

VALIDATION OF A FORCED DEGRADATION UPLC METHOD USING C₈ COLUMN FOR FLUOROMETHOLONE IN PARENTERAL DOSAGE FORMMohd Shafi*¹, Dr. Osman Ahmed¹ and Dr. Anas Rasheed²¹Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Hyderabad.²Chief Scientific Officer, Gaelib Medications Private Limited, Hyderabad.***Corresponding Author: Mohd Shafi**

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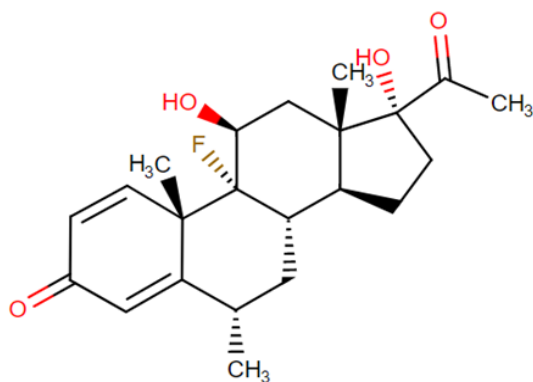
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ABSTRACT

A specific, precise, accurate and stability indicating UPLC method is validated for estimation of Fluorometholone in parenteral dosage form. The method employed, with C₈ column (250 ×4.6 mm id)—ACE Generix in gradient mode, with mobile phase of Acetonitrile with buffer 0.5M potassium dihydrogen orthophosphate (35: 65% v/v). The flow rate was 2 ml/min and effluent was monitored at 215nm. Retention time was found to be 5.167±0.015 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 0.3156 (µg/ml) and 0.95662(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, stability indicating method.**1. INTRODUCTION**

Fluorometholone, (1R,2S,8S,10S,11S,14R,15S,17S)-14-acetyl-1-fluoro-14,17-dihydroxy-2,8,15-trimethyltetracyclo[8.7.0.0.2⁷.0^{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.

**Fig. 1: Structure of Fluorometholone.**

A detailed literature revealed that several analytical methods have been reported for the determination of Fluorometholone in pharmaceutical parenteral dosage forms. In our present knowledge, there is no method reported for the estimation forced degradation studies of Fluorometholone using C₈ column in pharmaceutical parenteral dosage form by UPLC.

Hence, the aim was to develop a specific, rapid, sensitive, and accurate UPLC method which can estimate the forced degradation studies of Fluorometholone.

The present work aims with the developed method, only this mobile phase is sufficient for quantification of degraded products under stress.

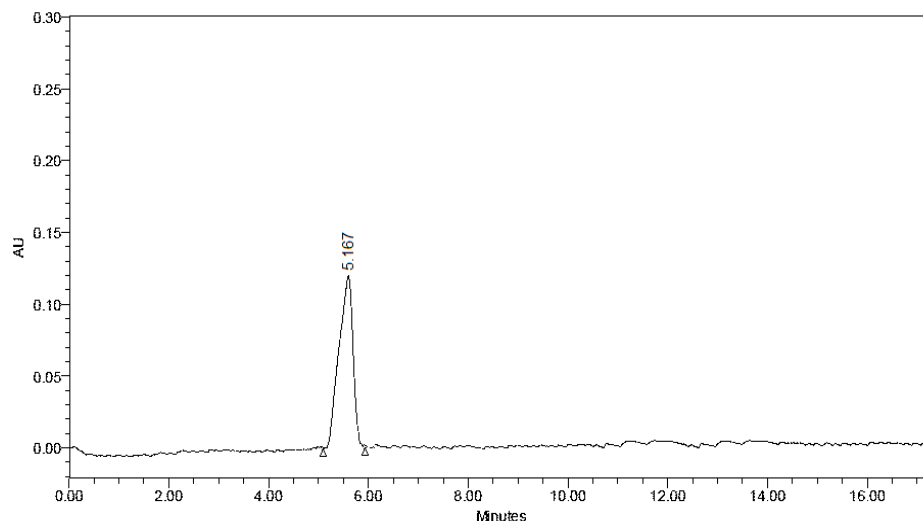


Fig. 2: Optimized chromatogram of Fluorometholone and internal standard using mobile phase of Acetonitrile with buffer 0.5M potassium dihydrogen orthophosphate (35: 65%v/v).

