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Development and Validation of RP-HPLC Method for Estimation of Salmeterol Xinafoate and Fluticasone in Pharmaceutical Dosage Form

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Abstract:

A simple, specific, accurate stability indicating RP-HPLC method was developed for assay of Salmeterol xinafoate and Fluticasone propionate in Mdi using Hypersil BDS C18 column (15 cm x 4.6 mm, 5 µm) Column and a mobile phase composing of Buffer: Acetonitrile: (35:65) v/v. The flow rate was 2.0 ml/min and the effluent was monitored at 220 nm. The retention time was 3.66 ±0.1 min and 9.225±0.1 minThe method was statistically validated for specificity, accuracy, precision, linearity, robustness and solution stability. Quantitative and recovery studies of the dosage form were also carried out and the % RSD was found to be less than 2. The developed method is simple, rapid and accurate and hence can be used for routine quality control analysis.

Introduction:

The chemical name of **fluticasone propionate** is S-Fluoromethyl 6, 9-difluoro-11ß-hydroxy- 16-methyl-3-oxo-17 -propionyloxy-androsta-1, 4-diene-17ß-carbothioate. It has a molecular formula of C25H31F3O5S and a molecular weight of 500.6.

The chemical name of **salmetrol xinafoate** is 4-Hydroxy'-[6-(4-phenylbutoxy)hexyl]amino]-methyl]-1,3-benzenedimethanol,1-hydroxyl-2-naphthoate. It has a molecular formula of C25H37NO4.C11H8O3 and a molecular weight of 603.8.

Fluticasone propionate is a white to off-white powder. It is freely soluble in dimethyl sulfoxide and dimethylformamide, sparingly soluble in acetone, dichloromethane, ethyl acetate and chloroform, slightly soluble in methanol and 95% ethanol, and practically insoluble in water.

Salmeterol xinafoate is a white to off-white crystalline powder. It is freely soluble in methanol, slightly soluble in ethanol, chloroform, and isopropanol, and sparingly soluble in water.

Literature surveys revealed that sensitive LC-MS methods are available for analysis of ant diabetic drugs and its metabolites in human plasma and urine. Several HPLC methods have been developed individually and combined dosage forms in human plasma. Even though stability indicating methods are developed for individual Anti asthametic drugs. Present study aimed for the easy specific precise and accurate earlier those than reported methods by reverse phase HPLC method. The method was validated according to the ICH (Q2A) guidelines.

Selection of wavelength

10µg/mLsolutionofSalmeterol xinafoate and10µg/mLsolutionFluticasone propionatewasprepared using methanol as solvent. The above mentioned solutions were scannedindividuallyfrom190to400nminUV-Visiblespectrophotometer.Theoptimalresponse for the overlain spectrum ofSalmeterol xinafoate obtained at 225 andFluticasone propionate was obtainedat259nm.

