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RESEARCH ARTICLE

SIMULTANEOUS DETERMINATION OF HALOBETASOL PROPIONATE AND SALICYLIC ACID RELATED SUBSTANCES IN OINTMENT FORMULATION AND IDENTIFICATION OF IMPURITIES.

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Abstract
A rapid and precise RP-HPLC method for determination of Halobetasol and Salicylic acid in bulk and pharmaceutical dosage forms. Halobetasol and Salicylic acid are found to be degraded together under different set of conditions as followed according to ICH guidelines and the degradants so formed along with Halobetasol and Salicylic acid are separated by using INERTSIL ODS C18 3V (250 x 4.6, 5µ) using 1ml orthophosphoric acid in one litre water and acetonitrile as mobile phase using a gradient program with a flow rate of 1ml/min with a detection wavelength of 231 nm for both the compounds with a injection volume of 10µl. The method was validated for selectivity, linearity, accuracy, robustness, precision and specificity. The results were indicating the method was selective in analysis of both Halobetasol and Salicylic acid in the presence of degradation products formed under various stress conditions

Keywords: Halobetasol, Salicylic acid, INERTSIL ODS C18, Orthophosphoric acid, Acetonitrile, ICH Guidelines

INTRODUCTION

Salicylic acid is a beta hydroxy acid with the formula C_6H_4 (OH) COOH, where the OH group is adjacent to the carboxyl group. This is colourless crystalline organic acid, widely used in organic synthesis and function as a plant hormone. It is highly soluble in water. Molecular weight is 138.12, melting point is 158^{0}C, boiling point is 211^{0}C, specific gravity is 1.44, and solubility in water is slight. Halobetasol propionate (Lunn G. 2005) is soluble in acetone, acetonitrile and sparingly soluble in methanol and insoluble in water, chemically halobetasol propionate is 21-chloro-6α, 9-difluoro-11β, 17-dihydroxy-16β-methylpregna-1, 4-diene-3-20-dione, 17-propionate, chemical formula is $C_{25}H_{31}ClF_2O_5$. In the literature, some high-performance liquid chromatographic (Nkam et al 2009) and spectrophotometric methods (Mostafa AA etal 2002) have been reported to assay halobetasol propionate.The United States Pharmacopoeia (2011) presents an HPLC method for halobetasol propionate drug substance, but as of now, no HPLC method in the literature has reported determination of the impurities present in these topical dosage forms. The proposed method is capable of quantitating the impurities present in halobetasol propionate ointments and creams.

Experimental:

Halobetasol and Salicylic acid reference standards was supplied by M/s Microlabs limited, Bangalore,India. HPLC grade Acetonitrile,methanol,orthophosphoric acid were purchased from Merck (Mumbai, India). All chemicals were of analytical grade.The determination was carried out on Waters HPLC 2690 equipped with PDA 996 as detector using data handling system – waters empower 2.0 software. The column used in the development for the determination is INERTSIL ODS C18 (250 x 4.6, 5µ). The detector wavelength was set at 231nm for both the components. A flow rate of 1ml/min was used for the determination of Halobetasol and Salicylic acid. The samples and standards were dissolved in diluent (water: ACN, 20: 80) and 10µL sample were injected into HPLC system at the column.

Mobile phase A: 1ml orthophosphoric acid in one litre water
Mobile phase B: Acetonitrile

Preparation of standard and sample solutions

Standard Preparation:

Standard Halobetasol propionate preparation (Stock solution-A): Weigh accurately about 25mg of Halobetasol propionate, transfer to 100ml volumetric flask. Add about 60 ml of tetrahydrofuran, sonicate to dissolve, allow cooling at room temperature. Make up volume with the same. (Concentration of Halobetasol propionate is 250 ppm) .

