Development and validation of RP HPLC method for determination of Halobetasol Propionate and preservative (Methyl Paraben and Propyl Paraben) in formulation.

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Abstract : A Simple, accurate, and precise reversed phase high performance liquid chromatographic method was develope for simultaneous determination of Halobetasol Propionate and preservative (Methyl Paraben and Propyl Paraben) in formulation. In RP- HPLC the separation was carried out using mobile phase consisting of Acetonitrile: Methanol: Buffer (60:15:25v/v/v) and the Buffer was 0.08 %(v/v) Triflouroacetic acid. The column used was Inertsil ODS 3V, C18, 250 X 4.6mm, 5µ with flow rate 1ml/min. using UV detector at 240 nm. The retention time of Methyl paraben, Propyl paraben and Halobetasol Propionate were found to be around 3.43min, 4.45min and 7.06min respectively. For Linearity, R2 value was found to be 0.9971 for Methyl Paraben, 0.9986 for Propyl Paraben and 0.9992 for Halobetasol Propionate. % Assay was found to be 102.00%, 102.91% and 100.94% for Methyl Paraben, Propyl Paraben and Halobetasol Propionate respectively. Percentage recovery for Methyl Paraben was 98.52– 100.84%, for Propyl Paraben, it was found to be 98.40– 100.06% and for Halobetasol Propionate it was 99.04-101.45%. The results of the study showed that the proposed RP-HPLC method was found to be simple, sensitive, precise and accurate and also useful for the routine analysis of Halobetasol Propionate and preservative (Methyl Paraben and Propyl Paraben) in dosage form.

Keywords: Halobetasol Propionate, Methyl Paraben, Propyl Paraben, RP-HPLC, Preservative.

I. INTRODUCTION

Halobetasol propionate Halobetasol propionate is superior high strength topical corticosteroid drug use for the treatment of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatitis, with molecular weight of 484.965g/mol and pKa value of 12.46 chemically it is (6S, 8S, 9R, 10S, 11S, 13S, 14S, 16S, 17R)- 17-(2-chloroacetyl)- 6,9-difluoro-11,17-dihydroxy-10, 13, 16-trimethyl-6, 7, 8, 11, 12, 14, 15, 16-octahydrocyclopenta[a] phenanthren-3-one. Halobetasol propionate is a strong corticosteroid that diffuses across cell layers to collaborate with cytoplasmic corticosteroid receptors situated in both the dermal and intra-dermal cells, in this way enacting quality articulation of calming proteins intervened by means of the corticosteroid receptor reaction component. Halobetasol is basically utilized for the treatment of various conditions like edema, erythema, and pruritus through its cutaneous impact on vascular enlargement and penetrability. [1]

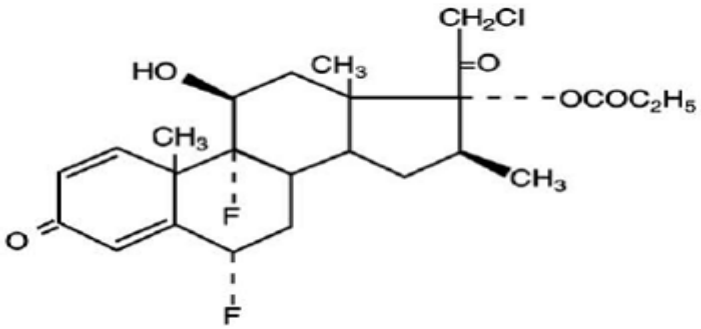

Fig. 1 Chemical Structure of Halobetasol Propionate

Methyl paraben Methyl paraben (CAS No. 99-76-3) is a methyl ester of p-hydroxybenzoic corrosive. It is a non-volatile, stable compound utilized as an antimicrobial additive in nourishments, medications and beauty care products for more than 50 years. Methyl paraben is promptly and totally consumed through the skin and from the gastrointestinal tract. methyl paraben is basically non-harmful by both oral and parenteral courses. In a population with normal skin, methyl paraben is non-irritating and non-sensitizing. In interminable organization considers, no-watched impact levels (NOEL) as high as 1050 mg/kg have been accounted for and a no-watched unfavorable impact level (NOAEL) in the rodent of 5700 mg/kg is placed. Methyl paraben isn't cancer-causing or mutagenic. It isn't teratogenic or embryotoxic and is negative in the uterotrophic measure. [2]

