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Application Note

Improving the Analysis of Betamethasone and Phosphate, Acetate, and Dipropionate Derivatives Using CORTECS™ Premier C_8 Columns

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Abstract

Newer column technologies can significantly improve analytical performance. This application note demonstrates the improved analysis of betamethasone and its phosphate, acetate, and dipropionate derivatives using CORTECS Premier C_8 Columns with MaxPeak™ High-Performance Surfaces (HPS) Technology. The inert hardware eliminates interactions with metal surfaces, enhancing peak area, shape, and symmetry, particularly for betamethasone phosphate, while maintaining excellent results for the other analytes.

Benefits

- Resolution of four steroid compounds under generic screening gradient conditions
- Narrower, more symmetrical peak and higher peak area for betamethasone phosphate obtained using an inert hardware CORTECS Premier C_8 Column compared to a CORTECS C_8 Column and a solid-core C_8

column from a different supplier

Introduction

While older column technologies may give adequate results for some analyses, using newer technology can provide better separations and future-proof methods to reduce the risk of time-consuming revalidation. As such, it is important to consider newer column technology like MaxPeak Premier Columns during method development, prior to validation. MaxPeak Premier Columns employ inert HPS hardware technology. The hardware mitigates interactions between analytes and the metal surfaces present in the column.[1–3] This technology is also available in liquid chromatography (LC) systems. Using both a system and a column with MaxPeak Premier Technology provides the most complete mitigation of these interactions.[4] The analytes most affected by metal surfaces in the column and system are generally those with acidic groups or other moieties than can bind metal ions. When using a mobile phase with a pH of less than about seven, the oxide layer on the surface of stainless steel has a positive charge. This acts as an anion exchange site that can interact with any negatively charged analytes like phosphates, sulfates, and some carboxylic acids.[5]

To demonstrate how MaxPeak Premier Columns can improve a separation, four steroid compounds were selected: betamethasone, betamethasone phosphate, betamethasone acetate, and betamethasone dipropionate. Separations were carried out using three different columns: a CORTECS Premier C_8 Column, a CORTECS C_8 Column, and a solid-core C_8 column from a different supplier. Peak areas, United States Pharmacopeia (USP) tailing factors, and peak width at 4.4% were monitored for all analytes. Comparisons were made across the three columns to assess how the inert hardware impacted the separation.

Experimental

Sample Description: Stock solutions were created at 1 mg/mL in 1:1 (v:v) water:acetonitrile. A sample mixture containing 50 µg/mL of each analyte was created using water as the sample diluent. The final solvent composition was 12.5% acetonitrile in water.

Method Conditions

LC system:	ACQUITY™ UPLC™ H-Class PLUS System with six column positions, PDA, QDa™ and a HPS flow path
Detection:	UV @ 254 nm
Columns:	CORTECS Premier C_8, 2.1 x 50 mm, 2.7 µm (p/n: 186011552) CORTECS C_8, 2.1 x 50 mm, 2.7 µm (p/n: 186008349) Vendor A C_8, 2.1 x 50 mm, 2.7 µm
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	1.0 µL
Flow rate:	0.5 mL/min
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase D1:	2% Formic acid in water
Gradient conditions:	A constant 5% D1 was used to maintain a 0.1% formic acid concentration. Starting conditions of 5% B, followed by a linear gradient to 95% B in 6.86 minutes. Hold at 95% B for 1.14 minutes, returning to starting conditions and re-equilibrating for 2.28 minutes. Total run time: 10.30 minutes.

Data Management

Chromatography software:	Empower Chromatography Data System (CDS)

Results and Discussion

Steroid phosphates, particularly betamethasone phosphate, have been studied previously and were shown to benefit from inert hardware, such as that used in MaxPeak Premier Columns.[6] However, no such analysis has been performed using CORTECS Columns, which employ solid-core silica particles that give higher column efficiencies.[7] To that end, four compounds were analyzed using a CORTECS Premier C_8 Column, a CORTECS C_8 Column, and a solid-core C_8 column from a different supplier. In addition to betamethasone phosphate, betamethasone and two other betamethasone derivatives were included. The structures of the analytes are shown in Figure 1. The compounds all contain the same betamethasone backbone, with alterations to position 21 or positions 17 and 21 in the case of betamethasone dipropionate.

