Pharmacophore 2014, Vol. 5 (2), 231-238 USA CODEN: PHARM7 ISSN 2229-5402

Pharmacophore

(An International Research Journal)

Available online at http://www.pharmacophorejournal.com/

Original Research Paper

SIMULTANEOUS ESTIMATION OF CLOBETASOL PROPIONATE AND FUSIDIC ACID IN CREAM DOSAGE FORM BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

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ABSTRACT

A simple, rapid, precise and accurate high performance liquid chromatography method was developed for simultaneous estimation of Clobetasol propionate and Fusidic acid in combined dosage form. The separation was obtained using a mobile phase consisting of acetonitrile and water in ratio of 80:20 and adjusting pH 5.0 with glacial acetic acid (10%) using Phenomenex-luna C18 (250 × 4.6 mm, 5 µm) column. The flow rate 1.0 mL min^{-1} and UV detection at 240 nm was employed. The retention time for Clobetasol propionate and Fusidic acid was 5.55 min and 7.48 min respectively. Linearity for Clobetasol propionate and Fusidic acid was found to be in the range of 4-12 µg/mL and 160-480 µg/mL respectively. The method was validated as per the ICH guidelines and the results were within the acceptance criteria for precision, linearity, specificity, stability of solution and robustness.

Keywords: Clobetasol propionate, Fusidic acid, RP-HPLC, Simultaneous estimation, Cream dosage form.

INTRODUCTION

The combination of Clobetasol propionate and Fusidic acid is available as creams formulation for topical use in psoriasis and eczema. Clobetasol propionate (21-Chloro-9-fluoro-11β-hydroxy-16β-methyl-3, 20-dioxopregna-1,4-dien-17-yl propanoate) is derivative of prednisolone with high glucocorticoid activity and low mineralocorticoid activity. It is reported in pharmacopoeias such as BP[1] and USP.[2] Fusidic acid [ent-(17Z)-16α-(Acetyloxy)-3β, 11β-dihydroxy-4β, 8, 14-trimethyl-18-nor-5β, 10α-cholesta-17(20), 24-dien-21-oic acid hemihydrate] is an antimicrobial substance. It is reported in pharmacopoeias such as BP[1], USP[2] and IP.[3] Several HPLC methods are reported in combination with other drugs for the determination of Clobetasol propionate and Fusidic acid in the literature for its assay.[4-18] However, no method is reported for simultaneous estimation of Clobetasol propionate and Fusidic acid by RP-HPLC in any literature. In the present investigation, a specific RP-HPLC method is described for the simultaneous estimation of Clobetasol propionate and Fusidic acid in pharmaceutical formulation.

MATERIAL AND METHODS

Instrumentation

The HPLC system used was isocratic HPLC Shimadzu LC-20AD, series equipped with a 20 µL sample loop, and SPD-20A detector. The output signal was monitored and integrated using Spinchrom version 2.4.1.93 software. Phenomenex-luna C18 (250 × 4.6 mm, 5µm) column was used for the separation.

Materials

The drug sample of Clobetasol propionate and Fusidic acid was obtained from Tripada Biotech, Ahmedabad and west-coast Pharma, Ahmedabad respectively. The creams were procured from market. Label claim for Clobetasol propionate and Fusidic acid were 0.05% w/w and 2.0% w/w per cream. Acetonitrile HPLC Grade (Rankem chemicals), HPLC Grade water (S D Fine chemicals), HPLC Grade glacial acetic acid (Rankem chemicals) are used in the study.

Chromatographic Conditions

The analysis was carried out on an isocratic HPLC system using a Phenomenex-luna C18 (250 × 4.6 mm, 5µm) column as a stationary phase with UV detection at 240 nm at ambient room temperatures using a 20µL injection volume.

Mobile Phase

A mixture of acetonitrile and water in ratio of (80:20) was prepared and adjusted to pH 5.0 using glacial acetic acid (10%), filtered, degassed and used. Glacial acetic acid (10%) was prepared by diluting 1 mL of concentrated glacial acetic acid in to 10 mL of HPLC grade water.

