ISSN: 2320-2831

International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.13 | Issue 1 | Jan - Mar -2024

www.ijpar.com

DOI : https://doi.org/10.61096/ijpar.v13.iss1.2024.12-22

Research

Development And Validation For The Simultaneous Estimation Of Fluticasone And Vilanterol In Bulk Form And In Its Pharmaceutical Dosage Form By Using RP-HPLC Method

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Published on: 03 Jan 2024

Published by:
DrSriram Publications

Abstract

A new, simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Fluticasone and Vilanterol in its pure form as well as in combined marketed formulation. Chromatography was carried out on a Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size column using a mixture of Methanol: Phosphate Buffer (pH-4.2) (37:63% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 275nm. The retention time of the Fluticasone and Vilanterol was found to be was 2.133, 3.692 ± 0.02min respectively. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Fluticasone and 10-30mg/ml of Vilanterol. The inter-day and intra-day precisions were found to be within limits. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Fluticasone and Vilanterol, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure. Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic

acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.[1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance.[2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

Different methods of analysis

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics. E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry). Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc.

HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance.

Instrumentation of HPLC

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

Mobile phase (solvent) reservoirs and solvent degassing

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity.

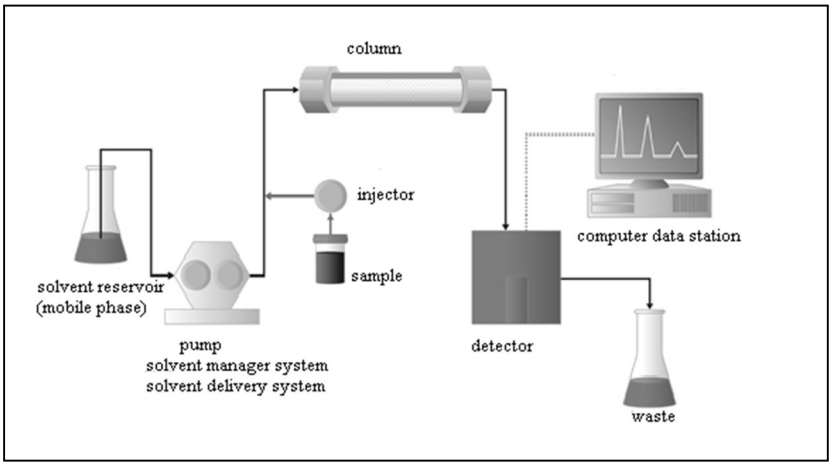

Fig 1: Components of HPLC instrument block diagram.

Mobile phase preparation

Mobile phases must be prepared from high purity solvents, including water that must be highly purified.

Mobile phases must be filtered through ≤1 µm pore size filters and be degassed before use.

Degassing of solvents

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

1. By warming the solvents
2. By stirring vigorously with a magnetic stirrer
3. By subjecting to vaccum filtration
4. By ultra sonication (using ultrasonicator)
5. By bubbling He gas through the solvent reservoir.

MATERIALS AND METHODS

Fluticasone -Sura labs, Vilanterol-Sura labs, Water and Methanol for HPLC- LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Potassium Dihydrogen Phosphate- Merck.

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Fluticasone and Vilanterol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.4ml of Fluticasone and 0.2ml of Vilanterol from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water, Methanol: Phosphate buffer and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v) in proportion 37:63 v/v respectively.

Optimization of Column

The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used	:	Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	:	35ºC
Column	:	Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Mobile phase	:	Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v)
Flow rate	:	1ml/min
Wavelength	:	245nm
Injection volume	:	10µl
Run time	:	6minutes

Method validation

Preparation of buffer and mobile phase

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.2)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.2 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of Mobile Phase

Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v)
Column : Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Column temperature : 35ºC
Wavelength : 245nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

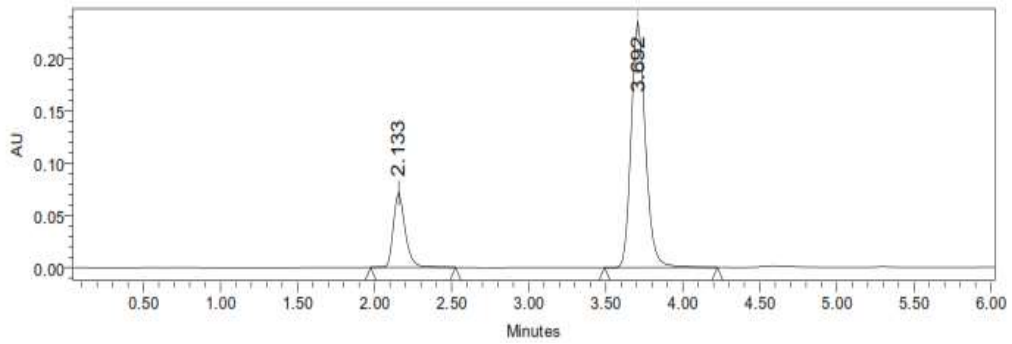

Fig 2: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Fluticasone	2.133	648367	84556	2.67	5499	9.4
2	Vilanterol	3.692	3899287	478673	2.88	6463	9.8

