ORIGINAL ARTICLE

# Study of forced degradation behavior of fluorometholone by reversed-phase high-performance liquid chromatography

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## Abstract

**Introduction:** The present proposed work describes the development and validation of a new liquid chromatographic method for the determination of fluorometholone (FM) in pharmaceutical products. **Materials and Methods:** Chromatography was performed on Shimadzu Model CBM-20A/20 Alite with C8 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) using 0.1 M ammonium formate and methanol as mobile phase (flow rate 0.8 ml/min) (ultraviolet detection at 241 nm). **Results and Discussion:** The method was validated. The linear regression equation was found to be y = 35,207x + 29,529 ($R^2$ = 0.9995). FM was exposed to forced degradation conditions - acidic, alkaline, and thermal and oxidation stress conditions - and found that the drug is highly resistant. **Conclusion:** The proposed method was found to be robust and specific and can be used for the assay of FM.

**Key words:** Fluorometholone, reversed-phase high-performance liquid chromatography, stability indicating, validation

## INTRODUCTION

Fluorometholone (FM) [Figure 1] is a corticosteroid usually administered after laser-based refractive surgery.[1] It is marketed in India with brand names Biflace Eye Drops (Alembic Pharma), Flurisone (Label claim: 0.1% and 0.25%) (MicroVision), and FML and FML Forte (Allergan India Ltd.). In literature, two liquid chromatographic methods[2,3] have been developed, and in the present study, a new stability indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method has been proposed for the determination of FM and validated.[4]

## MATERIALS AND METHODS

### Chromatographic Conditions

Chromatographic separation was achieved using Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with C8 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at 25º C. Isocratic elution was performed using 0.1M ammonium formate and methanol (20:80%, v/v), and the flow rate was 0.8 ml/min. The overall run time was 10 min. The detection was carried at 241 nm. 20 µL of sample was injected into the HPLC system, and all chromatographic conditions were performed at the room temperature (25° C±2° C).

### Chemicals and Reagents

Ammonium formate methanol, hydrochloric acid, sodium hydroxide, and hydrogen peroxide ($H_2O_2$) were purchased from Merck (India). All chemicals are of HPLC grade. All chemicals were of analytical grade and used as received.

The stock solution was prepared by transferring accurately 25 mg of FM into a 25 ml volumetric flask and diluting with mobile phase (1000 µg/ml), and further dilutions were made on daily basis from the stock solution with mobile phase as per the requirement and filtered through 0.45 µm membrane filter before injection.

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## Method Validation

### *Linearity*

A series of solutions (1–150 µg/ml) were prepared from the FM stock solution and 20 µL of each solution was injected into the HPLC system, and the peak area of the chromatogram was noted. A calibration curve was plotted by taking the concentration of the solutions on the X-axis and the corresponding peak area values on the Y-axis. The limit of quantification and limit of detection were calculated from calibration curve.

### *Precision, accuracy, and robustness*

The intra- and inter-day precision studies were performed (10, 50, and 100 µg/ml) (n=3). The % relative standard deviation (RSD) was calculated. The accuracy of the assay method was evaluated (80, 100, and 120%) and percentage recoveries were calculated. Robustness of the assay method was established 10 µg/ml by introducing minute changes in HPLC conditions that include wavelength (239 and 243 nm), percentage of methanol in the mobile phase (78 and 82%), and flow rate (0.7 and 0.9 ml/min).

### *Assay of FM ophthalmic preparations (eye drops)*

The available brands were procured from the local pharmacy store and extracted with mobile phase. The contents of the volumetric flask were sonicated for 30 min, filtered, and diluted with mobile phase as per the requirement, and 20 µL of these solutions were injected into the system only after filtering through 0.45-µm membrane and the peak area was recorded from the respective chromatogram.

