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Simultaneous Determination of Halobetasol Propionate and Fusidic Acid Related Substances by Reversed Phase High Performance Liquid Chromatographic Method

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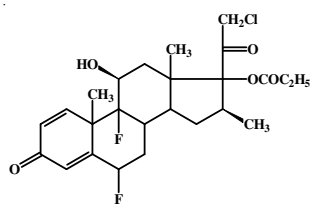
A simple fast, accurate, precise and cost effective isocratic RP-HPLC method is developed for simultaneous determination of halobetasol propionate and fusidic acid as well as its impurities in the cream formulation. The chromatography equipped with Zorbax SB phenyl (250 mm × 4.6 mm) column using a mobile phase of buffer (0.1 % triethylamine buffer pH adjusted to 2.0 with orthophosphoric acid) acetonitrile, methanol in the ratio of 40:35:25 v/v with a flow rate of 1.5 mL/min, with ultraviolet detector configured at 240 nm.

Key Words: Halobetasol, Fusidic acid, RP-HPLC.

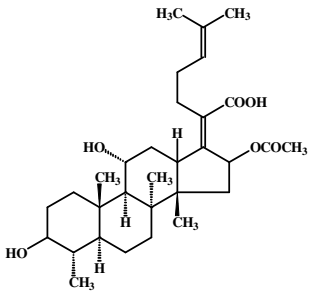
INTRODUCTION

Halobetasol propionate[1] (**1**) chemically is 2-chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione-17-propionate and having a molecular formula of ($C_{25}H_{31}O_5F_2Cl$). It is a super high potency corticosteroid indicate for the relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatitis.

Fusidic acid[2] (**2**), chemically is 2-(16-acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-2,3,4,5,6,7,9,11,12,13,15,16-dodecahydro-1*H*-cyclopenta[a]phenanthren-17-yelidene)-6-methyl-hept-5-enoic acid. It is often used topically in creams and eyedrops, but may also be given systemically as tablets or injections[3].



Halobetasol propionate (**I**)



Ketofusidic acid (**2**)

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Halobetasol propionate is a synthetic corticosteroid used to treat variety of skin condition. for example, eczema, dermatitis, allergies, rash in which reduces swelling itching and redness. It is also act as antiinflammatory and antipruritic agent[4]. Fusidic acid is bacteriostatic antibiotic which treat of primary and secondary skin infections caused by sensitive strains of *S. aureus*, *Strepto cocci* species and *C. minutissimum*[5]. Recently, various different topical formulation in combination of halobetasol and fusidic acid are available in the market. Hence, it is necessary to develop a sutiable method for the simultaneous determination of halobetasol propionate and fusidic acid related substances.

EXPERIMENTAL

Triethylamine, orthophosphoric acid, acetonitrile, methanol, all HPLC grade were purchased from Merck.

Chromatographic condition: Column: Zorbax SB Phenyl, 250 mm × 4.6 mm, 5 µ (Make: Agilent); Wavelength:240 nm; Flow rate: 1.5 mL/min; Injection volume: 50 µL; Column temperature: 30 ºC; Run time: 0.5 h.

Buffer solution: 1 mL of triethylamine in 1000 mL of HPLC water and adjust pH to 2.0 with orthophosphoric acid, mixed well and filtered through 0.45 µ filter.

Mobile phase: Mixed buffer acetonitrile and methanol in the ratio of 40:35:25 (v/v) filtered through 0.45 µ membrane filter and sonicated for 5 min.

Preparation of stock solutions: Prepared each solution separately having the concentration of halobetasol propionate 150 ppm, diflorasone 21 propionate 100 ppm, diflorasone 17 propionate 21 mesylate 100 ppm, fusidic acid 400 ppm, 3 keto fusidic acid 800 ppm, 11 keto fusidic acid 800 ppm, 16 disacetyl fusidic acid 800 ppm.

Mixed standard solution: 1 mL of halobetasol propionate stock solution, 1 mL each of all halobetasol impurities, 10 mL of fusidic acid stock solution and 1 mL each of 3 keto fusidic acid, 11 keto fusidic acid, 16 disacetyl fusidic acid are added to a 100 mL volumetric flask and make up to volume with diluent (80:20, acetonitrile: water).

(Concentration of halobetasol propionate is 1.5 ppm, fusidic acid 100 ppm, 3 keto fusidic acid, 11 keto fusidic acid and 16 disacetyl fusidic acid were in 80 ppm).

