STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FLUTICASONE PROPIONATE IN API AND PHARMACEUTICAL DOSAGE FORM BY HPTLC CHROMATOGRAPHIC TECHNIQUE

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ABSTRACT: ***A high performance thin layer chromatography (HPTLC) Stability indicating method was developed and validated for determination of anti-asthmatic drug .i. e. fluticasone propionate in API and Pharmaceutical formulation. Mobile phase consisting of Toluene:ethyl acetate (7:3) was used on pre-coated silica gel TLC plates for all studies. A TLC scanner in absorbance mode set at 239 nm was used for estimation of the chromatograms. ICH guidelines were used to validate developed method.Correlation coefficient (R^2) of calibration curve was found to be 0.9995 in the range of 100–500 ng spot^{-1} for fluticasone, respectively. Limit of detection (LOD) and Limit of quantification (LOQ) for fluticasone were 40.78 and 70.24 ng/spot Method had an accuracy of 99.5 ±2%. Force degradation studies were carried out for acidic, basic, oxidative and thermal conditions .There was no any interference of drug and degradant peaks. Method has been used to determine quality analysis of fluticasone from its dosage.***

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Keywords: Densitometric detection, HPTLC, fluticasone propionate.

INTRODUCTION

Fluticasone propionate (Fig. 1) is a neutral, highly potent trifluorinated corticosteroid based on the androstane nucleus. Asthma and allergic rhinitis are treated with fluticasone propionate because of its anti-inflammatory property. Thedrug is mainly available in market with other drugsin formulationas pressurized metered dose inhalers or dry powder inhalers.

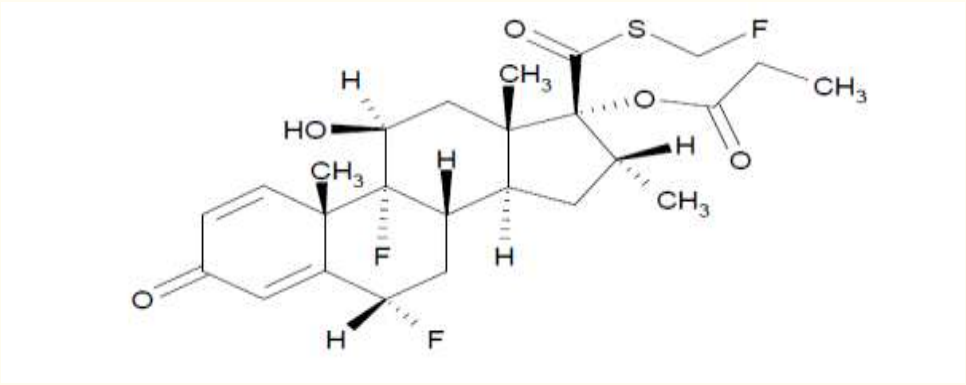

Fig 1: Chemical structure of fluticasone propionate.

Several methods have been reported for the analysis of salmeterol xinafoate and fluticasone propionate, like liquid chromatographic method with fluorescence detection, and spectrophotometric techniques.Fluticasone propionate in human plasma has been analysed by Liquid chromatographic technique coupled with APCI-MS.

As from above literature survey it was mainly accomplished that there is no Stability indicating method has been developed for estimation of Fluticasone propionate, hence there is a need for Stability indicating assay method that permits quantification of fluticasone propionate

The objective of this work was to develop a robust and sensitive HPTLC stability indicating assay method for determination of fluticasone propionate in bulk and Pharmaceutical formulation.

EXPERIMENTAL

Materials

Fluticasone propionate working Samples were mainly obtained as giftsamples from Wockhardt Research Centre, Aurangabad. Analytical grade Toluene (Fischer Scientefic), HPLC grade ethyl acetate (Fischer Scientefic), and HPLC grade methanol (Rankem), were all obtained from Avantor Performance India Ltd, Thane Maharashtra. The dosage formFlonase capsules (40mg) was purchased from retail out lets.

Instrumentation

Chromatographic separation of drug was performed by using TLC aluminium plates pre coated with silica gel 60 F254 (10 ×10) with 250 µm thickness. Samples were applied on the plate in the form of bands of width 6 mm using Hamilton microliter syringe (100ul), TLC scanner III (Camag, Muttenz, Switzerland), winCATS version 1.4.4.6337 software (Camag, Muttenz, Switzerland) were used in this study. Microsoft excel was also used to treat data statistically.

Standard Stock solution

10mg of fluticasone propionate was weighed and transferred to 10ml volumetric flask and dissolved with methanol. The resulting solution was sonicated for 10 minutes using the sonicator for uniform dissolution and volume was made up to the mark with methanol to get standard stock solution of fluticasone propionate.

Sample solutions

Working Standard solution
1ml of standard stock solution was withdrawn and transferred to 10ml volumetric flask and was diluted with methanol to 10ml to get 100µg/ml solution of fluticasone propionate.

Chromatographic conditions

6mm band of the sample was applied toTLC aluminium plates pre coated with silica gel 60 F254(10 ×10) with 250 µm thickness.10 ml mobile phase consisting of Toluene:ethyl acetate (7:3, v/v) was used in each chromatographic runin a twin trough chamber using ascending development techniques. 30 minutes saturation time was found to be optimum for the mobile phase used. The length of chromatogram run was 8 cm. WinCATS software was used for all measurements.The spots were scanned at 239nm using the TLC scanner 3 in the absorbance mode.

Force Degradation studies

Procedure for forced degradation study:

The force degradation study of the drug product was performed at acid, base hydrolytic, oxidative studies and at photolytic conditions.

Acidic Degradation

A Mixture of 5ml 0f fluticasone propionate and 5ml of 0.1N HCl was mixed together and was heated for 2Hrs at 60°C. the resultant solution was cooled at room temperature and the spots of the drug solution and degraded solution was applied on TLC plates with spot concentration equivalent to 300ng/spot and the plate was run with mobile phase Toulene:Ethylacetate. The plate was dried and scanned at 239NM usinfcamagwin software version 1.4.4.6337 and densitograms were recorded.

Basic Degradation

A Mixture of 5ml 0f fluticasone propionate and 5ml of 0.1N NaoH was mixed together and was heated for 2Hrs at 60°C. the resultant solution was cooled at room temperature and the spots of the drug solution and degraded solution was applied on TLC plates with spot concentration equivalent to 300ng/spot and the plate was run with mobile phase Toulene :Ethylacetate. The plate was dried and scanned at 239NM using camagwin software version 1.4.4.6337 and densitograms were recorded.

Oxidative Degradation

A Mixture of 5ml 0f fluticasone propionate and 5ml of 3% hydrogen peroxide was mixed together and was heated for 2Hrs at 60°C. the resultant solution was cooled at room temperature and the spots of the drug solution and degraded solution was applied on TLC plates with spot concentration equivalent to 300ng/spot and the plate was run with mobile phase Toulene:Ethylacetate. The plate was dried and scanned at 239NM using camagwin software version 1.4.4.6337 and densitograms were recorded.

Photolytic Degradation

5ml 0f fluticasone propionate was taken in an effendi tube and was exposed to photolytic light and the samples were withdrawn after 30min, 1hr, 2hr, 3hr time interval. the spots of the drug solution and degraded solution was applied on TLC plates with spot concentration equivalent to 300ng/spot and the plate was run with mobile phase Toulene:Ethylacetate. The plate was dried and scanned at 239NM using CAMAGwinsoftware version 1.4.4.6337 and densitograms were recorded.

Method validation

ICH guidelines were used for the following parametersfor validation of the developed method.[22-25]

Linearity

Linear relationship between peak area and concentration of the drugs were evaluated over the range of concentrations expressed in ng spot^{-1} by making five measurements at concentration levels in the range of 100–500 ng spot^{-1} for fluticasone propionate, respectively.

The procedure was repeated for three times (n=3) and the calibration curves were plotted by peak areas vs. concentration

Recovery studies

Recovery studies were carried out by spiking three different known amounts of the standard substances to the drug product by standard addition method. Hence, 80, 100, and 120 ng spot^{-1} of fluticasone propionate were spiked to the dosage form which containing concentration of 100 ng spot^{-1} of fluticasone propionate.

Precision

Precision of the developed method was studied by considering intra-day precision, inter-day precision and variation between analysts.To evaluate intraday and interday precision three spots of 3 µl, 4µl,and 5µl of standard solution were spotted on the TLC plate. Each spot was repeated for three times.(n=3)

Limits of detection and quantification

Determination of the detection and quantification limits was performed based on the standard deviations of the blank responses and the slope of the least square line parameters.

