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Analytical Method Development and Validation for Simultaneous Estimation of Moxifloxacin Hydrochloride and Fluorometholone Acetate in Ophthalmic Suspension by RP-HPLC

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ABSTRACT:

Moxifloxacin Hydrochloride (MOXI) is a fourth generation fluoroquinolone broad spectrum antibiotic agent used in conjunctivitis. Fluorometholone Acetate (FLM) is a corticosteroid employed for its steroidal anti inflammatory activity. The present study has established a specific, precise and accurate RP-HPLC method for simultaneous estimation of MOXI and FLM in ophthalmic suspension. The chromatographic system was equipped with Hypersil Gold BDS C_{18} column (25cm X 4.6mm, 5µ) with PDA detection set at dual wave length of 295nm for MOXI and 240nm for FLM in conjunction with a mobile phase of phosphate buffer (pH adjusted to 3 by OPA) and a combination of methanol : acetonitrile (40:60 % v/v) with a gradient elution at flow rate of 1ml min^{-1} and injection volume of 10µl with run time set for 20min. Optimum chromatographic separations were achieved at retention time of 5.1 min and 12.2 min for MOXI and FLM respectively. The method was validated in accordance with ICH guild lines. Response was a linear function over a concentration range of 50-150µg ml^{-1} for MOXI (r^2=0.9997) and 5-30µg ml^{-1} for FLM (r^2=0.9998) with a mean % recovery of 101% and 100.2% for MOXI and FLM respectively. The method resulted in good separation of analytes and degradation products with acceptable tailing and resolution. The results of the study showed that proposed RP-HPLC method was specific, precise and accurate which can be applied successfully for simultaneous determination of Moxifloxacin Hydrochloride and Fluorometholone Acetate in ophthalmic suspension for routine analysis and their stability studies.

Keywords: Moxifloxacin Hydrochloride, RP-HPLC, Method development, validation.

Introduction:

Moxifloxacin hydrochloride is known chemically as 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl-4-oxo-3 quinolinecarboxylic acid hydrochloride. The antibiotic is classified as a broad-spectrum agent, which functions through the inhibition of two essential enzymes, namely DNA gyrase and topoisomerase IV. These enzymes, belonging to the type II topoisomerase group, play a crucial role in the separation of bacterial DNA, ultimately impeding the process of cell replication. The antibiotic is employed in the treatment of bacterial conjunctivitis, keratitis, as well as for the prevention and management of eye infections before and after surgical procedures. Ketorolac tromethamine is chemically denoted as (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine.The compound is a carboxylic acid with a substituent consisting of an amino group and a hydroxymethyl group attached to the same carbon atom.The compound -1,3-propanediol, which is structurally similar to indomethacin, belongs to the class of pyrrolizine carboxylic acid derivatives. It is primarily utilized as a nonsteroidal anti-inflammatory drug (NSAID) with its main therapeutic application being pain relief. Various techniques have been employed to analyze both moxifloxacin hydrochloride and ketorolac tromethamine, both individually and in conjunction with other pharmaceutical compounds. The moxifloxacin hydrochloride can be analyzed using various analytical methods, such as spectrophotometry and hplc.

Materials & Methods:

Instrument:

The equipments used are double beam Uv-Visible spectrophotometer (Make: Schimadzu, Model: Uv 1700) equipped with Uv probe 2.10 software, Analytical balance (Make: Schimadzu), pH meter (Make: Eu Tech), Sonicator: Ultrasonic bath sonicator (Model: 19l500), Hot air oven (Make: Alpha Tempcon), water bath (Make: Alpha Tempcon). The chromatographic separation was carried out using HPLC Alliance Waters (2469) equipped with gradient system, connected to PDA detector. The data was acquired by Empower Pro.

Chemicals & Reagents:

Moxifloxacin working standard was used for analysis. Methanol, Acetonitrile used are of HPLC grade solvents. Chemicals Orthophosphoric acid, Potassium Dihydrogen ortho phosphate are of AR grade.

SELECTION Of WAVELENGTH

Selection of solvent

Mixture of Methanol and Water (70:30%v/v) was selected as the solvent for dissolving Moxifloxacin HCl and Fluorometholone Acetate.

Preparation of standard solutions

In order to prepare stock solution, 10 mg each Moxifloxacin HCl and Fluorometholone Acetate were accurately weighed and transferred into two separate 100 ml volumetric flasks respectively, about 70 ml of diluent was added to each flask and sonicated to dissolve, diluted up to mark with the diluent to obtain 100 µg/ml concentration each of Moxifloxacin HCl and Fluorometholone Acetate separately.

Determination of λmax

Both the standard solutions were scanned separately between 400nm to 200nm in 1cm cell against blank. The individual spectra for both drugs (Moxifloxacin HCl and Fluorometholone Acetate) were recorded as shown in Fig:-1&2.