**Table 1:** Linearity of FM

| Concentration (µg/ml) | *Mean peak area±SD | RSD (%) |
|---|---|---|
| 1 | 100,303±280.84 | 0.28 |
| 5 | 356,445±1211.91 | 0.34 |
| 10 | 732,937±3591.39 | 0.49 |
| 20 | 1,573,571±5979.56 | 0.38 |
| 50 | 3,446,133±20,332.18 | 0.59 |
| 100 | 6,917,045±64,328.51 | 0.93 |
| 150 | 10,251,886±88,166.21 | 0.86 |

*Mean of three replicates, RSD: Relative standard deviation, SD: Standard deviation

## Forced Degradation Studies[5]

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method (International Conference on Harmonization [ICH] guidelines, 2003). All solutions for stress studies were prepared at an initial concentration of 50 µg/ml of FM and refluxed for 60 min at 80° C and then diluted with mobile phase.

### *Acidic degradation*

Acidic degradation was performed by treating the drug solution (50 µg/ml) with 0.1 M HCl for 60 min in a thermostat maintained at 80° C. The stressed sample was cooled, neutralized with NaOH, and then diluted with mobile phase as per the requirement. 20 µL of this solution was injected into the HPLC system.

### *Alkaline degradation*

Alkaline degradation was performed by treating the drug solution (50 µg/ml) with 0.1 N sodium hydroxide for 60 min in a thermostat maintained at 80° C. The stressed sample was cooled, neutralized with HCl, and then diluted with mobile phase as per the requirement, and 20 µL of the solution was injected into the HPLC system.

### *Oxidation degradation*

Oxidation degradation was performed by treating the drug solution (50 µg/ml) with 30% $H_2O_2$ for 60 min in a thermostat maintained at 80° C. The drug solution mixture was cooled and then diluted with mobile phase as per the requirement, and 20 µl of the solution was injected into the HPLC system.

**Table 2:** Precision and accuracy studies of FM

| Concentration (µg/ml) | Intraday precision | Interday precision |
|---|---|---|
| | *Mean peak area±SD (% RSD) | *Mean peak area±SD (% RSD) |
| 10 | 732,937±1832.34 (0.25) | 754,897±5435.25 (0.72) |
| 50 | 3,446,133±26,190.61 (0.76) | 3,570,748±30,351.35 (0.85) |
| 100 | 6,917,045±57,411.47 (0.83) | 7,048,254±66,253.58 (0.94) |

**Accuracy**

| Spiked concentration (µg/ml) (%) | Total concentration (µg/ml) | *Mean peak area±SD (% RSD) | Drug found (µg/ml) | % Recovery |
|---|---|---|---|---|
| 4 (80) | 9 | 658,691±2568.89 (0.39) | 8.89 | 98.7 |
| 5 (100) | 10 | 731,951±3074.19 (0.42) | 9.98 | 99.8 |
| 6 (120) | 11 | 805,173±1932.41 (0.24) | 10.92 | 99.2 |

*Mean of three replicates, RSD: Relative standard deviation, SD: Standard deviation, FM: Fluorometholone

**Table 3:** Robustness study of FM

| Parameterww | Condition | *Mean peak area | *Mean peak area±SD (%RSD) |
|---|---|---|---|
| Flow rate (±0.1 ml/min) | 0.7 | 732,248 | 732,513±371 (0.05) |
| | 0.8 | 732,937 | |
| | 0.9 | 732,354 | |
| Detection wavelength (±2 nm) | 239 | 730,547 | 732,677±2012.6 (0.27) |
| | 241 | 732,937 | |
| | 243 | 734,547 | |
| Mobile phase composition (0.1M ammonium formate: methanol) (±2, v/v) | 18:82 | 735,824 | 732,436±3664.2 (0.50) |
| | 20:80 | 732,937 | |
| | 22:78 | 728,547 | |

*Mean of three replicates, RSD: Relative standard deviation, SD: Standard deviation, FM: Fluorometholone

**Table 4:** Analysis of FM in ophthalmic formulation

| Formulation | Labeled claim (%) | Amount found* (%) | Recovery* (%) |
|---|---|---|---|
| Brand I | 0.1 | 0.0988 | 98.8 |
| Brand II | 0.1 | 0.0992 | 99.2 |