LOD and LOQ was calculated using the following equation:

LOD= 3.3(SD)/S

LOQ= 10(SD)/S

Where,

SD= standard deviation of the response

S= slope of regression equation

Robustness

Change in the volume of the mobile phase, Saturation time and Scanning Wavelength were used in robustness study.±10% change in the volume of the mobile phase and ±20% change in timewas varied from optimized conditions to study robustness of the method developed.The effect of these changes on both the R_f values and peak areas were evaluated by calculating the relative standard deviations (RSD) for each parameter.

RESULTS AND DISCUSSIONS

Method optimization for the HPTLC-densitometric measurements

Several trials were made by using different solvent systems containing non-polar solvents, relatively polar solvents. Among the different mobile phase combinations tested Toluene:ethyl acetate (7:3, v/v) gave better resolution and sharper peaks with R_f values of 0.50 ± 0.04 for fluticasone propionate, respectively. Fig. 2 shows the HPTLC densitogram of the mixture using the optimal conditions.

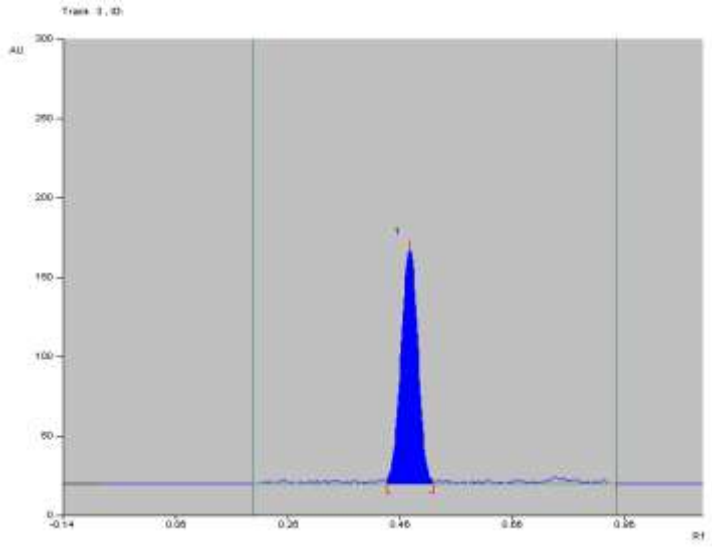

Fig 2 Typical densitogram of fluticasone propionate.

Method Validation

Linearity

Calibration graphs were constructed in the range of 100–500 ng spot^{-1} for fluticasone propionate.Peak areas were found to have good linear relationship with the concentration. For fluticasone propionate, linearity data has been presented in Table 1.The drug shows good linearity in the range of 100–500 ng spot^{-1}.

Limit of detection and quantitation

The limit of detection and quantification of the developed method were calculated using standard method mentioned in literature.(Eric-Jovanovic et al., 1998). The limits of detection and quantification were found to be 40.78 ng spot^{-1} and 70.24 ng spot^{-1}, respectively, for fluticasone propionate inTable.2

Table 1 Summary of linear regression data for calibration curves using peak areas.

Parameters	Fluticasone propionate
Linearity range	100–500 ng spot^{-1}
Linear regression equation	$y = 9.1392x + 792.94$
Slope ± SD	5.2956 ± 0.177
Intercept ± SD	1336.9 ± 105.3662
Correlation coefficient (r)	0.9989

Parameters	Fluticasone propionate
Determination coefficient (r^2)	0.9995
Limit of detection (LOD)	40.78 ng/spot
Limit of quantification (LOQ)	70.24 ng/spot

Precision

Repeatability and intermediate precision of the developed method were expressed in terms of coefficients of variation (CV) of the peak area. The results showed that intra- and inter-day variation of the results at three different concentration levels 300–500 ng spot^{-1} for fluticasone propionate were within the acceptable range (RSD less than 2%). (Table 2).

The percentage recovery at three levels (Argekar et al., 1996), in the range from 100 to 500 ng spot^{-1} for fluticasone propionate were studied and found to be satisfactory (Table 4). For fluticasone propionate, the recoveries were found between 98.1% and 102.5%.

Table 2 Intra- and inter-day precision for fluticasone propionate.