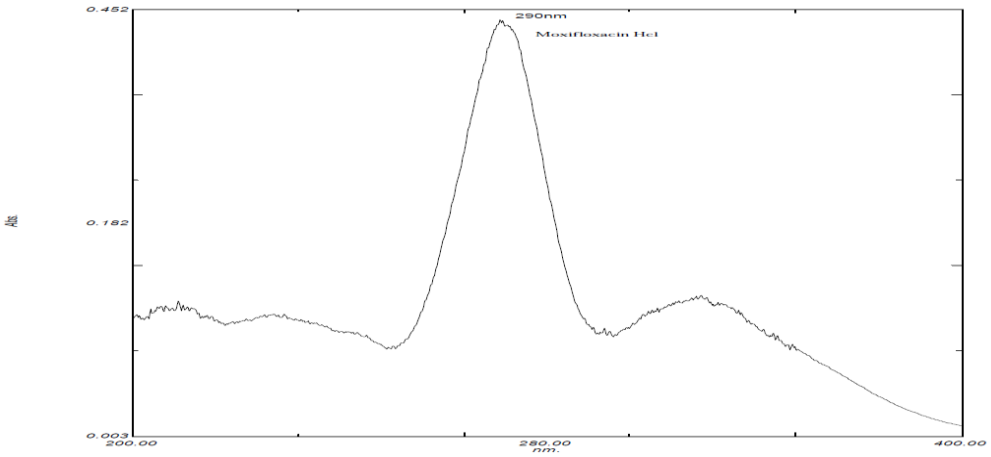

Fig.No.1: UV Spectrum of Moxifloxacin HCl showing λ max of 295nm

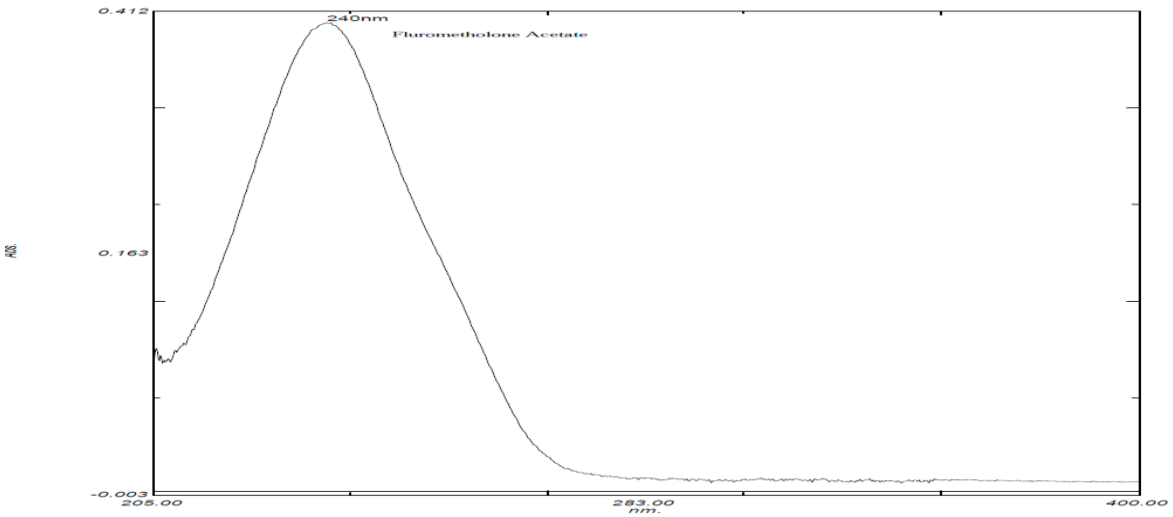

Fig.No.2: UV Spectrum of Fluorometholone Acetate showing λ max of 240nm

SYSTEM SUITABILITY

System suitability test is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard drug solution.

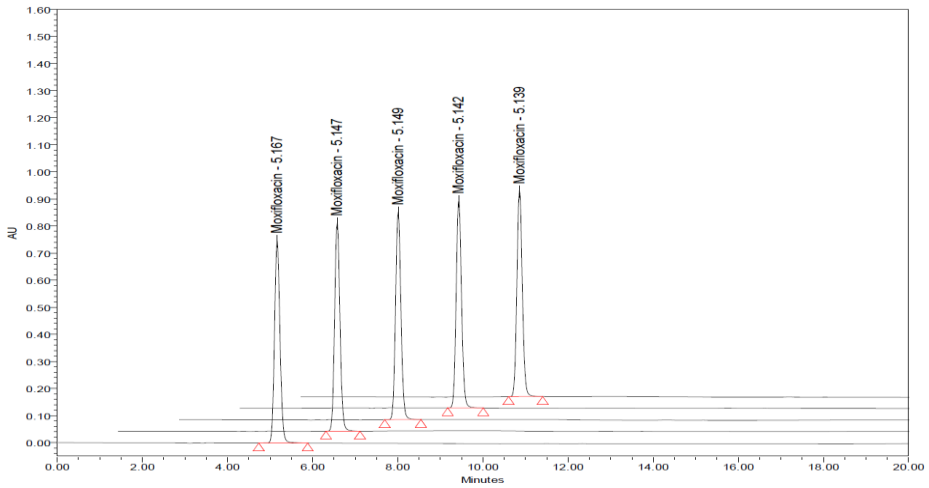

Fig.No.1: Chromatogram for System Suitability of Moxifloxacin Standard at 295nm

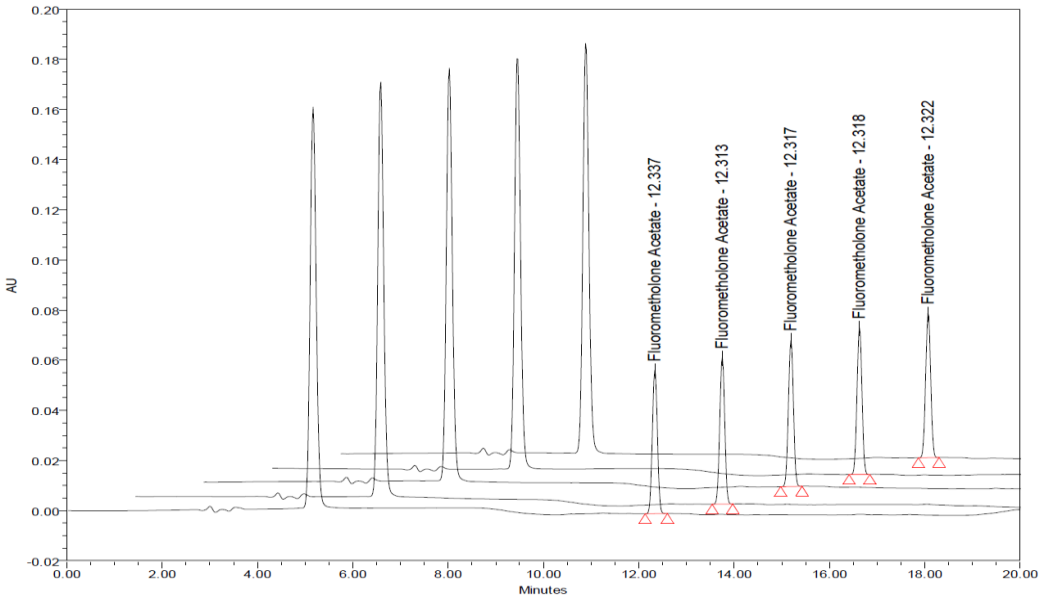

Fig.No.2: Chromatogram for System Suitability of Fluorometholone Acetate Standards at 240nm

Table No 2: Result and Statistical Data for System Suitability test of Moxifloxacin

Sample Name	**Retention Time (min)**	**Area**	**Plate Count**	**Tailing**
Standard 1	5.167	6708202	7668	1.1
Standard 2	5.147	6731189	7979	1.1
Standard 3	5.149	6735375	7911	1.1
Standard 4	5.142	6736760	7795	1.1
Standard 5	5.139	6721173	7734	1.1
Mean		6726538		
S.D		11928.24		
% RSD		0.177331		

Table No 3: Result and Statistical Data for System Suitability test of Fluorometholone Acetate.

