DIONEX

Application Brief 123

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UHPLC Separation of Nine Corticosteroids in Under Four Minutes

INTRODUCTION

Corticosteroids are a class of steroid hormones that are involved in the regulation of a variety of biological processes, and consequently are used to treat a wide range of maladies. For example, they are used to treat conditions caused by an overactive immune system (e.g., allergies), control inflammation, treat skin rashes, and reduce the growth of cancerous tumors. Corticosteroids are produced by the cortex of the adrenal gland, but there are synthetic compounds that act like a corticosteroid in the body, and are therefore also considered corticosteroids (e.g., dexamethasone).

This work shows separation of a set of nine corticosteroids: prednisone, prednisolone, cortisone, hydrocortisone, methylprednisone, betamethasone, dexamethasone, triamcinolone acetate, and cortisone acetate using an Acclaim® 120 C18 column. Good separation of these nine compounds is achieved using isocratic conditions with a water/THF/methanol gradient mobile phase. Different column formats can be used to execute this separation, depending on the analyst's needs for separation speed and mobile phase conservation.

This experiment first investigated a ternary gradient separation using the Acclaim 120 C18, 5 µm, 4.6 × 250 mm column with commonly used reversed-phase mobile phase components: water, methanol, and acetonitrile. Seven of the nine corticosteroids were separated, while cortisone and prednisolone coeluted (Figure 1).

Although these conditions may be acceptable if neither cortisone nor prednisolone are expected in the sample, an alternative method is needed to separate all nine compounds. To achieve separation of these steroids, acetonitrile was replaced by THF in the mobile phase.

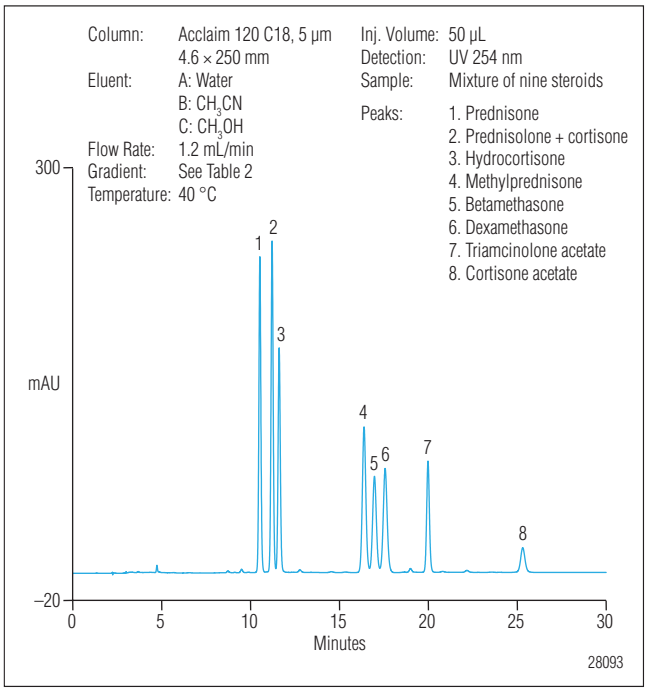

Figure 1. Separation of a mixture of nine corticosteroids using Condition 1.

Using isocratic conditions and increasing the column temperature to 50 °C, the nine steroids were well resolved (Figure 2). The same separation was achieved in less time and with reduced mobile phase consumption by switching to a 3 µm, 4.6 × 150 mm column (Figure 3). Figure 3 also shows that to achieve the separation, a 2 µL precolumn heater was necessary so that there was no thermal mismatch between the mobile phase and column.

To achieve a faster separation and further reduce mobile phase consumption, the separation was adapted to a 2.2 µm Acclaim Rapid Separation Liquid Chromatography (RSLC) 120 C18, 2.1 × 100 mm column (Figure 4). Because the heat exchange rate of the RSLC column is sufficiently fast due to the small internal diameter of 2.1 mm, a 2 µL precolumn heater was not needed. This separation required only four min and the column pressure was 8500 psi (586 bar). Chromatographic parameters from the four column formats are compared in Table 1 and show there is no sacrifice in performance in changing column format to reduce analysis time and mobile phase consumption.