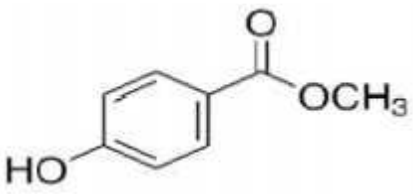

Fig.2 Chemical Structure of Methyl Paraben

Propyl Paraben Methylparaben and propylparaben are the most commonly used parabens and are frequently utilized together since they have synergistic impacts. It had been discovered that the antimicrobial activities of the parabens.[3]

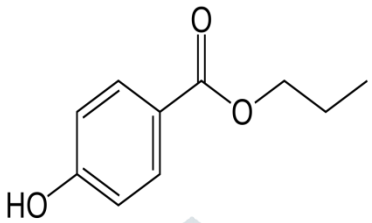

Fig. 3 Chemical Structure of Propyl Paraben

Literature surway state that different spectroscopic and chromatographic methods have been reported for estimation of Halobetasol Propionate with other drug and for Preservatives (Methyl paraben, Propyl paraben) . But there is not any HPLC method found for simultaneous estimation of Halobetasol Propionate along with its Preservatives.

1.1 Materials and Methods

1.1.1 Chemicals and solvants

Halobetasol propionate and Preservatives (Methyl Paraben, Propyl Paraben) were purchased from West Coast Pharmaceutical Works Ltd.

1.1.2 Instrumentation

Analysis was performed on Instrument Shimadzu LC- 2010C HT, with UV-VIS & PDA (Photodiod array detector) detector and CLASS-VDS software version 6.14 SPI, Singapore. UV- 1800, Shimadzu, Japan. K-EA 210, K-Roy Instrument Analytical balance.Other instruments like Ultra sonicator, Mechanical shaker & Filtration Assembly used.

1.1.3 Chromatographic Conditions

Column: ODS 3V, 250 X 4.6mm, 5µ or equivalent 250 C Column Temperature. The mobile phase was composed of Acetonitrile: Methanol: Buffer (60:15:25). In buffer 0.1M Potassium Dihydrogen phosphate buffer: At a flow rate 1.0 ml/min. Injection volume 10 µl and use methanol as a diluent The detection of drug carried out at 240nm.

1.1.4 Preparation of Buffer

Preparation of Buffer**:** 0.1M Potassium Dihydrogen phosphate buffer Filter it.

1.1.5 Preparation of Standard Solutions

Halobetasol propionate(40µg/ml), Methyl paraben(120µg/ml) and propyl paraben(40µg/ml) were prepared in methanol reanalysed after 12 hrs and 24 hrs time intervals and assay was determined for these Drug samples

1.1.6 Analysis of Formulation

Shake well and take 2.0 ml of homogenous lotion in 25.0 ml volumetric flask. Add 15.0 ml of methanol to it and sonicate for 20.0 minutes. Cool to room temperature and make the volume up to 25.0 ml with methanol and mix. Filter it. Take filterate as sample for analysis.

II VALIDATION METHOD

The HPLC process has been validated under ICH guidelines.The system precision of the method was confirmed by six replicate injections of standard solution containing Halobetasol propionate, Mehyl Paraben and Propyl Paraben. The method precision was performe for the analyte six times using the proposed method. Accuracy were performed by studies of percentage recovery at three different concentration levels. To the pre-analyzed sample solution of Halobetasol propionate, Mehyl Paraben, Propyl Paraben and a known amount of standard drug powders of Halobetasol propionate, Mehyl Paraben and Propyl Paraben were added at 80, 100, 120% level. The robustness of the method was studied for the sample. Sensitivity of the developed method were estimated in

respect of limit of detection (LOD) and limit of quantification. Ruggedness of the developed method were carried out by two altered analyst utilizing same environental and experimental conditions.[4]

2.1 Parameters for Validation of Developed Method:

1. Accuracy (Specificity)
2. Precision (repeatability and reproducibility)
3. Linearity
4. Range
5. Limit of detection (LOD)
6. Limit of Quantitation (LOQ)
7. Selectivity/ specificity
8. Robustness
9. Ruggedness
10. System Suitability Studies

III RESULTS AND DISCUSSIONS

For specificity there was no interference peak of blank, placebo solution with the peak of standard solution of Halobetasol propionate, Methyl Paraben and Propyl Paraben The proposed chromatographic system was found suitable for effective separation and good resolution, peak shapes and minimal tailing. Typical chromatogram of Standard and Sample were shown in figure 1 and 2.

The samples had followed linearity in the concentration range & Observed R2 value 0.9971 for Methyl Paraben, 0.9986 for Propyl Paraben and 0.9992 for Halobetasol Propionate The system precision, method precision and intermediate precision were evaluated on the basis of % RSDvalue and found. For system precision % of RSD value 0.06 for Methyl Paraben, 0.04 for Propyl Paraben and 0.11 for Halobetasol Propionate For method precision % of RSD value 0.26for Methyl Paraben, 0.06 for Propyl Paraben and 0.96 for Halobetasol Propionate For intermediate % RSD value 0.343 for Methyl Paraben, 1.17 for Propyl Paraben and 1.37 for Halobetasol Propionate The accuracy of method studied at three different levels i.e. 80%, 100%, 120% shows acceptable recoveries. Robustness of the method was studied by making

deliberate changes in the chromatographic conditions like flow rate (± 10%), wave length (± 2nm) and column Temperature (± 2C). The validation parameters were summarized in below Table. When the method was performed by two different analysts by under the same experimental and environmental conditions it was found to be rugged and %RSD (2%) indicates ruggedness of the method. The system suitability parameters such as number of

theoretical plates and tailing factor were studied. Stability of sample solution was established by the storage of sample solution at 250c for 24hr and sample was reanalyzed after 24 hr The results were shown in table.[5]

The developed method was considered suitable for estimation of Halobetasol propionate, Methyl Paraben and Propyl Paraben. with good peak shape and minimal tailing. The peak area of the drug was reproducible as shown by low coefficient of variance indicating the repeatability of the proposed method. The stated method has been validated with its recommendations in compliance with ICH standards. The method showing good selectivity and sensitivity. The %RSD values found less than 2 for intra and inter day variation studies show that the proposed method was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the methods was accurate. Low %RSD values of less than 2 in variation of flow rate, wavelength and mobile phase ratio specify the method was robust. When the method was execute by two altered analysts under the same experimental and environmental conditions and

%RSD were found to be less than 2 indicating the ruggedness of the proposed method. The solution Stability experiment confirmed that sample was stable up to 24 hour. during determination of assay. The sample recoveries of Halobetasol propionate, Methyl Paraben and Propyl Paraben from the commercial formulation were in good agreement with respective label claim showing that there was no interferences from the commonly used excipients and buffer used in analysis.[4]

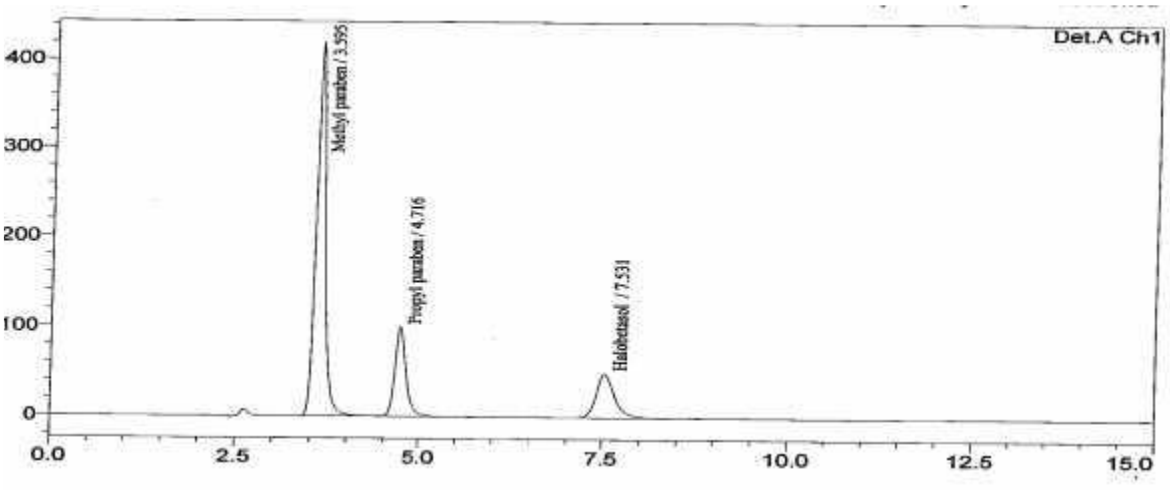

Fig. 4 Chromatogram of Mixed Standard

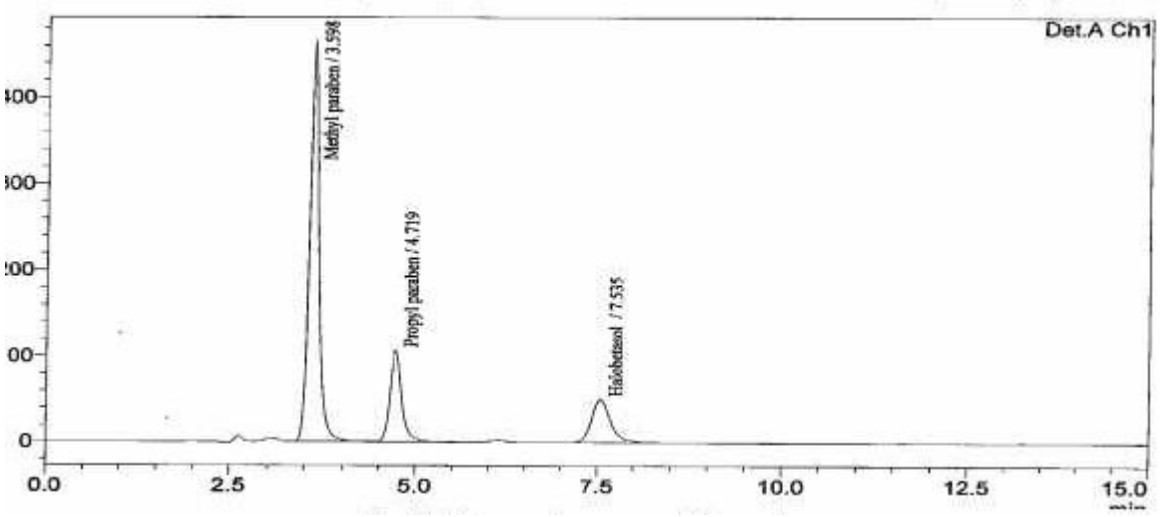

Fig. 5 Chromatogram of Sample

3.1 Precision

3.1.1 System Precision

Table 1 Precision Data for Halobetasol Propionate, Methyl Paraben and Propyl Paraben

Serial No.	Peak Area		
	Methyl paraben	Propyl paraben	Halobetasol
S1	3859282	1161914	883583
S2	3859000	1162015	886121
S3	3860073	1162810	884830
S4	3854574	1161397	8850988
S5	3856862	1161682	885981
S6	3859239	1161802	885620
AVG.	**3858262**	**1161937**	**885206**
STDEV	2134.34	478.24	937.70
% RSD	**0.06**	**0.04**	**0.11**

3.1.2 Method Precision

Table 2 Method Precision Data of Halobetasol Propionate, Methyl Paraben and Propyl Paraben

