many LC-MS analytical method development challenges for steroid phosphate, their well-known propensity to adsorb to metal surfaces in the LC flow path, due to their electron rich nature, is often the most problematic. This metal interaction negatively impacts chromatographic performance, often resulting in poor peak shape and issues with analyte recovery and reproducibility, ultimately limiting overall performance of the analytical method. Conditioning or passivation of LC systems and columns, using high concentrations of the analyte to block sites of adsorption, is often used. 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 presented herein demonstrates the improved analytical LC-MS quantitative performance using the Arc Premier System and XSelect Premier HSS T3 Column. The chromatographic systems and columns

enhanced with MaxPeak HPS Technology were specifically designed to prevent non-specific adsorption due to ionic interactions for metal-sensitive analytes, significantly improving peak shape, recovery, and overall reproducibility of the analytical method without the need for system or column passivation.

Results and Discussion

Chromatographic performance for dexamethasone, dexamethasone acetate, betamethasone sodium phosphate, dexamethasone sodium phosphate, and hydrocortisone phosphate triethylamine was evaluated using a standard ACQUITY Arc System and standard stainless-steel XSelect HSS T3 Column and compared to the Arc Premier System and XSelect Premier HSS T3 Column. MS detection and quantification were performed using a Xevo TQ-S micro Triple Quadrupole Mass Spectrometer.

On the Arc Premier System and XSelect Premier HSS T3 Column, with a 5 µL injection of the steroids (10 ng/mL), injection #3 in the sequence queue, all analytes were easily detected. This is illustrated in Figure 1A. However, the same sample, run on the same day, with the sample set queue, on the standard ACQUITY Arc System and standard stainless-steel XSelect HSS T3 Column, the non-phosphate steroids were not detected, while dexamethasone and dexamethasone acetate were easily detected with peak response comparable to the Arc Premier System and XSelect Premier HSS T3 Column. Results are illustrated in Figure 1B.

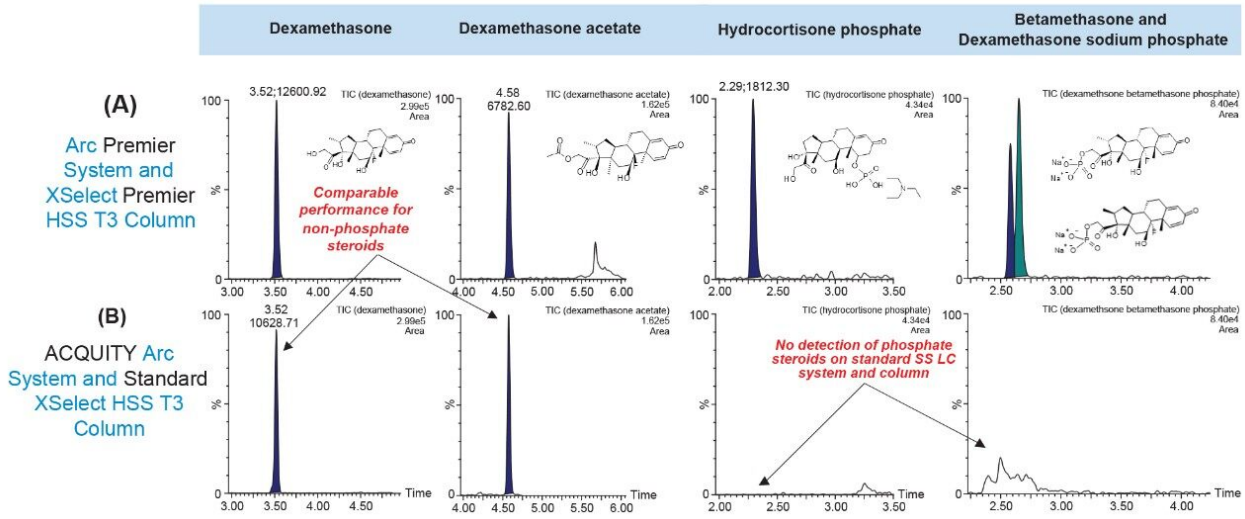

Figure 1. Illustration of chromatographic performance for dexamethasone, dexamethasone acetate, hydrocortisone phosphate, and betamethasone and dexamethasone phosphate using the Arc Premier System and XSelect Premier HSS T3 Column (A) versus the standard ACQUITY Arc System and standard XSelect HSS T3 Column (B). LC-MS system: Arc Premier System and standard ACQUITY Arc System coupled to a Xevo TQ-S micro Triple Quadrupole Mass Spectrometer. Column: XSelect Premier Column or standard XSelect HSS T3 Column, 2.5 µm, 2.1 x 100 mm at 35 °C. Injection volume: 5 µL. Mobile phase A: Water with 0.1% formic acid in water. Mobile phase B: Acetonitrile with 0.1% formic acid. Flow rate: 0.5 mL/min. Gradient: Initial at 85% A, gradient to 40% A in 4.5 min, followed by a 0.5 min flush at 5% A, return to initial conditions at 5.5 minutes (total analysis time 6.5 min).