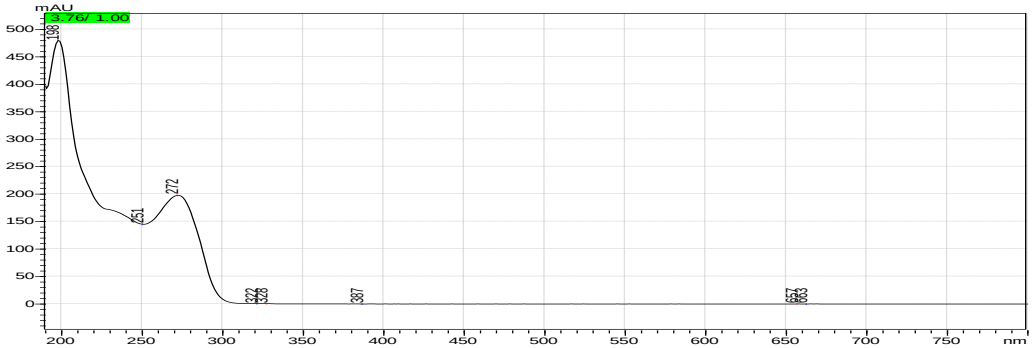

Figure No.1 :UVSpectrumofSalmeterol Xinafoate

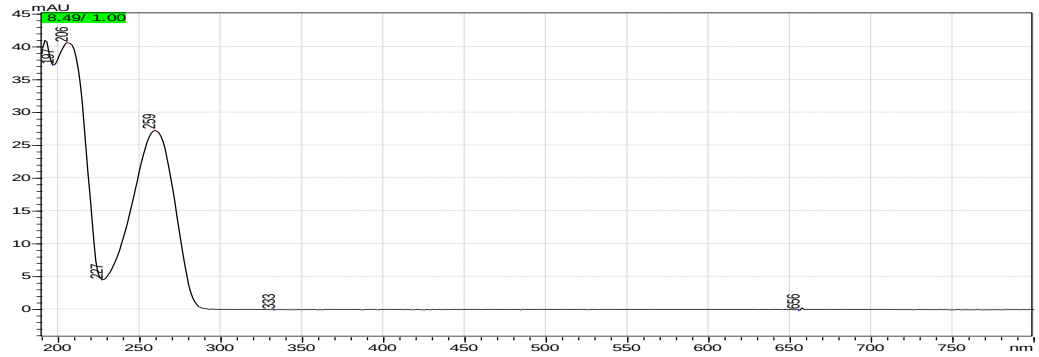

Figure No. 2:UVSpectrumofFluticasone Propionate

Several trials were made to get good peak resolution, acceptable plate count andtailing factor. Method was optimized for the simultaneous estimation of Salmeterol xinafoate andFluticasone propionate pharmaceutical dosageform.

OPTIMIZEDMETHOD

Mobilephase **:**phosphatebuffer(pH2.8):acetonitrile35:65v/v)
Diluent **:**Mobilephasewasusedasdiluent.

Chromatographicconditions

Flowrate **:**1ml per min
Column **:**Hypersil BDS C18 column (15 cm x 4.6 mm, 5 µm).
Detectorwavelength **:** 225nm
Columnoven **:**Ambient
Injectionvolume **:**20 ul
Runtime **:**10 min

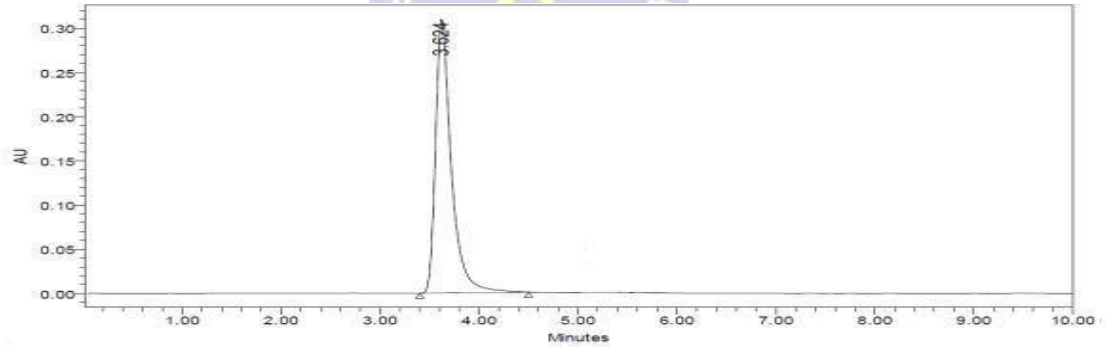

Figure No. 3: StandardChromatogramfor Salmeterol Xinafoate

Table No. 1 : Standard Chromatogramfor Salmeterol Xinafoate

Name	RetentionTime	Area	USP Tailing	USP Platecount
Salmeterol Xinafoate	3.661	3463416	1.5	4890

