RESEARCH ARTICLE

Stability-indicating Reversed-phase High-performance Liquid Chromatography Method for the Determination of Fluorometholone in Bulk and Pharmaceutical Formulation

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Abstract

Introduction: A new stability-indicating liquid chromatographic method has been established for the determination of Fluorometholone. Fluorometholone is used for the treatment of eye diseases. **Materials and Methods:** The Shimadzu Model CBM-20A/20Alite high-performance liquid chromatography (HPLC) system was monitored at detection wavelength 241 nm on isocratic mode with flow rate 0.8 mL/min, and the total run time is 10 min. Chromatographic separation was achieved through Phenomenex Luna C_8 column (250 mm × 4.6 mm i.d., 5 µm particle size). The method was validated and stress degradation studies were conducted. **Results and Discussion**: Fluorometholone has obeyed Beer-Lambert's law over a concentration range 0.5–100 µg/mL with linear regression equation, y = 70155x + 31667 and correlation coefficient of 0.9996. The limit of detection and limit of quantitation are found to be 0.1617 µg/mL and 0.4502 µg/mL, respectively, and the % RSD in precision, accuracy, and robustness studies was found to be less than 2%. Fluorometholone was found to be highly resistant toward all degradation conditions such as acidic, alkaline, thermal, and oxidation. **Conclusions:** It is concluded that the proposed reversed-phase HPLC method is accurate, precise, sensitive, and reproducible for the determination of Fluorometholone in pharmaceutical formulations, and the method was validated as per ICH guidelines.

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

INTRODUCTION

Fluorometholone [Figure 1] is a corticosteroid used after laser-based refractive surgery.[1] Fluorometholone (FLM) is a glucocorticoid employed in the treatment of allergic and inflammatory conditions of the eye. It is available with brand names FLOSOFT (Cipla), flurisone (Label claim: 0.1% and 0.25%) (MicroVision), and FML (Allergan India Ltd) eye drops. Only one high-performance liquid chromatography (HPLC) method[2] is available in the literature and the authors have developed a stability-indicating reversed-phase HPLC (RP-HPLC) method for the determination of FLM in the present study and the method was validated.[3]

MATERIALS AND METHODS

Chemicals and reagents

Methanol, sodium hydroxide, hydrochloric acid, acetic acid, and hydrogen peroxide (H_2O_2) were purchased from Merck

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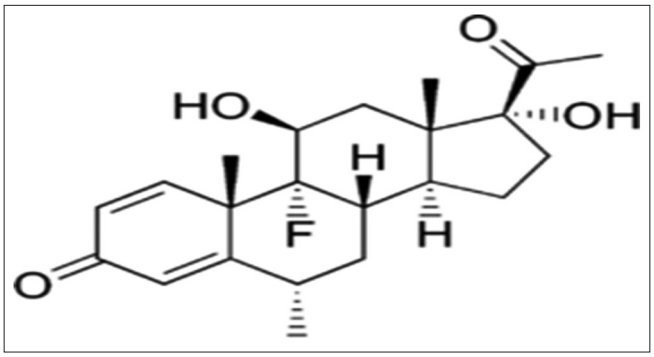

Figure 1: Structure of Fluorometholone

(India). All chemicals are of HPLC grade. All chemicals were of analytical grade and used as received.

Instrumentation and chromatographic conditions

Chromatographic separation was achieved using Shimadzu Model CBM-20A/20Alite HPLC system equipped with SPD-M20A prominence photodiode array detector with C_8 Phenomenex Luna column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at 25°C. Isocratic elution was performed using 0.1% acetic acid and methanol (20:80%, v/v) and the flow rate was 0.8 mL/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 room temperature (25°C±2°C). Stock solution of Fluorometholone (1000 µg/mL) was prepared with mobile phase, and further dilutions were made after filtering through 0.45 µm membrane filter.

Method validation

A series of solutions (0.5–100 µg/mL) were prepared from Fluorometholone stock solution, and 20 µL of each solution was injected into the HPLC system. The peak area of the chromatogram was noted, and 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 intraday precision and the interday precision studies were conducted at three concentration levels (10, 20, and 50 µg mL) on three different days, that is, day 1, day 2, and day 3, and the % RSD was calculated. The accuracy of the assay method was evaluated (80%, 100%, and 120%) using standard addition method and recovery experiments. The robustness of the assay method was established by introducing small changes in the HPLC conditions such as 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) with 10 µg mL of Fluorometholone. The limit of quantification and limit of detection were based on the standard deviation of the response and the slope of the constructed calibration curve (n = 3), as described in ICH guidelines Q2 (R1).[3]

Stress degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.[4] All solutions for stress studies were prepared at an initial concentration of 50 µg/mL of FLM and refluxed for 60 min at 80°C and then diluted with mobile phase.