Sample Name	Retention Time (min)	Area	Plate Count	Tailing
Standard 1	12.337	424055	61028	1.03
Standard 2	12.313	428199	62111	1.04
Standard 3	12.317	428486	60331	1.04
Standard 4	12.318	428048	61410	1.03
Standard 5	12.322	426945	61693	1.02
Mean		427146.6		
S.D		1824.3282		
% RSD		0.427096		

System Suitability Acceptance Criteria:

1. Relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0 %.

2. Theoretical plates of analyte peak in Standard chromatograms should not be less than 2000.

3. Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 2.0

Data interpretation:

It was observed from the data tabulated above that all the system suitability parameters meet the predetermined acceptance criteria as per the test method and indicates the suitability of the selected system.

LINEARITY AND RANGE:

Procedure:

Linearity was performed by diluting standard stock solution. From stock solution aliquots of 1.25, 2.5, 3.75, 5, 6.25, 7.5ml diluted to 25ml with diluent such that the final concentration of Moxifloxacin in the range of 50 to 150 µg/ml and Fluorometholone Acetate in the range of 5 to 30 µg/ml. 10 µl of each sample injected in duplicate for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration. The observations and calibration curve is shown below.

$$\frac{\text{Weight of Std taken in mg}}{100} \times \frac{\text{Vol pipette out}}{25} \times \frac{\text{Potency}}{100} \times \frac{\text{Factor 1} \times 1000}{\text{Factor 2}}$$

Calculation:

Final Conc. = (µg/ml)

Table No 4: Result and Statistical data for Linearity of Moxifloxacin

Sr .No	Linearity Level (%)	Vol. of stock Taken	Diluted	Concentration (ppm)	Avg. Area
1	25	1.25	25	23.9378	1698671
2	50	2.5	25	47.8757	3385855
3	75	3.75	25	71.8135	5171442
4	100	5	25	95.7514	6715986
5	125	6.25	25	119.6892	8574270
6	150	7.5	25	143.6270	10089220
		Correlation Coefficient			**0.9997**
		Slope (m)			**70495**
		Intercept (y)			**32988**

Table No 5: Result and Statistical data for Linearity of Fluorometholone Acetate

Sr. No	Linearity Level (%)	Vol. of stock Taken	Diluted	Concentration (ppm)	Avg. Area
1	25	1.25	25	4.9644	107301
2	50	2.5	25	9.9288	213225
3	75	3.75	25	14.8932	325691
4	100	5	25	19.8576	425495
5	125	6.25	25	24.8220	543212
6	150	7.5	25	29.7864	645203
		Correlation Coefficient			**0.9998**
		Slope (m)			**21751**
		Intercept (y)			**1239**

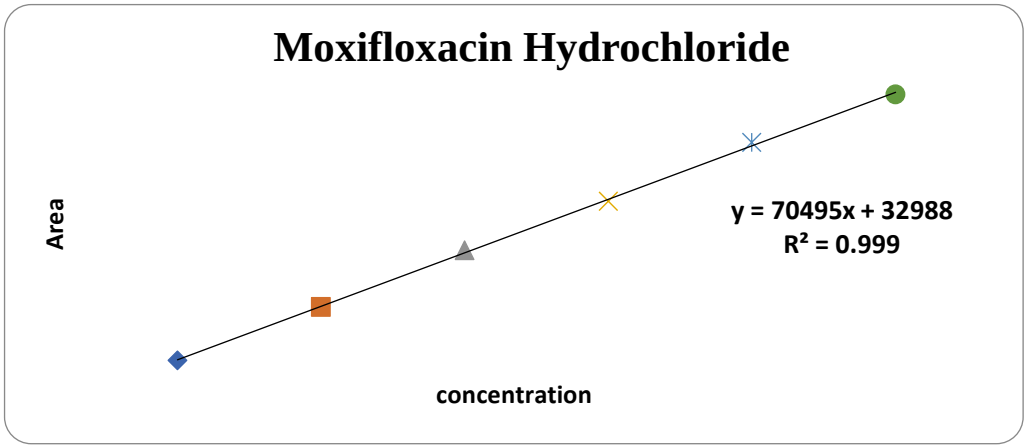

Fig.No.5: Linearity Plot of Moxifloxacin

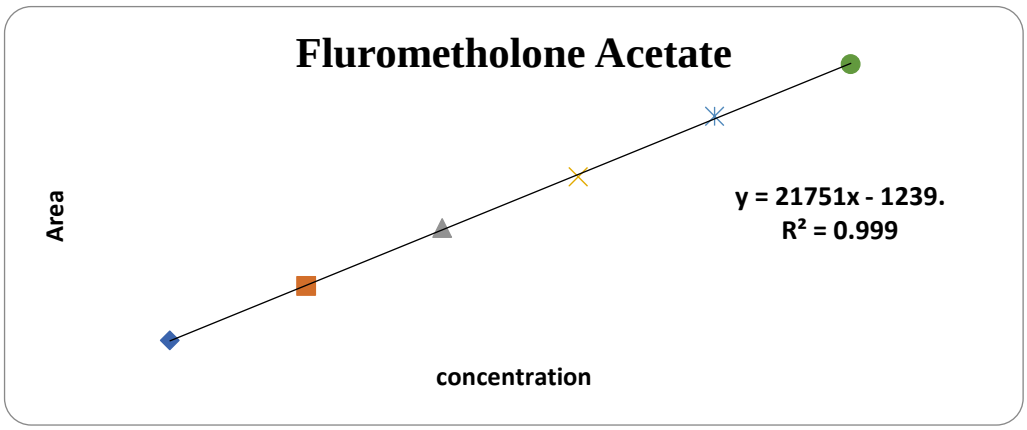

Fig.No.6: Linearity Plot of Fluorometholone Acetate

Acceptance Criteria:

The correlation coefficient should be NLT 0.999

***Data Interpretation*:**

The *Correlation coefficient* for Moxifloxacin and Fluorometholone Acetate was found to be **0.9997** and **0.9998** respectively, which indicates that the peak responses were linear. This concluded that the method was linear throughout the range selected.