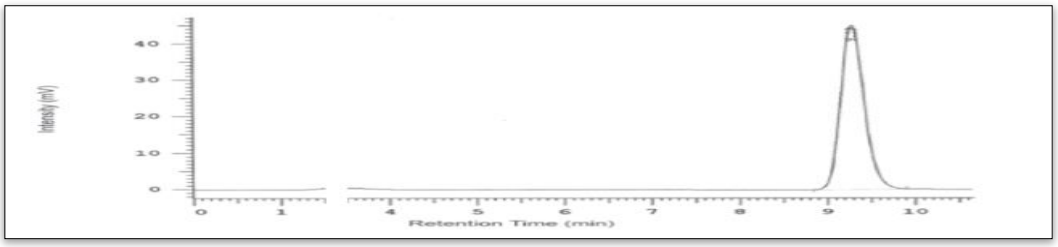

Figure No. 4: StandardChromatogramfor Fluticasone Propionate

Table No. 2: StandardChromatogramfor Fluticasone Propionate

Name	Retention Time	Area	USP Tailing	USP Platecount
Fluticasone Propionate	9.225	3043425	1.2	36568

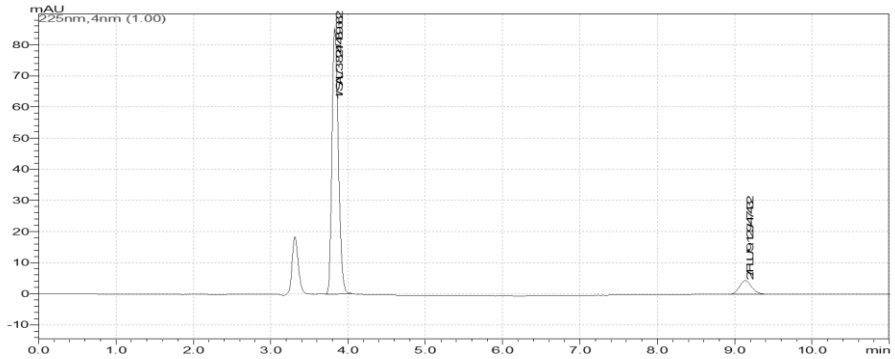

Figure No. 5: Standard Chromatogram for Optimized Method

Table No. 3: Standard Chromatogram for Optimized Method

Name	Retention Time		Area	USP Resolution	USP Tailing	USP Platecount
Salmeterol Xinafoate	3.824		489382		1.5	4890
Fluticasone Propionate	9.129		47432	5.1	1.4	3586

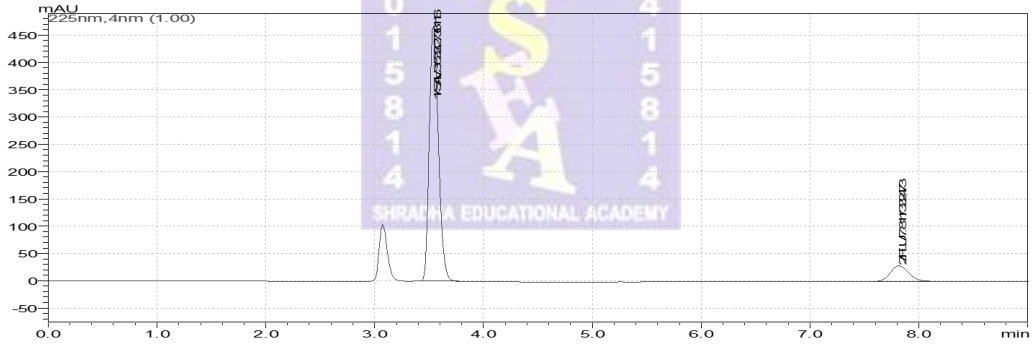

Figure No. 6 :SampleChromatogramforOptimizedMethod

Table No. 4:SampleChromatogramforOptimizedMethod

Name	Retention Time	Area	USP Resolution	USP Tailing	USP Platecount
Salmeterol Xinafoate	3.533	2736116		1.5	4874
Fluticasone Propionate	7.811	332473	5.1	1.4	3579