Placebo and sample preparation: 5 g of placebo in a 50 mL volumetric flask, added 20 mL of acetonitrile and kept on water bath at 80 ºC for 15-20 min and then cooled to room temperature. Added 5 mL of water to the above solution and mixed well, chilled the sample in ice-bath and finally filtered through 0.45 µ Teflon filter. Similarym the sample solution is also prepared in the same manner.

Procedure for injection: Injected equal volumes of diluent, placebo preparation, standard preparation separately in six replicates and sample once in to equilibrated HPLC system and recorded the chromatograms and measured the response in terms of peak area.

System suitability parameters maintained during method validation are theoretical plates not less than 2000, tailing factor not more than 2.0, relative standard deviation for six replicates of standard solution is not more than 5.0 %.

RESULTS AND DISCUSSION

The developed method was capable to quantify the impurities present in the drug product derived from both halobetasol propionate as well as from fusidic acid.

Specificity/selectivity: Individual halobetasol (free base) 100 ppm, diflorasone 21 propionate 100 ppm, diflorasone 17 propionate 21 mesylate 100 ppm, halobetasol propionate 150 ppm, fusidic acid 1000 ppm, 3 keto fusidic acid 800 ppm, 11 keto fusidic acid 800 ppm, 16 disacetyl fusidic acid 800 ppm were injected all the peaks are not only well resolved from each and also free from placebo interference.

Accuracy: The accuracy of an analytical method expresses the closeness of agreement between the value, which is accepted either as a conventional true value or as an accepted reference value and the experimental value. Accuracy is calculated as percentage recovery of the analyte by the assay of known added amount of the analyte in the sample[6].

Accuracy was studied at four concentration level at LOQ level, 50 % level, 100 % level and 150 % level of working concentration. Each level was studied in triplicate. Each preparation was prepared independently by spiking analyte in the placebo. Per cent recovery was calculated by comparing response obtained in spiked sample with those obtained in standard (Table-1).

Precision: To check the system precision (repeatability) for peak response obtained with six replicates of dilute standard at limiting concentration. To check repeatability (method precision) of method for independent 6 different sample preparations were injected and % RSD with six sample preparations found to be within 5.0 %, to demonstrate intermediate precision (ruggedness) of the method by comparing method precision (in terms of absolute difference) on two different days on two different systems.

Linearity: All the impurities were spiked in their limiting concentrations and linearity established, the correlation coefficient was given in Table-2.

Solution stability: To demonstrate stability of the standard solution by comparing data of absolute difference in % impurities at each interval with respect to initial values for impurities. The standard and sample solutions are stored at room temperature and analyzed over the time period of initial, 6, 12 and 24 h. The absolute difference between % impurities values should be within 5 % with respect to the initial value. From the experimental study, standard and sample solutions are found stable up to 24 h at room temperature.

System suitability: The relative standard deviation for the impurity peak area in standard solution is NMT 5.0 %.

Ruggedness and robustness: The ruggedness of the method was studied by analyzing the sample on two different days on two different systems and two different columns by same manufacturer, results found to be well acceptable limits.

Robustness of an analytical method was studied by changing the pH of the buffer and found to acceptable for organic addition, the 5 % change in organic was influencing the retention time of peaks, hence method validation advice to be cautious while adding organic phase.