2. EXPERIMENTAL

Materials

Fluorometholone (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 Fluorometholone. All chemicals used were of pharmaceutical or special analytical grade.

Chromatographic Conditions

Table 1: Chromatographic Conditions of the validating method.

Parameter	Value
Column	C8 column (250 \times 4.6 mm id)—ACE Generix
Mobile Phase	Acetonitrile with buffer 0.5M potassium dihydrogen orthophosphate (35: 65, v/v)
Flow rate	2.0 ml/min
Run time	16 Min.
Column Temperature	Maintained at 25°C
Injection volume	20 μL
Detection wavelength	215 nm
Diluent	Mobile Phase

Preparation of Standard Stock Solution

Stock standard solution of Fluorometholone (0.5 mg mL⁻¹) was prepared in methanol. Four milliliters were accurately transferred from FML® eye drops to a 100-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 20 mg of prednisolone working standard and transfer to 40 ml volumetric flask,

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 C8 column (250 \times 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.

add 100 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 μm membrane filter.

Stability Indicating Studies

Stability Indicating studies like acid hydrolysis, basic hydrolysis, dry heat degradation, wet heat degradation and oxidative degradation were carried out.

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 $\mu\text{g/mL}$, for Fluorometholone with correlation coefficient of 0.9958. A typical calibration curve has the regression equation of $y = 346.32x + 1497.233$ for Fluorometholone. Results are given in Table 2.

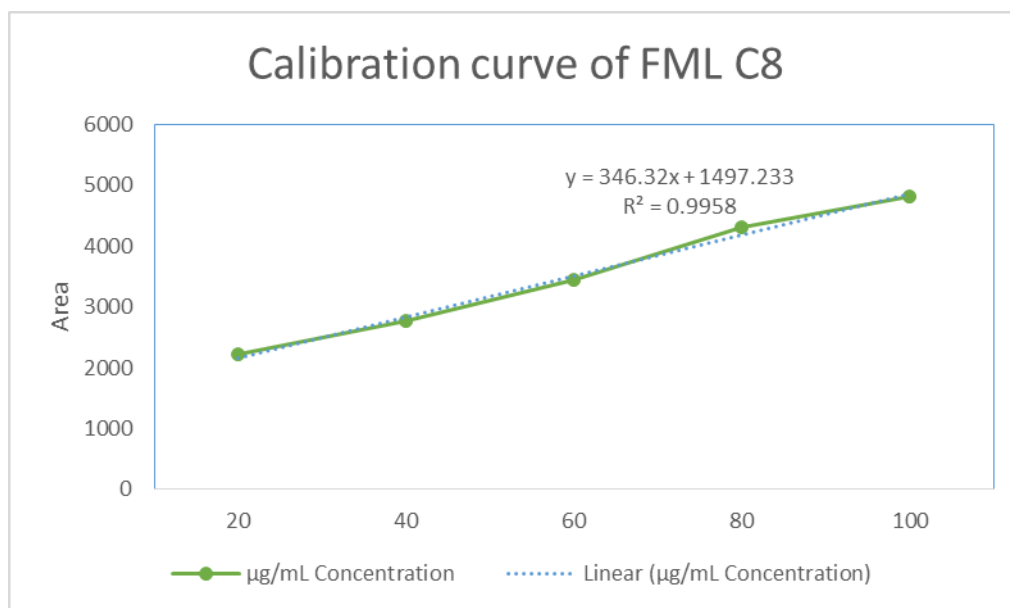


Fig. 3: Calibration curve of Fluorometholone.

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

The LOD and LOQ of Fluorometholone were calculated by mathematical equation. $\text{LOD} = 3.3 \times \text{standard deviation}$

$\div \text{slope}$ and $\text{LOQ} = 10 \times \text{standard deviation} \div \text{slope}$. The LOD of Fluorometholone was found to be 0.3156 ($\mu\text{g/ml}$) and the LOQ of Fluorometholone was found to be 0.95662 ($\mu\text{g/ml}$). Results are given in Table 2.