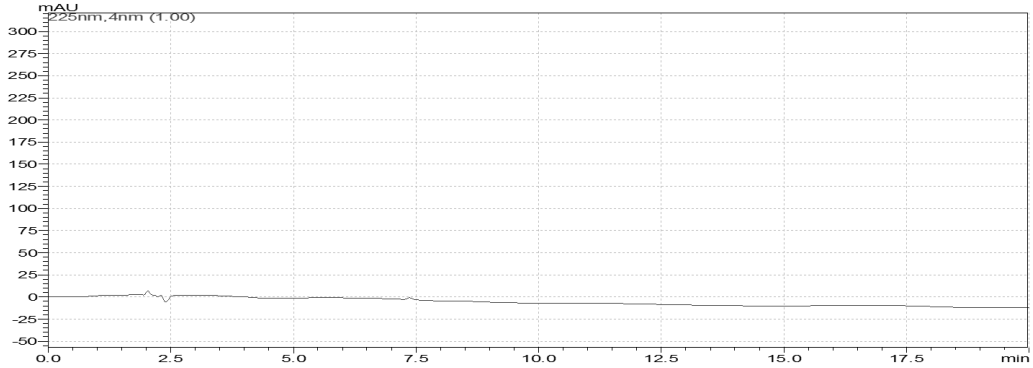

Figure No. 7: BlankChromatogramforOptimizedMethod

Table No. 5: BlankChromatogramforOptimizedMethod

Table No. 04: Results for Standard and Samples

Sr.No.	Nameofthe drug	Concentration	Area	RetentionTime
1.	Salmeterol Xinafoate Standard	100µg/ml	3463416	3.661
2.	Fluticasone Propionate Standard	100µg/ml	3043425	9.225
3.	Salmeterol XinafoateandFluticasone Propionate Standard Solution	100µg/ml	489382and 47432	3.824 and 9.129
4.	Cyplos Powder for *Inhalation*	100µg/ml	2736116 and 332473	3.533 and 7.811

TheretentiontimesforSalmeterol xinafoateand Fluticasone propionate standard solution werefoundtobe3.824 and 9.129respectively.PercentagepurityofSalmeterol xinafoateand Fluticasone propionate foundtobe98.7%w/wand98.8%w/wrespectively.Resolutionbetweentwoanalytesis good.Nopeakasymmetrywasobserved.Nootherimpurityinterferencewasseen.Alltheresultswerefoundtobewithintheacceptancecriteria.Hencethemethodwasconsideredtobe optimized.Resultsaregivenin TableNo. 4.

METHODVALIDATION:

SPECIFICITY:

Thechromatogramsofstandardandsampleareidentical withnearly sameretention time.Nointerferenceduetoplaceboandsampleattheretentiontime of analyte which shows that the method was specific. The chromatogramsfor specificity studies sample are representedasFigure No.17.

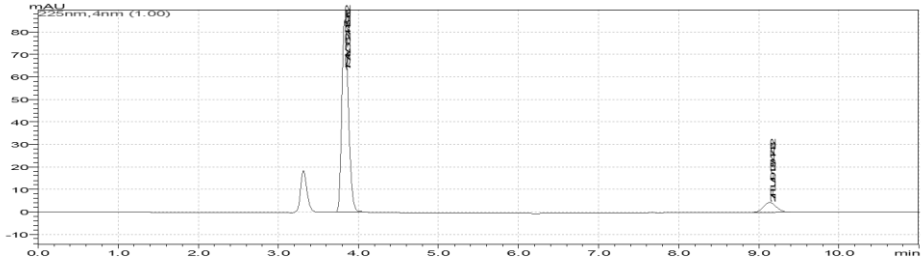

Figure No. 8: Standard Chromatogram for Salmeterol XinafoateandFluticasone Propionate Identification

Figure No. 6: Standard Chromatogram for Salmeterol XinafoateandFluticasone Propionate Identification

Name	Retention Time	Area	USP Resolution	USP Tailing	USP Platecount
Salmeterol Xinafoate	3.824	489382		1.5	4890
Fluticasone Propionate	9.129	47432	5.1	1.4	3586

LINEARITY:

Linearity study was performedin theconcentrationrange of10-50µg / ml. TheChromatograms for the linearity are shown in Figure No. 18. The linearity curve isplotted and shown in Fig.No.10 and 11. The data of linearity is tabulated in TableNo.5and6.

