Development and Validation of HPLC method for determination of Methylprednisolone aceponate in cream

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1. Introduction

Methylprednisolone aceponate (MPA) is an active pharmaceutical ingredient (API) used as a potent topical glucocorticoid in the treatment of various types of eczema and psoriasis. Compared to other glucocorticoids, MPA has high efficiency and reduced application (once a day).

The need to develop and validate a method for routine content determination of MPA in MPA cream, arose due to the lack of individual monograph, both for the active ingredient and for the pharmaceutical dosage form, in any of the official editions of different pharmacopoeias.

Therefore, the aim of our study was to develop and validate a simple and rapid reversed-phase HPLC method for routine determination of MPA in MPA cream.

2. Materials and methods

The method was performed using Waters—Alliance HPLC system equipped with quadruple pump, separation module e-2695, and automatic sampler (Waters corporation, USA). The detection wavelength was optimized with Waters 2489 UV/Vis Detector. All data were processed with the Empower® software.

The separation was achieved using the LiChrospher® RP-18 100 mm × 4 mm, 5 µm column, at 40°C, and with isocratic elution. Mobile phase consisted of 55 volumes of acetonitrile (ACN, Fischer Chemical) and 45 volumes of ultra-pure water produced in a laboratory on our department. The flow rate was 1 mL/min and the injection volume was 10 µL. The temperature of the injector was set at 25°C. The run time was 15 minutes, and the detection was performed at 240 nm.

Standard and sample preparation

The standard solution was prepared by dissolving the API, MPA Reference standard (99.4%), in a mixture of equal volumes of ACN and methanol. The working concentration of the standard and the sample solution was 0.1 mg/mL. To extract MPA from the cream, a quantity of the cream containing equivalent of 5 mg MPA, together with the solvent, was added to a volumetric flask and treated on vortex mixer and ultrasonic bath. The solutions were cooled down to room temperature and the rest of the solvent was added. Before the injection in the HPLC system, the standard and sample solutions, were filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter.

Table 2. Summary table of validation parameters

	Parameter	Acceptance criteria	Results
3.1.	**Specificity (selectivity)**	There should be no overlapping between the peaks from the API, the blank and the other substances present in the sample	There are no overlaps between the peaks
3.2.	**Linearity**	$R^2 > 0.9999$	0.9999
	Range	80—120%	80—200%
3.3.	**Precision**		
	System precision	RSD ≤ 2%	0.22
	Intermediate precision	Total RSD < 2%	0.50
		F critical value < 5.05	1.67
	Method precision	RSD ≤ 2%	0.65
3.4.	**Accuracy**	Recovery, 97—103%	97.79—100.77%
		Arithmetic mean of recovery, 98—102%	98.84—99.84%
		RSD ≤ 2%	0.65—2.00%
3.5.	**Robustness**		
	Change of column temperature	RSD ≤ 2%	Meet acceptance criteria
	Change of flow rate	RSD ≤ 2%	Meet acceptance criteria
	Change of mobile phase composition	RSD ≤ 2%	Meet acceptance criteria

3. Results and discussion

3.1. Specificity (selectivity)

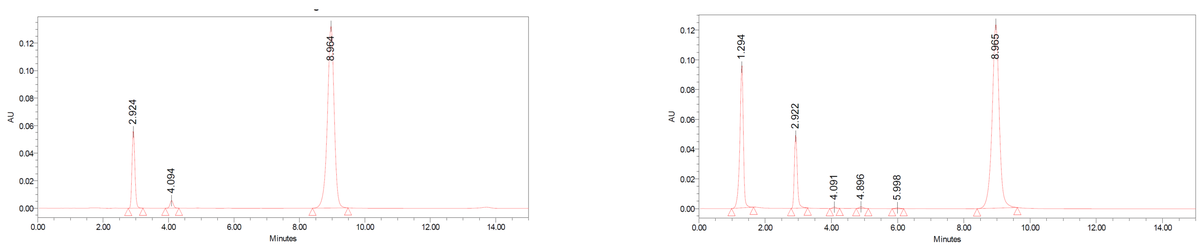

Figure 1. Chromatograms obtained from mixture of standards (left) and sample, MPA cream (right)

3.2. Linearity and range

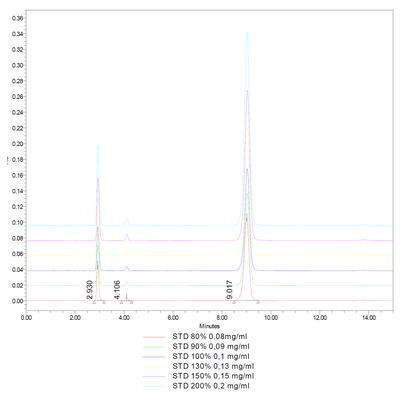

Figure 2. Linearity: Overlay chromatograms of standard solutions ranging from 80—200%

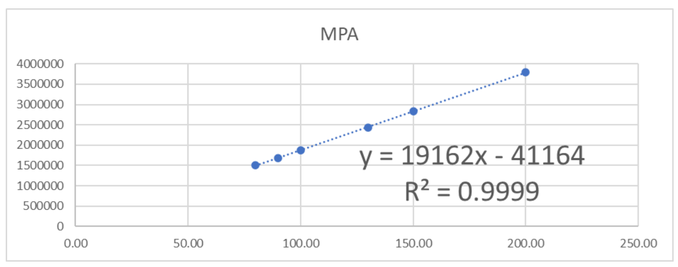

Figure 3. Graphic plot of regression analysis, least square method

3.3. Precision

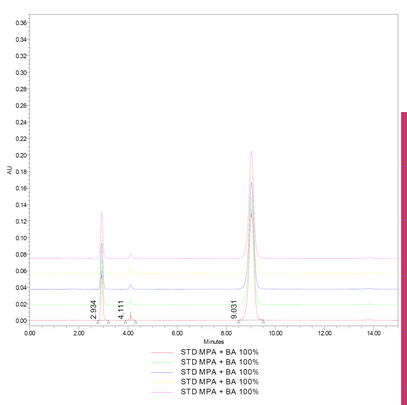

Figure 4. System precision: Overlay chromatograms of six consecutive injections of standard solutions

Table 1. Intermediate precision: Calculation according to F-test

Variance 1st day (SD^2)	0.401
Variance 2nd day (SD^2)	0.240
F (larger variance / smaller variance)	**1.67**
Arithmetic mean (12 samples, 2 days)	98.34
Standard deviation (12 samples, 2 days)	0.598
Total Relative Standard Deviation (12 samples, 2 days)	**0.61**

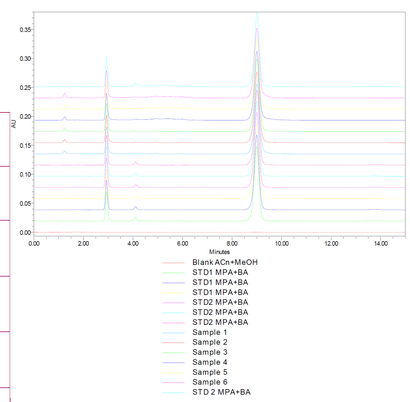

Figure 5. Method precision: Overlay chromatograms of six consecutive injections of standard solutions and samples