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 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 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.

Assay of Fluorometholone

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

RESULTS AND DISCUSSION

Method development and optimization

A simple stability indicating RP-HPLC method has been developed for the determination of Fluorometholone. During the optimization process the column, flow rate, mobile phase composition was selected based on the system suitability parameters. Enable C18 column has shown good number of theoretical plates [Figure 2], but the peak is not sharp and symmetrical due to its tailing factor (>2), and therefore, Phenomenex Luna C_8 Column [Figure 3] was tried where the system suitability parameters were within acceptable

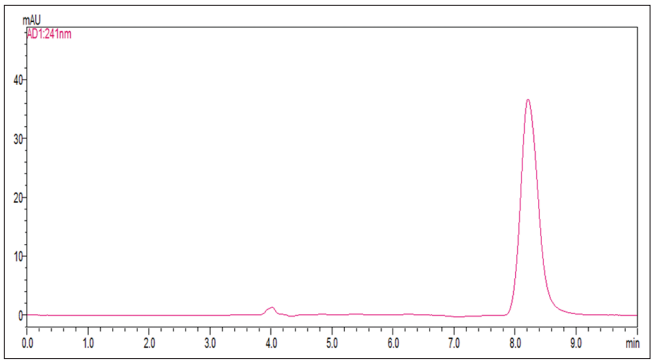

Figure 2: Enable C18 column (Rt8.220)

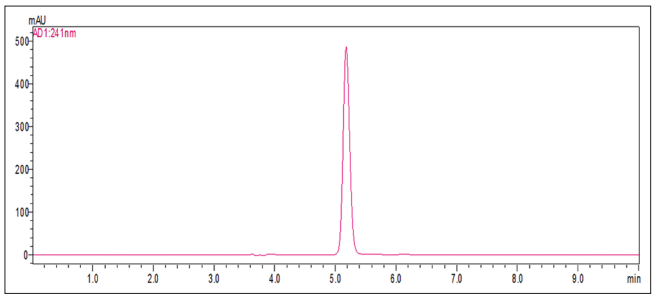

Figure 3: Phenomenex Luna C8 column (Rt 5.158) (method optimized)

criteria [Table 1]. Then, the mobile phase composition [Table 2 and Figure 4] and flow rates [Table 3 and Figure 5] were monitored. Mobile phase containing 0.1% acetic acid: Methanol (20:80, v/v) with flow rate of 0.8 mL/min has shown a sharp peak at 5.158 min for Fluorometholone.

Method validation

Fluorometholone has shown linearity 0.5–100 µg/mL [Table 4] with % RSD 0.18–0.93 and the chromatographic response was shown in Figure 6. The linear regression equations were found to be y = 70155x + 31667 (R^2 = 0.9996). The % RSD in intraday and interday precision and accuracy were found to be 0.20–0.86, 0.66–0.82, and 0.30–0.81, respectively, with a percentage recovery 98.88–99.5 [Table 5]. The % RSD value in robustness study was also found to be <2.0% (0.62–1.04) indicating that the method is robust [Table 6].

Analysis of ophthalmic formulations

The proposed method was applied for the determination of FLM in marketed formulations[Figure 7]. The % recovery was found to be 98.0–99.0 [Table 7].

Table 1: Optimization – selection of columns

Column used	Retention time (min)	Mean peak area	Tailing factor	Theoretical plates	Remarks	Figure
Enable C_{18} column (150×4.6 mm, 5 µm)	8.220	165790	2.579	3904.627	Blunt peak tailing factor >2	2
Phenomenex Luna C_8 column (250×4.6 mm, 5 µm)	5.158	715363	1.172	8948.819	Peak is sharp Rt is<6 min tailing factor <2	3

Table 2: Optimization – selection of mobile phase composition

Trials	Mobile phase composition (v/v)	Flowrate (mL/min)	Retention time (min)	Peak area	Tailing factor	Theoretical plates	Comment
1	0.1% acetic acid:methanol (40:60)	0.8	16.05	214687	1.388	10635.83	Broad peak
2	0.1% acetic acid:methanol (30:70)	0.8	8.023	314019	1.262	11175.22	Peak tailing
3	0.1% acetic acid:acetonitrile (25:75)	0.8	8.042	1254786	1.625	4257.94	Peak tailing
4	0.1% acetic acid:methanol (20:80)	0.8	5.158	715363	1.172	8948.82	Sharp peak method optimized