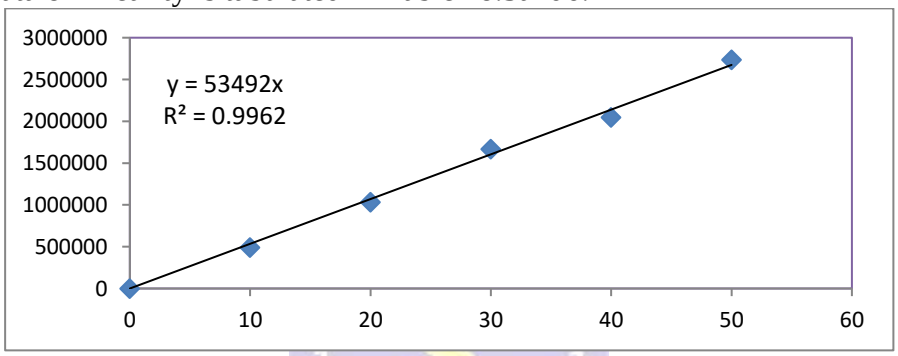

Figure No. 9 :CalibrationCurveofSalmeterol Xinafoate

Table No. 7: LinearityresultsforSalmeterol Xinafoate

Concentration (µg/ml)	Area
10	489382
20	1031852
30	1666491
40	2046833
50	2736116

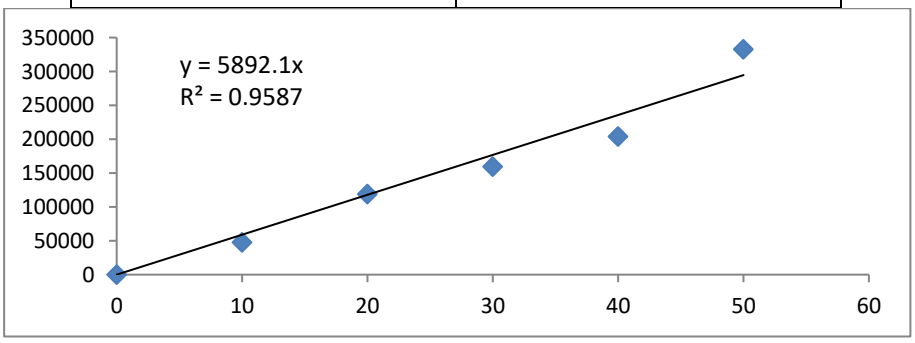

Figure No.10:CalibrationCurveofFluticasone Propionate

Table No. 8: LinearityresultsforFluticasone Propionate	
Concentration (µg/ml)	**Area**
10	47432
20	118971
30	159217
40	203813
50	332473

ACCURACY:

The percentage recoveries of pure drug from the analyzed solution of formulation arecalculatedintherecoveryrangefrom50%to150%.Standardandsamplechromatograms for linearity are shown in Fig.No.11. The summary of accuracyresultsarefurther tabulated.

