World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

# ANALYTICAL STABILITY INDICATING UPLC ASSAY AND VALIDATION USING C18 COLUMN FOR 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 04/07/2018**

## ABSTRACT

A pecific, precise, accurate and stability indicating UPLC method is validated for estimation of Fluorometholone in parenteral inhaler 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 5.215±0.005 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, 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$^{2}$,$^{7}$.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.

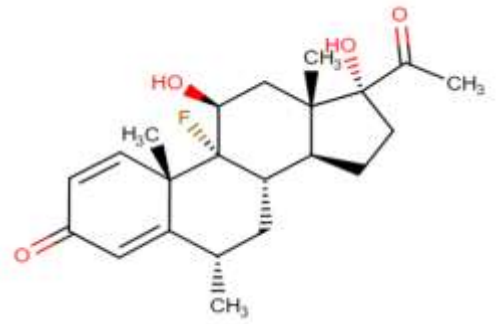


**Fig. 1: Structure of Flurometholone.**

Regulatory agencies recommend the use of stability indicating methods (SIMs) for the analysis of stability samples. This requires stress studies in order to generate the potential related impurities under stressed conditions, method development and validation. With the evident of the International Conference on Harmonization (ICH) guidelines, requirements for the establishment of SIMs have become more clearly mandated.[14] Environmental conditions including light, heat and the susceptibility of the drug product towards hydrolysis or oxidation can play an important role in the production of potential impurities. Stress testing can help identifying degradation products and provide important information about intrinsic stability of the drug product. Therefore, herein we report the results of stability study of Fluorometholone with the aim of determining the extent of the influence of different stress conditions on the stability of the parenteral product.

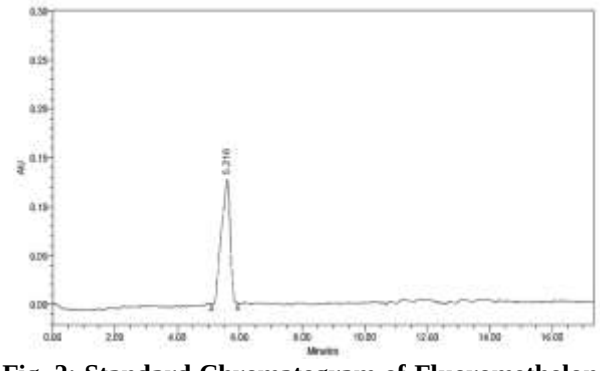

**Fig. 2: Standard Chromatogram of Fluorometholone, using mobile phase of Methanol–water (62: 38 v/v).**

## 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.

### 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 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.

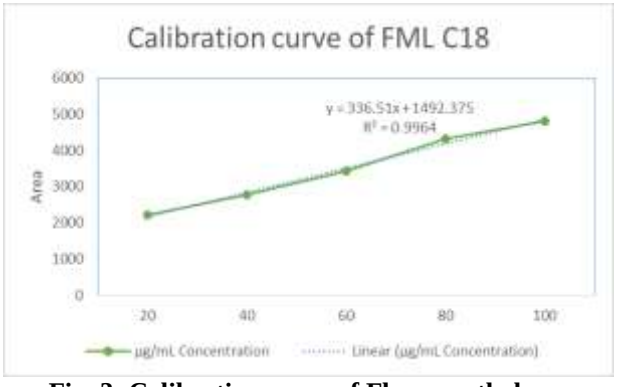


**Fig. 3: Calibration curve of Fluorometholone.**

### 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) |

**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 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 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 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 | | | |

**Table 4: 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 % |

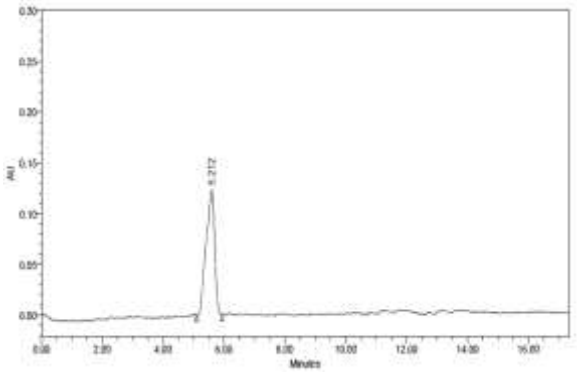

**Fig. 4: Chromatogram showing accuracy results.**

**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 | |

**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 Methanol–water (62: 38 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 Methanol–water (62: 38 v/v) after optimizing the mobile phase composition, chromatogram was recorded.

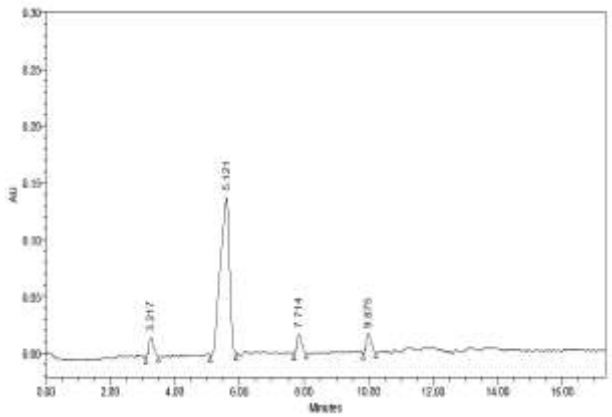

**Fig. 5: Chromatogram showing the degraded products in Acidic degradation.**

**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 Methanol–water (62: 38 v/v) after optimizing the mobile phase composition, chromatogram was recorded.

