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Method Development and Validation for the Determination of Fluticasone and Salmeterol in API and Combined Tablet Dosage Form by RP-HPLC

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ABSTRACT

Analytical Method Development and Validation forSalmeterol and Fluticasone in bulk and Combined Dosage Form by RP-HPLC, New method was established for simultaneous estimation ofSalmeterol and Fluticasone by RP-HPLC method. The chromatographic conditions were successfully developed for the separation ofSalmeterol and Fluticasone by using Phenomenex Luna C18 (4.6mm×250mm, 5µm) particle size, flow rate was 1.0 ml/min, mobile phase ratio was (40:60 v/v) Acetonitrile: TEA buffer pH-4.2 (pH was adjusted with orthophosphoric acid), detection wavelength was 220nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detectors. The retention times were found to be 2.246mins and 5.461mins respectively. The % purity ofSalmeterol and Fluticasone was found to be 101.27% and 99.76% respectively. The system suitability parameters forSalmeterol and Fluticasone such as theoretical plates and tailing factor were found to be 5387, 0.97 and 5398 and 1.26, the resolution was found to be 2.97. The linearity study nSalmeterol and Fluticasone was found in concentration range of 30µg-70µg and 60µg-140µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.14% and 100.56%, %RSD for repeatability was 0.1 and 0.5, % RSD for intermediate precision was 0.1 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 0.56 and 1.2, and LOQ value was 1.7 and 3.6 respectively. Hence the suggested RP-HPLC method can be used for routine analysis ofSalmeterol and Fluticasone in API and Pharmaceutical dosage form.

Keywords: Salmeterol and Fluticasone, Method Development, Validation, Accuracy.

INTRODUCTION

Pharmaceutical analysis comprises those procedures necessary to determine "identity, strength, quality and purity of the drug substances and drug products. Pharmaceutical analyst plays a major role in all quality controlling divisions of industry. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. The number of new drugs is constantly growing. This requires new methods for controlling the quality. Modern pharmaceutical analysis must need the following requirements [1].

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of the Pharmacopoeia.
3. The analysis should be performed with a minimal cost.
4. Precision and selectivity of the selected method should be good.

Typical Instrumental Techniques [2, 3]

The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones of them, physical and physicochemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of

turbidity, colour density or specific gravity (for liquids), moisture content, melting, freezing and boiling points. Physicochemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physicochemical methods are optical refractometry, polarimetry, emission and fluorescent methods of analysis, photometry including photocolorimetry, spectrophotometry, nephelometry and turbidometry, electrochemical (potentiometry, amperometry, coulometer, polarography) and chromatography (column, paper, thin layer, gas, high performance liquid) methods are generally preferable.

Methods involving nuclear reactions such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming more popular. The combination of mass spectroscopy with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures, which are based on complex formation, acid-base and precipitation and redox reactions. Titrations in non-aqueous media and complexometry have been widely used in pharmaceutical analysis whenever the existing amounts are in milligram level and the interference is negligible. The methods (LC-MS,[4] HPLC, GLC, NMR and Mass Spectroscopy) of choice for assay involve sophisticated equipment that are very costly and pose problems of maintenance. Hence, they are not in the reach of most laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations.

The visible Spectrophotometric methods which fall in the wavelength region 400-800 nm and fluorimetric methods (may fall in UV & Visible regions) are very simple, cheap and easy to carry out estimations of drugs in bulk form and their formulations. The limitations of many colorimetric or fluorimetric methods of analysis lie in the chemical reactions upon which the procedures are based rather than the instruments available. Many of the reactions involve colour or fluorescence of a drug are quite selective or can be rendered selective through the introduction of masking agents, control of PH, use of solvent extraction technique, adjustment of oxidation states or by prior removal of interfering ingredients with the aid of chromatographic separation.

1. This is preferably followed by general methodology for UV-Visible and HPLC method developments.
2. Followed by literature of drugs used in Analysis

Instrumentation

The essential parts of the High Performance Liquid Chromatography are:

1) Solvent reservoir and Treatment system
2) Mobile phase
3) Pump system
4) Sample Injection System
5) Column
6) Detector

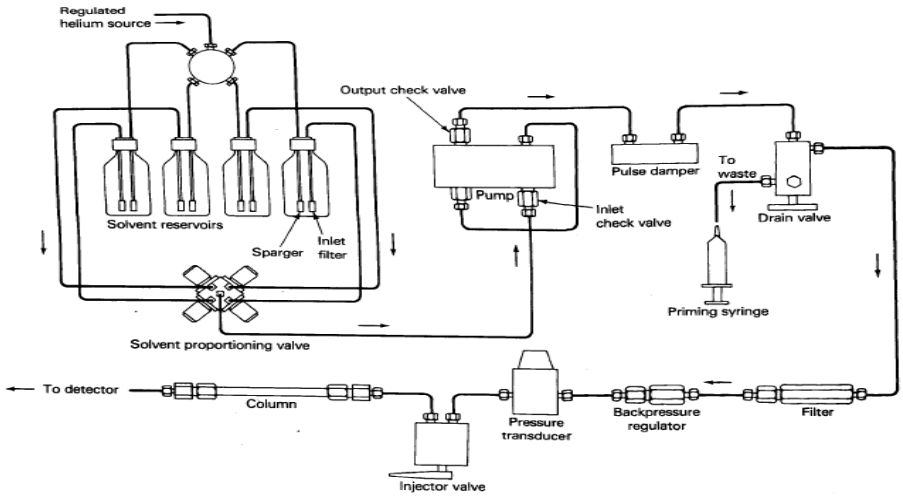

Fig 1: Typical diagram of HPLC

The primary objective of proposed work is

- To develop new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Fluticasone and Salmeterol.
- To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Fluticasone and Salmeterol dosage form.

MATERIALS AND METHODS

Table 1: Drug details

S. No	Drug name	Formulation	Manufacturer	Procurement
1	Salmeterol	_	_	Sura labs
2	Fluticasone	_	_	Sura labs

Table 2: Instruments and Equipments

S. No	Instruments	Software	Model	Company
1	HPLC	Empower 2	Alliance 2695 separation module. 996 PDA detector.	Waters
2	Weighing Balance	N/A	XEX 200	Sartorius
3	Sonicator	N/A	SE60US	Labman

Table 3: Chemicals and Reagents

S. No	Chemical	Brand names
1	Water for HPLC	LICHROSOLV (MERCK)
2	Methanol for HPLC	LICHROSOLV (MERCK
3	Acetonitrile for HPLC	Merck

METHOD DEVELOPMENT

Selection of initial conditions for method development

A. **Determination of solubility of drug**

Table 4: Solubility of Fluticasoneand Salmeterol

Solvent		Salmeterol
Water	Slightly Soluble	Freely soluble
Methanol	Soluble	Soluble
Acetonitrile	Soluble	Soluble

B. **Selection of chromatographic methods:**

The proper selection depends upon the nature of the sample, (ionic or ion stable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography was selected.

C. **Optimization of Column:**

The method was performed with various columns like HypersilC_{18} column, X- bridge column and Symmetry C_{18} (4.6 x 150mm, 5µm), X-terra (4.6 ×150mm, 5µm particle size) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

D. **Mobile Phase Optimization**:

Initially the mobile phase tried was Water: Methanol and Water: Acetonitrile with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetonitrile: Water in proportion (50:35:15%v/v) respectively.

Estimation ofFluticasoneand Salmeterol in pharmaceutical dosage form

Procedure

Preparation of mobile phase: Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation:

Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: TEA Buffer (pH-4.2) (40:60v/v)
Column : Phenomenex Luna C18 (4.6mm×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 220 nm
Column temp : Ambient
Injection Volume : 20 µl
Run time : 10 minutes

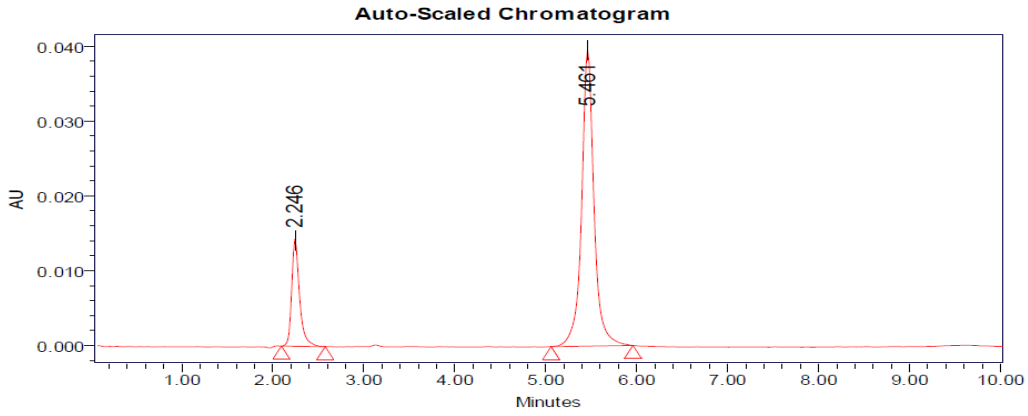

Fig 2: Optimized Chromatogram

Table 5: Peak Results for Optimized Chromatogram

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Salmeterol	2.246	765789	69584		0.97	5587.0
2	Fluticasone	5.461	2532158	190049	2.97	1.26	5398.0

From the above chromatogram it was observed that theSalmeterol and Fluticasone peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

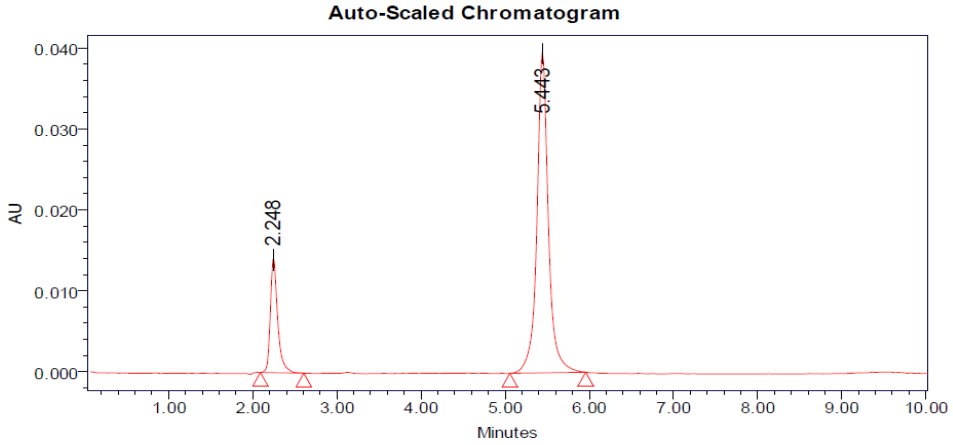

Fig 3: Optimized Chromatogram (Sample)

Table 6: Optimized Chromatogram (Sample)

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Salmeterol	2.248	775684	13124		0.99	6365.0
2	Fluticasone	5.443	2658478	937405	5.06	1.23	7458.0

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.

VALIDATION

System suitability

Table 7: Results of system suitability forSalmeterol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Salmeterol	2.247	765843	69587	5589	1.9
2	Salmeterol	2.246	766594	69854	5576	1.6
3	Salmeterol	2.248	765487	70211	5658	1.6
4	Salmeterol	2.252	765928	69213	5642	1.7
5	Salmeterol	2.248	765426	69558	5685	1.6
Mean			765855.6			
Std. Dev			466.6522			
% RSD			0.060932			

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 8: Results of system suitability for Fluticasone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Fluticasone	5.452	2534658	190058	5365	1.2	2.07
2	Fluticasone	5.484	2536854	190052	5348	1.4	2.05
3	Fluticasone	5.491	2535879	190078	5389	1.5	2.0
4	Fluticasone	5.482	2533564	190035	5347	1.6	2.01

5	Fluticasone	5.491	2534214	190085	5364	1.6	2.01
Mean			2535034				
Std. Dev			1183.309				
% RSD			0.046678				

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

SPECIFICITY

Table 9: Peak results for assay standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Salmeterol	2.256	759868	71255		1.7	5689	1
2	Fluticasone	5.427	2458754	215654	2.04	1.6	5362	1
3	Salmeterol	2.249	759458	72541		1.7	5748	2
4	Fluticasone	5.430	2465885	226565	2.00	1.6	5452	2
5	Salmeterol	2.248	759245	72584		1.7	5584	3
6	Fluticasone	5.443	2489578	221542	2.04	1.6	5456	3

Table 10: Peak results for Assay sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Salmeterol	2.247	756985	68958		0.98	7253	1
2	Fluticasone	5.452	2569856	198564	2.06	1.23	8836	1
3	Salmeterol	2.246	758745	69857		1.05	6530	2
4	Fluticasone	5.461	2598654	195682	2.04	0.99	7270	2
5	Salmeterol	2.243	756848	69588		1.7	7586	3
6	Fluticasone	5.466	2587454	192541	2.04	1.6	8371	3

$$\%\text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity ofSalmeterol and Fluticasone in pharmaceutical dosage form was found to be 99.76 %.

LINEARITY

Salmetero

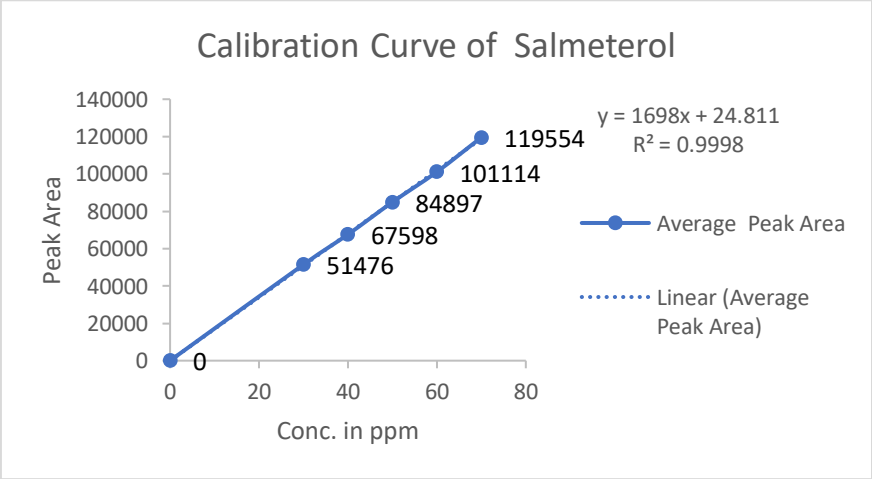

Fig 4: Calibration graph forSalmeterol

Correlation Coefficient (r) is 0.99, and the intercept is 24.811. These values meet the validation criteria.

Fluticasone

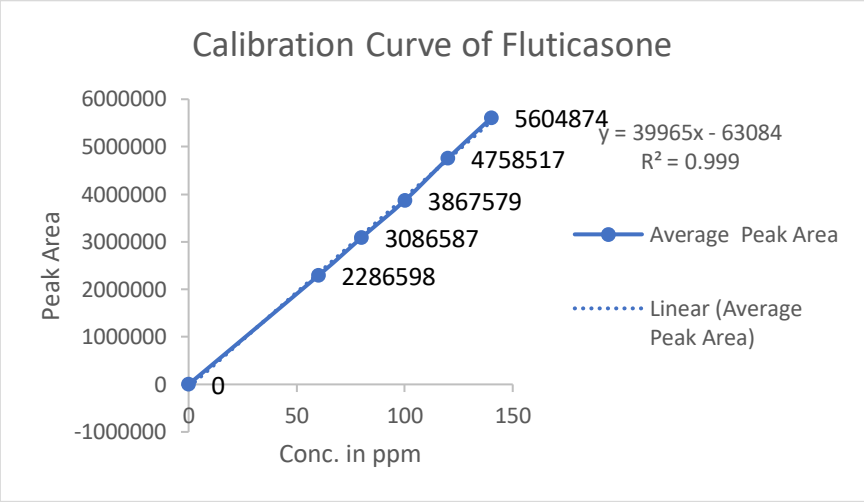

Fig 5: Calibration Graph for Fluticasone

Correlation Coefficient (r) is 0.99, and the intercept is 63084. These values meet the validation criteria.

Precision

REPEATABILITY

Table 11: Results of Repeatability for Salmeterol:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Salmeterol	2.269	766854	702564	5685	1.6
2	Salmeterol	2.255	765884	698789	5584	1.4
3	Salmeterol	2.252	765842	701235	5521	1.6
4	Salmeterol	2.267	768985	700124	5525	1.9
5	Salmeterol	2.260	765845	698986	5578	1.7
Mean			766682			
Std. Dev			1357.973			
% RSD			0.177123			

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 12: Results of method precision for Fluticasone:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Fluticasone	5.274	2569865	2231111	5365	1.6
2	Fluticasone	5.266	2578474	2674210	5425	1.6
3	Fluticasone	5.265	2568985	2231261	5368	1.5
4	Fluticasone	5.278	2586845	2421301	5359	1.5
5	Fluticasone	5.305	2545898	2324710	5498	1.6
Mean			2570013			
Std. Dev			15309.45			
% RSD			0.595695			

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 13: Results of Intermediate precision Day 1for Salmeterol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Salmeterol	2.248	758955	68986	5785	1.6
2	Salmeterol	2.245	759869	68957	5698	1.4

3	Salmeterol	2.242	758985	68545	5689	1.6
4	Salmeterol	2.239	756894	68952	5781	1.9
5	Salmeterol	2.243	759854	68595	5785	1.7
6	Salmeterol	2.246	756985	68952	5693	1.6
Mean			758590.3			
Std. Dev			1339.793			
% RSD			0.176616			

- %RSD of Six different sample solutions should not more than 2.