Figure 2 further demonstrates out-of-box chromatographic performance for low ng/mL steroid analysis of the betamethasone phosphate (4.37 min) and dexamethasone phosphate (4.54 min) using the Arc Premier System and XSelect Premier HSS T3 Column (A) vs the standard ACQUITY Arc System and standard XSelect HSS T3 Column (B). For this illustration, two solvent standard blanks were injected followed by six replicate 5 µL injections (10 ng/mL) of the steroid cocktail mix. Excellent chromatographic performance: analyte recovery, peak shape, resolution, and reproducible MS response were seen upon the first steroid injection. The performance for these phosphate-steroids was drastically different on the standard ACQUITY Arc System and stainless-steel column (2B), with much lower steroid analyte response, which increased with injection number. Additionally, there is more pronounced peak tailing and poor separation of betamethasone and dexamethasone phosphate.

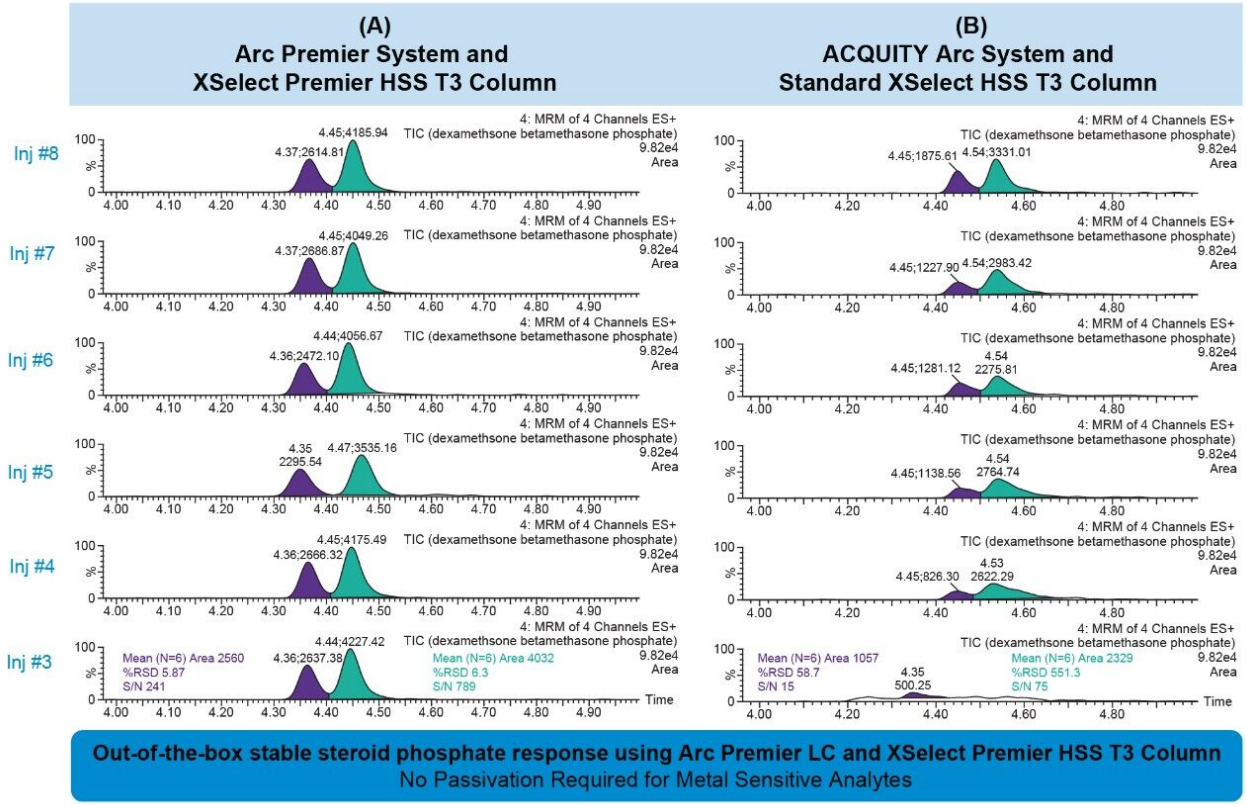

Figure 2. Demonstration of out-of-the-box betamethasone and dexamethasone phosphate steroid performance using the Arc Premier System and XSelect Premier HSS T3 Column (2.5 µm, 2.1 x 100 mm), panel A, versus the equivalent standard ACQUITY Arc System and standard stainless-steel XSelect HSS T3 Column, panel B. For this analysis, six 5 µL replicate injections of a 10 ng/mL hydrocortisone phosphate solution were compared using new columns and clean/flushed LC systems. Excellent chromatographic performance: analyte recovery, peak shape, resolution, and reproducible MS response were seen on the first steroid injection for the Arc Premier System and XSelect Premier HSS T3 Column (A), while the standard ACQUITY Arc System and standard XSelect HSS T3 Column showed pronounced hydrocortisone phosphate loss with poor resolution of betamethasone and dexamethasone phosphate (B), indicating that the standard system and column are passivating with each subsequent injection.

