APPLICATION BRIEF

Liquid Chromatography

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HPLC Analysis of Betamethasone Dipropionate Using a Quasar C18 Column in Accordance with the United States Pharmacopeia

Introduction

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]

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

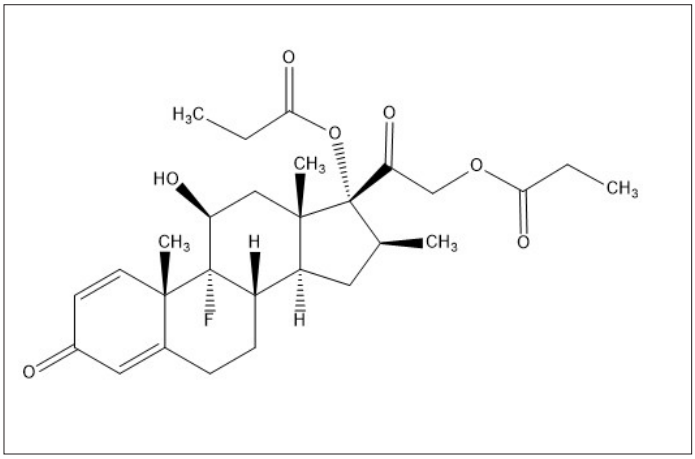

Figure 1. Structure of betamethasone dipropionate.

Experimental Conditions

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC method parameters.

Instrument	PerkinElmer Flexar™ with PDA Plus™ Detector			
Quasar C18	250 mm	4.6 mm	5 µm	N9308801
Mobile Phase	Water: Acetonitrile 35:65			
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 0.45 µm PTFE filter, p/n: 02542909.

Two stock solutions were prepared in acetic acid and methanol (1 in 1000) 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 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 of L1 packing (300 mm x 4.0 mm). Betamethasone dipropionate is successfully analysed with its internal standard (beclomethasone dipropionate) in just under 18 minutes using the Quasar C18 (250 mm, 4.6 mm, 5 µm) column (P/N: N9308801) 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 USP specifications.

The Quasar C18 is ideally suited to the analysis of small molecules, such as betamethasone, whilst providing excellent efficiency and peak shape. This is due to Quasar's optimised ligand bonding technology and ultra-high purity silica base, that minimize unwanted silanol interactions. As specified by the USP, tailing factor was calculated at 5% peak height and gave a value of 1.15. The results are summarised in Table 2.

The USP method requires the betamethasone dipropionate standard to be within 2% of the highest and lowest peak area ratios for three successive injections. 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 0.15% which is significantly less than required by the USP.

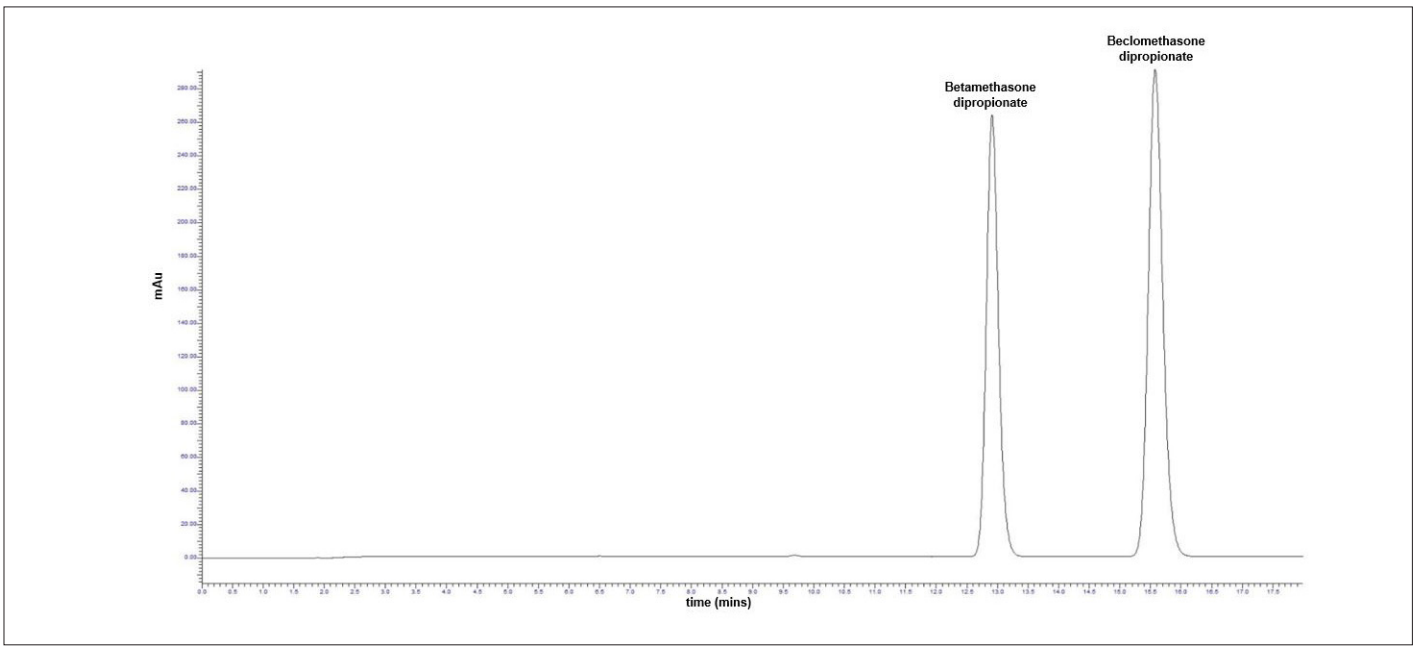

Figure 2. Analysis of betamethasone with beclomethasone internal standard.

Table 2. Results Summary.

Quasar C18 Column	Retention Time Betamethasone (min)	Tailing Factor (5% Height)	Column Efficiency (Plates)	Peak Area (µV*sec)
Betamethasone Dipropionate	12.90	1.15	19987	3644589
Beclomethasone Dipropionate	15.58	1.15	19953	4828423

Table 3. Peak area ratio summary for betamethasone dipropionate and beclomethasone dipropionate standard solution.

Agreement of Highest and Lowest Peak Area Ratios (%)	
Quasar	0.15
USP Requirement	≤ 2.0

Conclusion

- The Quasar C18 HPLC phase offers a repeatable and efficient separation of betamethasone dipropionate, well within USP requirements.
- The ultra-high purity silica base and low residual silanol activity yields excellent peak shape.
- Run time could be reduced by using a shorter Quasar 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.

Consumables

Component	Description	Part Number
Column	Quasar C18 (250 x 4.6 mm, 5 µm)	N9308801
HPLC Vials	2 mL Amber 9 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, Pack of 100	02542890
Syringe Filters	0.45 µm PTFE syringe filter	02542909
PEEK Fittings	Finger-tight for 1/16″ OD PEEK tubing	09920513

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