Table 3: Optimization – selection of flow rate

Trials	Flow rate (mL/min)	Mobile phase composition (v/v)	Rt (min)	Peak area	Tailing factor	Theoretical plates	Comments
1	0.6	0.1% acetic acid:methanol (20:80)	16.05	214687	1.388	11175	Broad peak
2	0.7	0.1% acetic acid:methanol (20:80)	8.042	1254786	1.625	4258	Peak tailing
3	0.8	0.1% acetic acid:methanol (20:80)	5.158	715363	1.172	8949	Method optimized sharp peak

Stress degradation studies

The overlay typical chromatogram obtained following the assay of stressed samples was shown in Figure 8. Very slight decomposition (<5%) was observed when FLM drug was exposed to alkaline, thermal, acidic, and oxidative degradations [Table 8]. The 3D chromatograms were shown in Figure 9. The system suitability parameters are within acceptable criteria.

Table 4: Linearity of Fluorometholone

Concentration (µg/mL)	*Mean peak area±SD	RSD (%)
0.5	51458±157.357	0.18
1	102917±257.292	0.25
5	393813±1260.20	0.32
10	716538±3439.38	0.48
20	1411702±3105.74	0.22
50	3645878±21510.68	0.59
100	6999612±65096.39	0.93

*Mean of three replicates

CONCLUSION

The proposed stability-indicating liquid chromatographic method can be applied for the determination of Fluorometholone in eye drops, and the drug is highly resistant toward all degradations.

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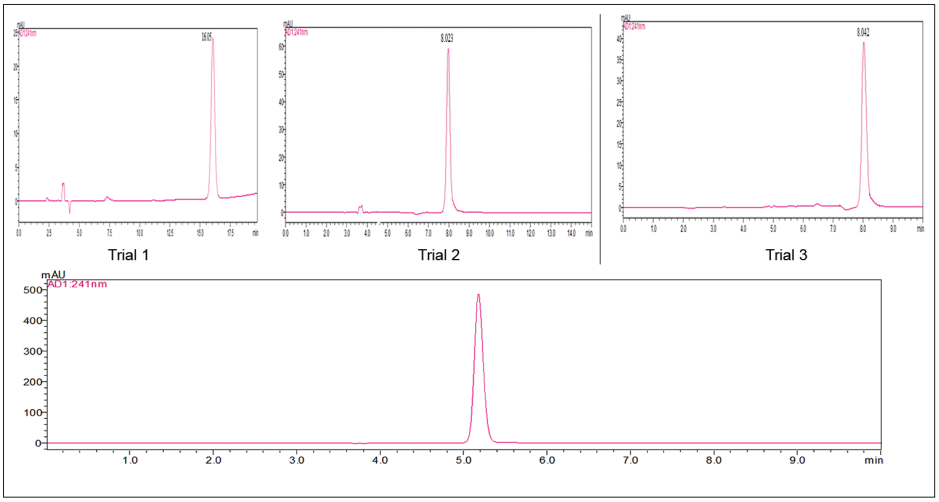

Figure 4: Chromatograms of Fluorometholone (Trial 4) (method optimized)

