INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

STABILITY-INDICATING HPTLC METHOD DEVELOPMENT AND VALIDATION OF FLURANDRENOLIDE IN API AND IT'S LOTION

Kalal Chetan [1], Janhavi R. Rao [1]* and Chaitali Dhale [2]
[1]Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India
[2]Department of Quality Assurance, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India
*Corresponding Author Email: raojanhavi@rediffmail.com

Article Received on: 07/09/18 Approved for publication: 12/10/18

DOI: 10.7897/2230-8407.0911262

ABSTRACT

HPTLC stability indicating method has been developed for determination of anti-psoriasis drug, flurandrenolide in API and its formulation. The method was constructed on high performance thin layer chromatography separation of the drug followed by densitometric measurements of their spots at 238 nm. The separation was carried out on Merck TLC aluminium sheets of silica gel 60F_{254} using toluene: acetone in the ratio of 6: 4 *v/v* as a mobile phase. The method was validated in accordance with the requirements of ICH guidelines. Calibration curves were linear in range of 200-1200 ng/µl for flurandrenolide and correlation coefficient of calibration curve was found to be 0.9997 respectively. Limit of detection and limit of quantification were found to be 29.7ng/µl and 90.0ng/µl. Method had an accuracy of 100.09% for flurandrenolide in lotion. The developed simple, accurate, precise, specific and reproducible HPTLC method is successively applied to determine flurandrenolide in its lotion formulation.

Keywords: Flurandrenolide, method validation, Densitometric detection, HPTLC

INTRODUCTION

Flurandrenolide also known as fludroxycortide chemically known as 6α-Fluoro-16α-hydroxyhydrocortisone 16, 17-acetonide; 6α-Fluoro-11β, 16α, 17α, 21-tetrahydroxypregn-4-ene-3, 20-dione16, 17-acetonide is anti-psoriasis and anti-inflammatory agent[1].

Flurandrenolide is a corticosteroid that is accessible in ointment, cream, lotion, and tape dosage formulations for the treatment of steroid responsive dermatoses. The sorting of these products, constructed on their vasoconstrictor action, ranges from class V lotion, cream to class I Tape. There is a good correlation between vasoconstrictor activity and clinical efficacy of topical corticosteroids[2-3]. Flurandrenolide is official in British Pharmacopoeia; European Pharmacopoeia and United State Pharmacopeia[4-5]. The structures of the drugs are shown in Fig.1[6].

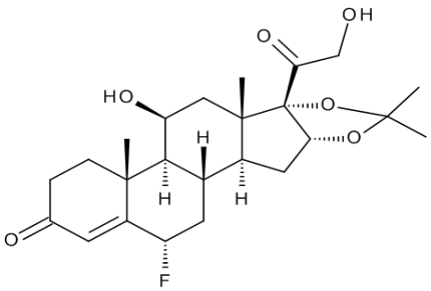

Fig. 1: Structure of Flurandrenolide

Literature survey revealed that very rare analytical methods have been reported for estimation of Flurandrenolide in pharmaceutical dosage forms like lotion, cream, tape[7-12]. So far no HPTLC method developed and its stability studies are reported for determination of Flurandrenolide in bulk and pharmaceutical dosage form[13-28]. The principal objective of this study is to develop a unique, simple, cost-effective, selective, precise, reproducible, and stability indicating HPTLC method with a wide linear range and excellent sensitivity for flurandrenolide study in the bulk and pharmaceutical formulations. In addition, the method will be inexpensive and not requires certain types of stationary phases. Thus, it can represent another good alternative for the already existing HPLC methods especially that the detectors used for HPLC methods are not present in most of the laboratories

MATERIALS AND METHODS

Materials and Reagents

Flurandrenolide were kindly supplied as a gift sample by Glenmark Pharmaceuticals Ltd., New Mumbai. Toluene and acetone were used as solvents to prepare the mobile phase. All the reagents used for development are analytical grade supplied from Sigma Aldrich Ltd, Mumbai, hplc grade ethyl acetate Merck Specialties Ltd, Mumbai, Laboratory grade acetone, chloroform, triethylamine from Rankem Ltd, Mumbai and hplc grade methanol from Rankem Ltd, Mumbai. The dosage form flurandrenolide lotion 0.05% USP was supplied by Taro Pharmaceuticals Ltd.