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

Optimized Chromatogram

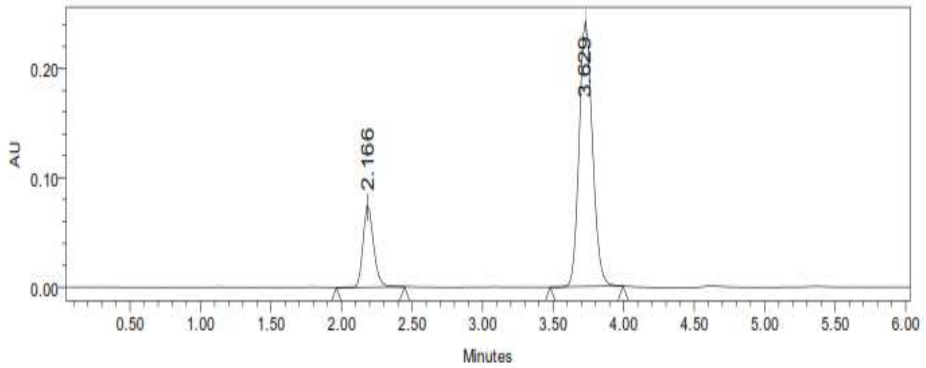

Fig 3: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Fluticasone	2.166	536587	77464	1.57	5789	9.8
2	Vilanterol	3.629	1695846	378564	1.80	8795	10.01

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, It was found from above data that all the system suitability parameters for developed method were within the limit.

System Suitability

Table 3: Results of system suitability for Fluticasone

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Fluticasone	2.152	537449	87699	6796	1.67
2	Fluticasone	2.157	537659	87365	6763	1.68
3	Fluticasone	2.141	537865	87699	6738	1.67
4	Fluticasone	2.133	537699	87356	6792	1.68
5	Fluticasone	2.166	535985	87632	6752	1.67
Mean			537357.5			
Std. Dev.			798.444			
% RSD			0.149617			

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

Table 4: Results of system suitability for Vilanterol

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Vilanterol	3.674	1793932	1797969	9769	1.67	9.8
2	Vilanterol	3.631	1793837	1796856	9786	1.68	9.9
3	Vilanterol	3.625	1798472	1796532	9793	1.56	9.8
4	Vilanterol	3.692	1793921	1796353	9753	1.68	9.8
5	Vilanterol	3.629	1794927	1796475	9746	1.69	9.8
Mean			1683907				
Std. Dev.			1982.03				
% RSD			0.117704				

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

Assay (Standard)

Table 5: Peak results for assay standard of Fluticasone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Fluticasone	2.152	637469	97699	1.77	6799	1
2	Fluticasone	2.198	637695	67895	1.68	6798	2
3	Fluticasone	2.179	639769	97364	1.67	6749	3

Table 6: Peak results for assay standard of Vilanterol

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Vilanterol	3.646	2798699	476989	1.91	9769	1
2	Vilanterol	3.604	2796998	476965	1.89	9798	2
3	Vilanterol	3.610	2896985	479965	1.91	9786	3

Assay (Sample)

Table 7: Peak results for Assay sample of Fluticasone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Fluticasone	2.152	647969	98673	1.69	6891	1
2	Fluticasone	2.150	643765	98776	1.69	6893	2
3	Fluticasone	2.187	643796	98576	1.69	6871	3

Table 8: Peak results for Assay sample of Vilanterol

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Vilanterol	3.646	2799679	489673	1.92	9869	1
2	Vilanterol	3.651	2799683	486958	1.90	9896	2
3	Vilanterol	3.601	2799658	487695	1.92	9856	3

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

= 99.89%

The % purity of Fluticasone and Vilanterol in pharmaceutical dosage form was found to be 99.89%

