Improvements in the bioanalytical method performance for steroid phosphates using a hybrid surface barrier to minimize analyte-metal surface interactions

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INTRODUCTION

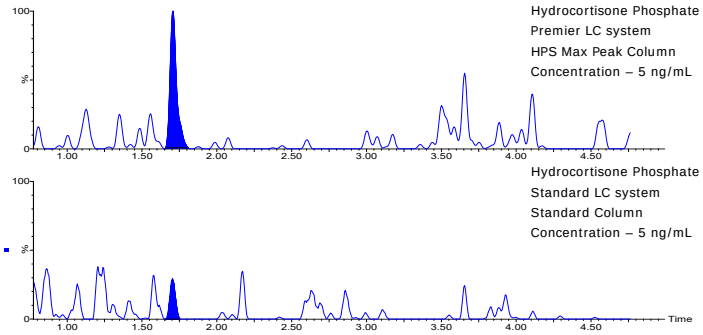
Analyte classes containing phosphorylated groups, uncharged amines, hydroxyls, and deprotonated carboxylic acids are electron rich and are easily absorbed onto metal surfaces such as the stainless steel used in LC systems. This can have a deleterious effect on bioanalytical methods resulting in complicated method development, time consuming data analysis, and insufficient assay sensitivity and reproducibility. This study evaluated the benefits of the Acquity Premier technology which comprises of an inert hybrid surface on the metallic components in the LC flow path, column frits and column wall to mitigate these interactions for the bioanalysis of two steroid phosphate drugs (dexamethasone phosphate and hydrocortisone phosphate).

EXPERIMENTAL

LC System	ACQUITY Premier UPLC
Detector	Xevo TQ-XS
Column	2.1 x 50mm ACQUITY MaxPeak HSS T3, 1.8 µm
Column Temp.	60ºC
Sample Temp.	5ºC
Inj Volume	10µL
Flow Rate	600 µL/min
Mobile Phase A	0.1% Formic acid in Water
Mobile Phase B	0.1% Formic acid in Acetonitrile
Gradient	5-75% B over 2.5 minutes

Hydrocortisone Phosphate	443.19>327.15
Dexamethasone phosphate	473.32>435.16

RESULTS & DISCUSSION



	Linear Range (ng/mL)	Weighting	R^2	Slope	Intecept
Hydrocortisone Phosphate	5-1000	1/x	0.993	0.043	0.054
Dexamethasone Phosphate	1-1000	1/x	0.992	0.119	0.129

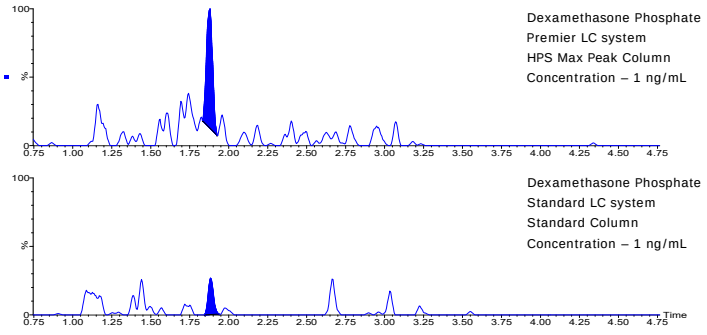
Table 1a – Calibration curve statistics for Hydrocortisone Phosphate and Dexamethasone Phosphate

	Expected Concentration (ng/mL)	Mean Observed Concentration (ng/mL)	Precision (%)	Accuracy (%)
LLOQ	5	5.03	9.39	100.67
LQC	10	10.20	6.86	102.00
MQC	75	73.90	10.53	98.53
HQC	750	743.63	7.13	99.15

Table 1b – Precision and accuracy statistics for Hydrocortisone Phosphate

	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 1c – Precision and accuracy statistics for Dexamethasone Phosphate



The Acquity Premier LC system improved the peak width and peak response for both hydrocortisone phosphate and dexamethasone phosphate. The LLOQ for hydrocortisone phosphate was improved by 10 fold, whereas that for dexamethasone phosphate was improved by 7.5 fold. The calibration curve statistics for both analytes were acceptable, as shown in table 1a. Across 4 QC levels (LLOQ, LQC, MQC and HQC), the assay precision was <11% with accuracies between 98.5-102 % for hydrocortisone phosphate. Similarly, for dexamethasone phosphate, the assay precision was <8% with accuracies between 92.6-111 % across the 4 QC levels.

CONCLUSIONS

Quantifying metal sensitive compounds on standard LC and column hardware suffer from poor peak shape and lack of reproducibility, resulting in inability to achieve desired LLOQ's. Using ACQUITY Premier LC with MaxPeak HPS columns for Hydrocortisone Phosphate and Dexamethasone phosphate we achieved

- Sensitivity improvements of 10x (Hydrocortisone Phosphate) and 7.5x (Dexamethasone phosphate)
- Assay precision of <11% (Hydrocortisone Phosphate) and <8% (Dexamethasone phosphate) across LLOQ, LQC, MQC and HQC levels