INSTRUMENTATION

The samples were applied in the form of spots of width 6 mm with a Camag 100 µL (Hamilton, Bonaduz., Switzerland) syringe on pre-coated silica gel alluminium plate 60 F_{254} [20×10] with 250µm thickness, (E Merck, Darmstadt, Germany), provide by

Anchrom technologists, (Mumbai) by means of a Camag Linomat V (Switzerland).

Chromatographic conditions

The thin layer chromatography (TLC) plates were pre-washed by methanol 100% and activated at 110^{0}C for 10 minutes, prior to chromatography. A continuous application rate of 15µl^{-1} was utilized. The slit dimension was kept at 5 *mm* × 0.45 *mm* and 10 *mm* spot^{-1} scanning speed was employed. The mobile phase consists of toluene: acetone (6: 4, *v/v*). Linear ascending development was carried out in 20 *cm* × 10 *cm* twin trough glass chamber (Camag, Muttenz, Switzerland). The optimized chamber saturation time for mobile phase was 30 min; at room temp. (25^{0}C± 2); at relative humidity 60% ± 5; the length of chromatogram run was 8 *cm* and TLC plates were air dried. Densitometric scanning was performed on Camag TLC Scanner 3 equipped with wincats software version 38. The source of radiation utilized was deuterium lamp, constantly emits UV spectrum between 200 *nm* to 400 *nm*. Evaluation was via peak area with linear regression.

Preparation of Standard stock solution

Weighed and transferred accurately about 10 mg of flurandrenolide into 10ml volumetric flask, add 6 ml methanol vortex and sonicate to dissolve with intermittent shaking, cool to room temperature and the volume was made up to the mark with methanol to get standard stock solution of flurandrenolide (1000 µg/ml) respectively.

Preparation of Working Standard solution

From 1ml of standard stock solution of flurandrenolide was diluted with methanol to 10 ml to get final working standard solution of Flurandrenolide (100 µg/ml).

METHOD VALIDATION

The developed method was validated in according to ICH guidelines. The following parameters were used for validation of the developed method[29-33].

Linearity

Linearity response for flurandrenolide was assessed in the concentration range of the drugs expressed in ng/ µl. Working standard solution 2, 4, 6, 8 10 and 12µl of Flurandrenolide was applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator to obtain the concentration of 200, 400, 600, 800, 1000 and 1200 ng/ µl. The concentration range was studied with three replicate readings of each concentration.

Accuracy studies (Recovery)

To the pre analysed sample a known amount of standard solution of pure drug flurandrenolide was added at three different levels i.e. 80, 100, 120%. Hence, 480, 600, and 720 ng/µl of flurandrenolide were spiked to the dosage form that contained 600 ng/µl of flurandrenolide, respectively. These solutions were subjected to analysis by proposed method.

Precision

The Precision of the developed method was studied in terms of intra-day and inter-day variation. %RSD was determined by analyzing standard drug solutions within the calibration range, three times on the same day for intra-day precision and three individual days over a period of one week for inter-day precision.

Sensitivity

The sensitivity of measurement of flurandrenolide by the use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The LOQ and LOD were calculated by the use of the equation:

$$LOD = 3(SD)/S$$
$$LOQ = 10(SD)/S$$

Where, SD = standard deviation, S = Slope of the Linear Regression equation.

Robustness

Robustness is an indication of the reliability of the method. Robustness studies were done by making small, deliberate changes in optimized condition like mobile phase composition ±0.5ml; saturation time ±5min and development distance ±5 *nm* and wavelength ±2*nm*. The effect of these changes on both the R_f value and peak area was estimated by calculating the % relative standard deviations (RSD) for each parameter.The acceptance criterion for %RSD was NMT 2%.