Linearity

Chromatographic data for linearity study of fluticasone

Table 9: Chromatographic Data for Linearity Study of Fluticasone

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

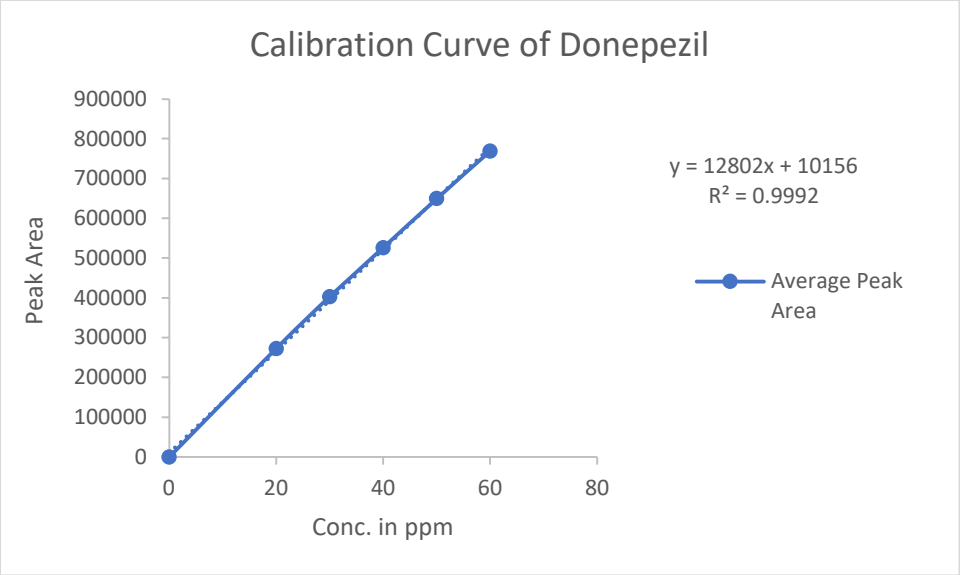

Fig 4: Calibration Curve of Fluticasone repeatability

Chromatographic data for linearity study of vilanterol

Table 10: Chromatographic Data for Linearity Study of Vilanterol

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

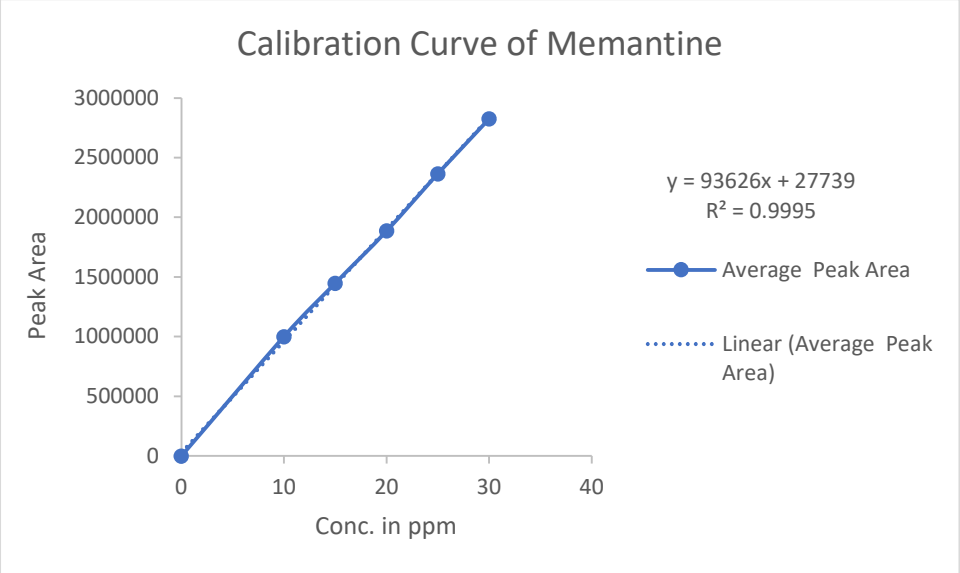

Fig 5: Calibration Curve of Vilanterol

Repeatability

Table 11: Results of repeatability for Fluticasone

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Fluticasone	2.157	526358	86598	5689	1.56
2	Fluticasone	2.159	524856	86542	5687	1.57
3	Fluticasone	2.186	526985	86578	5684	1.56
4	Fluticasone	2.160	528654	86354	5689	1.56
5	Fluticasone	2.170	528457	86958	5639	1.56
Mean			527062			
Std.dev			1569.114			
%RSD			0.297709			

%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 Repeatability for Vilanterol