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

PARAMETER	FLUOROMETHOLONE
Linearity	20 – 100 $\mu\text{g/ml}$
Intercept (c)	1497.233
Slope (m)	346.32
Correlation coefficient	0.9958
LOD	0.3156 ($\mu\text{g/ml}$)
LOQ	0.95662 ($\mu\text{g/ml}$)

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 3.

Precision

The Precision of the method was studied in terms of intraday and interday precision of sample injections (20 $\mu\text{g/ml}$). 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 4.

Robustness

Small deliberate changes in chromatographic conditions such as change in temperature ($\pm 2^\circ\text{C}$), flow rate ($\pm 0.1\text{ml/min}$) and wavelength of detection ($\pm 2\text{nm}$) 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 accuracy study.

FML C8						
Level %	Amount added ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% Recovery	Mean recovery (%)	Std.Dev	% RSD
50	10.05	10.02	99.50	99.55%	0.0914	0.08%
100	20.15	20.05	99.65			
150	30.24	30.12	99.57			

Table 4: Results of Precision Studies.

Replicate	FML C8		
S.No.	Concentration Taken ($\mu\text{g/ml}$)	Area	%LC
1	20	2236.54	98.97%
2		2237.87	98.95%
3		2242.32	98.97%
4		2246.18	98.92%
5		2256.65	98.92%
6		2261.28	98.91%
Average			99.94%
Std.Dev			0.0268
% RSD			0.03%
Standard weight			20 mcg
Standard potency			98.00 %

Table 5: Results of Robustness Studies.

Robustness Studies			
Parameter	Value	Peak Area	% RSD
Flow Rate	Low	2239.38	0.01%
	Actual	2240.87	
	Plus	2243.92	
Temperature	Low	2248.37	0.04%
	Actual	2247.58	
	Plus	2246.79	
Wavelength	Low	2248.44	0.02%
	Actual	2247.88	
	Plus	2247.59	

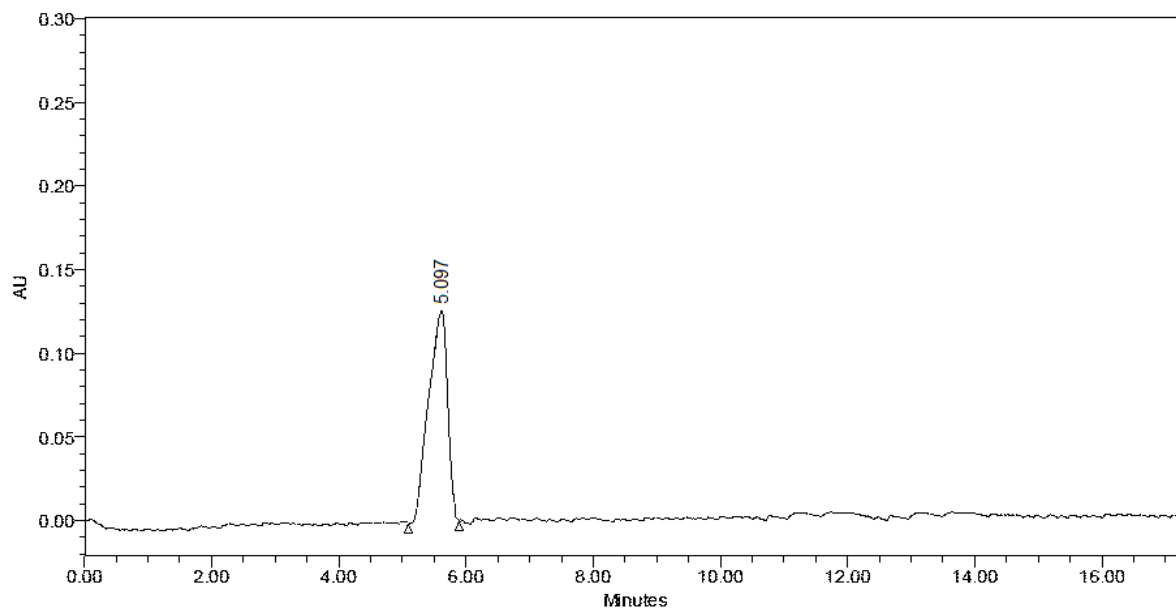


Fig. 4: Chromatogram Showing accuracy results.

