ejpmr, 2018,5(8), 319-324

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANALYTICAL SEPARATION AND CHARACTERISATION OF DEGRADATION PRODUCTS METHOD FOR THE ESTIMATION OF IMPURITIES IN FLUOROMETHOLONE IN PARENTERAL DOSAGE FORM

Mohd Shafi*[1], Dr. Osman Ahmed[1] and Dr. Anas Rasheed[2]

[1]Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Hyderabad.
[2]Chief Scientific Officer, Gaelib Medications Private Limited, Hyderabad.

***Corresponding Author: Mohd Shafi**

Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Hyderabad.

Article Received on 23/05/2018 **Article Revised on 13/06/2018** **Article Accepted on 03/07/2018**

ABSTRACT

A short selective, precise, accurate and sensitive LC-MS/MS^n method was developed for the quantitative determination of process-related impurities and degradation products of Fluorometholone in pharmaceutical parenteral dosage formulations. During the study, the degradation products of Fluorometholone were well-resolved from Fluorometholone and its impurities and the mass balances were found to be satisfactory in all the stress conditions, thus proving capability of the method. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection and quantification, accuracy, precision, ruggedness, and robustness. During the stability analysis of the drug product, three unknown impurities were detected by the above method. The flow rate was 1.5 ml/min and effluent was monitored at 240nm. Retention time was found to be 5.205±0.012 min. The LOD and LOQ values for were found to be 0.3245 (µg/ml) and 0.983 (µg/ml)respectively.

KEYWORDS: Fluorometholone, LC-MS/MS^n, Validation, stability indicating method, degradation products.

1. INTRODUCTION

Fluorometholone, (1R,2S,8S,10S,11S,14R,15S,17S)-14-acetyl-1-fluoro-14,17-dihydroxy-2,8, 15-trimethyltetracyclo[8.7.0.0^2,^{7}.0^{11},^{15}]heptadeca-3,6-dien-5-one (Fig. 1). Fluorometholone glucocorticoid employed, usually as eye drops, in the treatment of allergic and inflammatory conditions of the eye. It has also been used topically in the treatment of various skin disorders. The analytical data are a prerequisite for correct interpretation of any dosage form. The objective of UPLC method development and validation of Fluorometholone in parenteral dosage form procedure is to provide information about potency. The validation of a specific method must be demonstrated through laboratory experiments by routinely analysing samples.

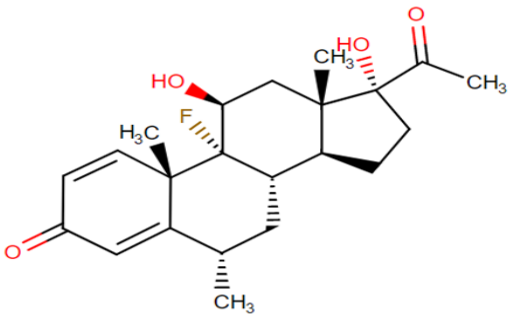

Fig. 1: Structure of Flurometholone.

As per the stringent regulatory requirements recommended by the ICH and regulatory agencies, it is mandatory and important to identify and structurally characterize any impurity formed during production and stability testing, exceeding the identification threshold. Various analytical instruments and advanced hyphenated techniques are routinely used to carry out the impurity profile study.

The present work aims with the developed method to separate the degradation product by preparative UPLC and subjected to ESI-MS/MS. The present study describes the separation of different impurities of Fluorometholone, as well as the development and validation of a RP-UPLC method for the estimation of degradation and process-related impurities of Fluorometholone. These studies were performed in accordance with established ICH guidelines.

2. EXPERIMENTAL

Materials

Flurometholone (99.50% purity) used as analytical standard was procured from Gaelib Medications (Hyderabad). UPLC grade methanol, Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were analytical grade. The

parenteral - dosage (FML Forte 0.5 mg mL−1) labelled to contain 0.5 mg per 1 mL of container for Flurometholone. All chemicals used were of pharmaceutical or special analytical grade.

Instrumentation

Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis. The data was collected using Empower 2 software. The column used was C18 column (250 ×4.6 mm id)—ACE Generix. A Band line sonerex sonicator was used for enhancing dissolution of the compounds. A Bandline sonerex sonicator was used for pH adjustment.

