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

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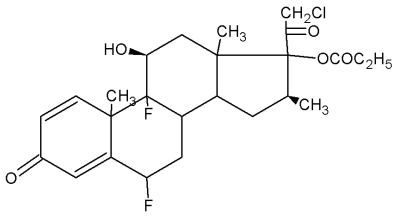
A simple fast, accurate, precise and cost effective reverse phase high performance liquid chromatographic method is developed for simultaneous determination of halobetasol propionate and salicylic acid impurities in the ointment formulation. The chromatography equipped with inertsil C-8 (250 mm × 4.6 mm) column using a mobile phase of buffer (0.1 % of orthophosphoric acid) and acetonitrile with a flow rate of 1.5 mL/min, with ultraviolet detector configured at 231 nm. The method is found to be linear in the range of 0.118-17.734 µg/mL for 4-hydroxy benzoic acid, 0.120-9.036 µg/mL for 4-hydroxy isophthalic acid, 0.12-3.586 µg/mL for phenol, 0.103-8.987 µg/mL for salicylic acid, 0.069-1.480 µg/mL for diflorasone 21 propionate, 0.071-1.472 µg/mL for diflorasone 17 propionate 21 mesylate and 0.057-1.514 µg/mL for halobetasol propionate.

Key Words: Halobetasol, Salicylic 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. It is a superior high potency corticosteroid indicated for the relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatitis.



(**1**) Halobetasol propionate

Salicylic acid is a β-hydroxy acid with the formulae $C_6H_4(OH)CO_2H$, widely used in organic synthesis and function as a plant hormone[2,3]. It is derived from the

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metabolism of salicylin. It is probably best known as compound that is chemically similar to but not identical to the active component of aspirin (acetylsalicylic acid) in fact salicylic acid is a metabolite of aspirin, the product of esterase hydrolysis in the liver. Salicylic acid is a keratolytic (peeling agent) it will destroy all type of skin cells[4]. Salicylic acid causes shelding of the outer layer of skin and it is used in the treatment of acne, dandruff, corns and warts[4].

EXPERIMENTAL

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

Chromatographic conditions: Column: Inertsil, C-8, 250 mm × 4.6 mm, 5 µ; Wavelength: 231 nm; Flow rate: 1.5 mL/min gradient; Injection volume: 50 µL; Column temperature: 40 ºC; Run time: 40 min.

Retention time: 4-Hydroxy benzoic acid: 3.1 min; 4-Hydroxy isophthalic acid: 4.6 min; Phenol: 5.0 min; Salicylic acid: 8.3 min; Diflorasone 21 propionate: 20.2 min; Diflorasone 17 propionate 21 mesylate: 22.7 min; Halobetasol propionate: 23.9 min.

Buffer solution: 1 mL orthophosphoric acid added to 1000 mL of water, mixed and filtered through 0.45 µ filter.

Diluent: Mixed water, acetonitrile and methanol in the ratio of 65:25:10 (v/v) and filtered through 0.45 µ filter.

Preparation of stock solutions: Prepared each solution separately having the concentration of 4-hydroxy benzoic acid 600 ppm, 4-hydroxy isophthalic acid 300 ppm, phenol 120 ppm, salicylic acid 300 ppm, diflorasone-21-propionate 10 ppm, diflorasone-17-propionate-21 mesylate 10 ppm, halobetasol propionate 10 ppm.

Mixed standard solution: To a volumetric flask, added 10 mL of halobetasol propionate and its related compounds and 2 mL of each 4-hydroxy benzoic acid, 4-hydroxy isophthalic acid, phenol, salicylic acid and make up to volume with diluent.

(Concentration of 4-hydroxy benzoic acid 12 ppm, 4-hydroxy isophthalic acid 6 ppm, phenol 2.4 ppm, salicylic acid 6 ppm, diflorasone-21-propionate 1 ppm, diflorasone-17-propionate-21 mesylate 1 ppm, halobetasol propionate 1 ppm).

Placebo preparation: Place 5 g of placebo in a 50 mL volumetric flask, added 25 mL of diluent and kept on water bath at 80 ºC for 15-20 min and cooled to room temperature. Mixed the solution well and chilled the sample in ice bath and filtered through 0.45 µ Teflon filter.

Sample preparation: Place 5 g of sample in a 50 mL volumetric flask, added 25 mL of diluent and kept on water bath at 80 ºC for 15-20 min and cooled to room temperature. Mixed the solution well and chilled the sample in ice bath and filtered through 0.45 µ Teflon filter.

Procedure for injection: Injected equal volumes of diluent, placebo preparation, standard preparation in 6 replicates separately and sample once in equilibrated HPLC system and recorded the chromatograms and measured the response in terms of peak area.

RESULTS AND DISCUSSION

Specificity: The sample and standard are subjected for forced degradation and the % impurities generated are calculated and reported values were given in Tables 1 and 2.