Sample No.	% Assay of Methyl Paraben	% Assay of Propyl Paraben	% Assay of Halobetasol Propionate
T1	101.93	102.95	100.67
T2	102.08	102.99	100.59
T3	101.71	102.90	100.59
T4	102.19	102.91	102.91

T5	102.36	102.87	100.48
T6	101.71	102.82	100.38
AVG.	**102.00**	**102.91**	**100.94**
STD DEV.	0.2627	0.0609	0.9705
% RSD	**0.26**	**0.06**	**0.96**

3.1.3 Intermediate Precision

Table 3 Intermediate Precision Data of Halobetasol Propionate, Methyl Paraben and Propyl Paraben

Sample No.	% Assay of Methyl Paraben	% Assay of Propyl Paraben	% Assay of Halobetasol Propionate
T1	101.43	102.82	100.11
T2	102.26	102.64	99.86
T3	102.39	104.89	102.75
T4	102.18	105.11	102.97
T5	101.56	105.59	103.30
T6	102.49	105.58	103.28
AVG.	**102.05**	**104.44**	**102.05**
STD.DEV	0.4460	1.3523	1.6115
%RSD	**0.4371**	**1.29**	**1.58**

Table 4 Comparison between method precision & intermediate precision (Halobetasol propionate):

Method Precision		**Intermediate Precision**	
Sample	**(%)Halobetasol**	**Sample**	**(%)Halobetasol**
1	100.67	**1**	100.11
2	100.59	**2**	99.86
3	100.59	**3**	102.75
4	102.91	**4**	102.97
5	100.48	**5**	103.30
6	100.38	**6**	103.28
AVG.	**100.94**	**AVG.**	**102.05**
STD.DEV	0.9701	**STD.DEV**	1.6115
% RSD	**0.96**	**% RSD**	1.58
Overall Average	**102.05**		
Overall STDEV	1.6115		

Overall %RSD	**1.37**

Table 5 Comparison between method precision & intermediate precision (Methyl Paraben)

Method Precision		**Intermediate Precision**	
Sample	**(%)Methyl Paraben**	**Sample**	**(%)Methyl Paraben**
1	101.93	**1**	101.43
2	102.08	**2**	102.26
3	101.71	**3**	102.39
4	102.19	**4**	102.18
5	102.36	**5**	101.56
6	101.71	**6**	102.49
AVG.	**102.00**	**AVG.**	**102.05**
STD.DEV	0.2627	**STD.DEV**	0.4460
% RSD	**0.2576**	**% RSD**	**0.4371**
Overall Average	102.02		
Overall STDEV	0.3502		
Overall %RSD	0.3432		

Table 6 Comparison between method precision & intermediate precision (Propyl paraben):

Method Precision		**Intermediate Precision**	
Sample	**(%)Propyl paraben**	**Sample**	**(%)Propyl paraben**
1	102.95	**1**	102.82
2	102.99	**2**	102.64
3	102.90	**3**	104.89
4	102.91	**4**	105.11
5	102.87	**5**	105.59
6	102.82	**6**	105.58
AVG.	**102.91**	**AVG.**	**104.44**
STD.DEV	0.0609	**STD.DEV**	1.3523
% RSD	**0.06**	**% RSD**	**1.29**
Overall Average	**103.67**		
Overall STDEV	1.2133		
Overall %RSD	**1.17**		

3.2 Accuracy Data

Table 7 Observation data of Halobetasol Propionate for Recovery

Recovery Levels	Mean	% RSD
80%	99.04	0.36
100%	101.45	0.46
120%	100.82	0.52

Table 8 Observation data of Methyl paraben for Recovery

Recovery Levels	Mean	% RSD
80%	98.52	0.12
100%	100.84	0.18
120%	99.27	0.07

Table 9 Observation data of Propyl paraben for Recovery

Recovery Levels	Mean	% RSD
80%	98.88	0.54
100%	100.06	0.25
120%	98.40	0.21

3.3 Robustness

3.3.1 Change in wavelength ±2 nm

Table 10 Data for Robustness (Change in wavelength)