Results of Stability Indicating Studies

According to Singh and Bakshi, the stress testing suggests a target degradation of 20-80% for establishing stability indicating nature of the method. UPLC study of samples obtained on stress testing of Fluorometholone under different conditions using mixture Acetonitrile with buffer 0.5M potassium dihydrogen orthophosphate (35: 65, v/v) as a mobile solvent system suggested the following degradation behaviour.

a. Acid hydrolysis

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF).

30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of “Acetonitrile with buffer 0.5M potassium dihydrogen orthophosphate (35: 65, v/v) after optimizing the mobile phase composition, chromatogram was recorded.

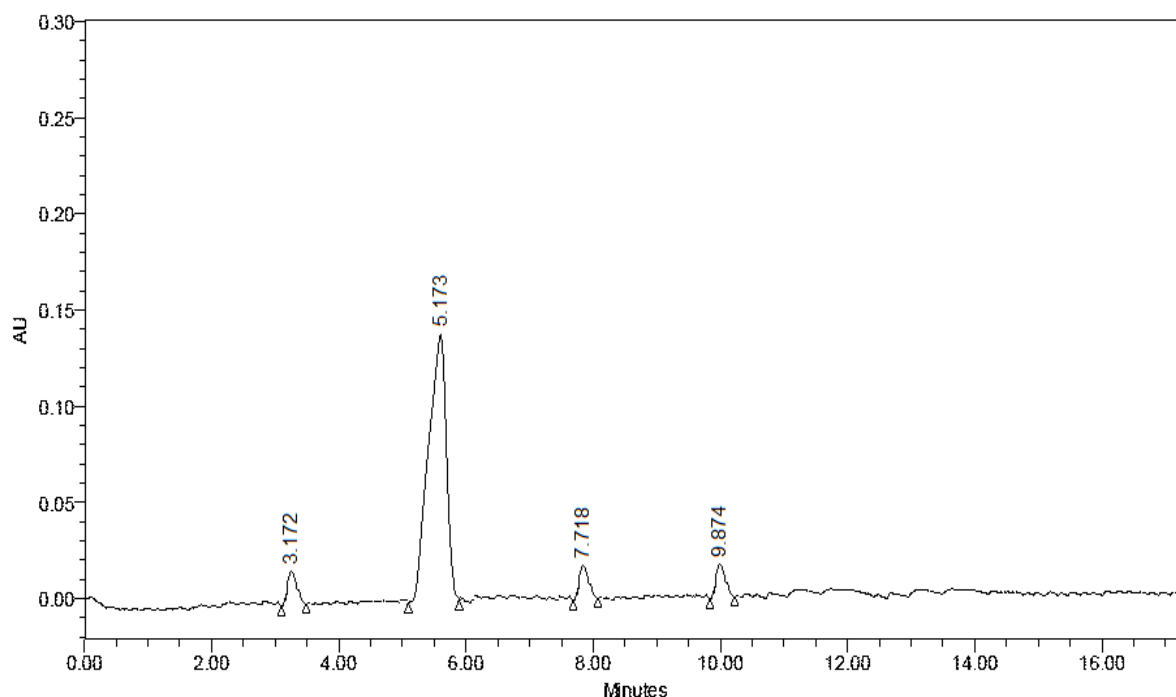


Fig. 5: Chromatogram showing the degraded products in Acidic degradation.

b. Basic hydrolysis

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N

HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of "Acetonitrile with buffer 0.5M potassium dihydrogen orthophosphate (35: 65, v/v) after optimizing the mobile phase composition, chromatogram was recorded.