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vilanterol	3.603	1687589	367859	8659	1.79
2	Vilanterol	3.608	1685987	368547	8679	1.80
3	Vilanterol	3.600	1685987	367985	8645	1.80
4	Vilanterol	3.696	1685754	365874	8695	1.79
5	Vilanterol	3.629	1685985	364589	8625	1.79
Mean			1686260			
Std.Dev			749.493			
%RSD			0.044447			

Intermediate precision

Table 13: Results of Intermediate precision Day 2 for Fluticasone

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Fluticasone	2.198	536854	8758	5789	1.58
2	Fluticasone	2.196	536985	8795	5726	1.59
3	Fluticasone	2.178	536587	8746	5742	1.58
4	Fluticasone	2.142	532546	8754	5746	1.59
5	Fluticasone	2.177	534587	8725	5798	1.58
6	Fluticasone	2.177	538598	8726	5785	1.59
Mean			536026.2			
Std. Dev.			2131.492			
% RSD			0.397647			

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

Table 14: Results of Intermediate precision Day 2 for Vilanterol

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Vilanterol	3.611	1678598	356875	8875	1.82	9.9
2	Vilanterol	3.623	1678985	358985	8856	1.83	10.01
3	Vilanterol	3.684	1678984	358754	8862	1.82	9.9

4	Vilanterol	3.697	1678985	352412	8849	1.83	10.01
5	Vilanterol	3.684	1678549	358987	8873	1.82	9.9
6	Vilanterol	3.684	1678984	358986	8842	1.83	10.01
Mean			1678848				
Std. Dev.			212.8048				
% RSD			0.012676				

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

Accuracy

Table 15: The accuracy results for Fluticasone

%Concentration(at specification Level)	Area	Amount Added(ppm)	Amount Found(ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

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

Table 16: The accuracy results for Vilanterol

%Concentration(at specification Level)	Area	Amount Added(ppm)	Amount Found(ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

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

Robustness
Fluticasone

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	637499	2.133	6889	1.67
Less Flow rate of 0.9 mL/min	653796	2.210	6375	1.65
More Flow rate of 1.1 mL/min	637594	2.184	6537	1.63
Less organic phase	627965	2.200	6274	1.68
More Organic phase	617909	2.172	6199	1.62

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

Vilanterol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2798396	3.692	9796	1.81
Less Flow rate of 0.9 mL/min	2836579	4.498	9376	1.79
More Flow rate of 1.1 mL/min	2763958	3.505	9526	1.61
Less organic phase	2798596	4.504	9437	1.72
More organic phase	2795635	3.512	9526	1.74

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 of Fluticasone and Vilanterol in bulk drug and pharmaceutical dosage forms. This method

was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Fluticasone was found to be freely soluble in chloroform, soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane. Vilanterol (hydrochloride) was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, soluble in water. Methanol: Phosphate Buffer (pH-4.2) (37:63 v/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 of Fluticasone and Vilanterol in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Krishna Teja Pharmacy College, Tirupathi. for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

REFERENCES

1. Weston A, Phyllisr. Brown, HPLC principle and practice. 1st ed. Academic press; 1997. P. 24-37.
2. Kazakevich Y, Lobrutto R. HPLC for pharmaceutical scientists. 1st ed. Wiley Interscience A JohnWiley & Sons, Inc Publishing House; 2007. P. 15-23.
3. Chromatography [online]. Wikipedia. Available from: http://en.wikipedia.org/wiki/Chromatography.
4. Meyer VR. Practical high-performance liquid chromatography. 4th ed. England: John Wiley & Sons Ltd; 2004. P. 7-8.
5. Sahajwalla CG a new drug development. Vol. 141. New York: Marcel Dekker, Inc; 2004. P. 421-6.
6. Snyder LR practical HPLC method development. 2nd ed. New York: John Wiley & sons; 1997. P. 180-2.
7. Skoog DA, West DM, Holler FJ. Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers; 1994. P. 1-5.
8. Sharma BK. Instrumental method of chemical analysis Meerut; 1999. P. 175-203.
9. Breaux J, Jones K. Understanding and implementing efficient analytical method development and validation. Pharm Technol. 2003;5:110-4.
10. Dr. Kealey, Haines PJ. Analytical chemistry. 1st ed. Bios Publisher; 2002. p. 1-7.
11. BraithWait A, Smith FJ. Chromatographic methods. 5th ed. Kluwer Academic Publishers; 1996. P. 1-2.
12. Introduction to column [online]. Available from: http://amitpatel745.topcities.com/index_files/study/columncare.pdf.
13. Detectors used in HPLC (online). Available from: http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC.
14. Detectors [online]. Available from: http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html.
15. Detectors [online]. Available from: http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf.