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Validated HPTLC Method for the Determination of Two Novel steroids in Bulk and Pressurized Metered-Dose Preparations

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Abstract: A sensitive, accurate, precise and reliable HPTLC method has been proposed for determination of Ciclesonide and Fluticasone propionate - two widely used novel steroids in bulk as well as in pressurized metered dose preparations. Ciclesonide and Fluticasone propionate resolved well in toluene: ethyl acetate: formic acid (7: 3: 0.1 v/v/v) with respective R_f values 0.46 ± 0.004 and 0.43 ± 0.012 and could be scanned at 242 nm and 236 nm respectively. Linearity was observed in the range of 400 – 1100 ng band^{-1} for Ciclesonide and 200 – 1000 ng band^{-1} for Fluticasone propionate. The method was validated as per ICH guidelines and successfully applied for quantification of Ciclesonide and Fluticasone propionate in their commercial metered dose formulations. The method was found to be less time consuming as well as cost effective for routine analysis.

Keywords: Ciclesonide; Fluticasone propionate; High Performance Thin Layer Chromatography; pressurized metered - dose preparations; Respiratory disorders

1. Introduction

Ciclesonide (Figure 1, Sub. A), chemically known as (11β, 16α) - 16, 17-[(R)- cyclohexylmethylene] bi (oxy)-11- hydroxy-21-(2-methyl-1- oxopropoxy) pregna-1, 4-diene-3, 20-dione, is a new corticosteroid. Fluticasone propionate (Figure 1, Sub. B), S- (fluoromethyl)-6α, 9-difluoro-11β, 17-dihydroxy-16α-methyl-3-oxoandrosta-1, 4-diene-17β-carbothioate, 17-propionate, is a synthetic steroid of the glucocorticoid family of drugs. Both the steroids are widely used for the treatment of various respiratory disorders.

Both Ciclesonide and Fluticasone propionate are Official in Indian pharmacopoeia - 2007. The official methods involve determination of Ciclesonide and Fluticasone propionate by liquid chromatography [1-2]. Other methods available for identification as well as determination of Ciclesonide include LC-MS/MS [3] and Uv-visible spectrophotometry [4]. Similarly for determination of Fluticasone propionate, methods like Uv-visible spectrophotometry [4], LC-MS/MS [5], HPLC [6] and visible spectroscopy [7] were reported. Similarly for determination of Fluticasone propionate, methods like Uv-visible spectrophotometry [4], LC-MS/MS [5], HPLC [6] and visible spec-

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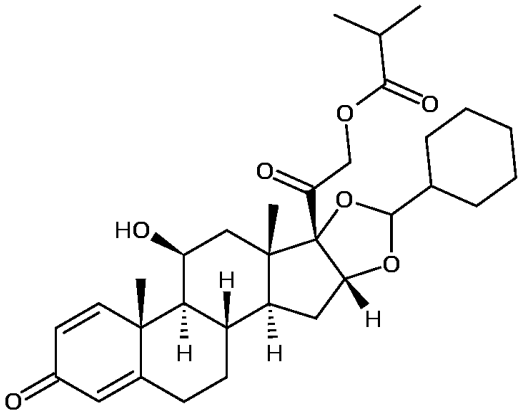
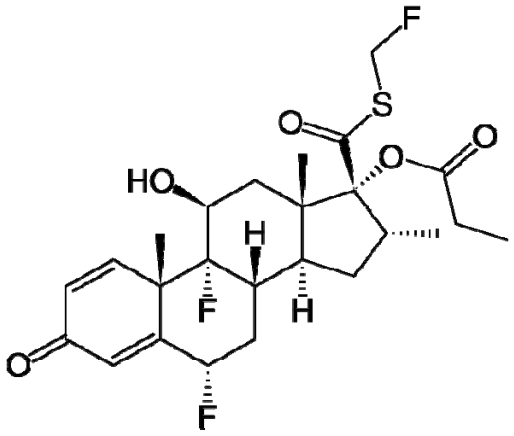
troscopy [7] were reported. Here we report HPTLC method for quantification of pure steroids and application of the developed method for determination of their metered dose commercial formulations. HPTLC methods are one of the sensitive, cost effective and less time consuming methods [8]. The method was successfully validated as per validation parameters of ICH guidelines [9].



