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# Novel HPTLC Method for Estimation of Fluocinonide

**Pooja Kumari Jaiswal[1], Dr Vandana Jain[2]**

Oriental college pharmacy Sanpada Navi Mumbai, India
Email: *vandana.jain[at]ocp.edu.in*

**Abstract:** ***A novel high-performance thin-layer chromatographic (HPTLC) method was developed and validated for estimation of fluocinonide. The HPTLC separation was achieved on a precoated silica gel 60$F_{254}$ aluminum sheet as a stationary phase using mixture of chloroform-methanol (19: 1 v/v) as mobile phase. The detection of spots was carried out at 245nm. The method was validated in terms of specificity, linearity, accuracy, precision, repeatability, and robustnessas per the International Conference on Harmonization guidelines. The calibration curve was found to be linear between 100-500 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, sensitive and selective for the analysis of fluocinonide. The method was successfully employed for the estimation of fluocinonide.***

**Keywords:** Estimation, analysis, Fluocinonide, Development, validation

## 1. Introduction

The quality of active pharmaceutical ingredients (API) and the drug products continue to be a challenge for the quality and effective analytical methods are need to assess the materials and finished products at release and during stability. Analytical methods are used to test and ensure the quality of drug substances / drug products. The most widely used methods for quantitative determination of drugs and metabolites are Gas chromatography (GC), High-performance liquid chromatography (HPLC), Thin layer chromatography (TLC), LC-MS, LC-MS-MS, GC-MS, and GC-MSMS, techniques like NMR is used for structure identification. High Performance Thin Layer Chromatography (HPTLC) gives much greater resolution and separation of components than normal TLC. It uses chromatographic stationary phases with excellent separation efficiency and employs state of the art instrumentation. This includes precise sample application, standardized reproducible chromatogram development and software-controlled evaluation. HPTLC is a rapid cost-effective method as less solvent is used for mobile phase in HPTLC as compared to other chromatography techniques.

Fluocinonide (6α, 9-difluoro-11β, 16α, 17, 21-tetrahydroxypregna-1, 4-diene-3, 20-dione, cyclic 16, 17-acetal with acetone, 21-acetate) is a high potency corticosteroid. It is used topically as an anti-inflammatory agent for the treatment of skin disorders such as eczema and dermatitis. Chemical Formula is $C_{26}H_{32}F_2O_7$ andMelting Point is 309 °C stability is 3years at 25°C and 2 years at 80°C. Dosage of fluocinonide is 0.05% and 0.1%. The literature survey revealed that although few HPLC methods have been reported for analysis of this drug alone or in combination with other drugs, no HPTLC method has yet been reported for estimation of Fluocinonide, this drug is given for the treatment of skin irritation and inflammation. The present work describes a novel, HPTLC method for estimation of selected compound.

## 2. Material

**Reagents and chemicals used:** Methanol and Chloroform of HPLC grade were procured from Merck Life Science Private Limited.

**Equipment:** XS205 Dual Range of mettle Toledo Weighing balance, borosil pipette, HPTLC system (Camag, Muttenz, Switzerland) consisted of a camag visualizer 1 and 2, a camag TLC sampler 4. Densitometric analysis was carried out using a camag TLC scanner with visionCATS software and TLC Silica gel 60 $F_{254}$ aluminum sheets (20× 20cm and 10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used, for the present study.

## 3. Methods

**1. Preparation of standard solution:** The stock solution of 10mg/mL was prepared by dissolving 10mg of fluocinonide in 10mL methanol and sonicated for 5 minutes, from the stock solution, 0.05mg/mL (50µg/mL) was prepared by taking 0.5mL in a 10mL volumetric flask and volume made up to the mark with methanol.

### 1.1 Preparation of sample solution

20gm of fluocinonide cream equivalent to 10mg of drug was weighed and transferred to the 200mL volumetric flask and dissolved with methanol up to the mark and sonicated and labeled as 0.05mg/mL solution.

