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USP Method Transfer and UPLC Method for Analysis of Mometasone Furoate Ointment

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Abstract

In this study, a compendial assay method for mometasone furoate 0.1% ointment written in the United States Pharmacopeia (USP) was transferred from HPLC to UPLC using sub-2 µm particle technology with reduced time and narrower peak shape for better chromatography.

Benefits

- 78% reduction in run time
- 94% reduction in mobile-phase consumption and 94% reduction in sample consumption
- Scalability of columns

Introduction

Mometasone furoate ointment is a prescription skin medication that belongs to the group of synthetic corticosteroids and is used to relief inflammation and itching caused by various skin problems, such as dermatitis, psoriasis, and eczema. It is applied directly on the affected areas of the skin and it works by reducing inflammation and suppressing an overactive immune system.[1] Mometasone furoate 0.1% ointment is available in brand and generic forms.

In this study, a compendial assay method for mometasone furoate 0.1% ointment written in the United States Pharmacopeia (USP)[2] was transferred from HPLC to UPLC using sub-2 µm particle technology with reduced time and narrower peak shape for better chromatography. The routine use of the method was conducted over 3000 injections using standard and sample preparations. The goal of transferring methodology to UPLC is to provide cost reductions for manufacturing and improvements in method robustness for routine analysis.

Experimental

HPLC Conditions

LC System:	Alliance HPLC with 2489 UV/Visible detector
Column:	Waters XBridge Shield RP18, 4.6 x 250 mm, 5 µm, part number 186003010
Column Temp.:	25 °C
Sample Temp.:	15 °C
Injection Volume:	20 µL
Flow Rate:	2.0 mL/min
Mobile Phase A:	100% water
Mobile Phase B:	100% acetonitrile
Separation Mode:	Gradient
Wash Solvents:	70:30 water:acetonitrile
Detection:	UV at 254 nm

Time (Minutes)	Solvent A (%)	Solvent B (%)
Initial	70	30
2	70	30
45	45	55
46	70	30
50	70	30

UPLC Conditions

LC System:	ACQUITY UPLC with PDA detector

Column:	ACQUITY UPLC BEH Shield RP18, 2.1 x 75 mm, 1.7 µm, part number 186005605
Column Temp.:	25 °C
Sample Temp.:	15 °C
Injection Volume:	1.3 µL
Flow Rate:	0.55 mL/min
Mobile Phase A:	100% water
Mobile Phase B:	100% acetonitrile
Separation Mode:	Gradient
Weak wash (600 µL):	70:30 water:acetonitrile
Strong wash (200 µL):	5:95 water:acetonitrile
Detection:	UV at 254 nm
Data Management:	Empower 2 CDS

Time (Minutes)	Solvent A (%)	Solvent B (%)
Initial	70	30
0.34	70	30
10.12	45	55
10.34	70	30
11.25	70	30

USP System Suitability Criteria

For 5 replicate injections

- Tailing factor of mometasone furoate peak: Not More Than (NMT) 1.5
- Relative standard deviation (RSD): NMT 2.0%

Sample Description

Internal standard, stock standard, working standard, and sample solutions were prepared as per the assay method defined in the USP Monograph for Mometasone Furoate Ointment.

Internal Standard Preparation

Diethyl phthalate internal standard was prepared in acetonitrile at approximately 1.4 mg/mL concentration.

Stock and Working Standard Preparation

Mometasone furoate stock standard was prepared by dissolving an accurate amount of mometasone furoate reference material in Diluent A (100:1 tetrahydrofuran:acetic acid) to make a solution at approximately 0.2 mg/mL concentration. Internal standard and mometasone furoate stock were diluted with Diluent B (50:50:1 acetonitrile:water:acetic acid) to obtain a working standard of approximately 0.05 mg/mL of mometasone furoate and 0.35 mg/mL of diethyl phthalate, respectively.

Sample Preparation

Mometasone Furoate Ointment USP, 0.1% manufactured by Perrigo was used in this study and prepared according to the USP methodology. Approximately 1 gram of ointment dissolved in Diluent A (100:1 tetrahydrofuran:acetic acid) was mixed with internal standard, diluted with Diluent B (50:50:1 acetonitrile:water:acetic acid), centrifuged, and syringe filtered through 0.2 µm polyprolylene filter prior to chromatographic analysis. The final concentrations were approximately 0.05 mg/mL of mometasone furoate and 0.35 mg/mL of diethyl phthalate, respectively.

Results and Discussion

The USP monograph for mometasone furoate ointment specifies a gradient separation at 2.0 mL/min. Standard and sample solutions are injected with an amount of 20 µL on a column with dimensions of 4.6 mm x 250 mm, 5 µm particle size and L60 packing. Column temperature is maintained at 25 °C and the detection

wavelength is 254 nm for analysis of standard and sample solutions. The USP methodology for mometasone furoate ointment was tested, as written, using Alliance HPLC and appropriately selected Waters column guided by the Waters Reversed-Phase Column Selectivity Chart. The HPLC analysis was performed with a Waters XBridge Shield RP18 column (4.6 x 250 mm, 5 µm), meeting all the system suitability requirements specified in the USP compedial method. See Table 1 for system suitability results acquired using Alliance HPLC system. The HPLC methodology was transferred to UPLC using the Waters ACQUITY UPLC Columns Calculator as described in the Application Note 720003721EN.[3] The resulting method was run on ACQUITY UPLC instrumentation, resulting in narrower peaks and reduced analysis time. The data obtained for the mometasone furoate standard solution acquired using Alliance HPLC and ACQUITY UPLC systems are displayed in Figure 1.

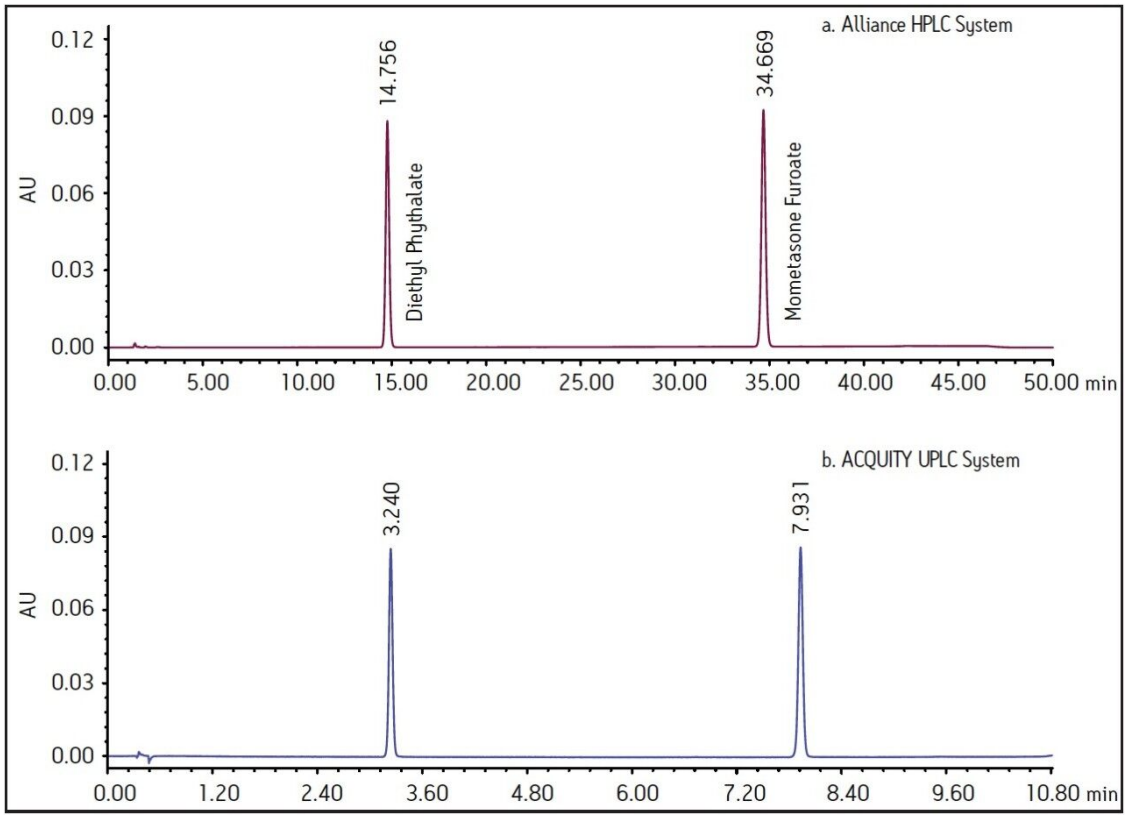

Figure 1. Mometasone furoate standard solution.