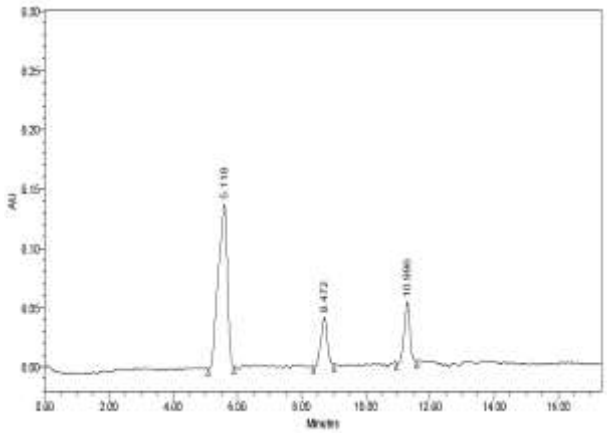

**Fig. 6: Chromatogram showing the degraded products in Basic degradation.**

**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 Methanol–water (62: 38 v/v) after optimizing the mobile phase composition, chromatogram was recorded.

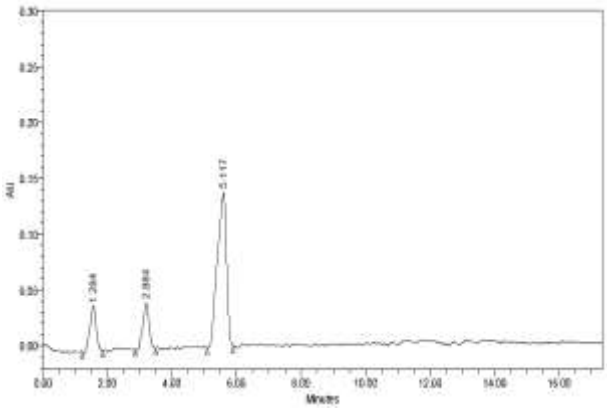

**Fig. 7: Chromatogram showing the degraded products in Wet heat degradation.**

**d. Oxidation with (3%) $H_2O_2$**

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% $H_2O_2$ 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. The chromatogram was recorded and shown in Chromatogram no: 204.

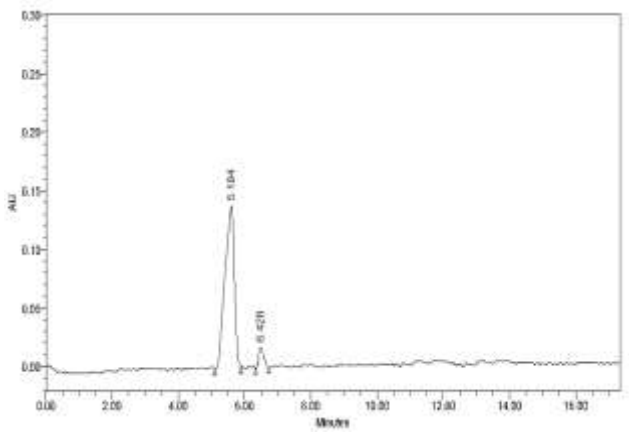

**Fig. 8: Chromatogram showing the degraded products in Oxidative degradation.**

**Table 6: Stability Indicating study for the developed method.**

| Nature of Stress | Degradation condition | Time (h) | Number of degradation products (Rt) |
|---|---|---|---|
| Acidic | 60°C | 4 | 3 (3.217, 7.714, 9.875) |
| Basic | 60°C | 4 | 2 (8.472, 10.996) |
| Oxidative | RT | 24 | 1 (6.428) |
| Wet Heat | 105°C | 6 | 2 (1.284, 2.884) |

## 4. CONCLUSION

A selective 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.

## ACKNOWLEDGEMENT

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

## 5. REFERENCES

1. International Conference on Harmonization Guideline on Validation of Analytical Procedures, Text and Methodology: Q2 (R1), 2005.
2. Nguyen D.T., Guillarme D, Rudaz S, Veuthey J. L., Fast Analysis In Liquid Chromatography Using Small Particle Size And High Pressure. J Sep Sci., Aug, 2006; 29(12): 1836-48.
3. Katharina Sterz, Gerhard Scherer, Josef Ecker. A Simple and Robust UPLC-SRM/MS Method To Quantify Urinary Eicosanoids. J Lipid Res., 2013; 1-28.
4. Ashok kumar, UPLC: A preeminent technique in pharmaceutical analysis. Acta poloniae pharmaceutica– drug research, 2012; 69(30): 371-380.
5. Michael E Swartz, Ultra performance liquid chromatography UPLC: an introduction. Separation science redefined, 2005; 1: 8-14.
6. Rasheed A et al., Analytical Method Development and Validation for the Simultaneous Estimation of Aspirin, Clopidogrel Bisulphate and Atorvastatin Calcium in Tablet Dosage Form. American Journal of Pharm Tech Research, 2014.
7. David A. Williams, William O. Foye, Thomas L. Lemke; Foye's principles of medicinal chemistry; 6th Ed Wolters Kluwer Health, 2008; 698-728.
8. Osman Ahmed, Anas Rasheed et.al; An Assay Method For The Simultaneous Estimation Of Acetaminophen And Tramadol Using Rp-Hplc Technology, Indo American Journal of Pharmaceutical Research, 2015; 5(07).
9. Anas Rasheed and Osman Ahmed, UPLC Method Development and Validation for the Determination of Chlophedianol Hydrochloride in Syrup Dosage Form, International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(2): 25-31.
10. Anas Rasheed and Osman Ahmed, Validation of A Uplc Method With Diode Array Detection For The Determination Of Noscapine In Syrup Dosage Form; European Journal of Pharmaceutical and Medical Research, EJPMR, 2017; 4(6): 510-514.