Sub. A. Structure of Ciclesonide



Sub. B. Structure of Fluticasone propionate

Figure 1. Structure of Ciclesonide (Sub. A) and structure of Fluticasone propionate (Sub. B).

2. Materials and Methods

2.1. Chemicals and reagents

All chemicals used were of analytical grade and double distilled water was used throughout. Pure Ciclesonide and Fluticasone propionate were of pharmacopeial grade. Various pressurized metered dose preparations of Ciclesonide and Fluticasone propionate were obtained from commercial source.

2.2. Preparation of the standard solutions for calibration curve

All reagents were tested for stability in solution and during the actual analyses; the behavior of the analytes remained unchanged up

to 72 h from the time of preparation at room temperature. The measurements were done at room temperature. Standard Ciclesonide and Fluticasone propionate solutions were prepared by dissolving 10 mg of each in 10 ml methanol (1 mg mL^{-1}). One mL of aliquots from these solutions were taken and diluted up to 10 mL using methanol (100 µg mL^{-1}). Five different concentrations of the stock solution of Ciclesonide representing 400 ng-1100 ng per band were applied on the TLC plate. Similarly five different concentrations of the stock solution of Fluticasone propionate representing 200 ng-1000 ng per band were applied on the TLC plate.

2.3. TLC method development

The solubility of the component was determined in various solvents. Different ratios of non polar/ semi polar solvents were tried with or without addition of organic acids.

2.4. HPTLC instrumentation and experimental conditions

HPTLC plates: Pre-coated silica gel aluminum plates 60 F_{254} (20 x 10) with 0.2 mm layer thicknesses (Merck, Darmstadt, Germany, Cat. No. 1.05548). The plates were pre-washed with methanol and activated at 60°C for 5 minutes prior to chromatography.

Spotter: Automated TLC sampler Linomat V (Camag, Muttenz, Switzerland) which was controlled by Win CATS software 1.3.3 (Camag, Muttenz, Switzerland).

Band width: 6 mm

Chamber: 20 x 10 cm twin trough glass chambers (Camag, Muttenz, Switzerland). The optimized chamber saturation time for mobile phase was 20 min at room temperature (25°C ± 2) at relative humidity of 60% ± 5

Mobile phase: toluene: ethyl acetate: formic acid (7: 3: 0.1) v/v/v. 20 ml mobile phase was used.

Source of radiation: D2 lamp emitting a continuous ultra violet radiation between 180 nm to 400 nm.

Scanner: Densitometric scanner III (Camag, Muttenz, Switzerland).

Slit widths and scanning speed: 6.0 mm x 0.45 mm and scanning speed of 10 mm S^{-1} was employed.

Detection: 242 nm for Ciclesonide and 236 nm for Fluticasone propionate. Evaluation was done by measuring peak areas with linear regression for each plate.

2.5. Procedure for pressurized metered dose commercial preparation

The preparation of the sample for metered dose formulation was done exactly as was described in Indian Pharmacopoeia – 2007 [10]. The samples from the metered dose formulation were collected in methanol and the contents were shaken well. Solutions were filtered through Whatman filter paper No. 41 and same HPTLC conditions were applied as mentioned above. All the brands of the pressurized metered dose preparations containing Ciclesonide and Fluticasone propionate were tested according to the procedure described above. The formulations were already available in the market and hence were procured from the registered medical store.

3. Method validation

3.1. Precision

Intraday precision and inter day precision for the developed methods were measured in terms of % RSD. The experiments were repeated six times a day for intraday precision and on six different days for interday preci-

sion. The concentration values for both intraday precision and interday precision were calculated six times separately and percent relative standard deviation were calculated. Finally the mean of % RSD (% RSD = [S/X] 100, where S is standard deviation and X is mean of the sample analyzed) was calculated.