Chromatographic Conditions

Table 1: Chromatographic Conditions of the validating method.

Parameter	Value
Column	C18 column (250 ×4.6 mm id)—ACE Generix
Mobile Phase	Methanol–water (62 : 38 v/v)
Flow rate	1.5 mL/min
Run time	16 Min.
Column Temperature	Maintained at 25°C
Injection volume	20 µL
Detection wavelength	240nm
Diluent	Mobile Phase

Preparation of Standard Stock Solution

Stock standard solution of Fluorometholone (0.5 mg mL−1) was prepared in methanol. Two milliliters were accurately transferred from FML® eye drops to a 50-mL volumetric flask and diluted to the mark with the mobile phase to get 20 µg mL−1 of FLU. The prepared solution was filtered through a 0.45-µm Millipore syringe membrane filter.

Preparation of internal standard solution

Weighed accurately about 10 mg of prednisolone working standard and transfer to 20 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 20 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

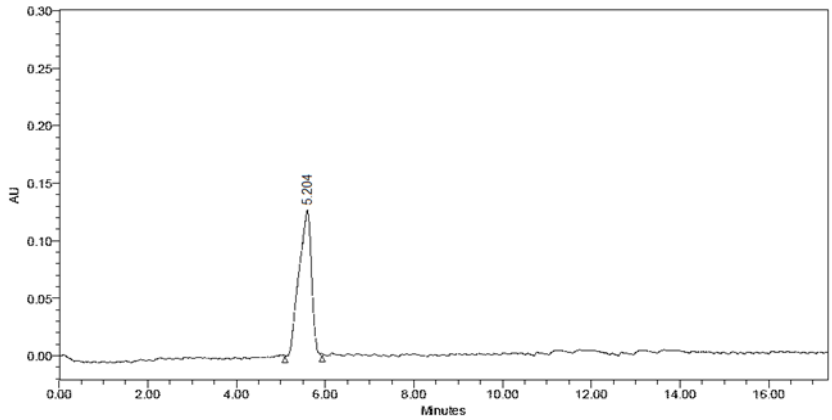

Fig. 2: Optimized chromatogram of Fluorometholone and internal standard using mobile phase of Methanol–water (62: 38 v/v).

3. RESULTS AND DISCUSSIONS

Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

Linearity and Range

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample. The calibration curve showed good linearity in the range of 20-100 µg/mL, for Fluorometholone with correlation coefficient of 0.9964. A typical calibration curve has the regression equation of y = 336.51x + 1492.375 for Fluorometholone. Results are given in Table 2.

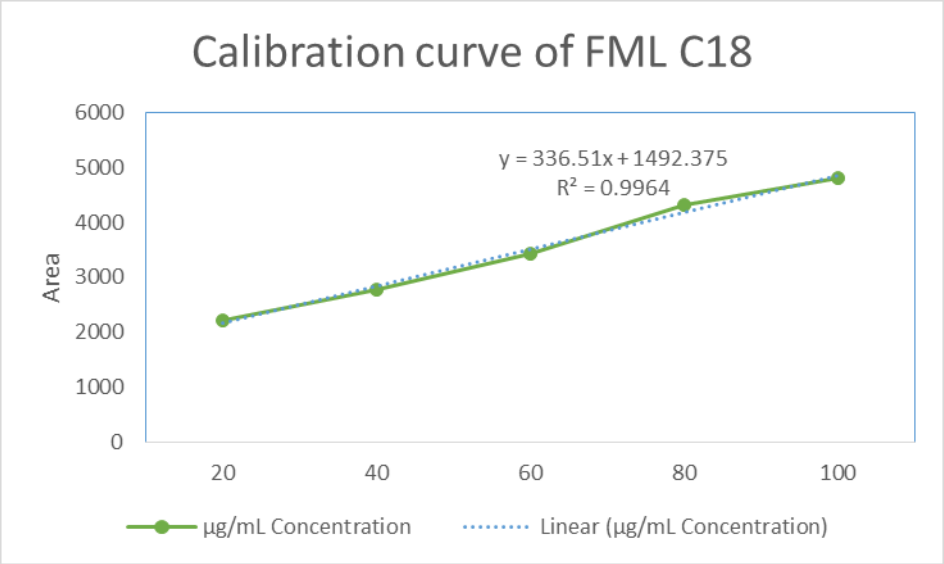

Fig. 3: Calibration curve of Fluorometholone.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Fluorometholone were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD of Fluorometholone was found to be 0.3245 (µg/ml) and the LOQ of Fluorometholone was found to be 0.983 (µg/ml). Results are given in Table 2.