a. Acquired using Alliance HPLC system with XBridge Shield RP18, 4.6 x 250 mm, 5 µm column.

b. Acquired using ACQUITY UPLC system with ACQUITY UPLC BEH Shield RP18, 2.1 x 75 mm, 1.7 µm column.

In summary, system suitability requirements for the mometasone furoate ointment on the Alliance HPLC

system and the ACQUITY UPLC system passed the USP criteria for the assay method and are summarized in the Table 1.

Parameter	USP Criteria	Alliance HPLC	ACQUITY UPLC
Tailing Factor	NMT 1.5	1.03	1.01
%RSD	NMT 2.0%	RT: 0.0% Areas: 0.2%	RT: 0.0% Areas: 0.1%

Table 1. System suitability results for five replicate injections of mometasone furoate standard for USP method transfer from Alliance HPLC to the ACQUITY UPLC system.

Routine Use Study

The routine use study of the UPLC assay method for mometasone furoate ointment was conducted after completion of method transfer to demonstrate that the method is suitable for long-term use within a quality control laboratory. The goal of the study was to evaluate the system performance, column behavior and robustness of the transferred method over 3000 injections of standard and ointment preparation solutions.

Three separate sample preparations proved to have good reproducibility of the sample preparation procedure. The sample set consisted of 2 injections of blank and 6 injections of sample solution, bracketed with two replicate injections of a mometasone furoate standard. The data obtained for the mometasone furoate ointment sample solution is displayed in Figure 2. The system suitability parameters for the 5 replicate injections of standard were evaluated throughout the course of the study and are summarized in Table 2. Overall, method performance was excellent and system pressure remained stable over the 3000 injections as displayed in Figure 3. At approximately 2500 injections, some increase in system pressure was observed, however replacing column in-line filter returned pressure to the original value. After an additional 200 injections, column temperature increased due to ambient temperature fluctuations in the laboratory, which then slightly reduced pressure until room temperature was controlled. The ACQUITY UPLC column demonstrated excellent performance for over 3000 injections, which is important for a long-term routine analysis in a quality control laboratory.

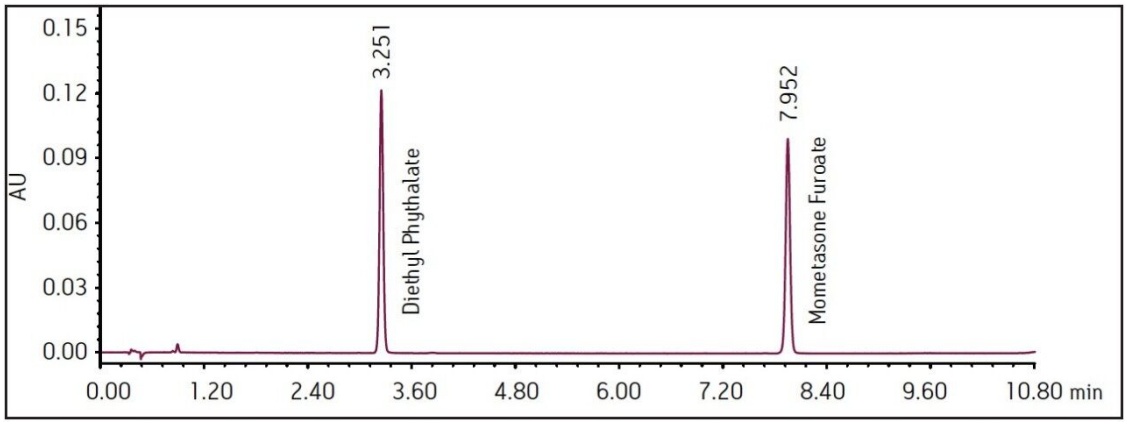

Figure 2. Mometasone furoate ointment sample solution acquired using ACQUITY UPLC system with ACQUITY UPLC BEH Shield RP18, 2.1 x 75 mm, 1.7 µm column.