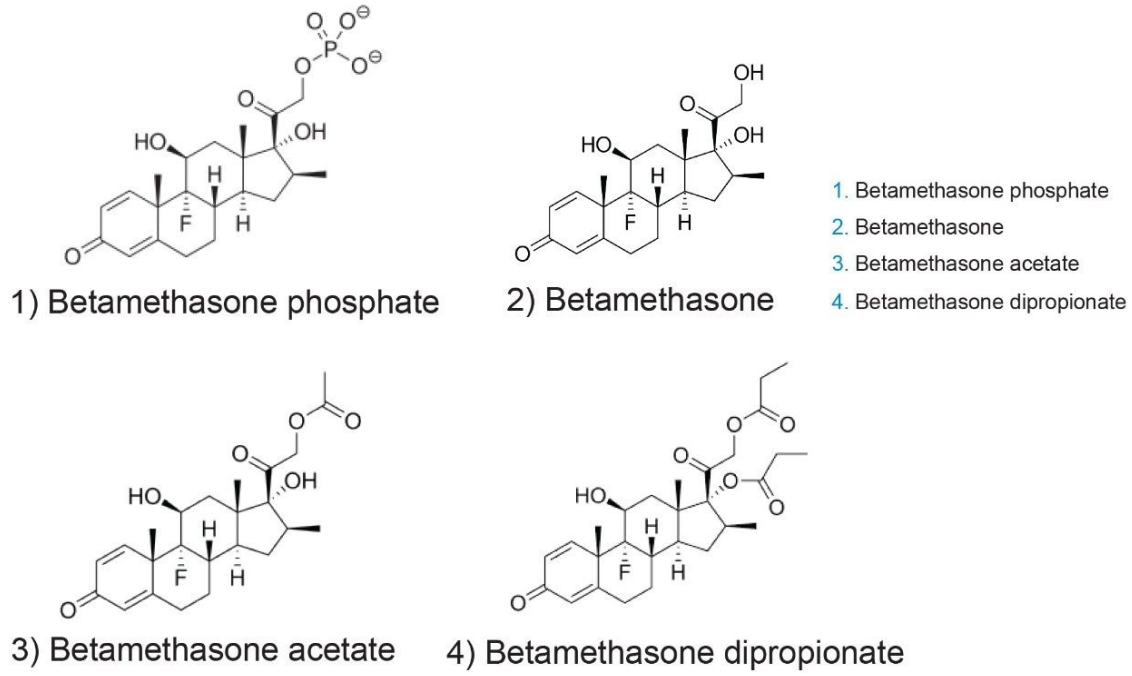

Figure 1. Chemical structures of the compounds tested.

Figure 2 shows the results obtained using the three columns. Betamethasone phosphate showed a narrower and more symmetrical peak with a higher area when using the MaxPeak Premier Column compared to the stainless steel columns. No other major changes in the separation were detected between the columns, which is confirmed by examining the peak areas, USP tailing factors, and peak width values for the analytes on both columns, as shown in Tables 1–3.

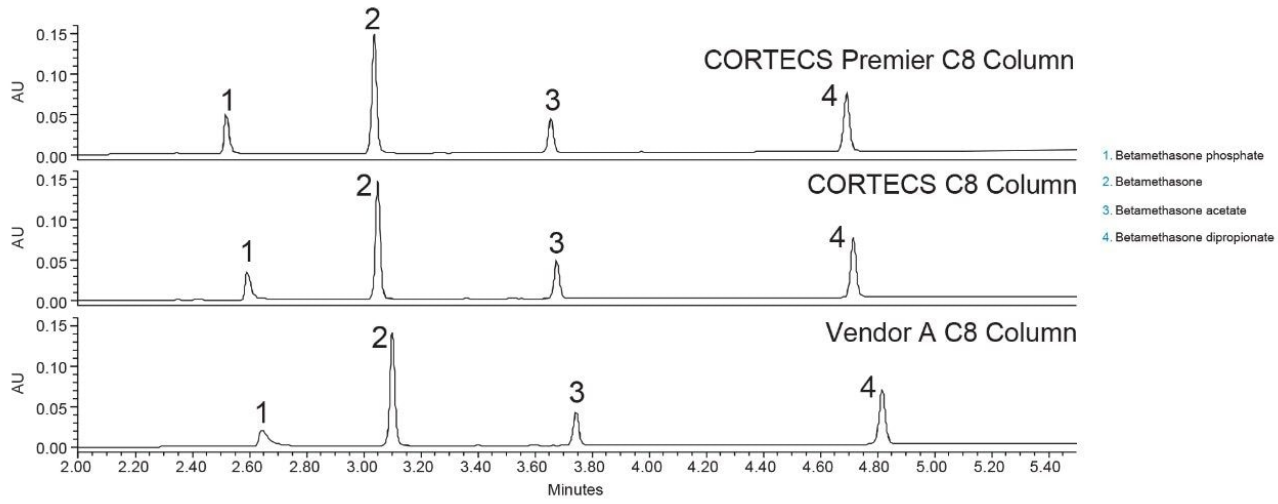

Figure 2. Chromatograms of betamethasone and derivatives obtained using an ACQUITY UPLC H-Class PLUS System with a PDA Detector and three different columns. Peak identification: 1) betamethasone phosphate, 2) betamethasone, 3) betamethasone acetate, 4) betamethasone dipropionate.

	CORTECS Premier C_8 Column	CORTECS C_8 Column	Vendor A C_8 Column
Betamethasone phosphate	62600	56000	56400
Betamethasone	177000	175000	175000
Betamethasone acetate	64000	62200	58000
Betamethasone dipropionate	101000	103000	102000

Table 1. Peak area values for all analytes on all columns tested.

	CORTECS Premier C_8 Column	CORTECS C_8 Column	Vendor A C_8 Column
Betamethasone phosphate	1.42	2.26	2.87
Betamethasone	1.04	1.07	1.01
Betamethasone acetate	1.01	1.02	0.97
Betamethasone dipropionate	0.99	1.02	0.96

Table 2. USP tailing factors for all analytes on all columns tested.

	CORTECS Premier C_8 Column	CORTECS C_8 Column	Vendor A C_8 Column
Betamethasone phosphate	0.0458	0.0717	0.1291
Betamethasone	0.0425	0.0421	0.0455
Betamethasone acetate	0.0467	0.0469	0.0506
Betamethasone dipropionate	0.0500	0.0498	0.0546

Table 3. Peak width @ 4.4% (minutes) for all analytes on all columns tested.

The numerical data confirm that only betamethasone phosphate was affected using MaxPeak Premier Column hardware. The best peak shape for betamethasone phosphate was obtained using the CORTECS Premier C_8 Column, with slightly worse peak shape on the CORTECS C_8 Column, followed by the Vendor A C_8 column. The other compounds, which are not susceptible to metal interactions, gave comparable results using the three inert columns.

Conclusion

MaxPeak Premier Columns employ inert HPS hardware to mitigate the interaction between analytes and metal surfaces in the column. These interactions can lead to peak tailing and loss of peak area in a chromatographic analysis. By standardizing on MaxPeak Premier Columns for all new method development activities, any possible

interactions between analytes and column hardware will be mitigated, elevating the quality of the data and future-proofing methods.

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