3.2. Robustness of the method

By introducing small changes in the proportion of the mobile phase, the effects on the results were examined. Mobile phases having different proportion were tried like toluene: ethyl acetate: formic acid (7:3:0.1) v/v/v, (7.1:2.9:0.1) v/v/v and (6.9:3.1:0.1) v/v/v were tried for both the drugs and chromatograms were run. The plates were pre-washed by methanol and activated at 60◦ C ± 5 for 5, 8 and 10 minutes prior to chromatography. Robustness of the method was done at three different concentration levels. Amount of mobile phase was varied and plates were developed in 10, 15 and 20 ml mobile phase. Time from spotting to chromatography and chromatography to scanning were also varied and RSD were determined.

3.3. Limit of detection and limit of quantitation

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank solution (methanol) was spotted six times following the same method as explained above. The signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting known concentrations of reference solution until the average responses were approximately three or ten times the standard deviation of the responses for six replicate determinations.

3.4. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The bands of samples were identified by comparing the R_f values and spectrum of the bands with those of the bands obtained from the standards. The purity of the peaks were tested by comparing the spectra at three different levels i.e. peak start (S), peak apex (M) and peak end (E) positions of the spot.

3.5. Recovery studies (Accuracy)

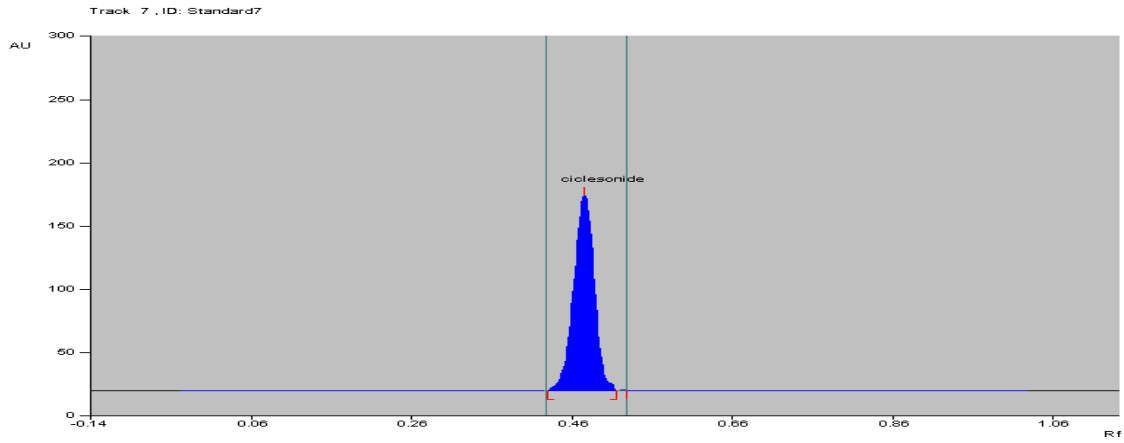
Accuracy of proposed method and interference from excipients was determined by recovery experiments. Recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of Ciclesonide and Fluticasone propionate to a known concentration of the pressurized metered dose formulations.