PRECISION:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement.

System precision

Standard solution was prepared as per the proposed method for system precision studies. Ten replicate injections were injected into the HPLC system. The % RSD for the peak responses of ten replicate injections should be ***NMT 1.0***.

Table No 6: Results and Statistical Data for System Precision of Moxifloxacin and Fluorometholone Acetate

Injection no.	Moxifloxacin		Fluorometholone Acetate	
	Retention Time (min.)	Area	Retention Time (min.)	Area
1	5.167	6708202	12.337	424055
2	5.147	6731181	12.313	428199
3	5.149	6735375	12.317	428486
4	5.142	6736760	12.318	428046
5	5.139	6721173	12.322	426945
6	5.138	6717112	12.338	425022
7	5.148	6720819	12.335	424125
8	5.149	6732231	12.312	428541
9	5.143	6734522	12.310	419085
10	5.138	6724678	12.339	420387
Mean		6726205		425289
S.D.		9364.205		3416.886
%RSD		0.1392		0.8034

Acceptance Criteria:

The % RSD for the ten determinations shall be NMT 1.0

Data interpretation:

It was observed from the data tabulated above, that the % RSD of the peak responses as peak area was found to be within acceptance criteria indicating an acceptance level of precision for system precision studies.

Method precision:

In method precision, a homogenous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results for a single batch. To each six 100 ml flask, 2 gm sample of Moxifloxacin Hydrochloride and Fluorometholone Acetate were transferred. % assay values and RSD of assay were calculated.

Table No 7: Results for Method Precision of Moxifloxacin

Test No	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Weight/ml	**1001.1**					
Wt. taken (in mg)	2014.5	2014.5	2014.5	2014.5	2014.5	2014.5
Area (Injection 1)	7048986	7202086	7189454	7188198	7199452	6953914
Area (Injection 2)	7046956	7245104	7154895	7165124	7125489	6980214
Average Area	7047971	7223595	7172175	7176661	7162471	6967064
% RSD	1.3592					
Assay(mg)	**4.99**	**5.11**	**5.07**	**5.08**	**5.07**	**4.93**
Assay (in %)	**99.8**	**102.2**	**101.4**	**101.6**	**101.4**	**98.6**
Average Assay		**In mg = 5.04**		**100.8 %**		

Table No 8: Results for Method Precision of Fluorometholone Acetate

Test No	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Weight/ml	**1001.1**					
Wt. taken (in mg)	2014.5	2014.5	2014.5	2014.5	2014.5	2014.5
Area (Injection 1)	437717	434568	437621	434523	435768	423178
Area (Injection 2)	437124	442015	432109	432712	438125	431021
Average Area	437421	438292	434865	433618	436947	427100
% RSD	0.9442					
Assay(mg)	**1.01**	**1.01**	**1.00**	**1.00**	**1.01**	**0.99**
Assay (in %)	**101.0**	**101.0**	**100.0**	**100.0**	**101.0**	**99.0**
Average Assay		**In mg = 1.00**		**100.3.%**		

Acceptance Criteria:

The % RSD for the six determinations shall be NMT 2.0

Data interpretation:

From the above results, it was concluded that the method was found to be precise.

ACCURACY:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy was performed in three different levels for Moxifloxacin and Fluorometholone Acetate. Spiked known quantity of Moxifloxacin and Fluorometholone Acetate Standard at 50%, 100% and 150% level into the placebo. Analyses of samples were done in triplicate for each level. From the results, % recovery was calculated.

Procedure for accuracy

Standard Preparation*:* Weighed accurately about 54.5mg of Moxifloxacin hydrochloride and 10mg of Fluorometholone Acetate in 100ml volumetric flask. To it added 50ml of diluent and sonicated for few minutes and then diluted upto mark with diluent.

Accuracy at 50 %: Accurately weighed 2001.2 mg of placebo, and spiked a known volume of standard preparation of 2.5ml was transferred to 25ml volumetric flask. To it 10 ml of diluent was added and sonicated for 5 minutes with occasional shaking and was cooled to room temperature then diluted upto the mark with diluent.

Accuracy at 100 %: Accurately weighed 2001.5 mg of placebo, and spiked a known volume of standard preparation of 5ml was transferred to 25ml volumetric flask. To it 10 ml of diluent was added and sonicated for 5 minutes with occasional shaking and was cooled to room temperature then diluted upto the mark with diluent.

Accuracy at 150 %: Accurately weighed 2001.3mg of placebo, and spiked a known volume of standard preparation of 7.5ml was transferred to 25ml volumetric flask. To it 10 ml of diluent was added and sonicated for 5 minutes with occasional shaking and was cooled to room temperature then diluted upto the mark with diluent.