Table 2: Summary of validation parameters for the proposed method.

PARAMETER	FLUOROMETHOLONE
Linearity	20 – 100 µg/ml
Intercept (c)	1492.375
Slope (m)	336.51
Correlation coefficient	0.9964
LOD	0.3245 (µg/ml)
LOQ	0.983 (µg/ml)

Precision

The Precision of the method was studied in terms of intraday and interday precision of sample injections (20 µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.11%. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.09%. Low % RSD values indicate that the method is precise. The results are given in table 3.

Accuracy

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

Robustness

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favour of (% RSD < 2%) the developed UPLC method for the analysis of Fluorometholone. The results are given in table 5.

Table 3: Results of Precision Studies.

Replicate	*FML*		
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	20	2246.72	98.97%
2		2247.29	98.95%
3		2252.84	98.97%
4		2256.87	98.92%
5		2266.89	98.92%
6		2271.16	98.91%
Average			99.94%
Std.Dev			0.0268
% RSD			0.03%
Standard weight			20 mcg
Standard potency			98.00 %

Table 4: Results of accuracy study.

FML						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	10.11	10.07	99.60	99.65%	0.0924	0.08%
100	20.30	20.25	99.75			
150	30.54	30.52	99.77			

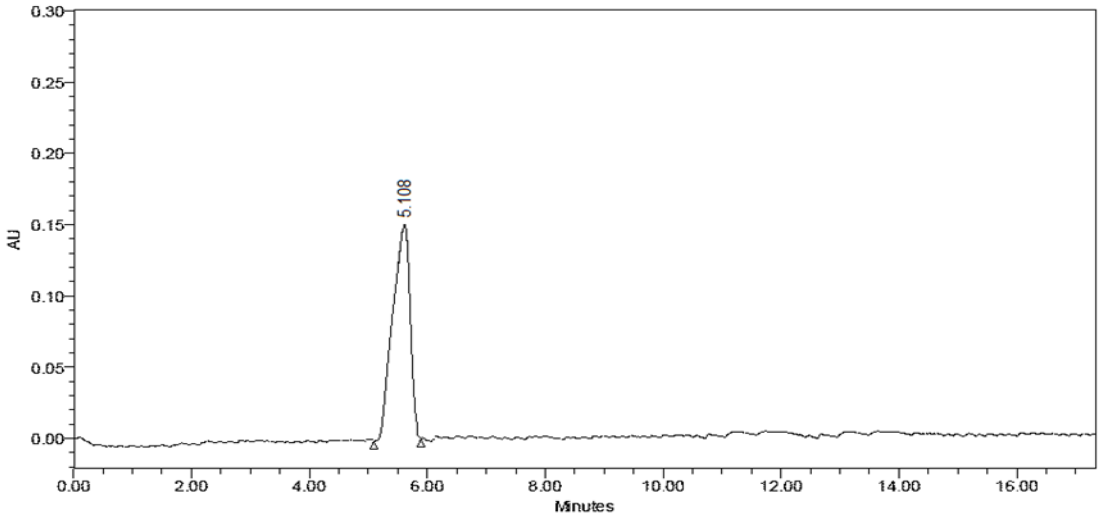

Fig. 4: Chromatogram Showing accuracy results.

Table 5: Results of Robustness Studies.