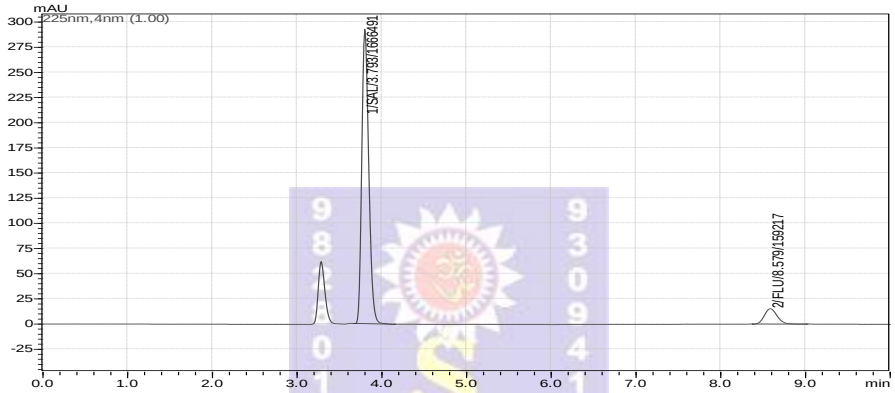

Fig No.11: StandardChromatogramforAccuracy

Table No.9: StandardChromatogramforAccuracy

Name	Retentiontime	Area	USP Resolution	USP Tailing	USP Platecount
SAL	3.793	1666491	91.2791	1.350	7931.765
FLU	8.579	159217	8.7209	1.181	14450.937

TableNo. 10:%RecoveryResultsforSalmeterol Xinafoate

Sample No.	Spike Level	Amount (µg/ml) added	Amount found (µg/ml)	% Recovery	Mean % Recovery
1	50%	5	4.96	99.2%	100.3%
		5	4.99	99.8%	
		5	5.1	102%	
2	100%	10	9.92	99.2%	99.4%
		10	9.94	99.4%	
		10	9.98	99.8%	

3	150%	15.3	15.1	98.6%	99.3%
		15.3	15.2	99.3%	
		15.3	15.3	100%	

TableNo.11:%RecoveryResultsforFluticasone Propionate

Sample No.	Spike Level	Amount (µg/ml) added	Amount found (µg/ml)	% Recovery	Mean % Recovery
1	50%	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100%	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150%	14.8	14.72	99.4%	99.69%
		14.8	14.79	99.9%	
		14.8	14.77	99.79%	

The % recovery for 50%, 100% and 150% accuracy level of Salmeterol xinafoate andFluticasone propionatewasfoundtobewithintherangeof99.3-100.3%and99.13-100%respectively(98.0to102.0%).

PRECISION: TheRSDof%RecoveryforSalmeterol xinafoate andFluticasone propionate chromatogramsofrepeatability precision and intermediate precision is calculated. It passes repeatabilityand intermediate precision.

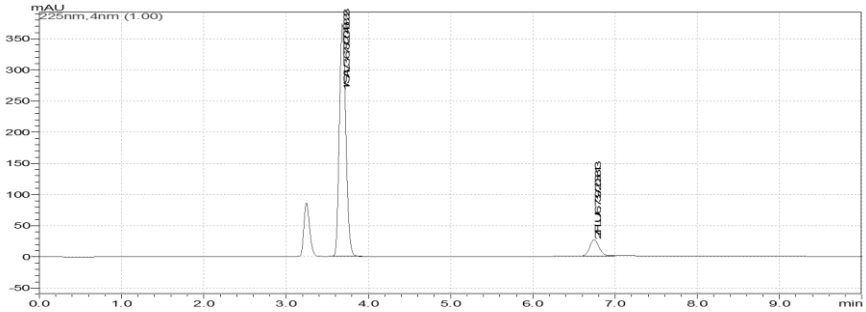

Figure No. 12: StandardChromatogramforPrecision

Table No. 12: StandardChromatogramforPrecision

Name	Retention Time	Area	USP Resolution	USP Tailing	USP Platecount
SAL	3.678	2046833	90.9442	1.252	8000.758
FLU	6.739	203813	9.0558	1.193	14609.097

LIMITOFDETECTION(LOD):

The limit of detection was calculated from the linearity curve method using slope, andstandard deviation ofintercepts ofcalibration curve. Limit ofDetection was found tobe 0.003µg/ml for Salmeterol xinafoate and 0.09 µg/ml for Fluticasone propionate.

