APPLICATION BRIEF

Liquid Chromatography

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HPLC Analysis of Betamethasone Dipropionate Using an Epic C18 Column in Accordance with the United States Pharmacopeia

Glucocorticoid steroids work by suppressing various aspects of the human immune system in conditions where hyperactivity can cause poor health through allergies, inflammation, and autoimmune dysfunction. Betamethasone dipropionate belongs to this class of steroids. It is used for its high potency as an anti-inflammatory and immunosuppressant in the treatment of diseases such as eczema, dermatitis, and psoriasis. Betamethasone dipropionate is classified as a 'super-potent' steroid in the treatment of psoriasis in comparison with betamethasone valerate (another common analogue of betamethasone) which is rated as upper mid-strength.[1]

This application brief describes the use of an Epic™ C18 column for the analysis of betamethasone dipropionate (Figure 1) in accordance with the official USP monograph.[2]

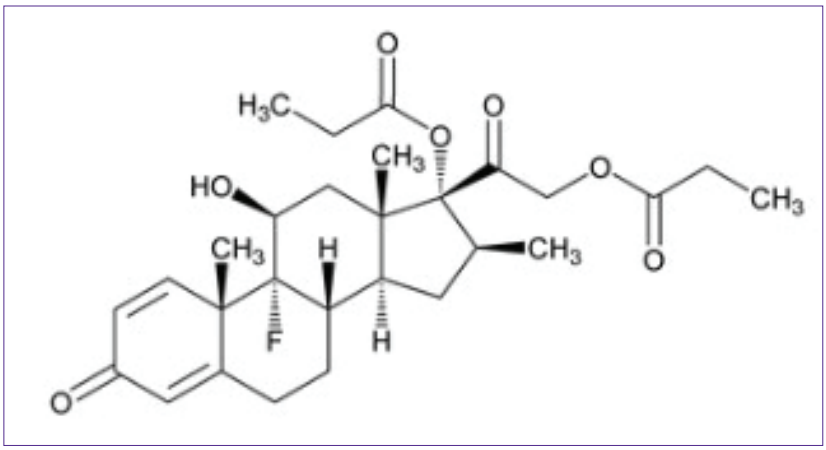

Figure 1. Structure of betamethasone dipropionate.

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Experimental Conditions

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC method parameters.

Instrument	PerkinElmer LC 300 HPLC System with LC 300 Multi-wavelength UV/Vis (MWD) Detector
Column	Epic C18 250 x 4.6 mm, 5 µm (P/N: 155291-EC18)
Mobile Phase	A: Acetonitrile B: Water **A**: 65% **B**:35%
Flow Rate	1.0 mL/min
Temp	23 °C
Wavelength	254 nm
Injection Volume	10 µL
Analyte	Betamethasone dipropionate

Solvents and Samples

All solvents were HPLC grade and samples were filtered using a 0.45 µm PTFE filter, P/N 02542909.

Two stock solutions were prepared in acetic acid and methanol (1 in 1,000) using USP betamethasone dipropionate (0.6 mg/mL) and USP beclomethasone dipropionate (0.9 mg/mL) as an internal standard. The standard solution was prepared by combining the two stock solutions, in equal parts as specified by the USP monograph, to concentrations of 0.3 mg/mL and 0.45 mg/mL respectively.

Results and Discussion

The USP method estimates the elution times of betamethasone and beclomethasone to be 14 and 18 minutes respectively under the specified conditions, using a column with L1 packing (300 mm x 4.0 mm). Betamethasone dipropionate was successfully analysed with its internal standard (beclomethasone dipropionate) in under 16 minutes using the Epic C18 (250 x 4.6 mm, 5 µm) column (P/N: 155291-EC18) as demonstrated in Figure 2. The change in column length and internal diameter from that stated in the monograph is within the allowed adjustments according to the USP <621> allowed changes. Mobile phase adjustments were also within the USP allowed adjustments.[3] The USP allows the minor components in the mobile phase to be altered by ±30% relative, while the change in any component cannot exceed 10% absolute. The change in water from 33% specified in the monograph, to 35% used in this application, is well within the 10% allowed change.

The Epic C18 is ideally suited to the analysis of small molecules, such as betamethasone, whilst providing excellent efficiency and peak shape. This is due to the superior base deactivation and high-density bonding technology of Epic which minimizes unwanted silanol interactions. As specified by the USP, tailing factor was calculated at 5% peak height and gave a value of 1.00. The results are summarised in Table 2.

The USP method requires the lowest and highest peak area ratios of three successive injections agree within 2.0%. The peak area ratios between betamethasone and its internal standard, beclomethasone, were calculated for each replicate. The difference between the highest and lowest ratio was 0.21%, which is significantly less than required by the USP.