TABLE-1
FORCED DEGRADATION OF SALICYLIC ACID IN THE SAMPLE

Name of impurity	4-Hydroxy benzoic acid	4-Hydroxy isophthalic acid	Phenol	Impurity at RRT 1.4	Impurity at RRT 1.54
			% Degradation		
Peroxide degradation	0.006	0.002	0.00	0.011	0.003
Acid degradation	0.000	0.008	0.00	0.013	0.005
Base degradation	0.007	0.000	0.00	0.00	0.000
Thermal degradation	0.000	0.000	0.00	0.011	0.003
Photo degradation	0.000	0.000	0.00	0.011	0.003
Humidity degradation	0.000	0.000	0.00	0.011	0.003

TABLE-2
FORCED DEGRADATION OF HALOBETASOL PROPIONATE IN THE SAMPLE

Name of impurity	Diflorasone 21 propionate	Diflorasone 17 propionate 21 mesylate	Impurity at RRT 1.02
Peroxide degradation	0.575	0.00	.0.184
Acid degradation	0.392	0.00	1.789
Base degradation	22.03	0.00	0.00
Thermal degradation	0.091	0.00	0.186
Photo degradation	0.080	0.00	0.182
Humidity degradation	0.086	0.00	0.177

Accuracy: 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 and obtained results are given in Table-3.

Precision: To demonstrate intermediate precision (ruggedness) of the method by comparing method precision (in terms of absolute difference) on two different days carried out by different analyst.

System precision: System suitability standard solution was analyzed in six replicates using test procedure under validation. Responses obtained due to impurity peak in each replicate of system suitability standard solution were recorded. % RSD for the response is calculated and obtained results are given in Table-4.

Method precision (repeatability): Six sample solutions will be prepared as described in methodology for impurities ointment. Calculate the % impurities, mean and % RSD for impurities and obtained results are given in Table-5.

TABLE-3
TOTAL ALL KNOWN IMPURITIES RECOVERY

Name of impurity	4-Hydroxy benzoic acid	4-Hydroxy isophthalic acid	Phenol	Diflorasone 21 propionate	Diflorasone 17 propionate 21 mesylate
			% Recovery		
Level-LOQ spl-1	104.9	97.3	103.5	97.0	104.4
Level-LOQ spl-2	96.7	96.8	101.2	92.0	95.0
Level-LOQ spl-3	103.6	95.5	103.9	96.4	101.1
Mean	101.7	96.5	102.9	95.1	100.2
S.D	4.4	0.92	1.45	2.73	4.76
%RSD	4.33	0.96	1.41	2.87	4.76
Level-50 spl-1	101.5	105.4	101.4	100.0	93.9
Level-50 spl-2	102.0	105.7	101.1	100.8	94.8
Level-50 spl-3	101.8	105.3	101.6	100.0	93.9
Level-100 spl-1	98.3	99.4	96.0	100.4	97.8
Level-100 spl-2	98.0	98.8	95.6	99.6	97.0
Level-100 spl-3	97.8	98.3	95.9	99.2	96.1
Level-150 spl-1	98.0	98.2	97.6	98.7	98.0
Level-150 spl-2	97.9	98.2	97.3	98.7	98.0
Level-150 spl-3	98.1	98.3	97.3	98.9	98.3
Mean	99.3	100.8	98.2	99.6	96.4
S.D.	1.88	3.48	2.47	0.76	1.81
% RSD	1.89	3.46	2.52	0.76	1.87

TABLE-4
PRECISION DATA OF SALICYLIC ACID, HALOBETASOL PROPIONATE AND RELATED SUBSTANCES

	4-Hydroxy benzoic acid	4-Hydroxy isophthalic acid	Phenol	Salicylic acid	Halobetasol propionate
Replicate-1	589795	879152	20656	589104	62117
Replicate-2	589866	880867	20977	589789	62431
Replicate-3	590643	881337	21399	590351	62111
Replicate-4	592426	885240	21436	590325	62819
Replicate-5	589396	873347	19845	588328	62779
Replicate-6	590293	874352	21215	588468	62453
Mean	590403	879049	20921	589394	62452
S.D	1080.21	4504.53	601.88	896.28	306.78
% RSD	0.1800	0.5100	2.8800	0.1500	0.4900

% RSD for impurities free base peak response in standard solution is maximum 2.88 % for phenol, which is well within the acceptance criteria not more than 5.0 %. % RSD also obtained for impurities is 5.83 % for the six determinations and is well within the acceptance criteria NMT 10.0 % (Table-6).