CalculationofS/NRatioforSalmeterol Xinafoate:

Averagebaselinenoiseobtainedfromblank: 52µV

SignalobtainedfromLOD solution(0.25%oftargetassayconcentration):154µVS/N=154/52=2.96

CalculationofS/NRatioforFluticasone Propionate:

Averagebaselinenoiseobtainedfromblank: 52µV

SignalobtainedfromLOD solution(0.3%oftarget assayconcentration:155 µVS/N=155/52=2.98

Limitofdetectionwasfoundtobe2.96for Salmeterol Xinafoate and2.98for Fluticasone Propionate.

LIMITOFQUANTIFICATION(LOQ):

Thelimitofquantificationwascalculatedfromthelinearitycurvemethodusingslope, and standard deviation of intercepts of calibration curve.

CalculationofS/NRatioforSalmeterol Xinafoate:

Averagebaselinenoiseobtainedfromblank: 52 µV

SignalobtainedfromLOQ solution(1%oftarget assayconcentration 522µVS/N=522/52=10.

CalculationofS/NRatioforFluticasone Propionate:

AverageBaselineNoiseobtainedfrom Blank:52µV

SignalObtainedfromLOQ solution(1.0%oftarget assayconcentration): 519µVS/N=519/52=9.98

Limitofquantificationwasfoundtobe10forSalmeterol Xinafoate and9.98forFluticasone Propionate.

ROBUSTNESS:

Table No. 13: RobustnessResultsforSalmeterol Xinafoate

Sr.No.	Flow Rate(ml/min)	SystemSuitabilityResults	
		USPPlatecount	SPTailing
1	0.4	4859	1.62
2	0.5	4890	1.58
3	0.6	4895	1.58

*Resultsforactualflow(0.5ml/min)havebeenconsideredfromassaystandard

Table No. 14: RobustnessResultsforFluticasone Propionate

Sr.No.	Flow Rate(ml/min)	SystemSuitabilityResults	
		USPPlatecount	SPTailing
1	0.5	3330.4	1.52
2	0.7	3437.6	1.47
3	0.9	3228.7	1.47

*Resultsforactualflow(0.5ml/min)havebeenconsideredfromassaystandard.

The%RSDofretentiontimeandasymmetrywerewithinlimitsforvariationin Flowrate(±0.1ml).

Table No. 15:RobustnessResultsforSalmeterol Xinafoate			
Sr.No.	ChangeinOrganic Composition in TheMobile Phase	SystemSuitabilityResults	
		USP Platecount	USPTailing
1	10%less	4899	1.52
2	*Actual	4857	1.52
3	10%more	4879	1.61

Table No. 16:RobustnessResultsforFluticasone Propionate			
Sr.No.	ChangeinOrganic Composition in TheMobile Phase	SystemSuitabilityResults	
		USP Platecount	USPTailing
1	10%less	3887	1.42
2	*Actual	3437	1.42
3	10%more	3985	1.51

*****Resultsforactualmobilephasecomposition(65:35acetonitrile:phosphatebuffer)hasbeenconsideredfromaccuracystandard.
The%RSDofretentiontimeandasymmetrywerewithinlimitsforvariation(±2%)incompositionofmobilephase.Hence themethodwasfoundtoberobust.
SYSTEMSUITABILITY: Fromthesystemsuitabilitystudiesitwasobservedthat %RSDofretentiontimewasfoundtobe0.2,%RSDofpeakareawasfoundtobe 0.2.Alltheparameterswerewithinthelimit.Theresults ofsystemsuitabilitystudies aretabulated.