Table 1: Gradient Program

Time(minutes)	Flow Rate ml/min	%Mobile Phase A	%Mobile Phase B
0	1.0	60	40
2	1.0	60	40
8	1.0	70	30
15	1.0	50	50
22	1.0	45	55
30	1.0	45	55
40	1.0	60	40

Table 2: Precision studies for Halobetasol and Salicylic acid

Component	Concentration (µg/ml)		% RSD
	LOD	LOQ	
Salicylic acid Imp-A	0.009	0.027	1.2
Salicylic acid Imp-B	0.010	0.032	1..0
Salicylic acid Imp-C	0.026	0.079	3.3
Salicylic acid	0.017	0.052	0.3
HalobetasolTrihydroxy Impurity	0.029	0.052	4.3
Halobetasol 11 propionate Impurity	0.066	0.199	2.2
Halobetasol propionate Impurity	0.021	0.063	6.8

Table 3: Recovery studies for Halobetasol and Salicylic acid

Mean % Recovered			
	Salicylic acid Imp-A	**Salicylic acid Imp-B**	**Salicylic acid Imp-C**
LOQ	96.3	104.87	102.94
100 %	100.0	97.36	106.90
150%	97.87	94.75	104.18
Mean % Recovered			
% Level	**HalobetasolTrihydroxy**	**Halobetasol 11 Propionate**	
LOQ	89.35	94.57	
100 %	107.00	105.87	
150 %	103.11	107.34	

Mix standard preparation: Weigh accurately about 75mg of Salicylic acid, transfer to 50ml volumetric flask. Add about 20 ml of tetrahydrofuran, sonicate to dissolve, add 5.0ml of stock solution A. Make up to volume with tetrahydrofuran and mix well. 5 ml of above mix stock solution make up to 50 ml with diluent Filter through 0.45µ Teflon filter, discarding first few milliliters. (Concentration of Halobetasol propionate and salicylic acid is 2.5 ppm and 150ppm respectively)

Sample Preparation: Placed 5 g of sample in a 50 ml volumetric flask, added 25 ml of diluent and kept on water bath at 80 C 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 u Teflon filter.

Results and Discussion:

The conditions tested for method development indicates that all the system suitability parameters according to ICH guidelines was achieved by using INERTSIL ODS C18 (250 x 4.6, 5µ) column using 1ml orthophosphoric acid in one litre water and acetonitrile as mobile phase using a gradient program with a flow rate of 1ml/min throughout the gradient program with a detection wavelength of 231 nm for both the compounds with a injection volume of 10µl. (Fig.1)

Table 4: Stability studies for Halobetasol and Salicylic acid

	Salicylic acid	
Condition	**Purity Angle**	**Purity Threshold**
Acid stress	8.617	11.690
Base stress	8.665	11.676
Peroxide stress	8.852	11.235
Photolytic stress	8.316	12.202
Thermal stress	8.852	11.235
Humidity stress	7.974	11.202
	Halobetasol	
Condition	**Purity Angle**	**Purity Threshold**
Acid stress	0.023	0.202
Base stress	0.022	0.201
Peroxide stress	0.025	0.201
Photolytic stress	0.023	0.206
Thermal stress	0.064	0.202
Humidity stress	0.026	0.202

Table 5: Robustness studies for Halobetasol and Salicylic acid

Conditions		**% RSD of Standard**	
		Salicylic Acid	**Halobetasol Propionate**
Wavelength	231 nm	0.0	0.2
	233nnm	0.1	0.3
	229 nm	0.1	0.3
Column oven Temperature	40°C	0.1	1.0
	45°C	0.2	0.2
	35°C	0.8	0.3
Flow rate	1.0 mL/min	0.4	1.9
	0.9 mL/min	0.3	0.6
	1.1 mL/min	0.9	0.3

To validate the RP-HPLC method, a series of tests were made using the most promising conditions. The linearity was evaluated by linear regression analysis that was calculated by the least square regression.The LOD, LOQ and precision (expressed as the relative standard deviation (RSD) was determined for Halobetasol and Salicylic acidand its impurities for repeated analysis and the values are presented in Table 2. The lower values of LOQ confirmed that the method was highly sensitive.The recovery experiment values obtained were performed by adding a fixed amount of drug to preanalysed formulation summarized in Table 3.

The stability of sample was checked by forced degradation in different conditions and % of degradation was calculated. The peak purity of the analyte was passed in all conditions (purity angle should be less than the threshold value). The following values in Table 4 indicate that any other impurity is not merging with the main peak (Fig 4-8). The analyte solution was stable up to 24hrs.The reliability of the method was determined by made small deliberate variations in method parameters and the RSD values (Table. 5) obtained, an indication of its reliability on normal usage.