*Mean of three replicates, FM: Fluorometholone

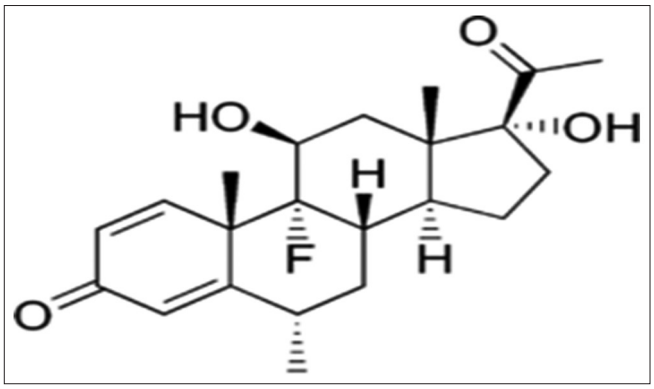


**Figure 1:** Structure of fluorometholone

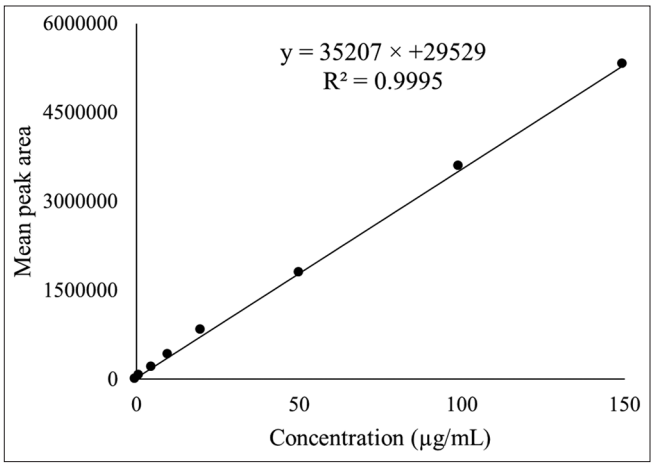


**Figure 2:** Calibration curve of fluorometholone

## RESULTS AND DISCUSSION

### Method Optimization

Initially, the stressed samples were analyzed using a mixture of 0.1M ammonium formate:methanol (40:60% v/v) with a flow rate of 0.8 ml/min, in which the peak was obtained at $R_t$ 8.85 min and also the resolution and peak symmetry were not satisfactory. The mobile phase ratio was changed to 30:70% v/v and the drug sample was injected into the loop where a sharp peak was eluted at 7.96 min with tailing. Finally, the mobile phase composition was modified as 20:80% v/v and the drug peak eluted was sharp and symmetrical (ultraviolet detection at 241 nm) with retention time 5.247±0.04 min.

### Method Validation

#### *Linearity*

FM shows linearity over a concentration range of 1–150 µg/ml [Table 1] with the % RSD of 0.28–0.93, and the chromatographic response is shown in Figure 2. The linear regression equations were found to be y = 35,207x + 29,529 ($R^2$ = 0.9995).

#### *Precision, accuracy, and robustness*

The % RSD for intraday precision (0.25–0.83), interday precision (0.72–0.94), accuracy (0.24–0.42) with recovery 98.7–99.8% [Table 2], and robustness (0.05–0.50) was <2.0%, indicating that the method is precise, accurate, and robust [Table 3].

#### *Analysis of ophthalmic formulations*

The proposed method was applied for the determination of FM in marketed formulations available. The % recovery was found to be 98.8–99.2 [Table 4].

### Stress Degradation Studies

The degradation of FM was found to be very similar for both the marketed formulation and API, and the chromatograms obtained following the assay of stressed samples are shown in Figure 3A-F. The three-dimensional chromatograms obtained are shown in Figure 4. A slight decomposition, i.e., <5% was observed, indicating that the drug is stable and resistant [Table 5] and the system suitability parameters are within acceptable criteria.