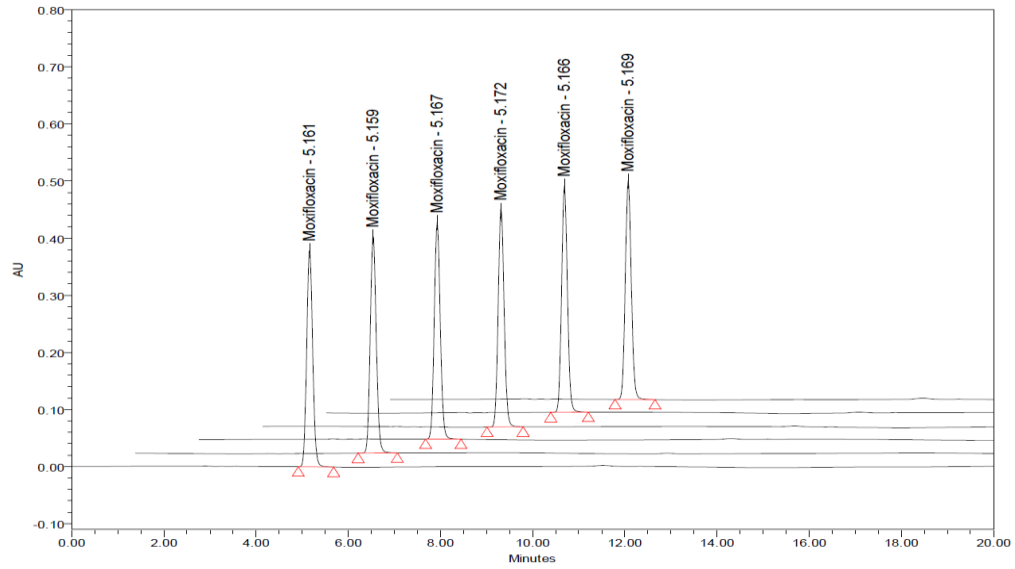

Fig.No.7: Chromatogram for Accuracy 50% of Moxifloxacin at 295nm

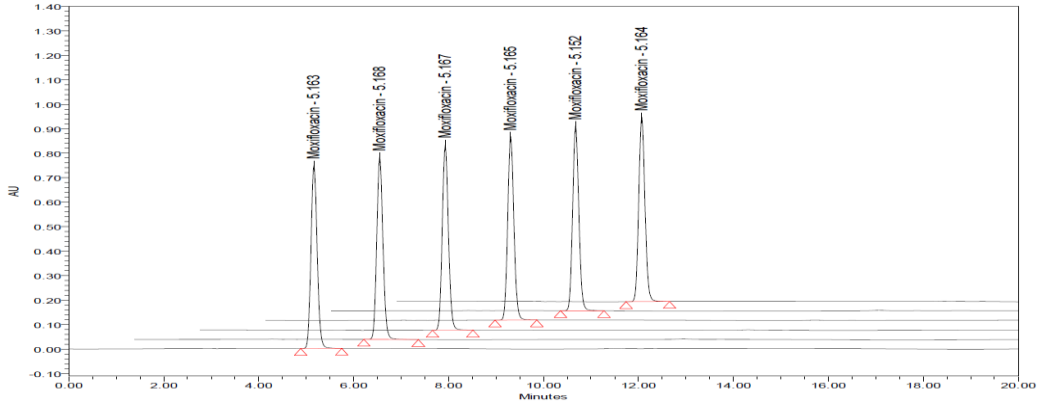

Fig.No.8: Chromatogram for Accuracy 100% of Moxifloxacin at 295nm

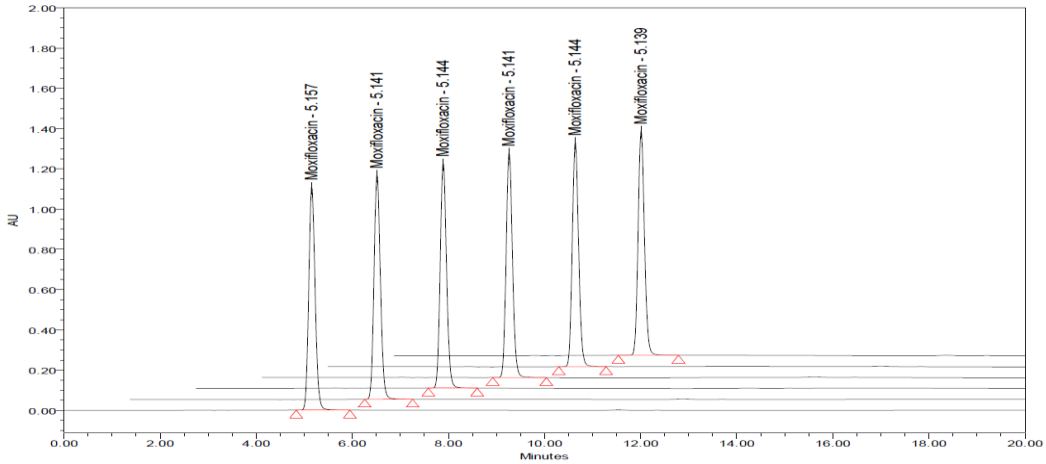

Fig.No.9: Chromatogram for Accuracy 150% of Moxifloxacin at 295nm

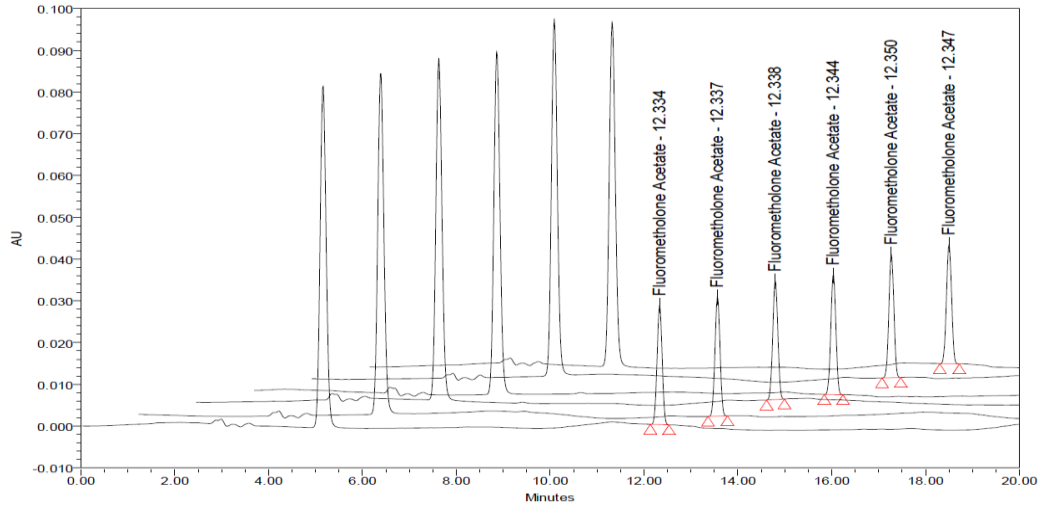

Fig.No.10: Chromatogram for Accuracy 50% of Fluorometholone Acetate at 240nm

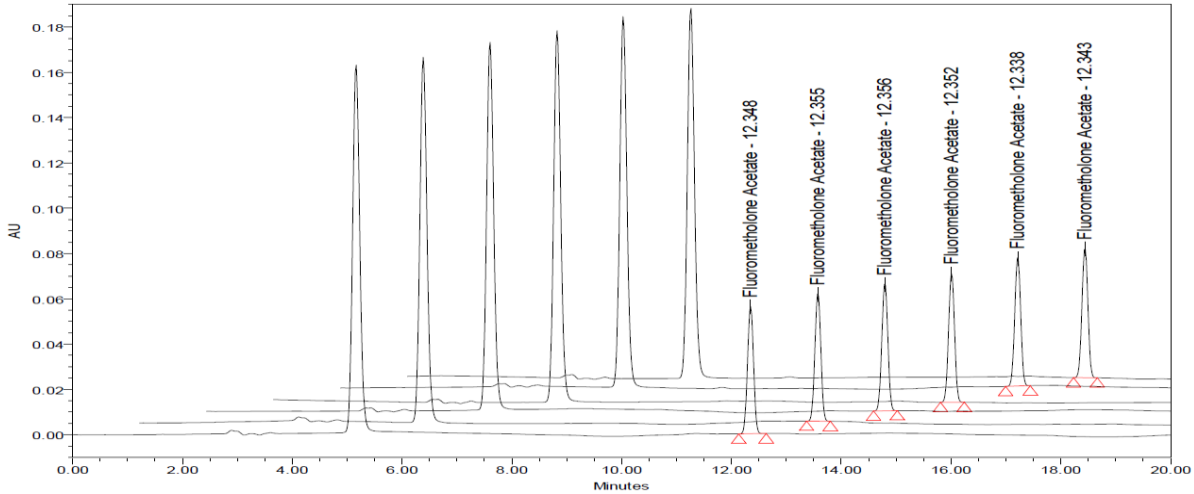

Fig.No.11: Chromatogram for Accuracy 100% of Fluorometholone Acetate at 240nm

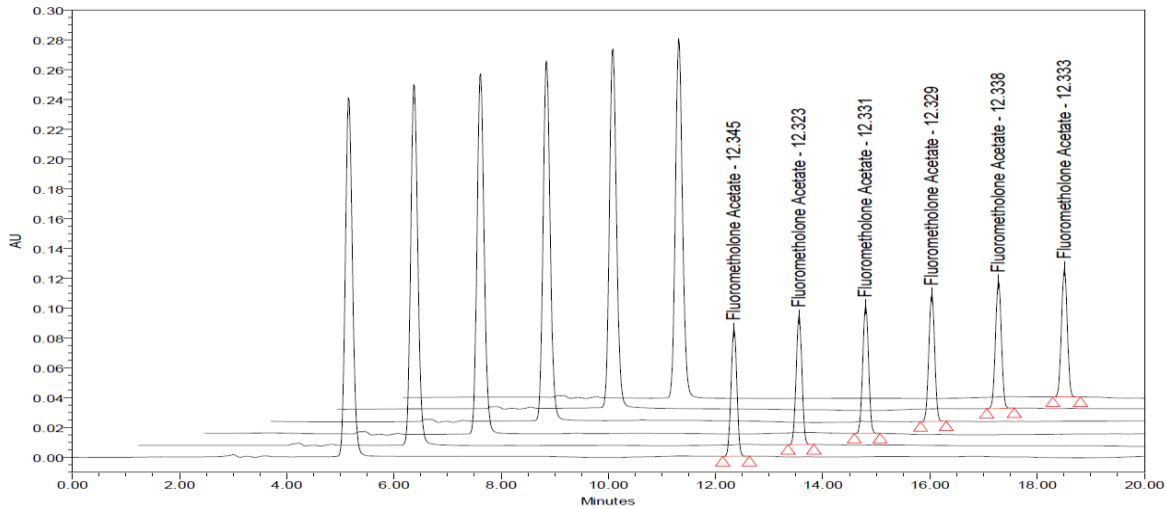

Fig.No.12: Chromatogram for Accuracy 150% of Fluorometholone Acetate at 240nm

Amount of drug Recovered was calculated using following formula

Table No 9: Result and Statistical data for Accuracy (Moxifloxacin)

Target Conc.	Vol.stk sol	Ppm Spiked	Area Inj 1	Area Inj 2	Avg Area	mg recov.	% Recovery	Avg Recovery	% RSD
50	2.5	52.8041	3464434	3444583	3454509	53.6408	101.6	**101.8**	**0.4**
50	2.5	52.8041	3451381	3458279	3454830	53.6457	101.6		
50	2.5	52.8041	3485817	3474087	3479950	54.0358	102.3		