3.4. Accuracy

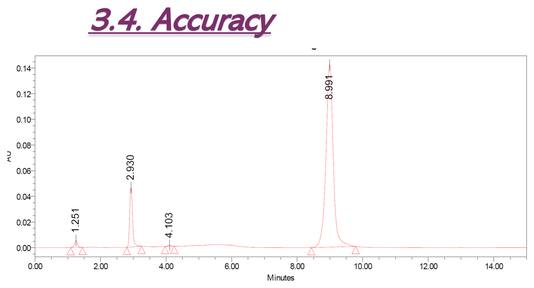

Figure 6. Chromatogram of spiked sample 120%

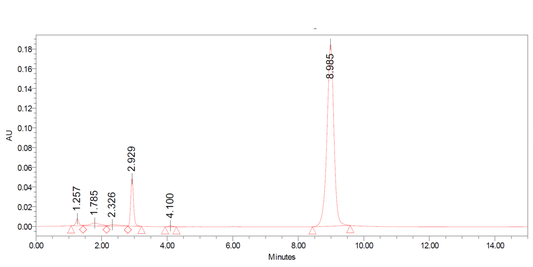

Figure 7. Chromatogram of spiked sample 150%

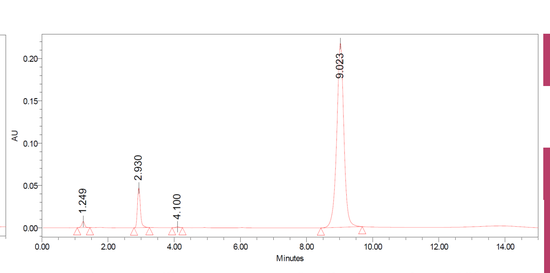

Figure 8. Chromatogram of spiked sample 180%

3.5. Robustness

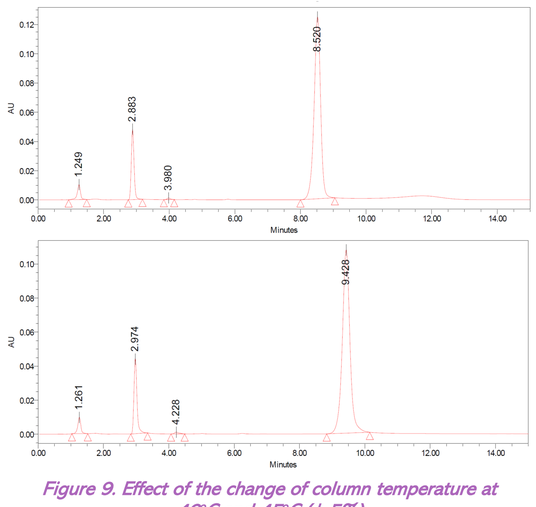

Figure 9. Effect of the change of column temperature at 40°C and 45°C (± 5%)

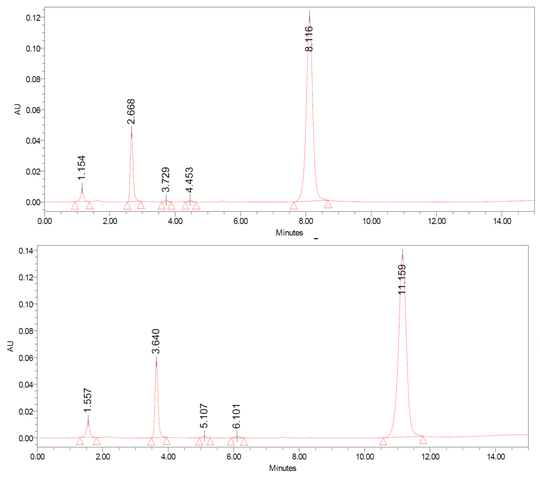

Figure 10. Effect of the change of flow rate (+ 10%, — 20%)

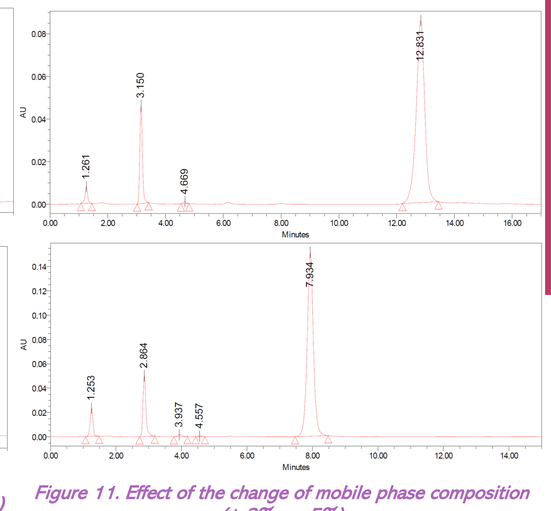

Figure 11. Effect of the change of mobile phase composition (+ 3%, — 5%)

System suitability

System suitability test is designed to evaluate the components of the analytical system to show that the performance of the system meets the standards required by the method.

In our case, the system suitability was evaluated through these parameters: capacity factor, resolution, symmetry factor and selectivity. Each of these test parameters was determined by injecting six consecutive replicas of the standard solution, prepared in the working concentration, and after calculating the arithmetic mean of the results obtained for each test parameter, we concluded that this HPLC system was suitable for content determination of MPA in MPA cream.

4. Conclusion

The validation results show that the method is accurate, precise, robust, selective, and linear in the given range.

It is easily applicable because it does not require complex sample preparation, or special preparation of the working environment.

Due to the easy availability of the organic solvents used as a mobile phase, the method is economically affordable.

This method offers important contribution to scientific knowledge, and it can be routinely used for content determination of MPA in MPA cream.

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