

## Liquid Chromatography

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## Comparison of Epic C18, Epic C18-SD, and Epic Polar Column for the HPLC Analysis of Hydrocortisone in Accordance With the United States Pharmacopeia

### Introduction

Hydrocortisone is the pharmaceutical term for cortisol used in oral administration, intravenous injection, or topical application. It is often used as an immunosuppressive

drug, given by injection, in the treatment of severe allergic reactions, such as anaphylaxis and angioedema. It is also used in place of prednisolone in patients needing steroid treatment that unable to take oral medication, as well as perioperatively in patients on long-term steroid treatment to prevent an adrenal crisis when treating immune, inflammatory, and neoplastic conditions.<sup>1</sup>

The United States Pharmacopeia (USP) provides monographs for the identification and impurity testing of the drug substances. This application brief describes use of PerkinElmer Epic C18, Epic C18 SD (highly base deactivated high carbon super dense phase), and Epic™ Polar (150 x 4.6 mm, 5 μm) columns, coupled with the PerkinElmer LC 300 liquid chromatography system and SimplicityChrom™ chromatography data system (CDS), for the assay analysis of hydrocortisone.

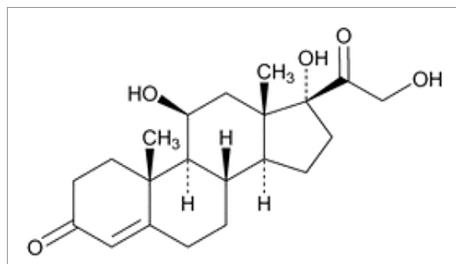


Figure 1: Chemical structure of Hydrocortisone.

## Experimental

All LC method parameters are shown in Table 1.

Instrument	PerkinElmer LC 300 UHPLC system with Photodiode Array (PDA) detector
Column	Epic C18, 150 x 4.6 mm, 5 µm (P/N: 522A91-EC18) Epic C18 SD, 150 x 4.6 mm, 5 µm (P/N: 135291-EC18 SD) Epic C18 Polar, 150 x 4.6 mm, 5 µm (P/N: 135291-EC18 Polar)
Mobile Phase	Isocratic method: Acetonitrile, methanol, and water (25:25:50)
Flow Rate	1 mL/min
Column Temp	25°C
Wavelength	Acquisition 254 nm, bandwidth 10 nm, reference 400 nm, bandwidth 10 nm
Injection Volume	10 µL (partial loop)

Table 1: LC Method Parameters.

### Solvents, Standards, and Samples

All solvents used were HPLC grade, and samples were filtered using a 0.45 µm nylon filter (P/N- 02542903).

The internal standard solution (propylparaben) was prepared by reconstituting propylparaben to a final concentration of 1 mg/mL in methanol. A standard stock solution (USP Hydrocortisone RS) was prepared by reconstituting to a final concentration of 1 mg/mL in methanol. The working standard solution was prepared using 2.0 mL of the internal standard solution and 2.0 mL of the standard stock solution, then diluting to 50 mL using methanol/water (1:1) as the diluent.

## Results and Discussion

The USP monograph specifies the use of an L1 column (150 x 4.6 mm, 5 µm). An L1 column is defined as octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 µm in diameter. Three PerkinElmer columns were evaluated for this work - an Epic C18, Epic C18 SD and Epic Polar. Each column complies with the USP monograph, and they are all ideal for the separation of Hydrocortisone, as can be seen in the results shown in Table 2.

The analysis of Hydrocortisone was carried out using an LC 300 UHPLC system, with a run time of 30 minutes (Table 2). Hydrocortisone was identified on the Epic C18 at 7.37 minutes, the Epic C18 SD at 7.34 minutes, and the Epic Polar at 5.90 minutes, as shown in Figure 2. The USP monograph requires that the area and RT relative standard deviation (RSD) of five replicate injections should not be no more than 2 %, that the tailing factor be no more than 1.2, the resolution not less than 9, and that the theoretical plate count be no less than 3000 N. When evaluating all three columns, the Epic C18 provided excellent peak shape and optimum column performance for the analysis of Hydrocortisone in comparison (Table 2). The Epic C18 SD and Epic Polar columns showed higher area % RSD, closer to the USP limit, but both performed within the USP requirements.

The resolution between Hydrocortisone and propylparaben using the Epic Polar column was 9.9, which is almost equal to the limit range of the USP requirement. Based on the study, it can be concluded that the Epic C18 column fulfils all the USP requirements, and is best suited for the analysis of hydrocortisone owing to its superior base deactivation and high-density bonding. Further, these results demonstrate that the Epic C18 column, along with the LC 300 UHPLC system, are appropriate for this method.

Suitability Parameter	Time (min)	Mobile Phase A (%)	Phase	USP Requirement*
Peak Area RSD (%)	1.08	1.92	1.24	NMT 2
RT RSD (%)	0.13	0.07	0.1	NMT 2
Tailing Factor (5% Peak Height)	1.06	1.07	0.898	NMT 1.2
Resolution Between Hydrocortisone and Propylparaben Peaks	14.5	13.3	9.9	NLT 9
Column Efficiency (N)	10157	8889	9744	NLT 3000

\*NLT- Not less than, NML- Not more than

Table 2: System suitability test for standard preparation (Hydrocortisone) using different Epic columns. RSD calculated from five successive injections.