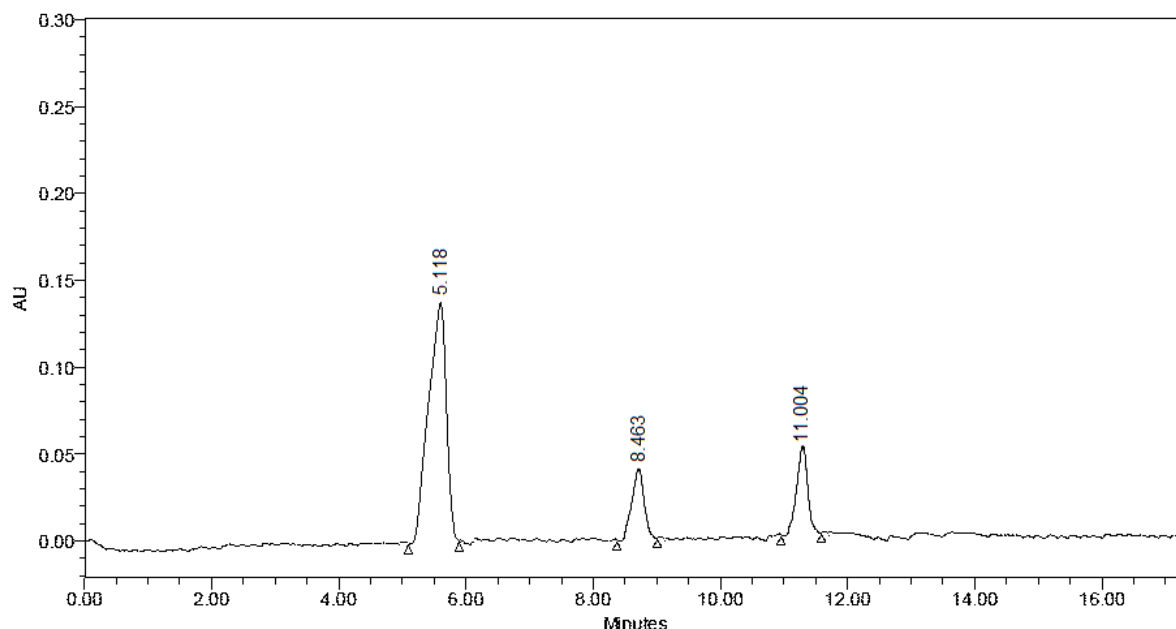


Fig. 6: Chromatogram showing the degraded products in Basic degradation.

c. Wet heat degradation

Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature.

Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of Acetonitrile with buffer 0.5M potassium dihydrogen orthophosphate (35: 65, v/v) after optimizing the mobile phase composition, chromatogram was recorded.