Table 14: Results of Intermediate precision Day 1for Fluticasone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Fluticasone	5.284	2659852	190025	5485	1.5	2.04
2	Fluticasone	5.293	2648574	190048	5421	1.6	2.03
3	Fluticasone	5.306	2659865	190054	5468	1.6	2.01
4	Fluticasone	5.319	2658547	190078	5487	1.6	2.05
5	Fluticasone	5.346	2648981	190016	5492	1.6	2.02
6	Fluticasone	5.352	2654652	190057	5463	1.6	2.03
Mean			2655079				
Std. Dev			5242.086				
% RSD			0.197436				

- %RSD of Six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Table 15: Results of Intermediate precision Day 2 forSalmeterol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Salmeterol	2.255	766895	69858	5586	1.5
2	Salmeterol	2.260	765988	69854	5636	1.6
3	Salmeterol	2.242	766532	69824	5432	1.6
4	Salmeterol	2.245	766214	69875	5468	1.6
5	Salmeterol	2.260	765897	69854	5546	1.9
6	Salmeterol	2.255	765245	69848	5507	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

- %RSD of Six different sample solutions should not more than 2.

Table 16: Results of Intermediate precision for Fluticasone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Fluticasone	5.266	2653254	190110	5428	1.6	7.98
2	Fluticasone	5.265	2648985	190058	5452	1.6	6.4
3	Fluticasone	5.306	2658213	190142	5498	1.6	8.9
4	Fluticasone	5.293	2653652	190031	5442	1.5	8.3
5	Fluticasone	5.265	2648978	190058	5489	1.5	7.5
6	Fluticasone	5.266	2658985	190047	5463	1.6	5.3
Mean			2653678				
Std. Dev			4313.355				
% RSD			0.162543				

ACCURACY

Table 17: The accuracy results forSalmeterol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	% Mean Recovery
50%	42594.67	25	25.070	100.280%	100.14%
100%	84867	50	49.965	99.930%	

150%	127654	75	75.164	100.218%	

Table 18: The accuracy results for Fluticasone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	% Mean Recovery
50%	2079124	50	50.445	100.890%	100.56%
100%	4082412	100	100.571	100.571%	
150%	6070195	150	150.309	100.206%	

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Salmeterol:

0.56µg/ml

Fluticasone:

1.7µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Salmeterol:

1.2µg/ml

Fluticasone:

3.6µg/ml

Robustness

Table 19: Results for Robustness of Salmeterol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765789	2.246	5387.0	0.97
Less Flow rate of 0.9 mL/min	758698	2.505	5458	0.96
More Flow rate of 1.1 mL/min	7689584	2.046	5696	0.94
Less organic phase	758412	2.505	5586	0.92
More organic phase	769852	2.046	5355	0.95

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table 20: Results for Robustness of Fluticasone

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532158	5.461	5398	1.26
Less Flow rate of 0.9 mL/min	2458692	5.599	5329	1.25
More Flow rate of 1.1 mL/min	2658642	4.576	5256	1.24
Less organic phase	2452148	5.599	5214	1.23
More organic phase	2653894	4.576	5524	1.22

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation ofSalmeteroland Fulticasone in bulk drug and pharmaceutical dosage forms. Salmeterolwas found to bepractically insoluble in water, slightly soluble in methanol, chloroform, dichloromethane, ethanol, toluene, benzene, 2-propanol, ethyl acetate, methanol and acetone,DMSO and dimethyl formamide. Fulticasonewas found to be practically insoluble in water, in ether and in chloroform; soluble in methanol; slightly soluble in alcohol. Acetonitrile: TEA Buffer (pH-4.2) (40:60v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for

RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination ofSalmeteroland Fulticasone in bulk drug and in Pharmaceutical dosage forms.

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