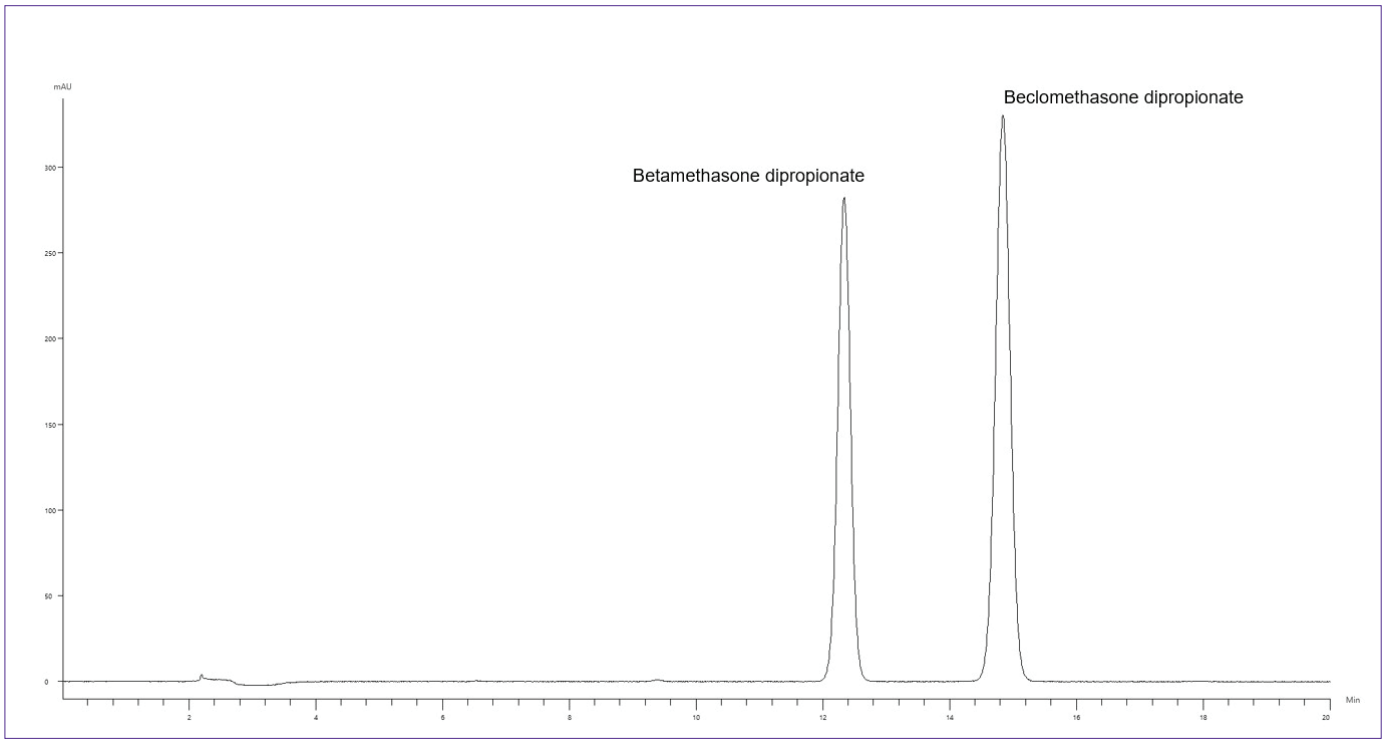

Figure 2. Analysis of betamethasone with beclomethasone internal standard.

Table 2. Results Summary.

Analyte	Retention Time (min)	Tailing Factor	Column Efficiency (N/m)	Peak Area (mAU*sec)
Betamethasone Dipropionate	12.60	1.00	72580	3962.3
Beclomethasone Dipropionate	15.11	1.01	74623	5390.9

Table 3. Peak Area Ratio Summary.

Peak Area Ratios (%)	
Epic C18 Column	USP Requirement
0.21	≤ 2.0

Conclusion

- The Epic C18 phase offers a repeatable and efficient separation of betamethasone dipropionate, well within USP requirements.
- The superior base deactivation and high-density bonding technology of the Epic C18 phase yields excellent peak shape.
- Run time could be reduced by using a shorter Epic C18 column.

References

1. National Psoriasis Foundation potency chart, https://www.psoriasis.org/about-psoriasis/treatments/topicals/steroids/potency-chart (accessed 22/01/2020)
2. Betamethasone Dipropionate, USP 35-NF30, United States Pharmacopeia, 2340-2341
3. USP <621>, https://online.uspnf.com/uspnf/document/1_GUID-6C3DF8B8-D12E-4253-A0E7-6855670CDB7B_1_en-US?source=Search%20Results&highlight=621, (accessed 14/05/21)

Consumables

Component	Description	Part Number
Column	Epic C18 (250 x 4.6 mm, 5 µm)	155291-EC18
HPLC Vials	2 mL, 8 mm Screw Top Vial with Write-on Patch and Fill Lines (100/pack)	N9307802
HPLC Vial Caps	9 mm Screw Top Blue (polypropylene) Cap with PTFE/Silicone pre-slit Septa (100/pack)	N9306203
Syringes	Syringe 1 mL BD Luer-Lok Disposable (100/pack)	02542890
Syringe Filters	0.45 µm PTFE syringe filter	02542909
PEEK Fittings	Finger-tight for 1/16" OD PEEK tubing	09920513
Stainless Steel Fittings	OptiTech Reusable Nut/Ferrule for UHPLC	N9306301

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