Compound	Amount (ng spot^{-1})	Intra-day precision (n = 3)			Inter-day precision (n = 3)		
		Mean peak area	SD	%RSD	Mean peak area	SD	%RSD
Fluticasone propionate	300	3464.333	46.24	1.33	3625.867	41.96	1.15
	400	4549.433	56.14	1.23	4517.267	72.96	1.51
	500	5341.4	66.43	1.24	5335.667	71.49	1.33

Accuracy/recovery

The percentage recovery at three levels (Argekar et al., 1996), in the range from 100 to 500 ng spot^{-1} for fluticasone propionate were studied and found to be satisfactory (Table 4). For fluticasone propionate, the recoveries were found between 98.1% and 102.5%.

Table 3 Recovery study of the method (using the standard addition method fluticasone propionate (n = 3).

Number of levels	Fluticasone propionate			
	Initial amount (ng spot^{-1})	Amount added (mg)	Amount added (ng spot^{-1})	% Recovery
1	300	80	220	98.83
2	300	100	200	100.33
3	300	120	180	101.67
Accuracy %				99.58

Repeatability

Repeatability of the method was checked by analysing the standard solution, M (Fluticasone 100µg/ml) after application of 3µl spot on TLC Plate (n=6). %RSD was found to be 1.08% for Fluticasone. %RSD was <2% which is in the acceptable range.(Table 4)

Table 4 Repeatability study for Fluticasone propionate

Sr. No.	Conc. (ng/spot)	Area	Avg. Area	Std. Deviation	%RSD
1	300ng/spot	3416.3	3352.533	36.29	1.08
2		3329.3			
3		3347.2			
4		3364.1			
5		3348.2			
6		3310.1			

Robustness

The standard deviations of peak areas were calculated for the mentioned four parameters Variation in composition of the mobile phase, volume of the mobile phase, time from spotting to development and time from development to scanning showed RSD less than 2% for peak areas indicating the method developed robust.(Table 5).

SD and %RSD were calculated from the peak areas of densitograms.

Table 5 Robustness study for the developed method (*n* = 5).

Parameter studied	Fluticasone propionate	
	SD	%RSD
Volume of mobile phase(±0.5ml)	51.38	0.24
Wavelength Change(±10nm)	51.38	1.43
Saturation Time(±20min)	34.48	0.94

SD and %RSD were calculated from the peak areas of densitograms.

Assay

To determine the content of Flutiacasone propionate in capsules (containing 250µg of Fluticasone propionate), 20 tablets were weighed accurately, crushed and average weight was calculated. The amount equivalent to 4capsules was taken and dissolved and diluted up to the mark in with methanol to obtain a concentration equivalent to 100µg/ml of fluticasone propionate. 3 µl spot of standard solution, (Fluticasone propionate 100µg/ml) and marketed formulation solution were spotted on TLC plate. The plate was dried and scanned at 239nm using Camagwin software version 1.4.4.6337 and densitograms were recorded and areas were reported. The drug content was calculated and possibility of excipient interference with analysis was examined. Table 6.

Table 6 Assay results of the commercial dosage form.

Drug	Rf	Drug Content	Mean %
Fluticasone propionate	0.50	100.41% 99.73%	99.90%

Forced Degradation Studies

Acid Degradation

The densitogram of acid degradation study (Fig.3) show peaks for Fluticasone propionate (300ng/spot)) at R*f* 0.51 respectively. On comparison with it showed additional peak of degradants at Rf 0.57and 0.63 in chromatogram which are well separated from principle peak of drug. There was 06.11% degradation of Fluticasone propionate with well resolved peak.

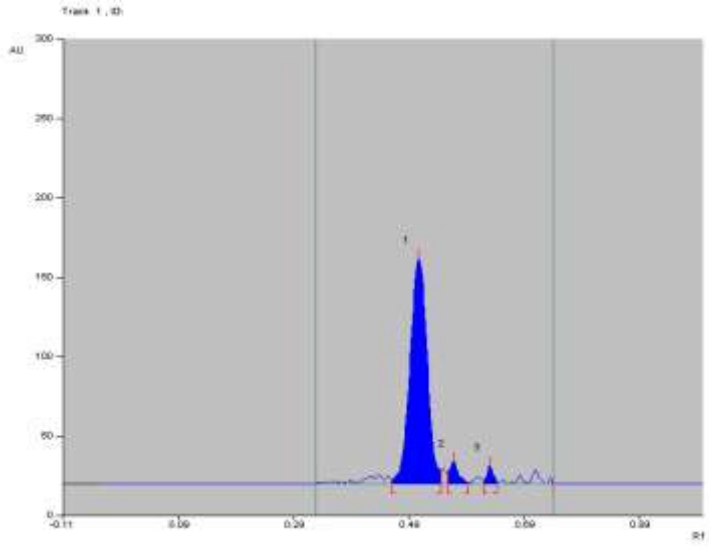

Fig. 3 Densitogram of Acid Degradation of Fluticasone Propionate.