4. Results and discussion

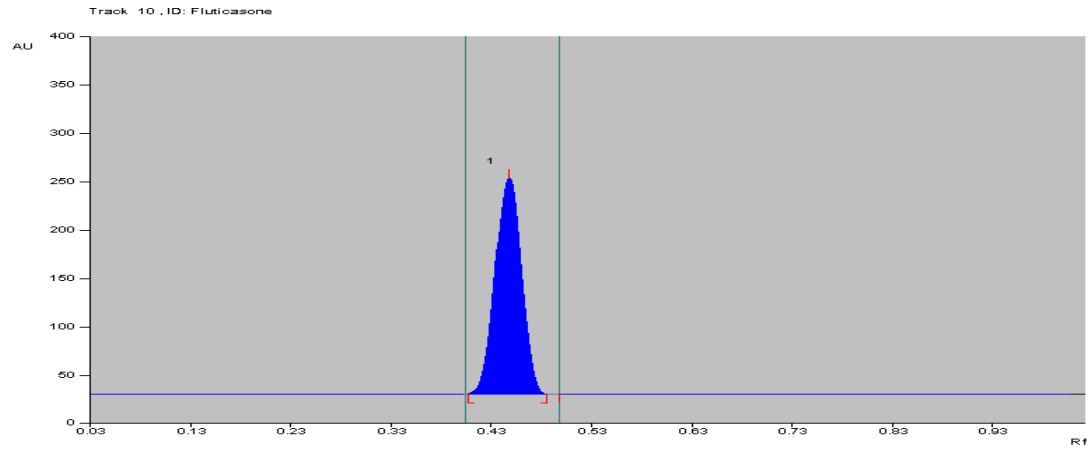
The TLC method was optimized first to develop the HPTLC method. It was observed that both Ciclesonide and Fluticasone propionate were soluble in methanol, ethyl acetate as well as in toluene. Out of number of permutations and combinations, Toluene: ethyl acetate (7:3) gave most satisfactory migration of the compounds on the TLC plates. However, the spots were spreading and broad. To address this problem, we incorporated 0.1ml of formic acid in the above mobile phase. This has significantly reduced the tailing resulting in clear concentrated bands. The above solvent system was found suitable for both Ciclesonide and Fluticasone propionate, when analyzed independently on different plates. The HPTLC Chromatogram was shown in Fig. 2 for both Ciclesonide and Fluticasone propionate. The developed method showed good linearity, precision, accuracy, sensitivity as well as robustness as can be seen from table 1. The analysis of the formulation showed results within the range as per Indian Pharmacopoeia – 2007 [10]. The recovery experiment

also gave the result of 99.35 ± 0.506 for Ciclesonide and 99.36 ± 0.909 for Fluticasone propionate. The detailed results are shown in table 2. The recoveries were calculated on the bases of actual results obtained for the formulations. The results of robustness testing were represented in table 3 and those for commercial formulation analysis were shown in table 4. The comparative study of pure drugs and commercial formulations were represented in table 5. The proposed method is valuable due to its simplicity, uniqueness as well as accuracy. To the best of our knowledge, this is the first report of HPTLC analysis for Ciclesonide and Fluticasone propionate and their metered dosage forms. The method doesn't need any cleanup process and there is no interference of the propellant or any other vehicles of the formulations.

Figure 2. HPTLC Chromatogram



Ciclesonide.



Fluticasone propionate.

Figure 2. HPTLC Chromatogram of Ciclesonide and HPTLC Chromatogram of Fluticasone propionate.

Table 1. Validation parameters of the proposed HPTLC method for estimation of Ciclesonide and Fluticasone propionate.

Validation Parameters	Ciclesonide	Fluticasone propionate
Linearity range (ng band^{-1})	400 – 1100 ng band^{-1}	200 – 1000 ng band^{-1}
Slop	3.485	5.088
Intercept	4.939	121.6
Correlation coefficient (R^2)	0.997	0.998
Accuracy (Recovery study)	99.35 ± 0.506	99.36 ± 0.909
LOD (ng band^{-1})	50 ng band^{-1}	25 ng band^{-1}
LOQ (ng band^{-1})	150 ng band^{-1}	75 ng band^{-1}
Precesion – Intraday Interday	0.7620 (%RSD) 1.5661 (%RSD)	0.4891 (%RSD) 1.2168 (%RSD)
% RSD of linearity of the method (n = 3 X 6)	1.12	1.46

RSD- Relative Standard Deviation

Table 2. Results of recovery studies of Ciclesonide and Fluticasone propionate by the developed HPTLC methods.

