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VALIDATION OF A UPLC METHOD WITH DIODE ARRAY DETECTION USING C_{18} COLUMN FOR THE DETERMINATION OF FLUOROMETHOLONE IN PARENTERAL DOSAGE FORM

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Abstract:
A selective, precise, accurate UPLC method is validated for estimation of Fluorometholone in parenteral dosage form. The method employed, with C18 column (250 ×4.6 mm id)—ACE Generix in gradient mode, with mobile phase of Methanol–water (62 : 38 v/v). The flow rate was 1.5 ml/min and effluent was monitored at 240nm. Retention time was found to be 5.05±0.03 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20- 100µg/ml respectively. The LOD and LOQ values for were found to be 0.3245 (µg/ml) and 0.983 (µg/ml) respectively. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of Fluorometholone in parenteral dosage form.

Keywords: *Fluorometholone, UPLC, Validation, parenteral dosage form.*

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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.

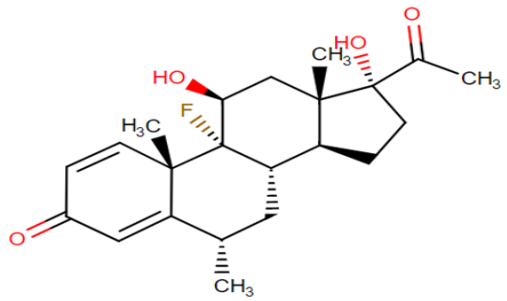

Fig. 1: Structure of **Flurometholone**

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.

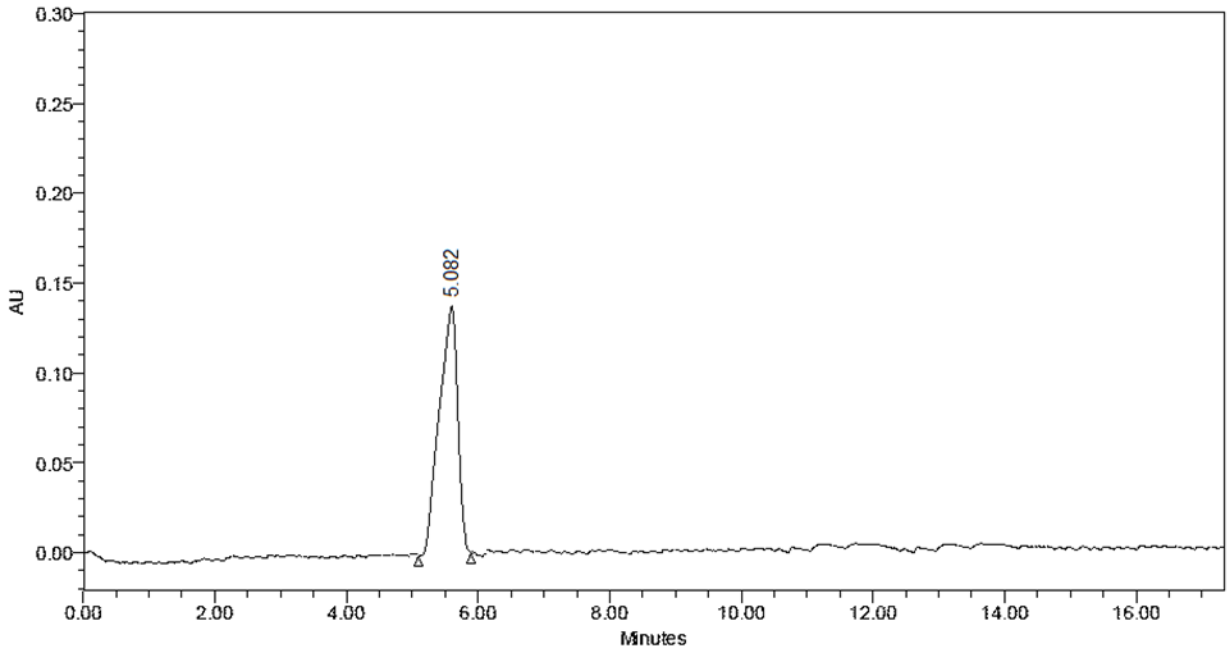

Fig. 2: Optimized chromatogram of Fluorometholone and internal standard using mobile phase of Methanol–water (62 : 38 v/v)

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.