Drug	Area(mV.s)(n=3)			Average	SD	%RSD
	238nm	240nm	242nm			
Halobetasol	880236	882135	898653	887008	10129.47	1.141981
	887956	886762	899895	891538	7262.243	0.814575
	887232	885587	898442	890420	6995.488	0.785639
Methyl paraben	3723122	3849921	3963232	3868758	38665.06	0.999418
	3766325	3863252	3998745	3889441	42726.44	1.098524
	3698989	3846652	3889633	3855091	31190.39	0.80907
Propyl paraben	1148234	1156623	1166978	1157278	9389.168	0.811315
	1149658	1154568	1166682	1156969	8762.36	0.757355
	1149893	1152236	1168952	1157027	10393.59	0.898301

3.3.2 Change in column temperature: ±2°C

Table 11 Data for Robustness (Change in column temperature)

Drug	Area(mV.s)(n=3)			Average	SD	%RSD
	23°C	25°C	27°C			
Halobetasol	878956	885632	894524	886370.7	7810.242	0.881148
	879962	879892	899654	886503	11389.44	1.284761
	875632	883231	899123	885995	11986.99	1.352941
Methyl paraben	3789624	3803221	3878931	3823925	48118.99	1.258366
	3776925	3812536	3885462	3824974	55327.25	1.446474
	3789665	3832133	3865241	3829013	37884.48	0.989406
Propyl paraben	1147234	1152314	1178958	1159502	17039.76	1.469576
	1148966	1154865	1176322	1160051	14396.48	1.241021
	1149896	1158695	1177695	1162095	14208.02	1.222621

3.3.3 Change in flow rate: ±10%

Table 12 Data for Robustness (Change in flow rate)

Drug	Area(mV.s)(n=3)			Average	SD	%RSD
	0.9ml/min	1ml/min	1.1ml/min			
Halobetasol	898952	881235	869256	883147.7	14940.11	1.691689
	899653	884135	868795	884194	15429.09	1.744988
	901325	881862	870996	884728	15366.23	1.736832
Methyl paraben	3920565	3812364	3798695	3843875	66766.5	1.736958
	3915623	3863562	3785462	3854882	65513.16	1.699485
	3904568	3845681	3795241	3848497	54717.86	1.421798
Propyl paraben	1173261	1156324	1139864	1156483	16699.07	1.443953
	1178952	1152365	1138695	1156671	20470.97	1.769818
	1177892	1154568	1136985	1156482	20520.53	1.774393

3.4 System suitability parameter

Table 13 System suitability for Halobetasol

Parameter	Theoretical plate	Tailing Factor	% RSD of replicates
Limit	NLT 2000	NMT 2.0	NMT 2.0 %
Change in wavelength ±2 nm	6362	1.52	0.914065

Change in column temperature: ±2°C	6383	1.49	1.17295
Change in flow rate: ±10%	6272	1.62	1.724503

Table 14 System suitability for Methyl Paraben

Parameter	Theoretical plate	Tailing Factor	% RSD of Replicates
Limit	NLT 2000	NMT 2.0	NMT 2.0 %
Change in wavelength ±2 nm	6286	1.36	0.969004
Change in column temperature: ±2°C	6457	1.28	1.231415
Change in flow rate: ±10%	6356	1.60	1.619413

Table 15 System suitability for Propyl Paraben

Parameter	Theoretical plate	Tailing Factor	% RSD of Replicates
Limit	NLT 2000	NMT 2.0	NMT 2.0 %
Change in wavelength ±2 nm	5968	1.35	0.82232
Change in column temperature: ±2°C	6125	1.52	1.31107
Change in flow rate: ±10%	6103	1.58	1.662721