Standard Stock Solution

Stock solution of Clobetasol propionate was prepared by dissolving 10 mg in 100 mL volumetric flask containing 70 mL of mobile phase, sonicated for about 10 min and the made up to volume with same mobile phase. Stock solution of Fusidic acid was prepared by dissolving 100 mg in 100 mL volumetric flask containing 70 mL of mobile phase, sonicated for about 10 min and the made up to volume with same mobile phase. Daily working standard solution of both Clobetasol propionate and Fusidic acid were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Sample Preparation

About 1.0 g of cream was weighed accurately in 50 mL beaker. To the prepared solution 10 mL of mobile phase (Acetonitrile and Water) and sonicated with maintaining the temperature about 60º- 65ºC for 10 min for complete dissolution. The above solution was cooled to room temperature and was transferred into a 50 mL volumetric flask and volume was adjusted by diluent. The solution was thoroughly mixed and filtered through whatmann filter paper. The resulting solution (10 µg/mL of Clobetasol propionate and 400 µg/mL of Fusidic acid) was filtered through 0.45 µm nylon membrane and injected into HPLC system.

Method Development

The mobile phase consisting of Acetonitrile and Water in varying proportions and change in pH was tried and finally ratio of 80:20 (pH-5.0 adjusted with diluted glacial acetic acid) was selected because it was found to give good separation for the peaks of Clobetasol propionate (R_t-5.55 min) and Fusidic acid (R_t-7.48 min), respectively as shown in the Figure.1. In addition to this, UV spectra of individual drugs were recorded at the wavelength range from 200 to 400 nm and the response for optimization was compared. The choice of wavelength 240 nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.

Method Validation

The method was validated in accordance with ICH guidelines.[19]

System suitability

System performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as retention time (R_t), number of theoretical plates (N), asymmetry factor, resolution factor were determined. The results are shown in table I.

Linearity

Mixed standard solution of Clobetasol propionate and Fusidic acid were prepared with mobile phase in such a way that the final concentration of Clobetasol propionate and Fusidic acid is in the range of 4-12 µg/mL and 160-480 µg/mL respectively. The peak area was recorded for all the peaks as shown in table II for linearity of Clobetasol propionate and Fusidic acid. The plots of peak area versus the respective concentration were found to be linear with regression coefficient (r^2=0.997) for Clobetasol propionate and (r^2=0.998) for Fusidic acid as shown in figure 2 and figure 3.

Accuracy

For accuracy study data from nine determinations over three concentrations at 80%, 100% and 120% of expected sample concentration covering the specified range was determined & expressed as recovery values. The results were shown in table III.

Precision

The method Precision was established by carrying out the analysis of two drugs using proposed analytical method in six replicates. It indicates the sample repeatability of the method. The results were shown in table IV.

Robustness

The robustness of method was determined to check the reliability of an analysis with respect to deliberate variation in method parameters.

The typical variations are given below:

Variation in wavelength by ± 2 nm,

Variation in mobile phase composition by ± 2 volume of solvent,

The robustness parameters for the method were shown in table V & VI.

Assay

The validated HPLC method was applied to simultaneous determination of Clobetasol propionate and Fusidic acid in marketed pharmaceutical dosage form, i.e. cream contains Clobetasol propionate (0.05 %w/w) and Fusidic acid (2.0 %w/w). An accurately weighed portion of cream i.e. about 1.0 g cream, which was equivalent to 0.5 mg of Clobetasol propionate and 20 mg Fusidic acid, was weighed accurately in 50 mL beaker. It was treated with 10 mL of mobile phase and sonicated with maintaining the temperature about 60º - 65°C for 10 min to dissolve it completely. This solution was mixed well and further diluted with mobile phase up to 50 mL to get a solution having concentration of 10 µg/mL of Clobetasol propionate and 400 µg/mL of Fusidic acid. Filter the final solution and 20 µL of this solution was injected into the chromatograph under the specified chromatographic conditions. The assay results, expressed as % assay of lable claim, are shown in table VIII.

RESULTS AND DISCUSSION

System Suitability

System suitability parameter such as retention time (R_t), number of theoretical plates (N), asymmetry factor, resolution factor were shown in table I.

Linearity

The linearity study was carried out for both drugs at five different concentration levels. The linearity of Clobetasol propionate and Fusidic acid was in the range of 4-12 µg/mL and 160-480 µg/mL respectively shown in the table II & calibration curve shown in figure 2 & 3.

Accuracy

Accuracy of the method was confirmed by recovery study from formulation at three levels of standard addition. The results were shown in table III.