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	2247.41	0.01%
	Actual	2246.95	
	Plus	2246.87	
Temperature	**Low**	2248.33	0.04%
	Actual	2247.98	
	Plus	2246.59	
Wavelength	**Low**	2248.24	0.02%
	Actual	2247.85	
	Plus	2247.53	

Mass Spectrometry Conditions for MS/MS

The samples (5 µL) is injected directly into the source by the flow injection method using Acetonitrile with buffer 0.5M potassium dihydrogen orthophosphate (35: 65, v/v) as mobile phase at a flow rate of 0.3 mL/min. The mass spectra were recorded in ESI positive mode. Ultra-high purity nitrogen and helium were used as curtain and collision gas, respectively. The typical ion source conditions were: nebulizer gas, 60 psi; dry temperature, 325˚C; dry gas, 5.0 mL/min; capillary voltage, 5kV; capillary current, 80.243 nA; vapourizer temperature, 400°C; dwell time, 200 ms. For the collision-induced dissociation (CID) experiments, the precursor ion was selected using the quadrapole analyzer and product ions were analyzed by the time-of-flight analyzer. HRMS data acquisition was performed by the following source conditions: capillary voltage, 5 kV; declustering potential (DP) and collision energy (CE) were −60 V and −10 V, respectively; focusing potential, 220 V; resolution 40,000 (FWHM).”

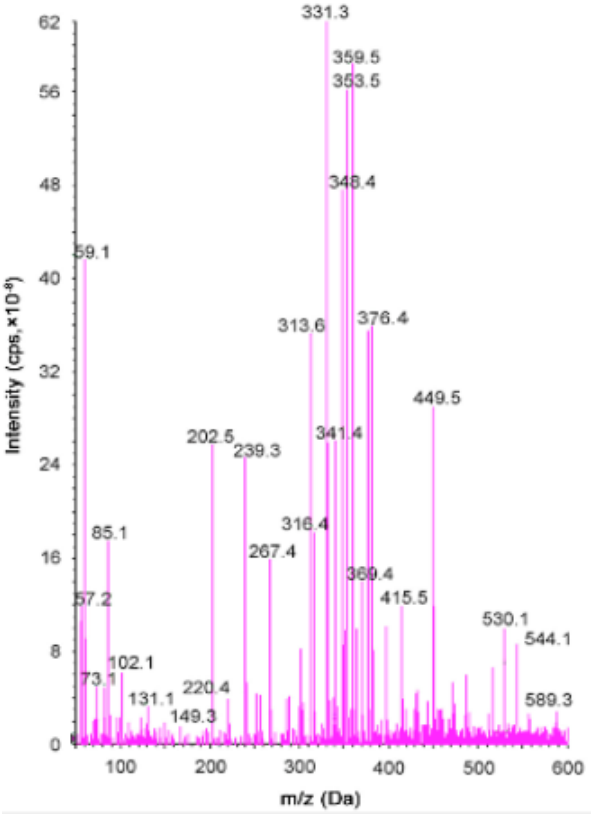

Fig. 5 MS/MS^n characterisation of impurities.

Elemental compositions of FML in MS/MS spectra

Table No. 6: Compositions in MS/MS spectra.

Analyte	Observed ion mass (Da)	Proposed formula	Calculated mass (Da)	Error (ppm)
FML	376.42	$C_{22}H_{29}FO_4$	376.37	0.015
	415.54	$C_{24}H_{32}FO_4$	415.42	0.021
	449.51	$C_{26}H_{33}FO_4$	449.47	0.026
	530.18	$C_{28}H_{35}FO_4$	530.07	0.010
	544.13	$C_{30}H_{36}FO_4$	544.09	0.056
	589.37	$C_{32}H_{36}FO_4$	589.26	0.015

4. CONCLUSION

This research paper describes the separation and characterization of impurities in Fluorometholone pharmaceutical parenteral dosage formulations. The impurities were isolated by preparative liquid chromatography and characterized by using spectroscopic techniques. A simple and efficient RP-UPLC method development and validation were discussed. The degradated products were formed during the study and was well-resolved from Fluorometholone by the proposed RP-UPLC method. The proposed structure of Fluorometholone was characterized by MS/MS^n analysis and was further confirmed to be accurate mass measurements.

ACKNOWLEDGEMENT

The authors would like to acknowledge Gaelib Medications Private Limited (Hyderabad, TS) for their constant support and help throughout our research study.

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