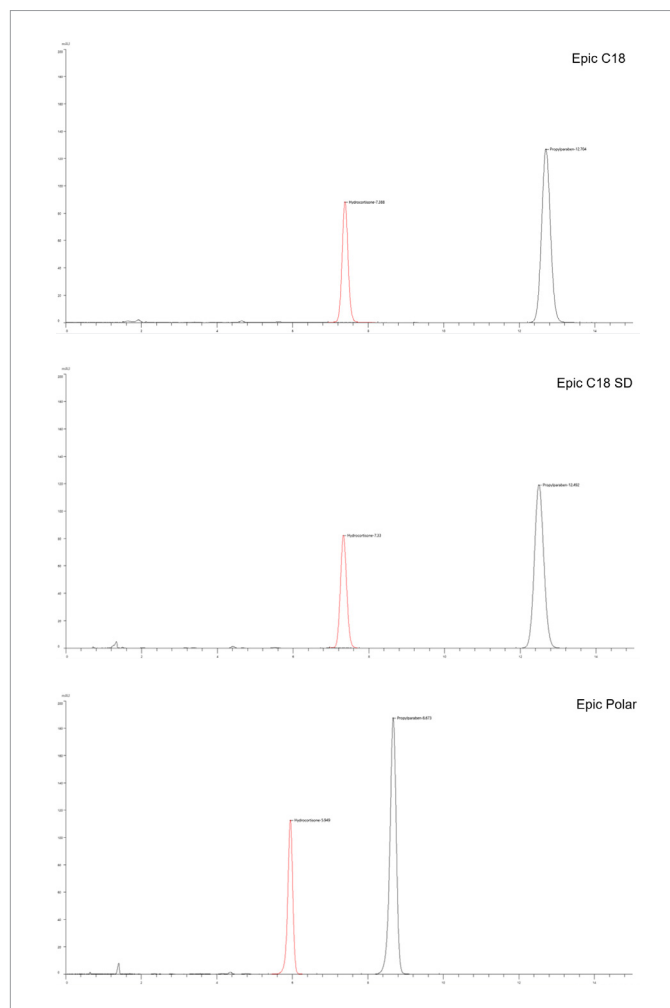


Figure 2: HPLC chromatograms of USP Hydrocortisone RS and propylparaben using 1) Epic C18, 2) Epic C18 SD and 3) Epic Polar.

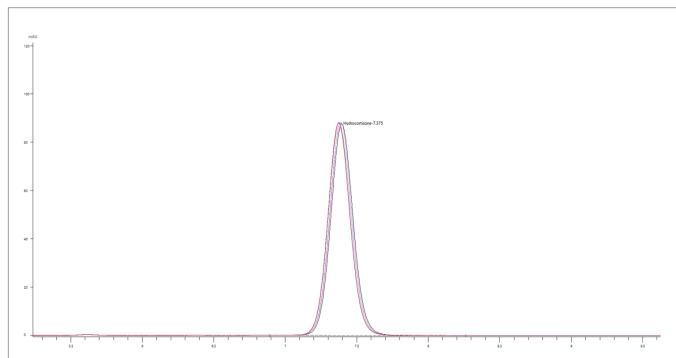


Figure 3: Overlay chromatogram of Hydrocortisone (5 injections) using an Epic C18

## Conclusion

In this work, the fast analysis of Hydrocortisone was carried out using three Epic columns. The Epic C18 showed the best peak shape, resolution, RSD and efficiency with excellent repeatability (Figure 3), meeting all requirements in the Hydrocortisone USP assay monograph. The excellent peak shape is attributable to the superior base deactivation and high-density bonding technology of the Epic C18 phase. The LC 300 UHPLC system showed excellent flow reproducibility and suitability for USP LC tests.

## References

1. "Hydrocortisone." Drugs.com. American Society of Health-System Pharmacists. February 9, 2015.
2. USP-NF <621> Chromatography general chapter.
3. Hydrocortisone, USP43-NF38, United States Pharmacopeia, 2228.

## Consumables

Component	Description	Part Number
Columns	Epic C18 column (150 x 4.6 mm, 5 µm)	<a href="#">522A91-EC18</a>
	Epic C18 SD column (150 x 4.6 mm, 5 µm)	<a href="#">135291-EC18-SD</a>
	Epic Polar column (150 x 4.6 mm, 5 µm)	<a href="#">135291-EPO</a>
HPLC Vials and Caps	2 mL Clear Write-On Vials w/ 9 mm Pre-Slit PTFE/Silicone Caps (pkg. 100)	<a href="#">N9300701</a>
Syringes	Syringe 1 mL BD Luer-Lok Disposable (pkg. 100)	<a href="#">02542890</a>
Syringe Filters	0.45 µm Nylon Filter, 13 mm Diameter	<a href="#">02542903</a>
PEEK Fittings	Finger-tight for 1/16" OD PEEK Tubing	<a href="#">09920513</a>
Stainless Steel Fittings	OptiTech Reusable Nut/Ferrule for UHPLC	<a href="#">N9306301</a>