Repeatability

Repeatability of sample application was evaluated by spotting concentration of 600 ng / µl for six times on a TLC plate followed by development of plate and recording the peak areas. The acceptance criteria for % RSD for peak areas values of flurandrenolide were NMT 2%, respectively.

FORCE DEGRADATION STUDIES

In order to develop stability indicating method for estimation of flurandrenolide forced degradation studies were carried out according to ICH guidelines. The force degradation study of the drug product was performed at acid/base hydrolytic, oxidative studies, photolytic conditions and thermal conditions.

Heat induced Acid hydrolysis

To the 10 ml volumetric flask, 10 mg of flurandrenolide was weighed and dissolved in 10 ml of methanolic solution of 1N HCl to perform heat induced acid hydrolysis. The flask was heated at 60°C for 1 hr and allowed to cool to room temperature. The 1 ml of above solution was taken and neutralized and diluted up to 10ml with methanol. The resultant solution was applied on TLC plate in triplicate (6 µl each, i.e. 600 ng/ µl) and the plate was run with developed mobile phase. The plate was dried, scanned at 238*nm* and densitograms were recorded.

Heat induced Base hydrolysis

To the 10 ml volumetric flask, 10 mg of flurandrenolide was weighed and dissolved in 10 ml of methanolic solution of 1N NaOH to perform heat induced base hydrolysis. The flask was heated at 60°C for 1 hr and allowed to cool to room temperature. The 1 ml of above solution was taken and neutralized and diluted up to 10ml with methanol. The resultant solution was applied on TLC plate in triplicate (6 µl each, i.e. 600 ng/ µl) and the plate was run with developed mobile phase. The plate was dried, scanned at 238*nm* and densitograms were recorded.

Oxidative degradation

The 10 mg of flurandrenolide was separately dissolved in 10 ml of hydrogen peroxide (3.0%, *v/v*). The solution was preserved for 3 hrs at 60°C in the dark in order to eliminate the possible degradative influence of light. The 1 ml of above solution were

taken and diluted up to 10 ml with methanol. The resultant solution was applied on TLC plate in triplicate 600 ng/ µl and the plate was run with developed mobile phase. The plate was dried, scanned at 238*nm* and densitograms were recorded.

Photolytic Degradation

The photolytic stability of the drug was correspondingly performed by exposing the working standard solution to direct sunlight for 8hrs. The resultant solution was applied on TLC plate in triplicate 600 ng/ µl and the plate was run with developed mobile phase. The plate was dried, scanned at 238*nm* and densitograms were recorded.

Dry heat degradation or Thermal Degradation

The powdered drug was stored in an oven at 72°C for 4h. A solution of 10 mg Flurandrenolide in 10 ml methanol was prepared from the dry heat treated sample. The 1 ml of above solution was diluted to 10 ml with methanol. The resultant solution was applied on TLC plate in triplicate 600 ng / µl and the plate was run with developed mobile phase. The plate was dried, scanned at 238*nm* and densitograms were recorded.