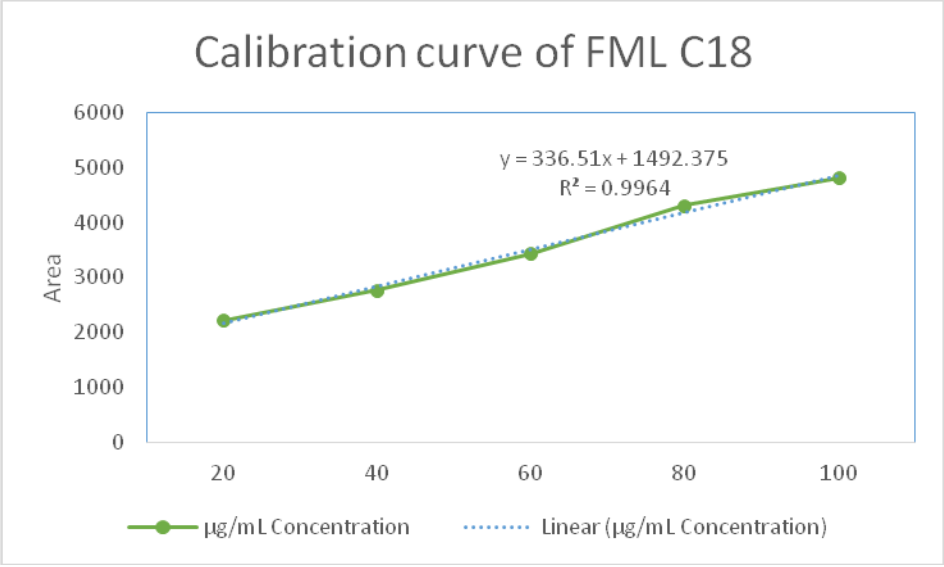

Fig. 3: Calibration curve of Fluorometholone

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)

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.

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 C18***		
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	20	2226.72	98.97%
2		2227.29	98.95%
3		2232.84	98.97%
4		2236.87	98.92%
5		2246.89	98.92%
6		2251.16	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 C18						
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			