### 1.2 Preparation of mobile phase

The mobile phase consisted of chloroform: methanol in the ratio of 19: 1 v/v.


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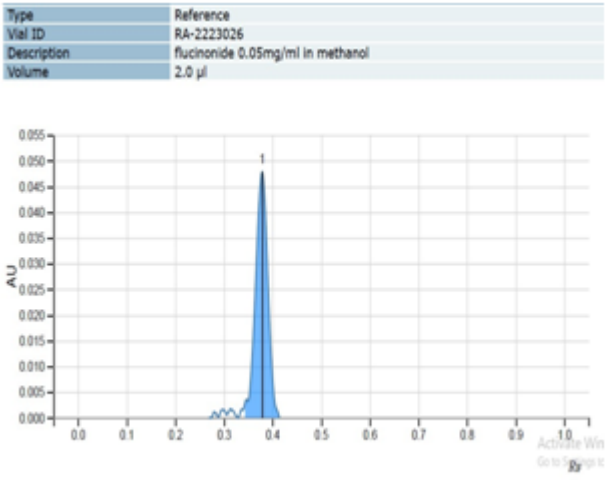


**Figure 1:** HPTLC densitogram of Reference

### 1.3Determination of λ max and selection of wavelength

The suitable wavelength for the HPTLC analysis was determined by the UV spectrum in the range of 200-400 nm for drug solution of fluocinonide. Hence 245 nm, the λ max of fluocinonide was selected as suitable wavelength. Since the drug has appreciable absorbance at that wavelength.

### HPTLC method and chromatographic conditions for estimation of fluocinonide

**Sample application**
The standard and sample of fluocinonide were spotted on precoated HPTLC plates in the form of narrow bands of lengths 8mm, with 8mm from the bottom and left margin and with 12mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL/s.

**Mobile phase and migration**
Plates were developed using mobile phase consisting of chloroform: methanol (19 +1 v/v). Linear ascending development was carried out in 20 × 20 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min at 25 ± 2°C. Ten milliliters of the mobile phase (10 mL in trough containing the plate and 10mL in other trough) was used for each development and allowed to migrate a distance of 70 mm, which required 20 min. After development, the HPTLC plates were dried completely.

**Validation of the method**
Validation of the optimized HPTLC method was carried out as per ICH guidelines with respect to the following parameters.

**Specificity**
The specificity of the method was determined by analyzing standard drug and test sample. The band for fluocinonide the sample and standard, diluent (methanol), and mobile phase was confirmed by comparing their Rf value.

**Linearity and Range**
Linearity of the method was evaluated by constructing calibration curves at five concentration levels. Calibration curves were plotted over a concentration range of 100-500 ng/band. 2, 4, 6, 8, and10$\mu$L/band of standard working solution was applied to the plate to give 100, 200, 300, 400 and 500ng/band concentration of fluocinonide and scanning was done at wavelength of 254 nm.

The calibration curve was prepared by plotting peak area versus concentrations.

**Accuracy**

Accuracy of the method was evaluated by carrying the recovery study at three levels. Recovery experiments were performed by adding three different amounts of standard drug, that is, 80%, 100%, and 120% to check the % recovery. Tracks were assigned on the plate of 20×10cm with different volume as shown in the Table no 3.

**Precision**

Precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling if same sample under the prescribed condition. Precision of the method was verified by system precision and method precision studies.

a) **System precision**
System precision (% RSD) was determined by analysis of fluocinonide at the concentration of 200ng/band of standard.
b) **Method precision**
Methodprecision (% RSD) was determined by analysis of fluocinonide at the concentration of 300ng/band of sample.

**Robustness of the method**
By introducing small changes in mobile phase concentration and chamber saturation time, the effects on the results were examined.

1) **Effect of saturation time:** Robustness of the method was determined in duplicate at a concentration level of 2µL/band i.e. 100ng/band and 5µL/band i.e. 250ng/band of standard and sample at 10min and 30min. The mean and %RSD of peak area were calculated.
2) **Change in mobile phase concentration:** Robustness was determined by applying bands of 100ng/band of two different concentrations at standard saturation time of 20min.