Figure 1: Sample chromatogram for Halobetasol and Salicylic acid

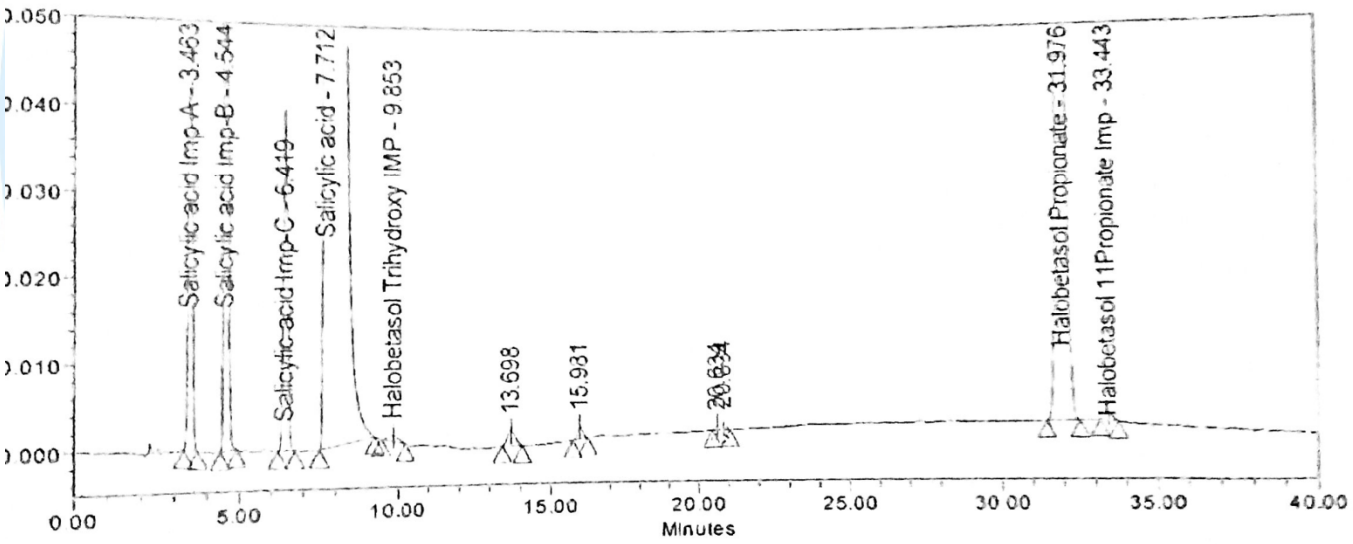
ANNEXURE – N

Chromatogram of Spiked Sample

Purity Plot 1

SAMPLE INFORMATION

Sample Name:	Spiked Sample	Acquired By:	Srinivasulu
Sample Type:	Unknown	Sample Set Name:	Halo_Sali_RS_SPE_140812
Vial:	13	Acq. Method Set:	Halo_Sali_RS_PDA
Injection #:	1	Processing Method	Halo Sali RS_PDA
Injection Volume:	20.00 ul	Channel Name:	231.0nm
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	PDA 231.0 nm
Date Acquired:	8/15/2012 2:45:46 AM IST	System Name	AD_HPLC_16



Peak Results

	Name	RT	Area (µV*sec)	Purity1 Angle	Purity1 Threshold	Purity1 Flag
1	Salicylic acid Imp-A	3.463	546575	0.054	0.222	No
2	Salicylic acid Imp-B	4.544	1464662	0.094	0.250	No
3	Salicylic acid Imp-C	6.419	354684	0.249	0.253	No
4	Salicylic acid	7.712	92342710	9.058	15.191	No
5	obetasol Trihydroxy	9.853	18091	1.056	2.147	No
6	lobetasol Propiona	31.976	2104646	0.019	0.209	No
7	etasol 11Propional	33.443	16238	1.182	1.698	No

CONCLUSION

The test method is validated for specificity, linearity, accuracy, precision and robustness. The method was found to meet all the predetermined acceptance criteria. The validated HPLC method for Assay, Chromatographic Purity of Halobetasol propionate and Salicylic acid Ointment is specific, linear, accurate, precise and robust. Based on the validation study results, it has been concluded that the HPLC method Assay, Chromatographic Purity of Halobetasol propionate and salicylic acid Ointment is stability indicating.

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