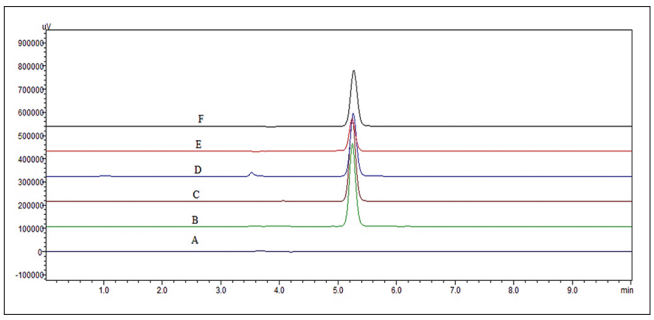


**Figure 3:** Chromatograms of (A) blank, (B) fluorometholone API (50 µg/ml), (C) thermal, (D) oxidation, (E) alkaline, (F) acidic degradations

## CONCLUSION

The proposed stability-indicating HPLC method was validated as per the ICH guidelines and applied for the determination of FM in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of FM formulations. It was observed that FM is stable toward the forced degradation studies as the drug decomposed is <5%.

## ACKNOWLEDGMENT

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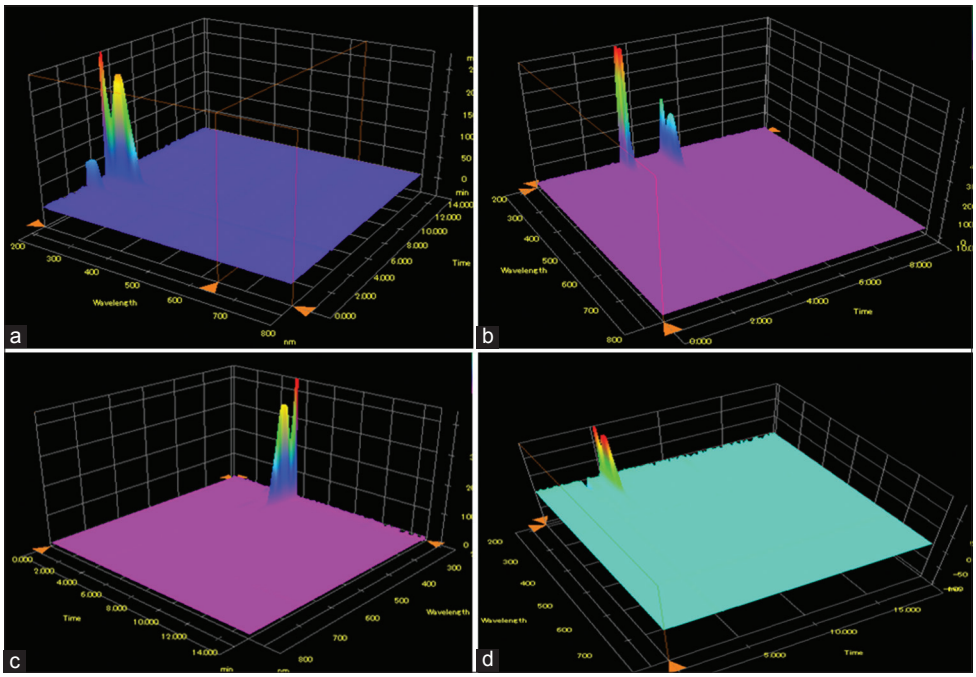


**Figure 4:** Three-dimensional chromatograms of fluorometholone in (a) acidic, (b) oxidation, (c) thermal, (d) alkaline degradations

**Table 5:** Stress degradation studies of FM

| Stress conditions | *Mean peak area | *Drug recovered (%) | *Drug decomposed (%) | Theoretical plates | Tailing factor |
|---|---|---|---|---|---|
| Standard drug (Untreated) | 3,446,133 | 100 | - | 9402.128 | 1.113 |
| Acidic degradation | 3,425,789 | 99.40 | 0.6 | 8107.232 | 1.089 |
| Alkaline degradation | 3,357,852 | 97.43 | 2.57 | 9242.865 | 1.146 |
| Oxidative degradation | 3,315,478 | 96.20 | 3.8 | 8702.447 | 1.103 |
| Thermal degradation | 3,382,487 | 98.15 | 1.85 | 8915.929 | 1.122 |

*Mean of three replicates, FM: Fluorometholone

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