## 4. Results and Discussion

**1) Specificity**
The band for fluocinonide in the sample was confirmed by comparing the Rf value and spectrum of the band with that of a standard. In the bands of diluent and mobile phase, no peaks


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were observed, hence no interference was found thus indicating the specificity of the method in the presence of various excipients.

**Table 1:** Results for specificity

| S. No. | Description | Vol (µL) | ng/band | Rf |
|---|---|---|---|---|
| 1. | Fluocinonide 0.05mg/mL in methanol (Standard) | 2.0 | 100 | 0.42 |
| 2. | Fluocinonide 0.05mg/mL in methanol (Sample) | 2.0 | 100 | 0.43 |
| 3. | Diluent (Methanol) | 5.0 | 250 | 0.02 |
| 4. | Mobile phase | 5.0 | 250 | 0.01 |

**2) Linearity**

Linear relationships were observed by plotting drug concentrations against peak areas for each compound fluocinonide shown linear response in the tested concentration range of 100-500ng/band. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted. Drug has shown a good correlation coefficient variation with $R^2$ of 0.9942.

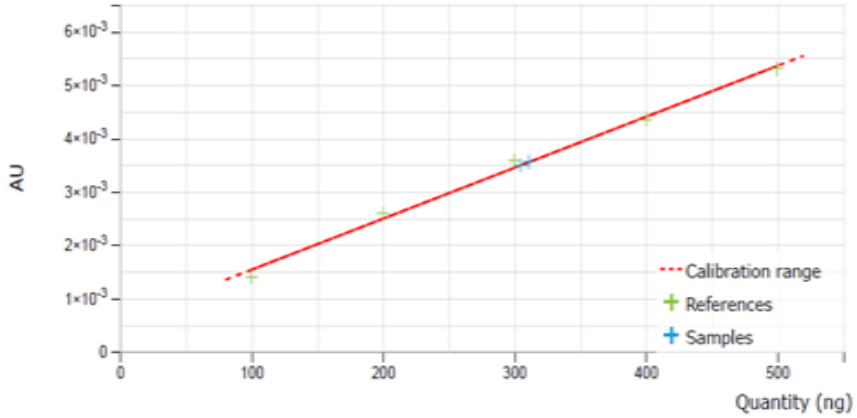


**Figure 2:** Calibration plot for fluocinonide

**Table 2:** Results of linearity study

| Vol (µL) | Range (ng/band) | Rf | Height | Area |
|---|---|---|---|---|
| 2 | 100 | 0.37 | 0.047 | 0.001403 |
| 4 | 200 | 0.36 | 0.092 | 0.002593 |
| 6 | 300 | 0.35 | 0.125 | 0.003579 |
| 8 | 400 | 0.32 | 0.163 | 0.004346 |
| 10 | 500 | 0.32 | 0.197 | 0.005297 |

**Table 2.1:** Linear regression data for the calibration curve

| Range (ng/band) | $R^2$ | CV | Slope | Intercept |
|---|---|---|---|---|
| 100-500 | 0.9942 | 1.42 | 0.000009527 | 0.0005841 |

**3) Accuracy**

The proposed method showed good percentage of recovery rates between 96-101% for fluocinonide with %RSD less than 2%.

**Table 3:** Results for recovery study

| Track * | Vol (µL) (Sample +standard) | ng/band (Sample +standard) | Area | Average area | SD | % RSD | Recovery % | Mean % recovery |
|---|---|---|---|---|---|---|---|---|
| 80% | 3.0+2.4 | 150+120 | 0.003692 | 0.003654 | 3.6 | 1.0 | 101 | 98.7 |
| | | | 0.003651 | | | | | |
| | | | 0.003619 | | | | | |
| 100% | 3.0+3.0 | 150+150 | 0.003921 | 0.003922 | 3.5 | 0.9 | 98 | |
| | | | 0.003958 | | | | | |
| | | | 0.003887 | | | | | |
| 120% | 3.0+3.6 | 150+180 | 0.004197 | 0.004243 | 5.1 | 1.2 | 96 | |
| | | | 0.004234 | | | | | |
| | | | 0.004298 | | | | | |

***n=3**


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**4) Precision**

**(a) System precision**: The method was found to be precise in case of system precision. The standard deviation was found to be 0.9313 and % RSD was found to be 1.0202 which is less the 2% as per ICH guidelines.