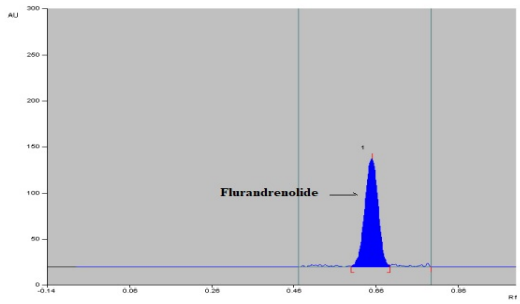

Fig. 2: Representative Densitogram of Flurandrenilode

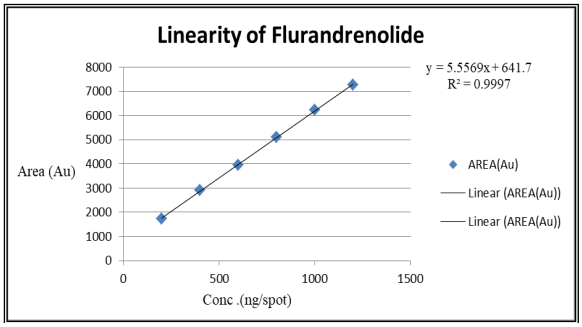

Fig. 3: Linearity Graph of Flurandrenolide

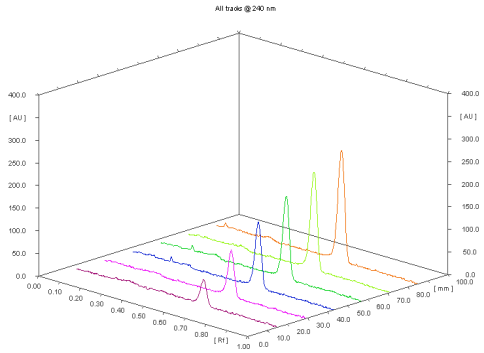

Fig. 4: 3D Overlay of chromatograms for Linearity of flurandrenolide

Table 1: Intra and Inter-day precision details of Flurandrenolide

Drug	Amount Applied (ng/spot^{-1})	Intraday Precision			Inter-day precision		
		Avg. area	SD	%RSD	Avg. area	SD	%RSD
Flurandrenolide	400	2967.50	27.40	0.923	2963.40	28.86	0.974
	800	5158.97	25.62	0.497	5162.40	38.49	0.746
	1200	7310.77	47.49	0.650	7358.97	37.86	0.515

*Mean of Three Estimations

Table 2: Recovery study of Flurandrenolide

Label Claim	% level	Initial amount (ng/spot)	Amount added (ng/spot)	Amount recovered (mg)	%Recovery (n=3)	%RSD
Flurandrenolide	80	600	480	1080.01	100.00%	0.91%
	100	600	600	1203.53	100.58%	0.88%
	120	600	720	1317.88	99.70%	0.65%

*Mean of Three Estimations

Table 3: Repeatability study of Flurandrenolide

Sr. No.	Conc. (ng/spot)	Area	Avg. Area	Std. Deviation	RSD	%RSD
1	600ng/spot	3850.2	3883.3	29.52	0.0076	0.760
2		3890.8				
3		3845.8				
4		3902.5				
5		3920.5				
6		3890.2				

*Mean of Six Estimations

Table 4: Robustness details of Flurandrenolide (n = 3)

Mobile Phase Ratio			(± 0.5 ml)			
5.5: 4.5	6.0:4.0	6.5: 3.5	Mean	SD	*Rf*	% RSD
3914.5	3988.5	3896.1	3930.09	48.78	0.65	1.24
3929.5	3975.8	3890.4			0.64	
3899.1	3888.7	3988.2			0.65	
Saturation Time(min.)			(± 5 min.)			
25	30	35	Mean	SD	*Rf*	% RSD
3920.5	3910.5	3899.7	3943.82	18.70	0.63	0.47
3908.4	3980.4	3988.2			0.65	
3970.5	3945.7	3970.5			0.64	
Development Distance (cm)			(± 0.5 cm)			
7.5	8	8.5	Mean	SD	*Rf*	% RSD
3955.7	3967.8	3905.7	3945.70	32.88	0.63	0.83
3978.5	3992.3	3913.7			0.64	
3985.4	3902.5	3909.7			0.65	
Wavelength (nm)			(± 0.2 nm)			
236	238	240	Mean	SD	*Rf*	% RSD
3978.5	3922.5	3971.8	3942.06	31.04	0.65	0.79
3939.7	3921.2	3899.1			0.63	
3977.5	3968.1	3900.1			0.65	

*Mean of Three Estimations

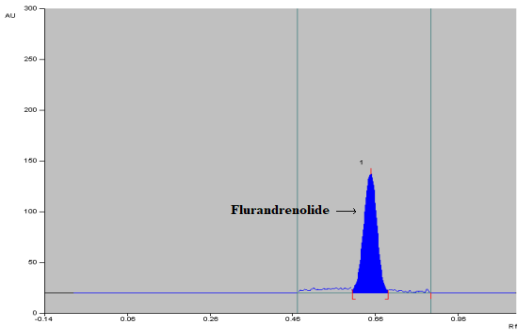

Fig. 5: Densitogram of Flurandrenolide in Flurandrenolide lotion 0.05% formulation