Precision

The data for the repeatability of the peak area measurement for the Clobetasol propionate and Fusidic acid, based on six measurements of same solution of Clobetasol propionate and Fusidic acid were shown in table IV.

Robustness

Robustness parameter determined by changing

Variation in wavelength by ± 2 nm,

Variation in mobile phase composition by ± 2 volume of solvent,

The robustness parameters for the method were shown in table V & VI.

Assay

Applicability of the proposed method was tested by analysing the formulation. The results were shown in table VIII.

CONCLUSION

The proposed HPLC method provides a rapid, accurate, precise and rugged assay with stability indicating potential for Clobetasol propionate and Fusidic acid in cream. In conclusion, the developed method is strongly recommended for the assay of Clobetasol propionate and Fusidic acid in marketed pharmaceutical dosage form i.e. cream.

ACKNOWLEDGEMENT

The authors are thankful to Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy, Ahmedabad, India for providing all the facilities to carry out the work. The authors are thankful to Tripada biotech Pvt. Ltd. Ahmedabad, India and west-coast Pharma, Ahmedabad, India for providing reference standard and sample of Clobetasol propionate and Fusidic acid respectively.

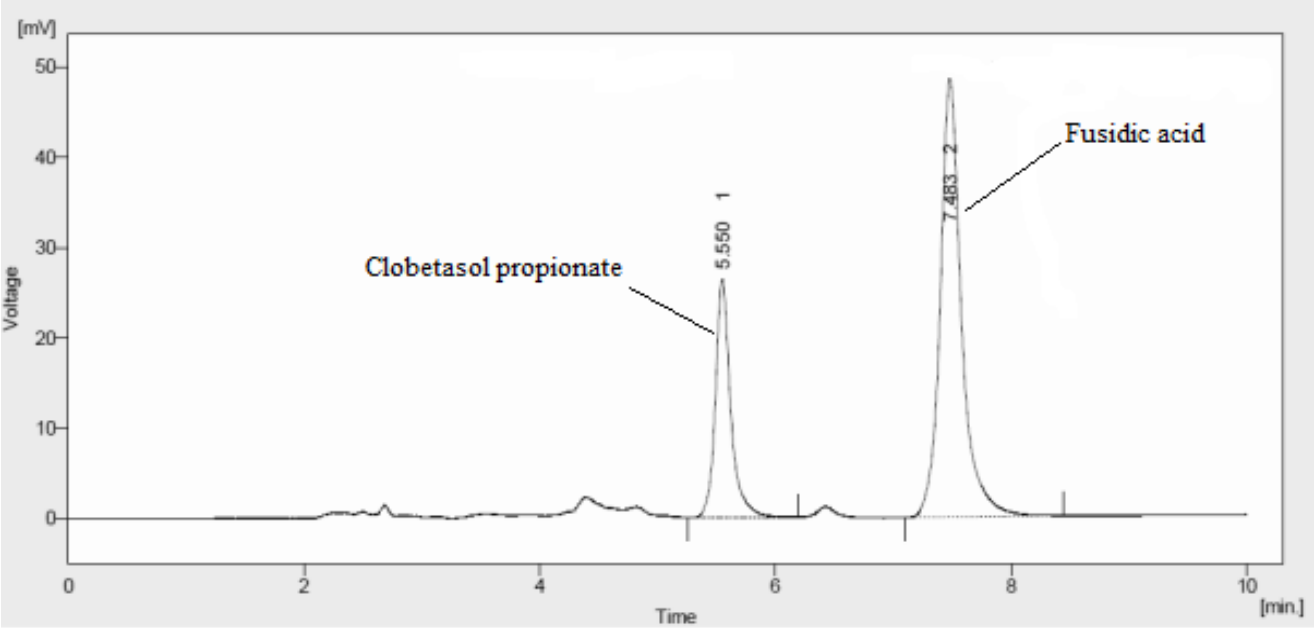

Figure 1: A typical chromatogram of mixture of Clobetasol propionate (4 µg/mL) and Fusidic acid (160 µg/mL)