Excess standard drug added to the formulation (%)		Estimated content (ng/band)		Amount of standard found (ng/band) [a]		% Recovery[a]	
Ciclesonide	Fluticasone propionate	Ciclesonide	Fluticasone propionate	Ciclesonide	Fluticasone propionate	Ciclesonide	Fluticasone propionate
0	0	465	277	----------	----------	----------	----------
80	80	837	498.6	828.9	496.79	99.03 ± 0.38	99.63 ± 1.12
100	100	930	554	926.85	548.34	99.66 ± 0.69	98.97 ± 0.43
120	120	1023	609.4	1016.52	606.16	99.36 ± 0.44	99.46 ± 1.19
Mean Recovery ± Standard deviation						99.35 ± 0.50	99.36 ± 0.90

[a] - mean value of three determinations

Table 3. Results of Robustness testing of the proposed HPTLC methods for determination of Ciclesonide and Fluticasone propionate.

Parameter	% RSD[a]	
	Ciclesonide	**Fluticasone propionate**
Mobile phase composition		
toluene: ethyl acetate: formic acid (7:3:0.1) v/v/v	1.95	1.84
1. (7:3:0.1) v/v/v	1.12	1.79
2. (7.1:2.9:0.1) v/v/v	1.47	1.92
3. (6.9:3.1:0.1) v/v/v		
Amount of mobile phase		
1. 10 ml	0.98	0.99
2. 15 ml	0.87	1.24
3. 20 ml	1.16	1.48
Plate pretreatment		
Pre – washed with methanol and dried at 60° C for:	0.76	0.45
1. 5 minutes	0.84	0.62
2. 8 minutes	0.69	0.47
3. 10 minutes		
Time from spotting to chromatography		
1. 1 minute	1.18	1.45
2. 3 minutes	1.23	1.37
3. 5 minutes	1.11	1.64
Time from chromatography to scanning		
1. 1 minute	0.95	0.79
2. 5 minutes	1.17	0.82
3. 10 minutes	1.24	0.89

[a] - mean value of three determinations

Table 4. Application of the developed HPTLC methods for determination of pressurised metered dose commercial formulations of Ciclesonide and Fluticasone propionate.

Formulation		Amount labeled (mcg/spray)		Amount found[a] (mcg/spray)		% Amount Found [a] ± S.D.	
Cicleson-ide	Fluticasone propionate	Ciclesonide	Fluticasone propionate	Ciclesonide	Fluticasone propionate	Ciclesonide	Fluticasone propionate
Brand A	Brand C	50	50	46.53	46.19	93.07 ± 0.46	92.39 ± 0.41
Brand B	Brand D	50	50	47.05	45.805	94.10 ± 0.41	91.61 ± 0.52

[a] - mean value of three determinations

Table 5. Comparative HPTLC analysis of Ciclesonide and Fluticasone propionate in their pure forms and commercial formulations.

Comparative Parameter	Ciclesonide[a]		Fluticasone propionate[a]	
	Pure Drug	Formulation	Pure Drug	Formulation
R_f value	0.46 ± 0.004	0.46 ± 0.011	0.43 ± 0.012	0.46 ± 0.025
Precision – Intraday	0.7620	0.435	0.4891	0.472
Interday	1.506	1.625	1.216	1.341
(In terms of %RSD)				

[a] - mean value of six determinations
RSD- Relative Standard Deviation

5. Conclusion

A simple, accurate, precise and reproducible HPTLC method was developed which could be applied for quantification of Ciclesonide and Fluticasone propionate and their metered dose formulations. The method eliminates the tedious process of sample clean up as well as derivatisation. Even though HPLC methods are available for analysis of these compounds, no HPTLC methods are reported till now. The statistical analysis proves that the developed methods are suitable for determination of Ciclesonide and Fluticasone propionate as bulk drugs and in the commercial pressurized metered dose preparations without any interference from the excipients. Since the metered dose formulation could also be analyzed with satisfactory precision, this method should have wider applicability in the routine analysis.

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