100	5.0	105.6081	6798090	6812420	6805255	105.6703	100.1	**100.4**	**0.4**
100	5.0	105.6081	6834992	6813602	6824297	105.9660	100.3		
100	5.0	105.6081	6834989	6875277	6855133	106.4448	100.8		
150	7.5	158.4122	10240510	10310805	10275658	159.5578	100.7	**100.7**	**0.2**
150	7.5	158.4122	10272369	10287123	10279746	159.6213	100.8		
150	7.5	158.4122	10246149	10269604	10257877	159.2817	100.5		
						Overall Recovery		**101.0**	**0.7**

Table No 10: Result and Statistical data for Accuracy (Fluorometholone Acetate)

Target Conc.	Vol.stk sol	Ppm Spiked	Area Inj 1	Area Inj 2	Avg Area	ppm recov.	% Recovery	Avg Recovery	% RSD
50	2.5	10.0800	216420	217659	217040	10.0900	100.1	100.6	0.4
50	2.5	10.0800	217595	218496	218046	10.1367	100.6		
50	2.5	10.0800	219484	218478	218981	10.1802	101.0		
100	5.0	20.1600	430870	428659	429765	19.9793	99.1	99.6	0.5
100	5.0	20.1600	432899	432256	432578	20.1101	99.8		
100	5.0	20.1600	431928	435404	433666	20.1607	100		
150	7.5	30.2400	655274	650271	652773	30.3467	100.4	100.4	0.1
150	7.5	30.2400	651573	654220	652897	30.3525	100.4		
150	7.5	30.2400	652469	652705	652587	30.3381	100.3		
						Overall Recovery		**100.2**	**0.5**