**Table 4:** Results for system precision

| S. No. | Vol (µL) | ng/band | Area | Rf | Average area | SD | % RSD |
|---|---|---|---|---|---|---|---|
| 1 | 4.0 | 200 | 0.00209 | 0.39 | 0.002125 | 0.9313 | 1.0202 |
| 2 | 4.0 | 200 | 0.00211 | 0.40 | | | |
| 3 | 4.0 | 200 | 0.00213 | 0.41 | | | |
| 4 | 4.0 | 200 | 0.00214 | 0.41 | | | |
| 5 | 4.0 | 200 | 0.00215 | 0.41 | | | |
| 6 | 4.0 | 200 | 0.00213 | 0.40 | | | |

**(b) Method precision**: Method was found to be precise in case of method precision with average area 0.0036, standard deviation was found to be 0.9833 and %RSD wasfound to be 1.0771 which is less than 2% as per ICH guidelines.

**Table 5:** Results for method precision

| S. No. | Vol (µL) | ng/band | Area | Average area | SD | %RSD |
|---|---|---|---|---|---|---|
| 1. | 6.0 | 300 | 0.00364 | 0.0036 | 0.9833 | 1.0771 |
| 2. | 6.0 | 300 | 0.00369 | | | |
| 3. | 6.0 | 300 | 0.00366 | | | |
| 4. | 6.0 | 300 | 0.00371 | | | |
| 5. | 6.0 | 300 | 0.00362 | | | |
| 6. | 6.0 | 300 | 0.00361 | | | |

**5) Robustness**

The method is found to be robust with mean and %RSD is less than 2% which is acceptable as per ICH guidelines.

**Table 6:** Results and statistical data for robustness

| S. No. | Robustness parameters | Parameters changed | Vol (µL) | Area | Average area | %RSD of area |
|---|---|---|---|---|---|---|
| 1. | Saturation time | 10min | 2.0 * | 0.00118 | 0.001195 | 1.775 |
| | | | 2.0# | 0.00121 | | |
| | | | 5.0 * | 0.00276 | 0.002725 | 1.816 |
| | | | 5.0 # | 0.00269 | | |
| | | 30min | 2.0 * | 0.00101 | 0.001 | 1.414 |
| | | | 2.0 # | 0.00099 | | |
| | | | 5.0 * | 0.00191 | 0.00189 | 1.496 |
| | | | 5.0 # | 0.00187 | | |
| 2. | Mobile phase | Ratio (19.5: 0.5) v/v | 2.0 | 0.00110 | 0.00112 | 1.52 |
| | | | 2.0 | 0.00113 | | |
| | | | 2.0 | 0.00114 | | |
| | | | 2.0 | 0.00112 | | |
| | | Ratio (19: 6: 0.4) v/v | 2.0 | 0.00109 | 0.00109 | 1.17 |
| | | | 2.0 | 0.00108 | | |
| | | | 2.0 | 0.00111 | | |
| | | | 2.0 | 0.00110 | | |

*Standard solution # sample solution

## 5. Conclusion

Validation requirements for HPTLC procedures are highly diversified, depending upon the actual type of analysis, as HPTLC can be used in wide range of applications. HPTLC represents a sophisticated analytical technique that provides essential benefits, such as simultaneous or separately screening of many samples, cost effectiveness, and time savings. In the present work a novel, rapid, sensitive, and sustainable HPTLC method has been developed that could be used for the identification of fluocinonide. This method can be used in research, or for routine quality control of formulations contains fluocinonide as active ingredient.

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