Table 5: Assay results of commercial dosage form (*Mean of Six Estimations)

Sr. No.	Conc. (ng/spot)	Area	%Assay
1	600	3929.1	98.38
2		3950.8	99.03
3		3981.6	99.96
4		3922.7	98.19
5		3991.2	100.24
6		3977.8	99.84
		Mean	99.27
		SD	0.87
		%RSD	0.87

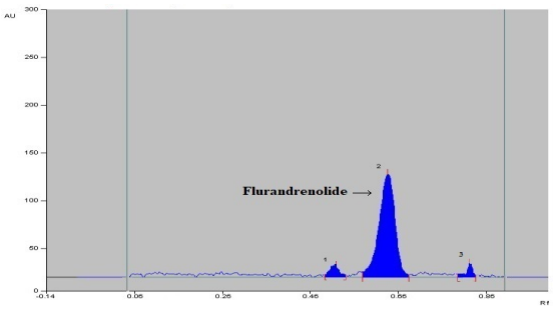

Fig. 6: Acidic Degradation of Flurandrenolide

Table 6: R*f* values of acid degradation study

Sr. No.	Peak	R*f*
1	Degradation Peak 1	0.49
2	Standard Flurandrenolide	0.64
3	Degradation Peak 2	0.80

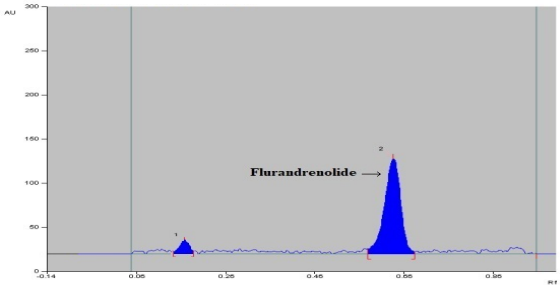

Fig. 7: Base Degradation of Flurandrenolide

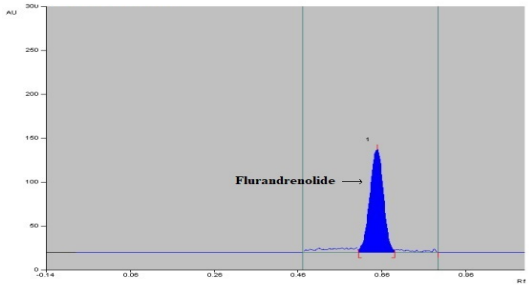

Fig. 8: Oxidative Degradation of Flurandrenolide.

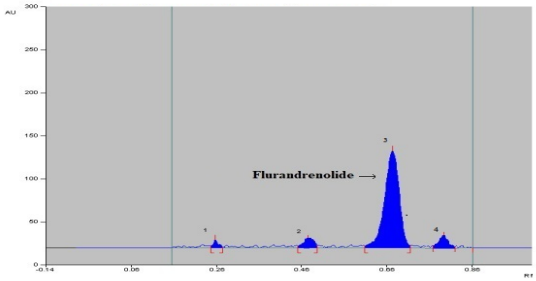

Fig. 9: Photolytic degradation of Flurandrenolide

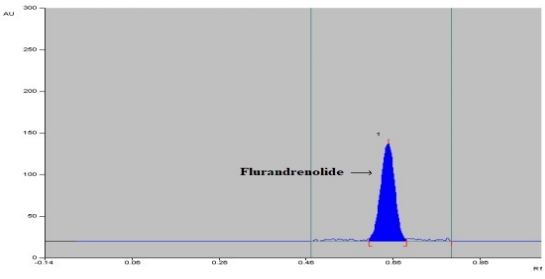

Fig. 10: Thermal degradation of Flurandrenolide

Table 7: R*f* values of base degradation study

Sr. No.	Peak	R*f*
1	Degradation Peak	0.12
2	Standard Flurandrenolide	0.64

Table 8: R*f* values of oxidative degradation study

Sr. No.	Peak	R*f*
1	Std. Flurandrenolide	0.65

Table 9: R*f* values of of Photolytic degradation study

Sr. No.	Peak	R*f*
1	Degradation Peak 1	0.25
2	Degradation Peak 2	0.48
3	Std. Flurandrenolide	0.66
4.	Degradation Peak 3	0.77