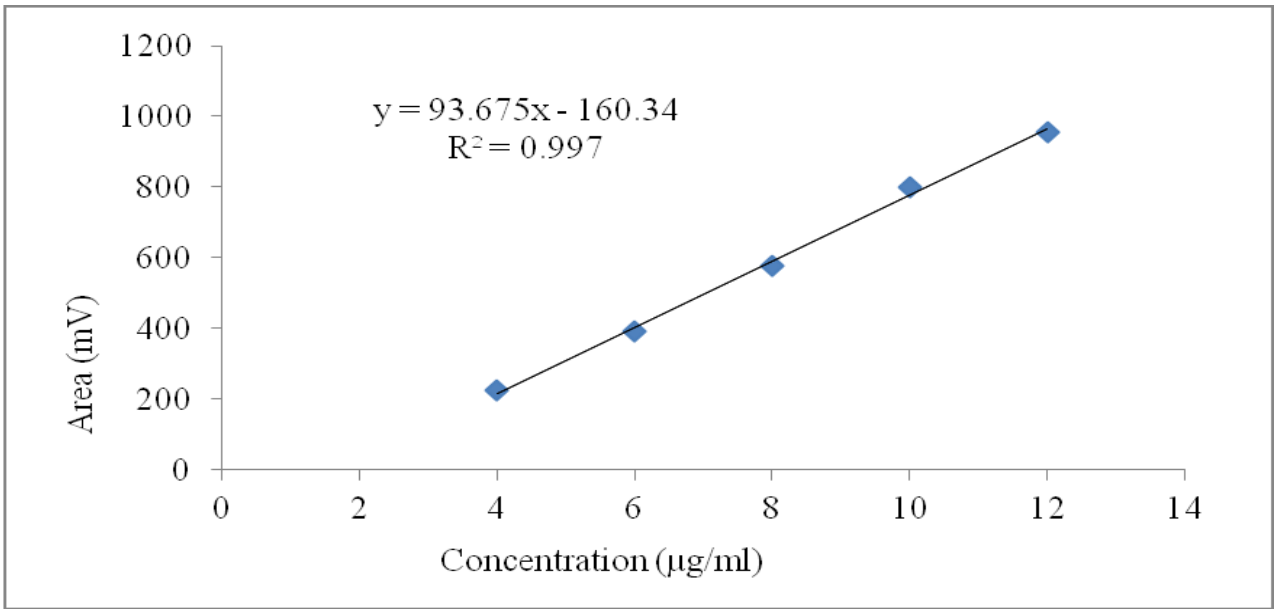

Figure 2: Calibration curve of Clobetasol propionate

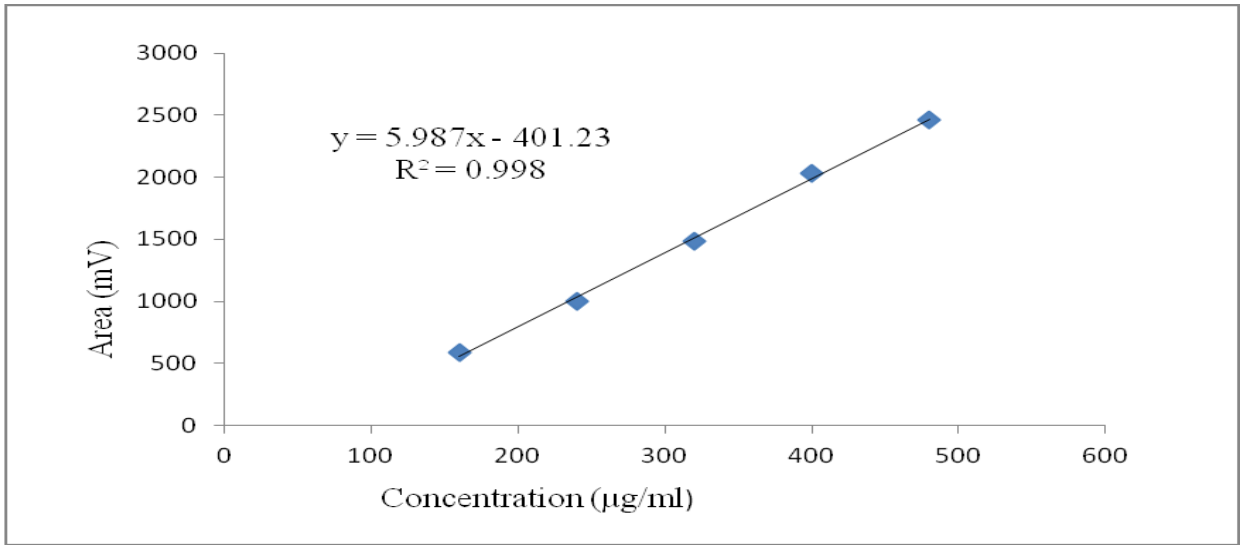

Figure 3: Calibration curve of Fusidic acid

Table 1: System suitability parameters

Parameters	Clobetasol propionate	Fusidic acid
Retention time (R_t)	5.55 min	7.48 min
Asymmetry factor	1.389	1.308
Theoretical plates	10636	9940
Resolution factor	-	7.501