Base Degradation

The densitogram of base degradation study (Fig. 4) show peaks for Fluticasone propionate (300ng/spot) at R*f* 0.50 respectively. On comparison with it showed additional peak of degradant at Rf 0.34 and 0.37gv in chromatogram which are well separated from principle peak of drug. There was 8.32% of Fluticasone propionate with well resolved peak.

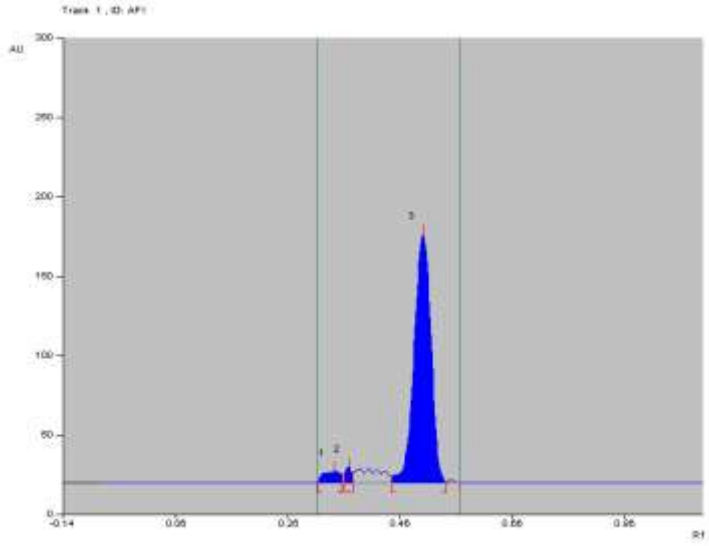

Fig 4 Densitogram of Base Degradation of Fluticasone propionate.

Oxidative Degradation

The densitogram of oxidative degradation study (Fig.5) show peaks for Fluticasone propionate (300ng/spot) at R*f* 0.49 respectively. On comparison with it showed additional peak of degradants at Rf 0.23 in chromatogram which are well separated from principle peak of drug. There was 14.78% degradation of Fluticasone propionate with well resolved peak.

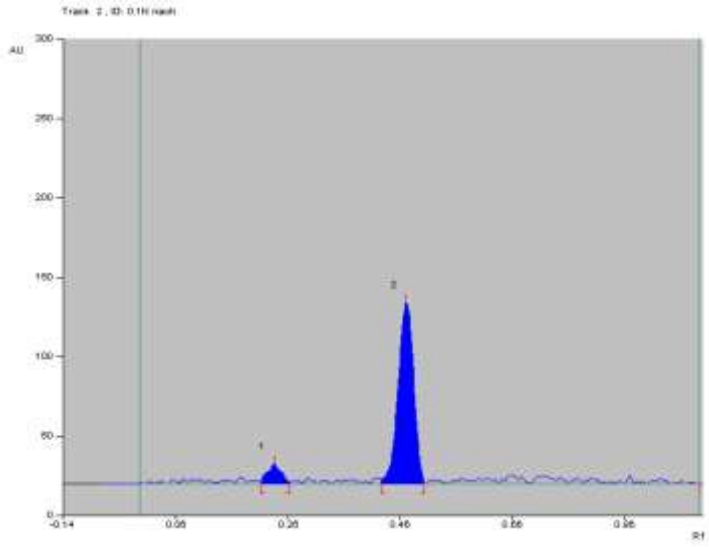

Fig 5 Densitogram of Oxidative Degradation of Fluticasone propionate.

Thermal Degradation

The densitogram of dry heat degradation study (Fig.6) shows peaks for Fluticasone propionate (300ng/spot) at R*f* 0.49 respectively**.** On comparison with it showed additional peak of degradants at Rf 0.80 in chromatogram which are well separated from principle peak of drug. There was 9.45% of degradation of Fluticasone propionate with well resolved peak.