Parameter	Injection Number					
	~500	~1000	~1500	~2150	~2500	~3000
Tailing Factor	1.02	1.04	1.05	1.05	1.07	1.06
%RSD						
■ RT	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%
■ Area	0.1%	0.2%	0.3%	0.2%	0.4%	0.0%

Table 2. System suitability results for routine use study of over 3000 injections on ACQUITY UPLC BEH Shield RP18 column and ACQUITY UPLC system.

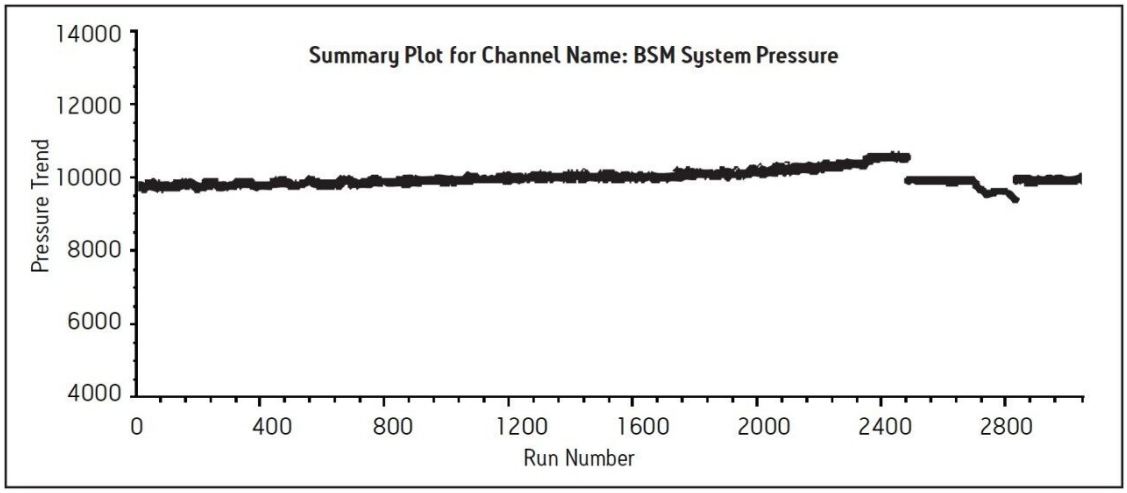

Figure 3. System pressure trend plot for routine use study of over 3000 injections on the ACQUITY UPLC BEH Shield RP18 column and ACQUITY UPLC system.

Conclusion

The transfer of this compendial method proves applicability of successfully transferring existing USP methods from HPLC to UPLC. The resulting UPLC method provides a 78% reduction in run time while maintaining the integrity of the specifications of the USP method. Furthermore, the amount of mobile phase used per injection equals to 6.2 mL compared to 100 mL for HPLC method, which accounts for 94% reduction in mobile-phase consumption per each injection. The other benefit through this transfer is 94% reduction in sample consumption. Overall, the resulting UPLC method provides cost savings for solvent and waste disposal. Moreover, approximately 3000 injections were performed onto the ACQUITY UPLC column with no decrease in method performance and no change in system pressure. The excellent method performance over the course of the study demonstrated that the UPLC assay method for mometasone furoate ointment is applicable for a long-term routine use in a quality control laboratory.

References

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2. USP Monograph, Mometasone Furoate Ointment, USP33-NF28, The United States Pharmacopeia Convention, official October/1/2010 – January/31/2011.

3. Jones M.D., Alden P., Fountain K.J., Aubin A. *Implementation of Methods Translation between Liquid Chromatography Instrumentation*, WatersApplication Note [2010], Part Number 720003721EN.

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