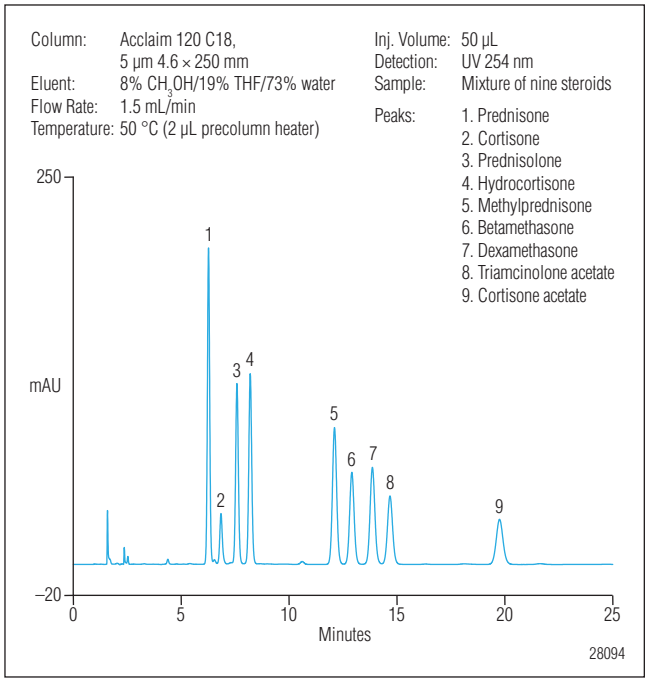

Figure 2. Separation of a mixture of nine corticosteroids using Condition 2.

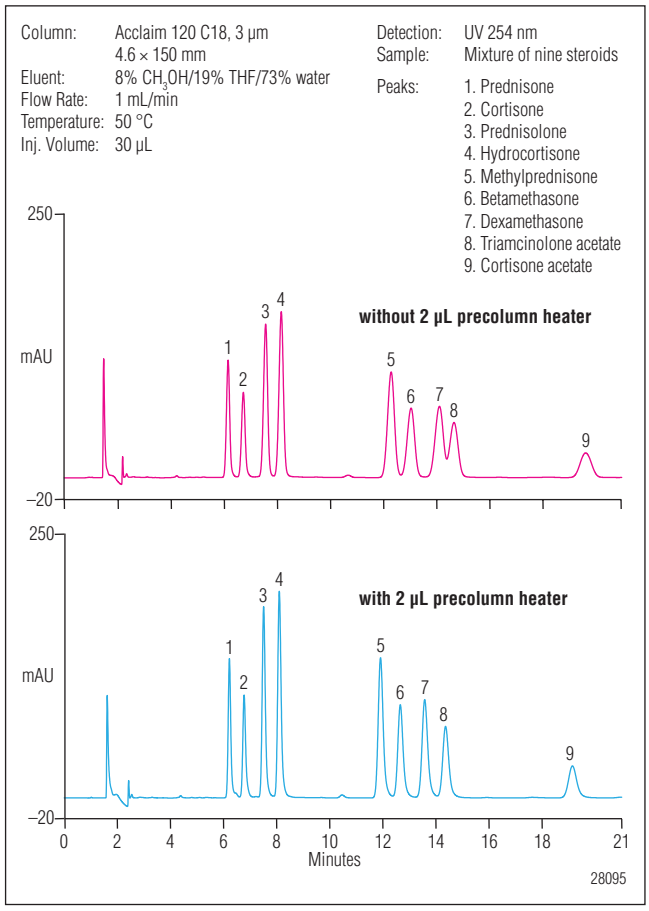

Figure 3. Separations of a mixture of nine corticosteroids using Condition 3 with and without a 2 µL precolumn heater.

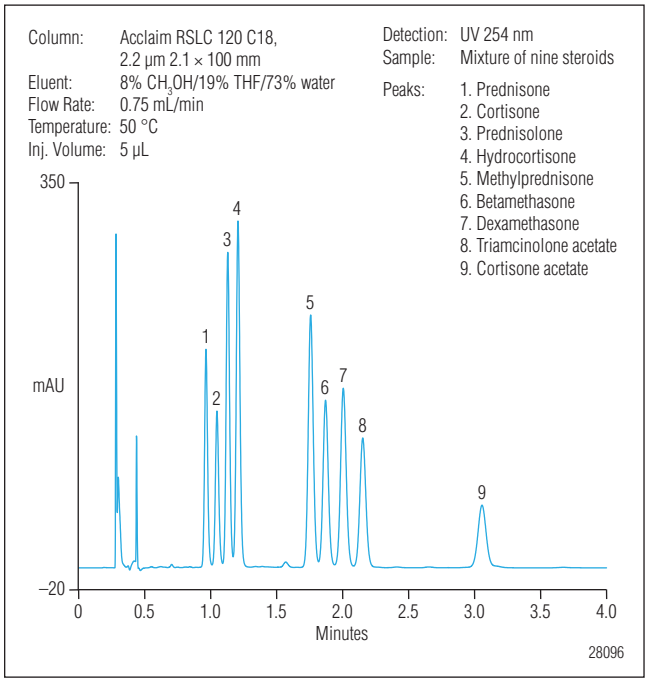

Figure 4. Separation of a mixture of nine corticosteroids using Condition 4.