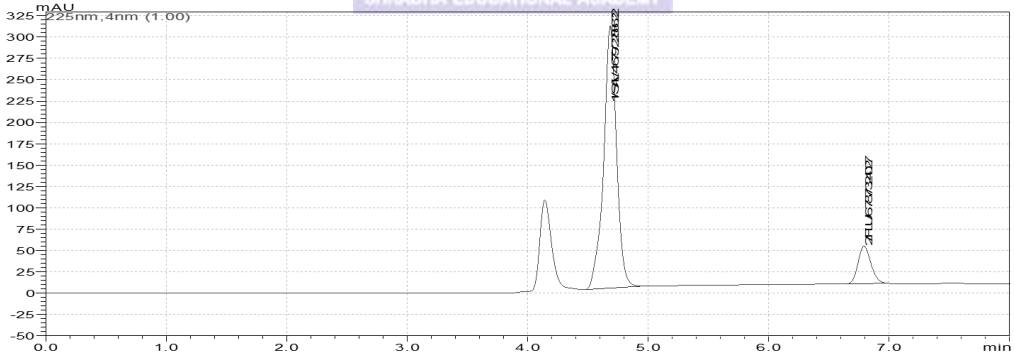

Figure No. 13: StandardChromatogramforSystem suitability

Table No. 17: StandardChromatogramforSystem suitability

Name	Retention Time	Area	USP Resolution	USP Tailing	USP Platecount
SAL	4.675	2208291	87.2155	1.119	8250.910
FLU	6.563	323703	12.7845	1.223	13383.445

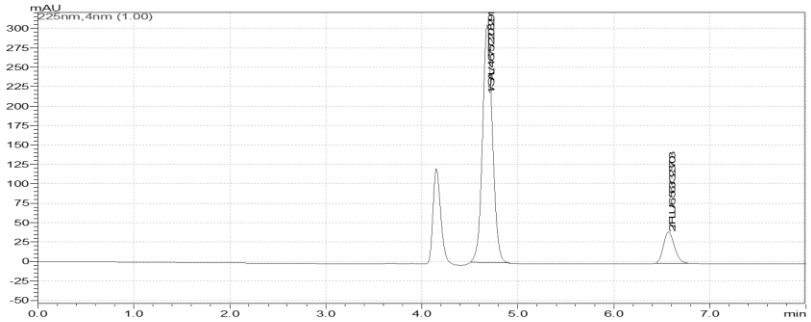

Figure No. 14: SampleChromatogramforSystem suitability

Name	Retentiontime	Area	USP Resolution	USP Tailing	USP Platecount
SAL	4.679	2288622	87.5978	0.978	7998.670
FLU	6.787	324027	12.4022	1.239	16419.708

Table No. 18 :Summary Of Results Of Method Validation For Salmeterol Xinafoate And Fluticasone Propionate

Sr. No.	Parameter	Requirement	Results		Acceptancecriteria
			SAL	FLU	
1.	Specificity	Nointerference	Pass	Pass	Nointerference
2.	Linearity	Correlationcoefficient	0.9998	0.9997	NLT0.999
3.	Accuracy	50% recovery	100.3%	100%	100± 2.0%
		100% recovery	99.4%	99.13%	
		150% recovery	99.3%	99.69%	
4.	Precision (repeatability)	%RSD	0.42	0.36	NMT2%
5.	Intermediate precision	%RSD	0.03	0.89	NMT1%
6.	Robustness	%RSD	0.43	0.36	NMT1%
7.	Systemsuitability	RT	4.679	6.787	-
8.		Tailingfactor	1.6	1.4	NMT2
9.		Platecount	4859	3330	NLT3000
10.		Assayvalue	98.7%	98.8%	100± 2.0%

Conclusion:

An reversed phase high performance liquid chromatographic method was developed and validated for the determination of Salmeterol and Fluticasone in pharmaceutical dosage form as a single component. This chromatographic assay fulfilled all the requirements needed for it to be

identified as a reliable and feasible method, including accuracy, recovery and precision data. It is highly accurate, precise and selective. The analytical procedure and its chromatographic run time is less than 10 min. Therefore, the HPLC method can be used as a routine sample analysis for stability study purposes

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