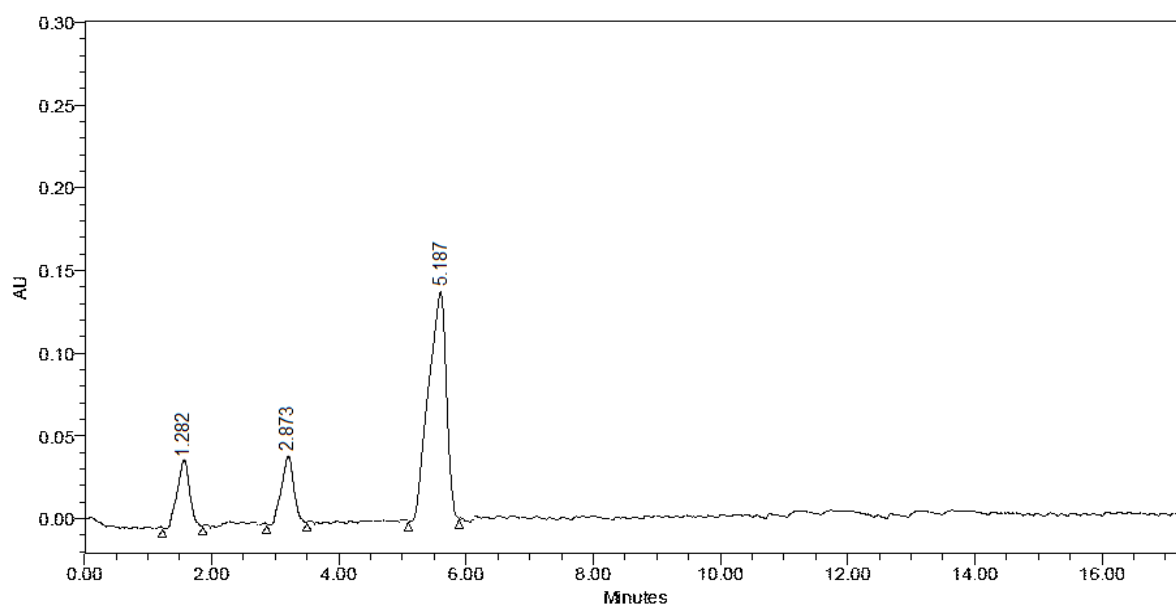


Fig. 7: Chromatogram showing the degraded products in Wet heat degradation.

d. Oxidation with (3%) H₂O₂

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H₂O₂ and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water

to prepare 100 ppm solution. The above sample was injected into the UPLC system.

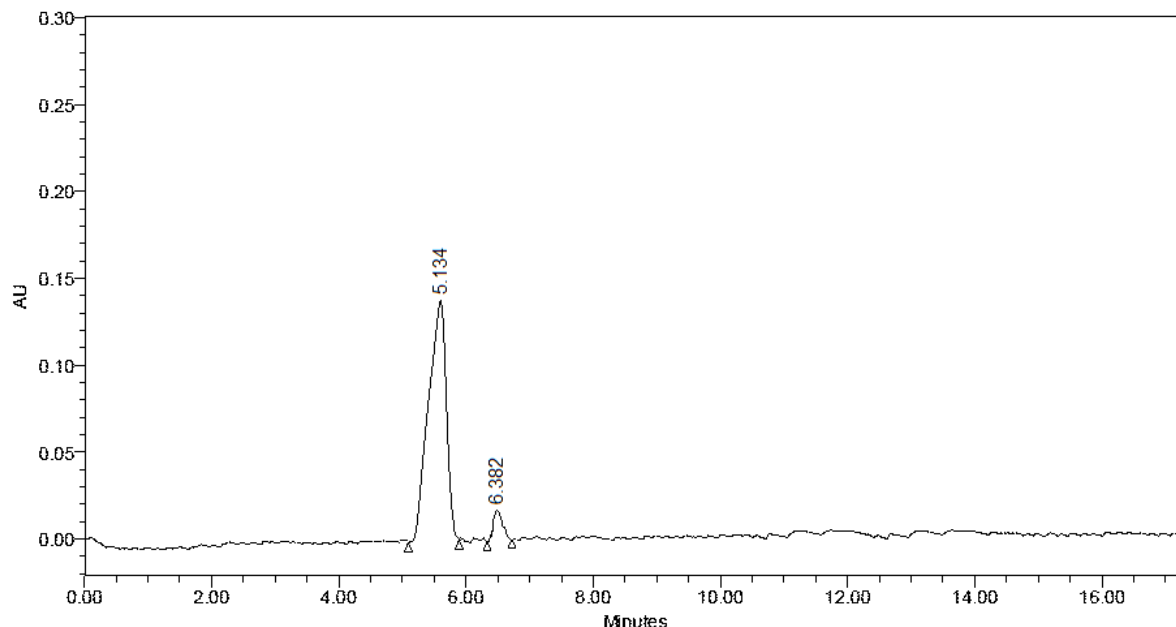


Fig. 8: Chromatogram showing the degraded products in Oxidative degradation.

Table No. 6: Summary of Forced Degradation Studies.

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)
Acidic	60°C	4	3 (3.172, 7.718, 9.874)
Basic	60°C	4	2 (8.463, 11.004)
Oxidative	RT	24	1 (6.382)
Wet Heat	105°C	6	2 (1.282, 2.873)

4. CONCLUSION

A specific and sensitive stability indicating UPLC method has been validated for the analysis of Fluorometholone in parenteral dosage form. Based on peak purity results, obtained from the analysis of stability indicating studying samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Fluorometholone indicated that the developed method is specific for the estimation of Fluorometholone in presence of degradation products. Further the proposed UPLC method has excellent precision, sensitivity and reproducibility. Even though no attempt has been made to identify the degraded products, proposed method can be used as stability indicating method for assay of Fluorometholone in commercial formulations.

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