SPECIFICITY AND SELECTIVITY:-

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is the procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

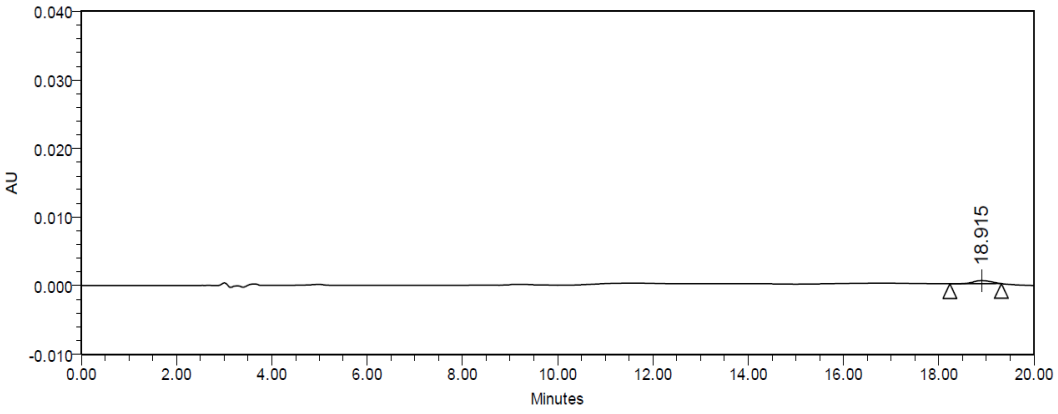

Fig.No.13: Typical Chromatogram for blank

Interference of Placebo:

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

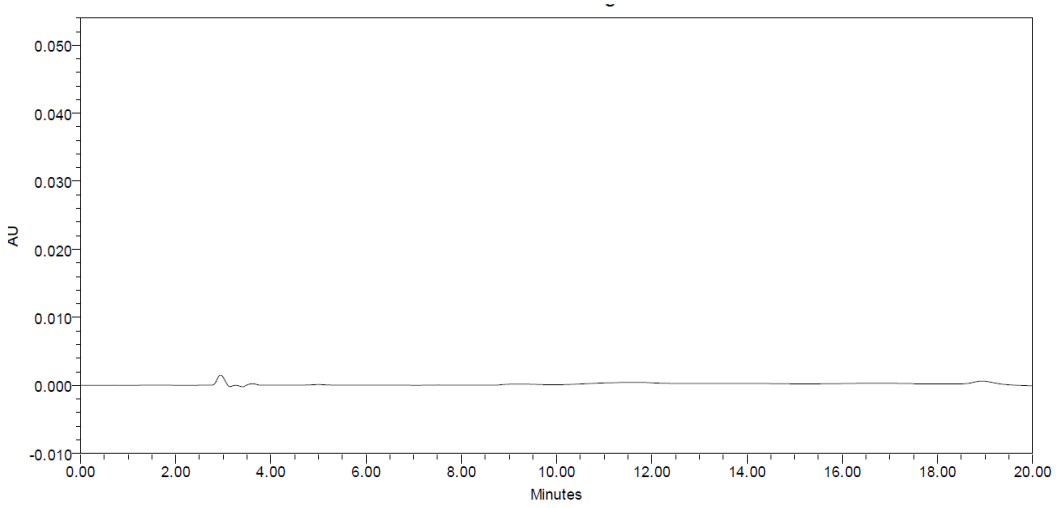

Fig.No.14: Typical Chromatogram for Placebo

INTERMEDIATE PRECISSION/ RUGGUDNESS:

Standard preparation and sample preparations were prepared and injected into the HPLC system by different analyst to record the chromatograms and measured the peak responses for the Moxifloxacin and Fluorometholone Acetate peaks.

Table no.11: Results for Analyst 1 Variability of Moxifloxacin

Test No	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Weight/ml	**1001.1**					
Wt. taken (in mg)	2000.2	2000.2	2000.2	2000.2	2000.2	2000.2
Average Area	7000347	7002835	7001795	7006522	7016254	7000438
% RSD	0.07945					
Assay(mg)	**4.99**	**4.99**	**4.99**	**4.99**	**5.00**	**4.99**
Assay (in %)	**99.8**	**99.8**	**99.8**	**99.8**	**100.0**	**99.8**
Average Assay(mg)		**In mg = 4.99**		**99.8 %**		

Table no.12: Results for Analyst 2 Variability of Moxifloxacin

Test No	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Weight/ml	**1000.2**					
Wt. taken (in mg)	2011.1	2011.1	2011.1	2011.1	2011.1	2011.1
Average Area	7013793	7016543	7020679	7050273	7128718	7001464
% RSD	0.66819					
Assay(mg)	**4.97**	**4.97**	**4.97**	**4.99**	**5.05**	**4.96**
Assay (in %)	**99.4**	**99.4**	**99.4**	**99.8**	**101.0**	**99.2**
Average Assay		**In mg = 4.99**		**99.7%**		

Table no.13: Results for Analyst 1 Variability of Fluorometholone Acetate

Test No	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Weight/ml	**1001.1**					
Wt. taken (in mg)	2000.2	2000.2	2000.2	2000.2	2000.2	2000.2
Average Area	427978	426148	427605	429914	421374	426706
% RSD	0.951					
Assay(mg)	**0.99**	**0.99**	**0.99**	**1.00**	**0.98**	**0.99**
Assay (in %)	**99.8**	**99.0**	**99.4**	**100**	**98.0**	**99.3**
Average Assay		**In mg = 0.99**		**99.0%**		

Table no.14: Results for Analyst 2 Variability of Fluorometholone Acetate

Test No	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Weight/ml	**1000.2**					
Wt. taken (in mg)	2011.1	2011.1	2011.1	2011.1	2011.1	2011.1
Average Area	431819	437646	419935	437764	429470	428869
% RSD	1.583					
Assay(mg)	**1.00**	**1.01**	**0.97**	**1.01**	**0.99**	**0.99**
Assay (in %)	**100.0**	**101.0**	**97.0**	**101.0**	**99.0**	**99.0**
Average Assay		**In mg = 1.00**		**99.5%**		

Different Column:

Standard preparation and sample preparations were prepared and injected into the HPLC system by using different column to record the chromatograms and measured the peak responses for the Moxifloxacin and Fluorometholone Acetate peaks.

