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Short communication

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

Paulina Apostolova[1*], Dino Karpicarov[1*], Marija Arev[1], Emilija Janevik-Ivanovska[1], Biljana Gjorgjeska[1], Zorica Arsova-Sarafinovska[1,2]

[1]*Faculty of Medical Sciences, Department of Pharmacy, Goce Delchev University of Shtip, Krste Misirkov No. 10-A, 2000 Shtip, Republic of North Macedonia*
[2]*Institute of Public Health of Republic of North Macedonia, 50-ta Divizija 6, 1000 Skopje, Republic of North Macedonia*

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) (Ruzicka, 2006).

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 the routine determination of MPA in MPA cream.

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 3® software.

The separation was achieved using the LiChrospher® RP-18 100 mm x 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 2 5ºC. The run time was 15 minutes and the detection was performed at 240 nm.

We used two standards, one for the API – MPA Reference standard (99.4%), and one for the antimicrobial preservative present in the dosage form – Benzyl alcohol, puriss. p.a., ACS reagent, ≥ 99.0%. The standards were purchased from Sigma Aldrich. The commercial cream samples were purchased from a local pharmacy.

Standard and sample preparation

The standard solution was prepared by dissolving the API 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.

Results and discussion

A variety of mobile phases and columns were investigated during the development of the HPLC method for analysis of MPA in MPA cream. The proposed method was defined on a basis of the system suitability test.

*paulina.apostolova@ugd.edu.mk

The method performance was fully validated according to the ICH Q2(R1) Guideline by a determination of accuracy, precision, specificity, linearity, and range.

The impact of the system or method changes on the obtained results, was evaluated through the robustness of the method (ICH Q2(R1), 2019).

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 (USP <1225>, 2018).

Specificity (selectivity)

The specificity of the method was demonstrated with acceptable resolution between the peaks from the API and the antimicrobial preservative (Rs = 13.81), and without any interference from placebo peaks.

Linearity and range

Linearity was determined using six standard solutions with concentrations ranging from 80 – 120% of the working concentration. By processing the data using the least squares method, we obtained the following equation: $y = 19162x - 41164$, and concluded that the method is linear in the given range, with a correlation coefficient of 0.9999 ($R^2 = 0.9999$).

Precision

The precision was determined in terms of system precision, method precision, and intermediate precision. On each level of precision, we calculated the standard deviation (SD) and the relative standard deviation (RSD) of the retention time, area under the curve (AUC), and percentage of content. Based on the obtained results for SD and RSD (RSD ≤ 2%), we concluded that the system precision and method precision meet the acceptance criteria. The intermediate precision was determined using six sample solutions, prepared in the same manner, with the same concentrations, and analyzed under the same conditions, on two different days. Based on the results from the F-test, we concluded that there is less than 5% probability that the difference in the results will be significant. Thus, we confirmed the acceptable intermediate precision of this method.

Accuracy

The accuracy of the method was determined by spiking the sample solution with known amounts of the standard solution, in order to produce three solutions with concentrations of 120%, 150% and 180% of the working concentration. The average recovery was 99.41% (98.84% – 99.84%), which confirmed the method accuracy.

Robustness

The robustness of the method was determined by monitoring the response of the method to the changes we delibrately introduced on the column temperature, flow rate, and composition of the mobile phase. We calculated the plate number and RSD for the tailing of the main peak. Considering that the above mentioned, parameters meet the acceptance criteria (RSD ≤2%), we concluded that the method is robust.

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. Also, 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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