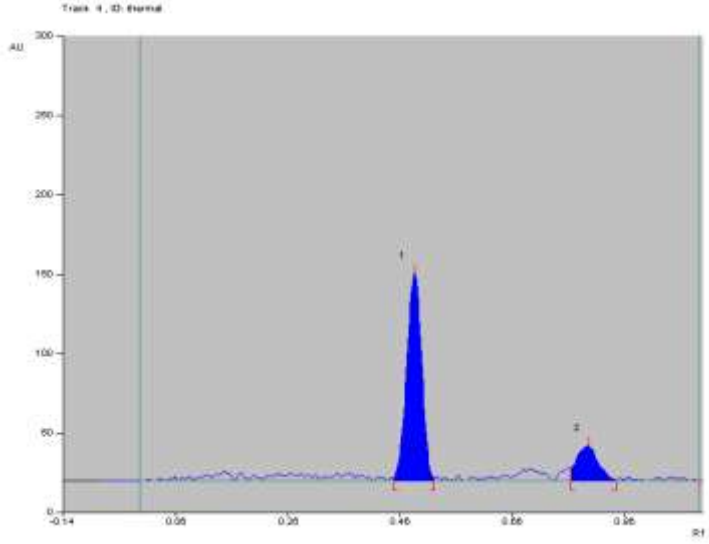

Fig 6 Densitogram of Thermal Degradation of Fluticasone propionate.

Neutral Degradation

The densitogram of neutral degradation study (Fig.7) shows peaks for Fluticasone propionate (300ng/spot) at R*f* 0.50 respectively**.** On analysis it was found that no degradant peak was found excluding the principle peak of fluticasone propionate.

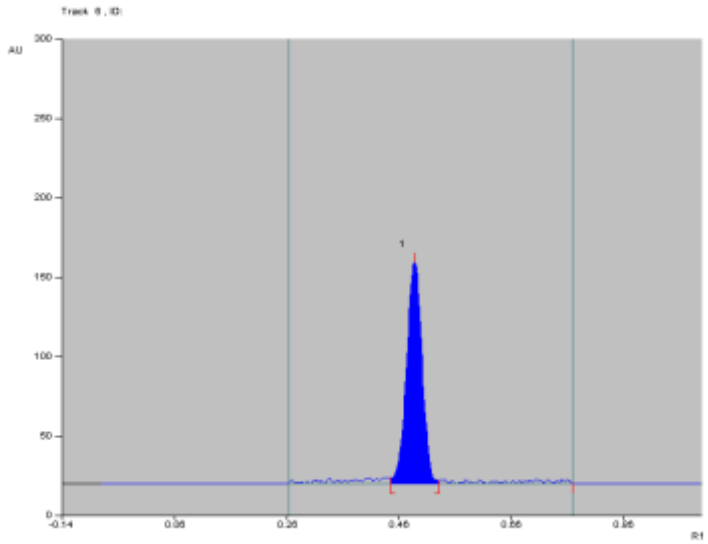

Fig 7. Densitogram of Neutral Degradation of Fluticasone propionate.

Sample solution stability study

Sample solution was prepared and was kept at room temperature (20 ± 2 °C) on a shelf protected from direct light. The solution was analyzed after 20 min, 3 h, 8 h, 24 h, 4 days and 8 days. Because of the time needed for sonication and filtration, the fastest possible analysis was carried out within 20 min and hence results of the remaining analysis times were compared with it. The average peak areas and the CV values are presented in Table 7. The average peak areas of fluticasone propionate were significantly varied from the reference time (p = 0.05, t stat. = 4.73, n = 6) after 24 h. Therefore, in order to decrease systematic errors because of test solution instability, the analysis should be carried out within 24 h of sample preparation.

Table 7 Solution stability study for fluticasone propionate.

Time of analysis	Fluticasone propionate	
	Average peak area (n = 6) ± SD	CV[a]
20 min	5615.95 ± 60.3	2.17
3 h	5742.12 ± 101.9	0.99
8 h	5524.711 ± 36.2	0.95
24 h	5498.48 ± 38.7	0.65
4 days	5266.6 ± 48.5	1.90

Time of analysis	Fluticasone propionate	
	Average peak area (n = 6) ± SD	CV[a]
8 days	5263.12 ± 57.9	1.20

[a]CV: coefficient of variation.

CONCLUSION

The developed HPTLC method was found to be simple, specific, and robustfor determination of fluticasone propionate in the pharmaceutical dosage form.The method is validated with statistical analysis.The method uses less costly and simple reagents and requires less time for the analysis of multiple samples. Hence this method developed is more suitable for regulatory quality control laboratories to facilitate the post-marketing surveillance program. Hence the developed method is an alternative for the reported expensive HPLC methods using PDA detector

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