Table 1. Peak Analysis Results of a Mixture of Nine Corticosteroids Obtained from Chromatography Conditions 1, 2, 3, and 4								
	Condition 1		Condition 2		Condition 3*		Condition 4	
Steroid	Resolution (USP)	Asymmetry (USP)	Resolution (USP)	Asymmetry (USP)	Resolution (USP)	Asymmetry (USP)	Resolution (USP)	Asymmetry (USP)
Prednisone	3.20	1.06	2.81	1.03	2.96	1.23	1.93	1.09
Cortisone	1.77	1.03	3.29	1.16	3.59	1.31	1.53	1.05
Prednisolone	1.77**	1.03**	2.54	1.05	2.64	1.22	1.57	1.08
Hydrocortisone	16.17	1.09	8.46	1.03	8.94	1.23	8.98	1.12
Methylprednisone	1.57	1.02	2.14	1.04	2.12	1.21	1.76	1.06
Betamethasone	1.55	1.05	2.38	1.05	2.45	1.20	1.71	1.05
Dexamethasone	7.02	1.09	1.92	1.05	1.96	1.18	2.05	1.06
Triamcinolone acetate	14.53	1.06	9.98	1.05	9.73	1.19	9.47	1.06
Cortisone acetate	n.a.	1.06	n.a.	1.05	n.a.	1.18	n.a.	1.08

*Condition 3 data relates to the separation with the 2 µL precolumn heater.

**With Condition 1, cortisone and prednisolone coelute.

EQUIPMENT

Dionex UltiMate® 3000 RSLC system including:

- SRD-3600 Integrated Vacuum Degasser
- DGP-3600RS Dual-Gradient Pump with 400 µL static mixer
- WPS-3000RS Split-Loop Sampler with 100 µL sample loop
- TCC-3000RS Thermostatted Column Compartment
- DAD-3000RS Diode Array Detector
- Semi-Analytical flow cell (5 µL, 7 mm) (P/N 6082.0200)
- Semi-Micro flow cell (2.5 µL, 7 mm) (P/N 6082.0300)

CHROMATOGRAPHIC CONDITIONS

Condition 1

Column: Acclaim 120 C18, 5 µm 4.6 × 250 mm

Mobile Phase: A; Water
B; CH_3CN
C; CH_3OH

Flow Rate: 1.2 mL/min

Gradient: See Table 2

Column Temp.: 40 °C

Inj. Volume: 50 µL

Detection: UV 254 nm
Semi-analytical SST flow cell (5 µL, 7 mm)
Data collection rate, 5 Hz
Response time, 0.5 s

Table 2. Gradient Table			
Time (min)	% A	% B	% C
–5	80	10	10
0	80	10	10
0.1	50	10	40
2	50	10	40
5	67	30	3
15	67	30	3
16	62	35	3
30	62	35	3

Condition 2

Column: Acclaim 120 C18, 5 µm 4.6 × 250 mm

Mobile Phase: 8% CH_3OH/19% THF/73% water

Flow Rate: 1.5 mL/min

Column Temp.: 50 °C (2 µL precolumn heater is required)

Inj. Volume: 50 µL

Detection: UV 254 nm
Semi-analytical (5 µL, 7 mm) SST flow cell
Data collection rate, 5 Hz
Response time, 0.5 s

Condition 3

Column:	Acclaim 120 C18, 3 µm 4.6 × 150 mm
Mobile Phase:	8% CH_3OH/19% THF/73% water
Flow Rate:	1.0 mL/min
Column Temp.:	50 °C (2 µL precolumn heater is required)
Inj. Volume:	30 µL
Detection:	UV 254 nm Semi-analytical SST flow cell (5 µL, 7 mm) Data collection rate, 5 Hz Response time, 0.5 s

Condition 4

Column:	Acclaim RSLC 120 C18, 2.2 µm 2.1 × 100 mm
Mobile Phase:	8% CH_3OH/19% THF/ 73% water
Flow Rate:	0.75 mL/min
Column Temp.:	50 °C
Inj. Volume:	5 µL
Detection:	UV 254 nm, Semi-Micro SST flow cell (2.5 µL, 7 mm) Data collection rate, 25 Hz Response time, 0.2 s

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