3.5 Stability of Analytical Solution

Table 16 Stability of Analytical Solution at Room Temperature

Time in minutes	Area	% Response from Initial	Area	% Response from Initial	Area	% Response from Initial
	HP		MP		PP	
Initial	891535	0.00	3869382	0.00	151359	0.00
4 hrs	896888	0.60	3867701	0.04	1152999	-0.14
8 hrs	895665	-0.46	3887440	-0.47	1153895	-0.22
12 hrs	896770	-0.59	3890656	-0.55	1153253	-0.16
16 hrs	904662	-1.47	3909901	-1.05	1152332	0.08
20 hrs	899774	-0.92	3885933	-0.43	1152907	-0.13
24 hrs	899382	-0.88	3883152	-0.36	1152131	-0.07

Table 17 Stability of Analytical Solution at 80c Temperature

Time in minutes	Area	% Response from Initial	Area	% Response from Initial	Area	% Response from Initial
	HP		MP		**PP**	
Initial	891535	0.00	3869382	0.00	1151359	0.00
4 hrs	899825	-0.93	3884164	-0.38	1152999	-0.14
8 hrs	896536	-0.56	3888609	-0.50	1153895	-0.22
12 hrs	887376	0.47	3887042	-0.46	1153253	-0.16
16 hrs	896096	-0.51	3882071	-0.33	1152332	-0.08
20 hrs	900459	-1.00	3885427	-0.41	1152907	-0.13
24 hrs	899718	-0.92	3881804	-0.32	1152131	-0.07

IV CONCLUSION

Halobetasol propionate is the drug, class Corticosteroid, thought to act through the induction of inhibitory proteins of phospholipas e A2, collectively called lipocortins. It is believe that these proteins regulate the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of specific arachidonic acid. Arachidonic acid is discharge from membrane phospholipids by phospholipase A2. It is used for relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses. Methyl paraben and Propyl paraben has been used as an antimicrobial preservative in the food,
cosmetic, and pharmaceutical industries. It is also used as a food additive and as an anti-fungal preservative agent. Literature surway stated that different spectroscopic and chromatographic methods have been reported for estimation of Halobetasol Propionate with other drug and for Preservatives (Methyl paraben & Propyl paraben) with other drug. But there is no any one HPLC method found for simultaneous estimation of Halobetasol Propionate along with these Preservatives.
Therefore it was thought to develop and validate a simple, economical, precise and accurate RP-HPLC method for simultaneous estimation of Halobetasol Propionate along these Preservatives in combined pharmaceutical dosage form. Further the RP-HPLC was validated in terms of Specificity, Linearity, Precision, Accuracy, Stability and Robustness
RP-HPLC method was developed and validated. The mobile phase used was Acetonitrile: Methanol: Buffer (60:15:25v/v/v) and the Buffer was 0.08 %(v/v) Triflouroacetic acid. The retention time of Methyl paraben, Propyl paraben and Halobetasol Propionate were found to be around 3.43min, 4.45min and 7.06min respectively. For Linearity, R2 value was found to be 0.9971 for Methyl Paraben, 0.9986 for Propyl Paraben and 0.9992 for Halobetasol Propionate. % Assay was found to be 102.00%, 102.91% and 100.94% for Methyl Paraben, Propyl Paraben and Halobetasol Propionate respectively. Percentage recovery for Methyl Paraben was 98.52– 100.84%, for Propyl Paraben, it was found to be 98.40– 100.06% and for
Halobetasol Propionate it was 99.04-101.45%.
The developed methods were simple, rapid, precise and accurate and so, it can be successfully apply for the simultaneous estimation of Halobetasol Propionate, Methyl Paraben and Propyl Paraben in formulation.
Propyl Paraben and Halobetasol Propionate respectively. Percentage recovery for Methyl Paraben was 98.52– 100.84%, for Propyl Paraben, it was found to be 98.40– 100.06% and for
Halobetasol Propionate it was 99.04-101.45%.
The developed methods were simple, rapid, precise and accurate and so, it can be successfully apply for the simultaneous estimation of Halobetasol Propionate, Methyl Paraben and Propyl Paraben in dosage form i.e., Lotion.[6,7]

V ACKNOWLEDGEMENT

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