Table no.15: Results for Column Variability of Moxifloxacin

Test No	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Weight/ml	**1000.2**					
Wt. taken (in mg)	2011.1	2011.1	2011.1	2011.1	2011.1	2011.1
Average Area	7103347	7065738	7067582	7099553	7004866	7045652
% RSD	0.5166					
Assay(mg)	**5.03**	**5.00**	**5.00**	**5.03**	**4.96**	**4.99**
Assay (in %)	**100.6**	**100.0**	**100.0**	**100.6**	**99.2**	**99.8**
Average Assay		**In mg = 4.99**		**99.9%**		

Table no.16: Results for Column Variability for Fluorometholone Acetate

Test No	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Weight/ml	**1000.2**					
Wt. taken (in mg)	2011.1	2011.1	2011.1	2011.1	2011.1	2011.1
Average Area	437728	419729	429624	423192	429316	415069
% RSD	1.7103					
Assay(mg/)	**1.01**	**0.97**	**0.99**	**0.98**	**0.99**	**0.96**
Assay (in %)	**101.0**	**97.0**	**99.0**	**98.0**	**99.0**	**96.0**
Average Assay		**In mg = 0.98**		**98.3%**		

Different Days:

InterDay Study:

The interday study was performed by applying the proposed method on same sample of solution on different days. The percentage label claim was calculated for the above observations.

Table no.17: Results for InterDay study of Moxifloxacin and Fluorometholone Acetate

Day	**Wt of SPL**	**wt/ml**	**Avg sample area**		**Assay In mg**		**%Label claim**	
			MOXI	**FLM**	**MOXI**	**FLM**	**MOXI**	**FLM**
0	2004.1	1000.2	7100575	424083	5.04	0.98	100.8	98
2	2006.4	1000.2	7110565	427317	5.05	0.99	101	99
4	2012.7	1000.2	7170187	438314	5.07	1.01	101.4	101
						%RSD	0.3022	1.5377

Intraday study:

The intraday study was performed by applying the proposed method on the same sample of the solution on the same day at two hours interval. The percent label claim was calculated from the obtained results

Table no.18: Results for InterDay study of Moxifloxacin and Fluorometholone Acetate

Time(hrs)	**Wt of SPL**	**wt/ml**	**Avg sample area**		**Assay In mg**		**%Label claim**	
			MOXI	**FLM**	**MOXI**	**FLM**	**MOXI**	**FLM**
0	2000.3	1002.5	7001820	424639	5.00	0.99	100	99
2	2000.3	1002.5	7001703	425938	5.00	0.99	100	99
4	2000.3	1002.5	7053212	419145	5.03	0.98	100.6	98
						%RSD	0.3457	0.5851

System variability:

The system variability study was performed by applying the proposed method on same sample of solution on different systems. The percentage label claim was calculated for the below observations.

Table no.19: Results for System Variability Results of Moxifloxacin

Test No	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Weight/ml	**1000.1**					
Wt. taken (in mg)	2007.1	2007.1	2007.1	2007.1	2007.1	2007.1
Average Area	7093032	7019193	7045632	7076280	7051400	7059826
% RSD	0.4166					
Assay(mg)	**5.03**	**4.98**	**5.00**	**5.02**	**5.00**	**5.01**
Assay (in %)	**100.6**	**99.6**	**100.0**	**100.4**	**100.0**	**100.2**
Average Assay		**In mg = 5.01**		**100.1%**		

Table no.20: Results for System Variability Results of Fluorometholone Acetate

Test No	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Weight/ml	**1000.1**					
Wt. taken (in mg)	2007.1	2007.1	2007.1	2007.1	2007.1	2007.1
Average Area	427031	432771	429255	434984	428312	426908
% RSD	0.8103					
Assay(mg)	**0.99**	**1.00**	**0.99**	**1.01**	**0.99**	**0.99**
Assay (in %)	**99.0**	**100.0**	**99.0**	**101.0**	**99.0**	**99.0**
Average Assay		**In mg = 0.99**		**99.5%**		

Table. No.21 Compatibility results of ruggedness for Moxifloxacin

S.No.	Method Precision	Analyst Variability	Different days	Column Variability	System Variability
1	99.8	99.6	100.8	100.6	100.6
2	102.2	99.6	101	100	99.6
3	101.4	99.6	101.4	100	100
4	101.6	99.8	100	100.6	100.4
5	101.4	100.5	100	99.2	100
6	98.6	99.5	100.6	99.8	100.2
Overall Avg.		100.2			
Overall %RSD		0.459			

Table. No 22 Compatibility results of ruggedness for Fluorometholone Acetate

S.No.	Method Precision	Analyst Variability	Different days	Column Variability	System Variability
1	101	99.9	98	101	99
2	101	100	99	97	100
3	100	98.2	101	99	99
4	100	100.5	99	98	101
5	101	98.5	99	99	99
6	99	99.2	98	99	99
Overall Avg.		99.2			
Overall %RSD		0.746			

Acceptance criteria:

The overall % RSD for the above determinations shall be NMT 2.0

***Data interpretation*:**

The overall % assay and overall % RSD for above determinations was found to be within acceptance criteria for the cumulative comparisons with data of method precision and ruggedness study. Hence, method was rugged.

CONCLUSION

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various columns, mobile phase compositions were tried, to separate titled ingredients. Column selection depends upon the structural integrity of the selected compounds. Since MOXI and FLM were having higher carbon to heteroatom ratio can be well separated by both C8 or C18 stationary phases which in turn depends upon their overall hydrophobicity. For optimization of chromatographic conditions and to obtain symmetrical peaks with better resolution and with no peak impurity various stationary phases with different packaging materials(Inertsil ODS C_{18}, Gemini NX C_{18}, Phenomenex C_8 , Hypersil gold BDS C_{18}) were applied. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with Hypersil Gold BDS C_{18} (250 X 4.6 X 5µ) as column and KH_2PO_4 having pH 3.0 buffer and methanol : acetonitrile as mobile phase at gradient flow rate of 1.0 ml min^{-1} was found to be quite robust.

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