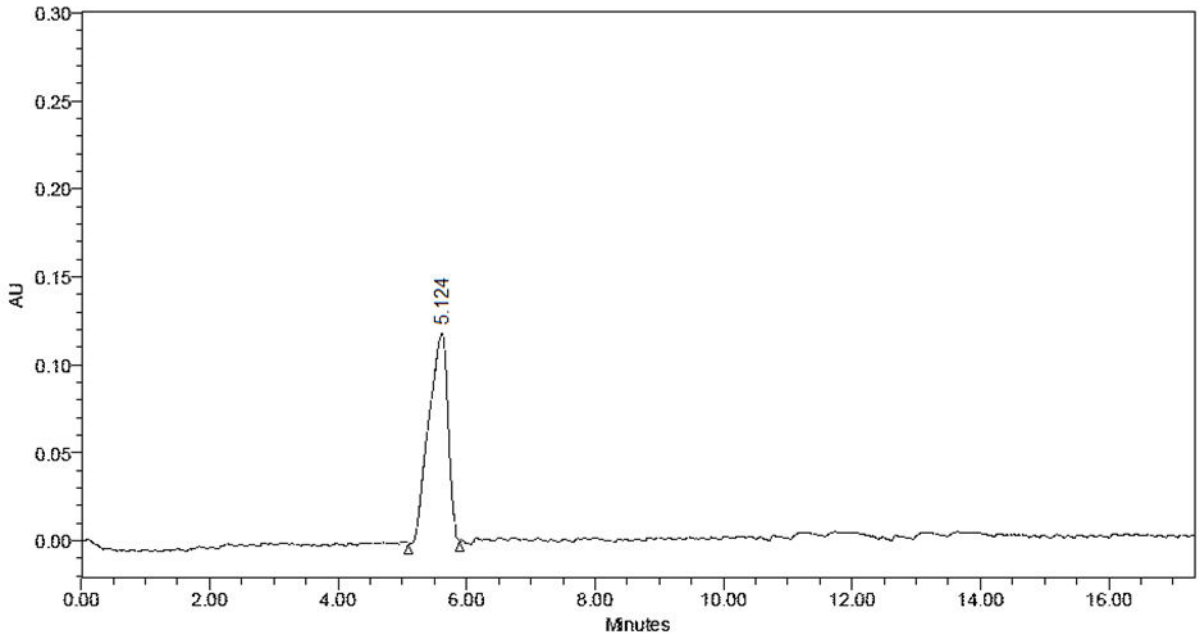

Fig. 4: Chromatogram Showing accuracy results

Table: 5, Results of Robustness Studies

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	2237.41	0.01%
	Actual	2236.95	
	Plus	2236.87	
Temperature	**Low**	2238.33	0.04%
	Actual	2237.98	
	Plus	2236.59	
Wavelength	**Low**	2238.24	0.02%
	Actual	2237.85	
	Plus	2237.53	

ANALYSIS OF FORMULATION

Assay studies for the analysis of parenteral - dosage formulation of Fluorometholone. Fixed chromatographic conditions were made use for the analysis of formulation and was found to be 98.412%.

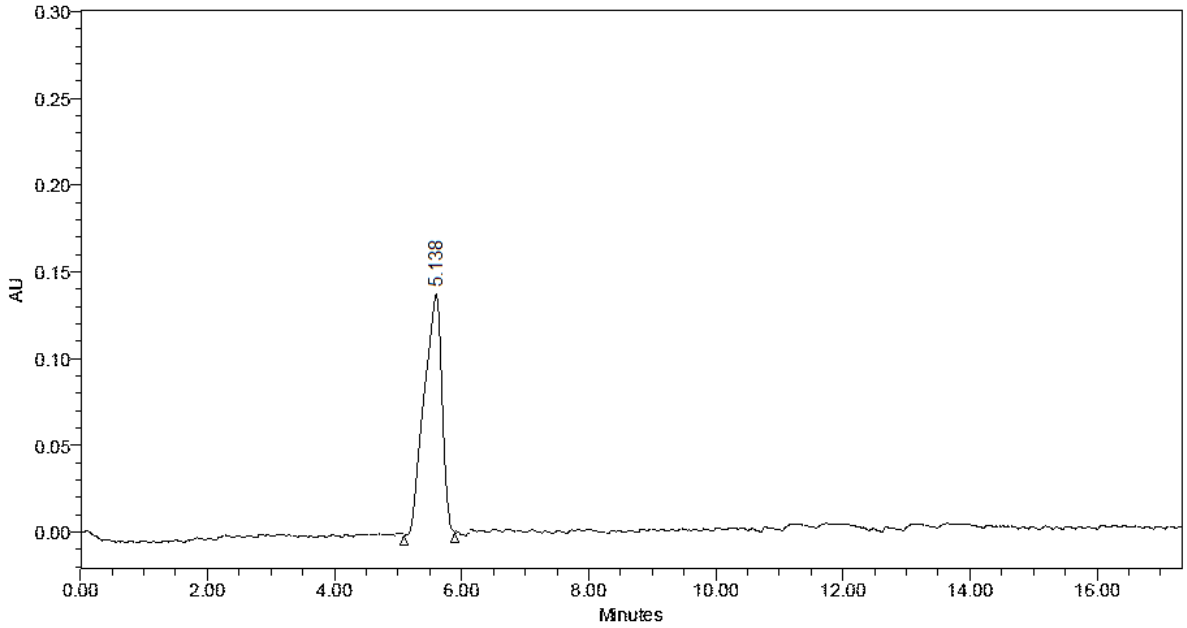

Fig. 5: Chromatogram of Assay Studies

4. CONCLUSION:

The method provides selective quantification of Fluorometholone without interference from blank affirming precise method. The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters.

The developed method was robust in the separation and quantification of Fluorometholone in parenteral dose. This method can be used for the routine analysis of production samples. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies. The current method is validated for the assay study of the formulation and was found to be satisfactory.

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