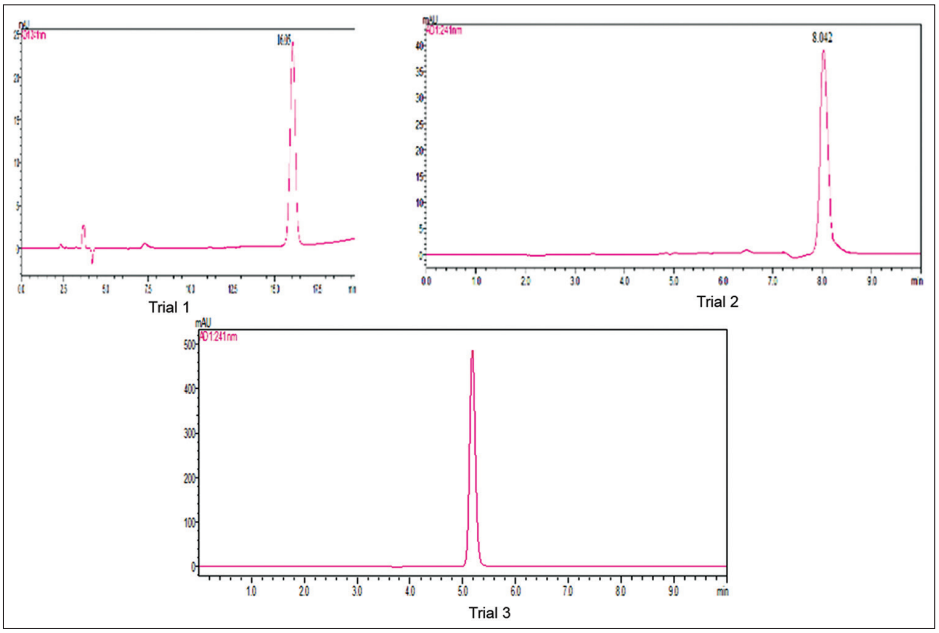

Figure 5: Chromatograms of Fluorometholone (Trial 3) (method optimized)

Table 5: Precision and accuracy studies of Fluorometholone

Concentration (µg/ml)	Intra-day precision	
	*Mean peak area ± SD (%RSD)	*Mean peak area ± SD (%RSD)
10	715363.24±1430.72 (0.20)	716252.00±5873.26 (0.82)
20	1425734.16±12261.31 (0.86)	1484341.00±9796.65 (0.66)
50	3658469.00±15731.41 (0.43)	3692472.67±28062.79 (0.76)

Accuracy

Spiked	Total	*Mean peak area±SD	Drug Found	%
0.8 (80)	1.8	327841.27±2655.51 (0.81)	1.78	98.88
1 (100)	2	357057.52±2178.05 (0.61)	1.99	99.5
1.2 (120)	2.2	477407.47±1432.22 (0.30)	2.18	99.09

*Mean of three replicates

Table 6: Robustness study of Fluorometholone

Parameter	Condition	*Mean peak area	*Mean peak area±SD (% RSD)
Flow rate (±0.1 mL/min)	0.7	705729	708280.33±4391.33 (0.62)
	0.8	716538	
	0.9	702574	
Detection wavelength (±2 nm)	239	708457	712947.33±6630.41 (0.93)
	241	716538	
	243	713847	
Mobile phase composition (0.1% acetic acid: methanol) (±2% v/v)	18:82	714873	713745±7422.94 (1.04)
	20:80	716538	
	22:78	709824	

*Mean of three replicates

Table 7: Analysis of Fluorometholone in ophthalmic formulation

Formulation	Labeled claim (%)	Amount found* (%)	Recovery* (%)
Brand I	0.1	0.098	98.0
Brand II	0.1	0.099	99.0

*Mean of three replicates

Table 8: Stress degradation studies of Fluorometholone

Stress conditions	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard drug (control)	3645878	100	-	9414.140	1.162
Acidic degradation	3545415	98.20	1.8	8047.210	1.228
Alkaline degradation	3439943	98.12	1.88	8123.215	1.136
Oxidative degradation	3466384	96.64	3.36	8445.845	1.166
Thermal degradation	3556739	99.06	0.94	8405.603	1.164

*Mean of three replicates

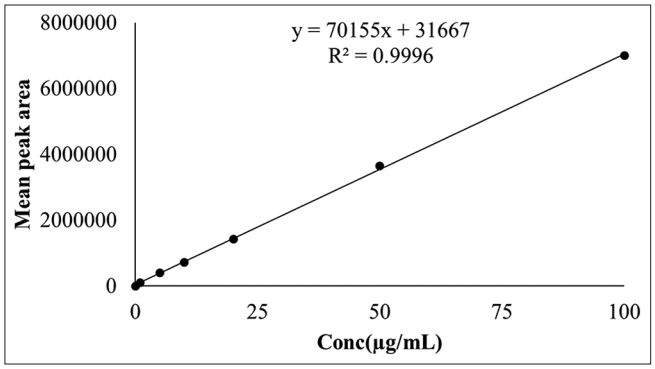

Figure 6: Calibration curve of Fluorometholone

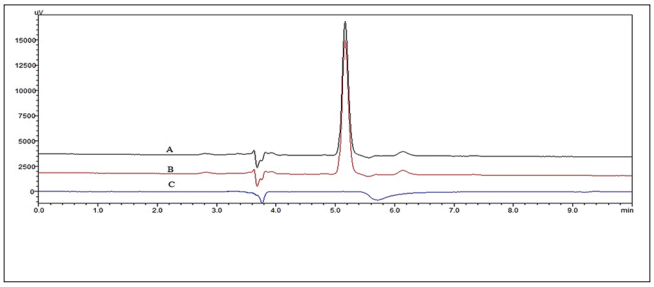

Figure 7: Typical chromatograms of (A) FLM standard (1 µg/mL) (Rt= 5.157) (B) blank (C) flurisone (label claim 0.1%) (1 µg/mL) (Rt= 5.164)