In accordance with small molecule analytical method development guidances,[1,2] a developed assay must be able to demonstrate linearity (correlation coefficient or $R^2 \geq 0.98$), accuracy (±15%), and precision (±15%). These criteria were easily achieved using the XSelect Premier HSS T3 Column (2.5 µm, 2.1 x 100 mm) and the Arc Premier System coupled to the Xevo TQ-S micro. Quantitative performance for all five steroids is highlighted in Table 1. Linearity (R^2) for all steroids was >0.99 using $1/x^2$ weighting with dynamic ranges from

1–250 ng/mL (dexamethasone, dexamethasone acetate, dexamethasone phosphate, and betamethasone phosphate) and 1–1000 ng/mL for hydrocortisone phosphate. Lower limits of quantification (LLOQ) of 1 ng/mL were easily achieved for all steroids evaluated, with signal-to-noise ratios (S/N) ranging from 13 to 278 for all steroids. This chromatographic performance for the LLOQ calibration points of dexamethasone sodium phosphate, betamethasone sodium phosphate, and hydrocortisone phosphate is illustrated in Figure 2.

Analyte	Molecular weight (g/mol)	MS (ESI+) MRM transition	LC retention time (min)	LLOQ peak width at base (sec)	LLOQ RMS S/N	Peak assymetry factor (b/a)	Calibration curve dynamic range (ng/mL)	Calibration curve weighting	Linear fit (r^2)	Calibration curve mean accuracy (%)
Dexamethasone	392.5	393.25 > 373.26	3.43	6.0	278	1.07	1-250	1/x2	0.995	100.0
Dexamethsone acetate	434.5	435.4 > 397.32	4.43	6.6	59	1.10	1-250		0.998	100.0
Dexamethasone sodium phosphate	516.4	473.31 > 43521	2.7	4.8	66	1.27	1-250		0.993	98.2
Betamethasone sodium phosphate	516.4	473.31 > 43521	2.62	5.4	35	1.06	1-250		0.998	97.2
Hydrocortisone phosphate triethylamine	543.6	443.3 > 327.23	2.33	6.0	13	1.32	1-1000		0.997	99.8

Table 1. Steroid quantification performance including linear dynamic range, mean calibration curve accuracy, S/N, and peak asymmetry.

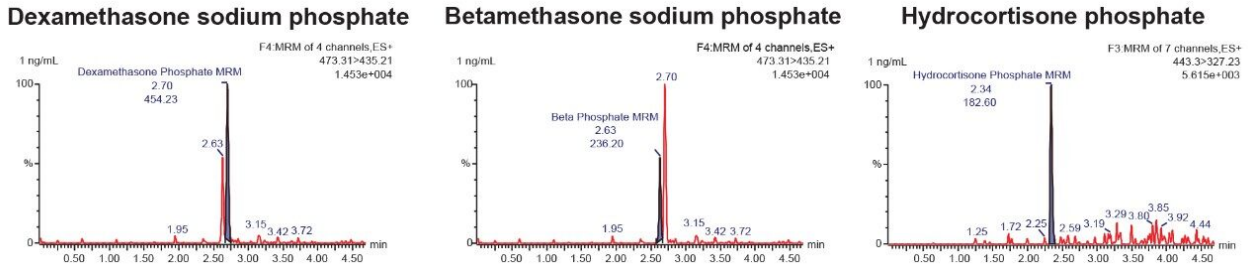

Figure 3. Demonstration of high sensitivity steroid quantitation, with 1 ng/mL LLOQ using the developed method with chromatographic separation with the Arc Premier System and XSelect Premier HSS T3 Column and detection with the Xevo TQ-S micro Triple Quadrupole Mass Spectrometer for dexamethasone sodium phosphate, betamethasone sodium phosphate, and hydrocortisone phosphate.

Conclusion

The use of the Arc Premier System and XSelect Premier HSS T3 Column enabled the development of a

sensitive quantitative MRM method for steroid pharmaceutics, achieving LLOQs of 1 ng/mL. The Arc Premier System and XSelect Premier Columns with MaxPeak HPS Technology greatly improved recovery of the steroid-phosphates, betamethasone, dexamethasone, and hydrocortisone, while demonstrating equivalent performance for the non-phosphate steroids, dexamethasone, and dexamethasone acetate. This proof of concept method shows great promise for accurate quantification of steroids in support of drug discovery, research, and manufacturing.

References

1. Viswanathan, C. T.; Bansal, S.; Booth, B.; DeStefano, A. J.; Rose, M. J.; Sailstad, J.; Shah, V. P.; Skelly, J. P.; Swann, P. G.; Weiner, R. Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays. *Pharm. Res.* 2007, 24, 1962–1973.
2. Bansal, S.; DeStefano, A. Key Elements of Bioanalytical Method Validation for Small Molecules. *AAPS J.* 2007, 9, E109–114.

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