TABLE-5
PRECISION DATA OF TOTAL IMPURITIES

Name of impurity	Diflorasone 21 propionate	Diflorasone 17 propionate 21 mesylate	Impurity at RRT 1.02	Total impurities
Precision set-1	1.002	0.964	0.185	2.151
Precision set-2	1.007	0.944	0.159	2.11
Precision set-3	1.007	0.942	0.174	2.123
Precision set-4	0.991	0.937	0.171	2.099
Precision set-5	0.987	0.939	0.159	2.085
Precision set-6	0.989	0.939	0.173	2.101
Average	0.997	0.944	0.170	2.112
S.D	0.009	0.010	0.010	0.023
% RSD	0.924	1.062	5.834	1.093

TABLE-6
INTERMEDIATE PRECISION DATA OF SALICYLIC ACID, HALOBETASOL PROPIONATE, RELATED SUBSTANCES AND IMPURITIES

	4 Hydroxy benzoic acid	4 Hydroxy isophthalic acid	Phenol	Salicylic acid	Halobetasol propionate	Total impurities
Replicate-1	0.197	0.100	0.040	0.012	0.003	0.352
Replicate-2	0.196	0.100	0.040	0.012	0.003	0.351
Replicate-3	0.195	0.100	0.039	0.012	0.003	0.349
Replicate-4	0.194	0.099	0.040	0.012	0.003	0.348
Replicate-5	0.194	0.099	0.039	0.012	0.003	0.347
Replicate-6	0.194	0.099	0.039	0.012	0.003	0.347
MEAN	0.195	0.100	0.040	0.012	0.003	0.349
S.D	0.000	0.000	0.000	0.000	0.000	0.000
% RSD	0.650	0.550	1.390	0.000	0.000	0.600

Linearity: Linearity is studied over the range of LOQ to 150 % of the test concentration at different levels. Linearity is studied at seven concentration level (LOQ, 50, 80, 90, 100, 110, 120 and 150 % of test concentration). A graph of concentration vs response was plotted. Correlation coefficient, slope, intercept were calculated (Tables 7-9).

TABLE-7
LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ) IN µg/mL

Name of compound	LOD	LOQ
4 Hydroxy benzoic acid	0.036	0.121
4 Hydroxy isophathlic acid	0.036	0.120
Phenol	0.036	0.120
Salicylic acid	0.031	0.104
Diflorasone 21 propionate	0.070	0.021
Diflorasone 17 propionate 21 mesylate	0.073	0.022
Halobetasol propionate	0.057	0.017

TABLE-8
LINEARITY OF HALOBETASOL PROPIONATE AND ITS RELATED SUBSTANCES

Name of compound	Diflorasone 21 propionate		Diflorasone 17 propionate 21 mesylate		Halobetasol propionate	
	Conc. in µg/mL	Area	Conc. in µg/mL	Area	Conc. in µg/mL	Area
Level-LOQ	0.069	1385	0.071	1153	0.057	1765
Level-50 %	0.493	29856	0.491	26915	0.505	32006
Level-80 %	0.790	47911	0.785	43360	0.807	51200
Level-90 %	0.888	54006	0.883	48813	0.908	57666
Level-100 %	0.987	59164	0.982	53424	1.009	63099
Level-110 %	1.086	65316	1.080	59035	1.110	69477
Level-120 %	1.184	72435	1.178	65376	1.211	77225
Level-150 %	1.480	90432	1.472	81048	1.514	96005
Slope	62672	–	57001	–	64395	–
Intercept	–2095	–	-2009	–	–1264	–
Correlation coefficient	0.9996	–	0.9996	–	0.9997	–

TABLE-9
LINEARITY OF SALICYLIC ACID AND ITS RELATED SUBSTANCES

Name of Compound	4-Hydroxy benzoic acid		4-Hydroxy isophthalic acid		Phenol		Salicylic acid	
	Conc. in µg/mL	Area	Conc. in µg/mL	Area	Conc. in µg/mL	Area	Conc. in µg/mL	Area
Level-LOQ	0.118	6415	0.120	9299	0.120	1606	0.103	6822.0
Level-50 %	5.911	311638	3.012	453251	1.195	14206	2.996	302869
Level-80 %	9.458	498793	4.819	726880	1.912	21961	4.793	485049
Level-90 %	10.64	561784	5.422	817451	2.151	24639	5.392	544673
Level-100 %	11.82	613916	6.024	878306	2.390	26889	5.992	596324
Level-110 %	13.00	678178	6.626	983895	2.629	29841	6.591	655896
Level-120 %	14.18	752253	7.229	1090428	2.868	32859	7.190	726542
Level-150 %	17.73	937076	9.036	1360643	3.586	40512	8.987	906572
Slope	52705	–	150761	–	11196	–	100890	–
Intercept	–989.0	–	–6904	–	481	–	–2136	–
Correlation coefficient	0.9998	–	0.9996	–	0.9998	–	0.9998	–

Solution stability: Sample was spiked with known amount of impurities and the same sample was monitored for stability of different time intervals. The results for % impurities of sample obtained at different pool time are compared with initial % impurities value (Table-10).

TABLE-10
SOLUTION STABILITY OF IMPURITIES

Name of impurity	4-Hydroxy benzoic acid	4-Hydroxy isophthalic acid	Phenol	Diflorasone-21-propionate	Diflorasone-17-propionate-21-mesylate
Initial	0.203	0.106	0.040	1.052	0.956
After 8 h	0.205	0.105	0.040	1.015	0.951
After 12 h	0.205	0.105	0.042	1.039	0.941
Mean	0.204	0.105	0.041	1.035	0.949
S.D	0.001	0.001	0.001	0.019	0.008
% RSD	0.565	0.548	2.830	1.810	0.805

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