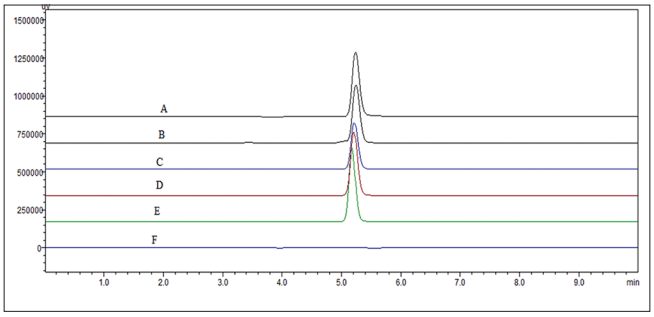

Figure 8: Overlay chromatograms of Fluorometholone acidic (A) alkaline (B) oxidation (C) thermal (D) standard (50 µg/mL) (E) and blank (F) degradations

REFERENCES

1. Budavari S. The Merck Index, An Encyclopedia of Chemicals, drugs and Biologicals. 14th ed. Whitehouse Station, NJ: Merck Research Laboratories Division of Merck and Co., Inc; 2006. p. 4175, 714.

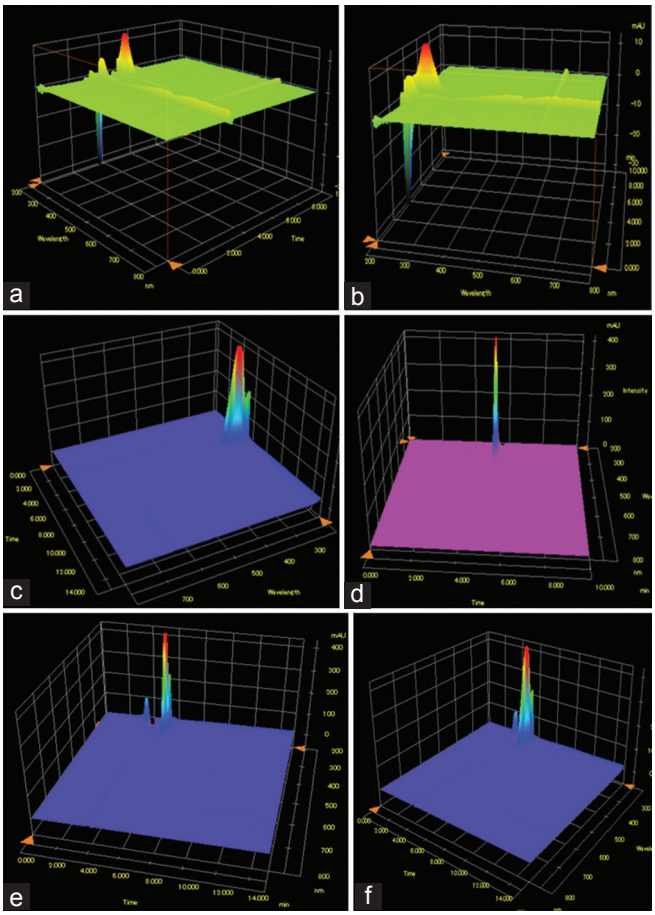

Figure 9: 3D chromatograms of Fluorometholone, (a) Fluorometholone standard (1 µg/mL), (b) flurisone, (c) acidic degradation, (d) thermal degradation, (e) alkaline degradation, (f) oxidation degradation

2. Jonvel P, Andermann G. Determination of Fluorometholone Purity by very high-performance liquid chromatography. Analyst 1983;108:411-4.
3. ICH Validation of Analytical Procedures: Text and Methodology Q2 (R1), International Conference on Harmonization; 2005.
4. ICH Guidelines, Q1A (R2): Stability Testing of New Drug Substances and Products (revision 2). International Conference on Harmonization; 2003.

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