Table 2: Linearity of Clobetasol propionate and Fusidic acid

Clobetasol propionate			Fusidic acid		
Concentration (µg/mL)	Peak area ± SD (n=5)	% RSD	Concentration (µg/mL)	Peak area ± SD (n=5)	% RSD
4	222.950±2.483	1.113	160	586.068±2.287	0.390
6	390.918±2.891	0.739	240	1001.607±3.843	0.383
8	575.647±2.246	0.390	320	1485.877±2.231	0.150
10	801.248±3.501	0.436	400	2035.631±3.921	0.192
12	954.537±3.271	0.342	480	2463.851±3.720	0.151

Table 3: Accuracy study parameter

Name of sample	Amount taken (µg/mL)	Amount added (µg/mL)	Recovered Concentration (µg/mL)	% Recovery ± SD (n=3)
Clobetasol propionate	5	4	8.803	97.81±1.447
	5	5	10.233	102.33±1.307
	5	6	11.104	100.95±1.416
Fusidic acid	200	160	359.331	99.81±1.012
	200	200	411.039	102.75±0.292
	200	260	437.272	99.38±0.428

Table 4: Precision study parameter

Clobetasol propionate			Fusidic acid		
Concentration (µg/mL)	Peak area ± SD (n=6)	% RSD	Concentration (µg/mL)	Peak area ± SD (n=6)	% RSD
8	575.286±2.203	0.383	320	1485.988±2.052	0.138

Table 5: Robustness parameter by changing wavelength

Change in wavelength (nm)	Clobetasol propionate			Fusidic acid		
	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay ±SD(n=3)	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay ±SD(n=3)
238	8	7.602	95.03 ±0.281	320	312.971	97.26 ±0.234
240	8	7.920	95.97 ±0.281	320	318.839	97.82 ±0.234
242	8	7.663	95.48 ±0.281	320	313.718	97.63 ±0.234

Table 6: Robustness parameter by changing mobile phase composition

Change in mobile phase composition (Acetonitrile: Water)	Clobetasol propionate			Fusidic acid		
	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay ±SD(n=3)	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay ±SD(n=3)
78:22	8	7.711	95.05 ±0.462	320	310.367	96.98 ±0.350
80:20	8	7.910	96.11 ±0.462	320	316.624	97.84 ±0.350
82:18	8	7.774	95.93 ±0.462	320	312.413	97.41 ±0.350

Table 7: Summary of Optical regression characteristics and validation parameter for Clobetasol propionate and Fusidic acid

Parameter	Clobetasol propionate	Fusidic acid
Linearity rang	4-12 µg/mL	160-480 µg/mL
Regression equation	y = 93.675x - 160.34	y = 5.987x - 401.23
Regression co-efficient (r^2)	R^2 = 0.997	R^2 = 0.998
Accuracy (%Recovery)	97.81-102.33	99.81-102.75
Repeatability (%RSD)	0.383	0.318
Detection limit (µg/ml)	0.176	2.596
Quantitation limit (µg/ml)	0.534	7.868
%Assay	96.69	97.17

Table 8: Assay study parameter

Clobetasol propionate			Fusidic acid		
Concentration (µg/mL)	Amount found (µg/mL)	% Assay ±SD (n=3)	Concentration (µg/mL)	Amount found (µg/mL)	% Assay ±SD (n=3)
10	9.669	96.69±0.816	400	388.700	97.17±0.951

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Cite This Article: Mehul A, Jakasaniya; Jignesh S, Shah and Dilip G, Maheswari (2014), "Simultaneous estimation of clobetasol propionate and fusidic acid in cream dosage form by reversed phase high performance liquid chromatographic method", ***Pharmacophore***, Vol. 5 (2), 231-238.