TABLE-1
ACCURACY OF HALOBETASOL AND FUSIDIC ACID IMPURITIES BY PLACEBO SPIKED RECOVERY METHOD

Accuracy				
	Halobetasol free base	Diflorasone 21 propionate	Diflorasone 17 propionate 21 mesylate	Halobetasol propionate
LEVEL		% Recovery		
LOQ	96.60	99.60	96.34	103.21
LOQ	98.20	96.20	98.60	102.42
LOQ	99.34	98.34	99.39	100.12
Mean	98.05	98.05	98.11	101.92
SD	1.38	1.72	1.58	1.61
% RSD	1.40	1.75	1.61	1.58
Level-50 %	101.26	99.21	100.23	101.23
Level-50 %	102.33	102.26	101.11	102.25
Level-50 %	99.15	101.43	98.26	100.43
Level-100 %	98.34	100.23	102.26	102.26
Level-100 %	98.23	102.26	98.60	101.23
Level-100 %	100.23	100.23	99.39	98.60
Level-150 %	102.26	101.23	102.26	99.39
Level-150 %	97.47	102.42	100.23	100.23
Level-150 %	102.11	100.76	100.23	100.23
Average	100.15	101.11	100.29	100.65
SD	1.92	1.10	1.42	1.23
%RSD	1.92	1.09	1.42	1.22
Accuracy fusidic acid impurities				
	3 keto fusidic acid	11 keto fusidic acid	16 Disacetyl fusidic acid	Fusidic acid
LEVEL		% Recovery		
LOQ	96.60	99.60	103.21	103.21
LOQ	103.21	103.21	102.42	102.42
LOQ	102.42	102.42	99.39	100.12
Mean	100.74	101.74	101.67	101.92
SD	3.61	1.90	2.02	1.61
%RSD	3.58	1.87	1.98	1.58
Level-50%	101.26	99.21	100.23	101.23
Level-50%	102.33	98.20	103.11	102.25
Level-50%	99.15	99.34	98.26	98.20
Level-100%	98.34	98.92	102.26	99.34
Level-100%	98.20	99.39	98.60	99.93
Level-100%	99.34	99.22	99.39	98.60
Level-150%	98.63	101.23	102.26	99.39
Level-150%	97.47	102.42	100.55	100.23
Level-150%	102.11	101.76	101.23	102.26
Average	99.65	99.96	100.65	100.16
SD	1.80	1.45	1.71	1.48
% RSD	1.80	1.45	1.69	1.48

TABLE-2
LINEARITY OF HALOBETASOL PROPIONATE, FUSIDIC ACID AND ITS RELATED SUBSTANCES

	Halobetasol		Diflorasone 21 propionate		Diflorasone 17 propionate 21 MSC		Halobetasol propionate	
Level	Conc. in ppm	Peak area	Conc. in ppm	Peak area	Conc. in ppm	Peak area	Conc. in ppm	Peak area
LOQ	0.03	1949	0.03	1963	0.03	1965	0.03	1863
Level-50 %	0.76	47079	0.76	46433	0.76	46481	0.78	45929
Level-75 %	1.14	74740	1.13	74290	1.13	74367	1.16	70505
Level-100 %	1.52	97432	1.51	98125	1.51	98226	1.55	93128
Level-125 %	1.90	122979	1.89	123853	1.89	123981	1.94	115680
Level-150 %	2.28	147707	2.27	146795	2.27	146946	2.33	139315
Level-200 %	3.04	195936	3.02	196348	3.02	196550	3.10	189137
Corr. coff.	0.9999	–	0.9998	–	0.99984	–	0.99986	–
R. square	0.9998	–	0.9997	–	0.99968	–	0.99971	–
	Fusidic acid		3 Keto fusidic acid		11 Keto fusidic acid		16 Disacetyl fusidic acid	
Level	Conc. in ppm	Peak area	Conc. in ppm	Peak area	Conc. in ppm	Peak area	Conc. in ppm	Peak area
LOQ	0.08	1275	0.08	1234	0.09	1394	0.09	1399
Level-50 %	20.06	314436	21.10	322878	22.10	364747	21.55	358892
Level-75 %	30.09	489059	31.65	473394	33.15	534781	32.33	522463
Level-100 %	40.12	637543	42.20	617122	44.20	697146	43.10	699321
Level-125 %	50.15	804707	52.75	778931	55.25	879938	53.88	882683
Level-150 %	60.18	979266	63.30	935557	66.30	1056873	64.65	1067164
Level-200 %	80.24	1282099	84.40	1247204	88.40	1408932	86.20	1420321
Corr. coff.	0.9998	–	0.9999	–	0.99992	–	0.99992	–
R. square	0.9997	–	0.9998	–	0.99984	–	0.99984	–

REFERENCES

1. G. Lunn, HPLC Methods for Recently Approved Pharmaceuticals, John Wiley & Sons, pp. 306-315 (2005).
2. A.J. O'Neill, A.R. Larsen,A.S. Henriksen and I. Chopra, *Antimicrob. Agents Chemother.*, **48**, 3594 (2004).
3. D. Livermore, D. James, G. Duckworth and P. Stephens, *The Lancet*, **360**, 806 (2002).
4. S. Yawalkar, I. Wiesenberg-Boettcher, J. Gibson, S. Siskin and W. Pignant, *J. Am. Acad. Dermat.*, **25**, 1137 (1991).
5. Y. Tveten, A. Jenkins and B.-E. Kristiansen, *J. Antimicrob. Chemother.*, **50**, 873 (2002).
6. L.R. Snyder, J.J. Kirkland and J.L. Glajch, Practical High Performance Liquid Chromatographic Method Development, Elsevier, Amsterdam (1997).

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