Table 10: R*f* values of thermal degradation study

Sr. No.	Peak	R*f*
1	Std. Flurandrenolide	0.65

RESULTS AND DISCUSSION

Initially, toluene and ethyl acetate mobile phase with different composition were tried for flurandrenolide. The spot were not developed properly and dragging was observed. Mobile phase composition has to be changed; for better optimization the volume of toluene was increased to 6 mL and 4 mL Acetone. Ultimately, mobile phase consisting of Toluene: Acetone (6:4 *v/v*) gave good resolution and sharper peak with R_f value 0.65± 0.03for flurandrenolide. The linear regression data for the calibration curves showed good linear relationship over the concentration range of 200- 1200 ng/spot for flurandrenolide and R^2 was found to be 0.9997. The data was subjected to regression analysis shown in Fig. 3 and 4.The intraday precision of the method %RSD was found to be 0.49%-0.92% and inter day precision %RSD was found to be 0.0515%-0.974% given in table 1.The Percentage for flurandrenolide recovery at three different levels was calculated and found to be 99.70-100.58% values obtained was given in the table 2 for flurandrenolide respectively. The limit of detection and quantification of the developed method were found to be 29.7ng/ul and 90.00ng/spot^{-1}, respectively. Repeatability and robustness of the method was found in between the acceptable range and results were given in the table 3 and table 4. The content of flurandrenolide in flurandrenolide lotion had Label claim 0.05% or 0.5mg/g of lotion shown no interferences from the excipients commonly present in the tablets. The results were shown in Table 5 and densitogram in fig. 5.

Force degradation studies for flurandrenolide such as heat induced acid hydrolysis and base degradation study were shown 15.6% and 14.2% degradation.

Flurandrenolide was found to be stable after exposing the drug to 3% of Hydrogen peroxide at 60°C for 3hrs. No any degradation was seen with additional peaks. Photolytic degradation showed 19.5% degradation of flurandrenolide with well resolved peak. The densitogram of thermal degradation study was found to be stable no any degradation was seen with no additional peaks. There was no degradation of flurandrenolide with well peak shape. Densitograms of flurandrenolide degradation studies were given in fig. 6-10 and results given in table 6-10.

CONCLUSION

The developed HPTLC densitometric method was found to be novel, simple, accurate, precise, specific and reproducible for estimation of flurandrenolide. The method was found to be repeatable and suitable for routine quality control and analysis of combined dosage form. Statistical analysis proved that the method is repeatable, accurate and specific for the analysis of flurandrenolide. The method can minimize the cost of reagents and time for analysis than other techniques such as HPLC and LC-MS. In addition, the method is inexpensive and not requires certain types of stationary phases. It also utilized the merit of applying several sample spots on HPTLC plate, which may be more advantageous for regulatory quality control laboratories specially to facilitate the post-marketing surveillance program. Thus, it can represent another good alternative for the already existing HPTLC methods.

ACKNOWLEDGEMENTS

The authors are thankful to Poona College of Pharmacy for providing necessary facilities and also gratitude towards Glenmark Pharmaceuticals Ltd., New Mumbai for providing gift sample.

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Cite this article as:

Kalal Chetan *et al*. Stability-indicating HPTLC method development and validation of flurandrenolide in API and it's lotion. Int. Res. J. Pharm. 2018;9(11):78-84 http://dx.doi.org/10.7897/2230-8407.0911262

Source of support: Nil, Conflict of interest: None Declared