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METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF VILANTEROL TRIFENATATE AND FLUTICASONE FUROATE USING RP-HPLC METHOD

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ABSTRACT

A new RP-HPLC method was developed and validated for the simultaneous estimation of Vilanterol trifenatate and Fluticasone furoate in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Waters BEH C18 column (4.6×150mm, 5µm), with a mobile phase consisting of methanol:acetonitrile buffer (60:20:20 v/v) at a flow rate of 1.0 ml/min, and detection at 280 nm. Retention times were 3.539 mins for Vilanterol trifenatate and 4.232 mins for Fluticasone furoate. The method exhibited high system suitability parameters, including theoretical plates, tailing factors, and resolution. The method was validated in accordance with ICH guidelines, demonstrating excellent linearity (r2=0.999 for both compounds), accuracy, precision, and robustness. The linearity range was 50µg-250µg for Vilanterol trifenatate and 5µg-25µg for Fluticasone furoate. Percentage recoveries were 99.56% and 99.48%, respectively, with %RSD for repeatability at 0.1 and 1.4. Intermediate precision %RSD for analysts 1 and 2 were within acceptable limits. LOD values were 0.39 µg/ml and 0.7 µg/ml, and LOQ values were 1.18 µg/ml and 2.12 µg/ml for Vilanterol trifenatate and Fluticasone furoate, respectively. The method was further validated through force degradation studies under various conditions, proving its stability-indicating capability. This RP-HPLC method is suitable for routine quality control, stability studies, and simultaneous analysis of Vilanterol trifenatate and Fluticasone furoate in pharmaceutical formulations.

Keywords: RP-HPLC, Vilanterol trifenatate, Fluticasone furoate, Method validation, Pharmaceutical analysis.

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) has emerged as one of the most potent tools in analytical chemistry due to its versatility and precision. HPLC can be applied to a wide array of samples, including pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals [1]. Among the various forms of HPLC, Reversed Phase HPLC (RP-HPLC) is particularly significant due to its use of a non-polar stationary phase and an aqueous, moderately polar mobile phase. [2] Common stationary phases include silica treated with RMe2SiCl, where R represents a straight chain alkyl group such as C18H37 or C8H17. [3] This configuration allows polar molecules to elute more readily while retaining non-polar molecules longer, with retention times being adjustable through the addition of polar or hydrophobic solvents to the mobile phase. In the pharmaceutical industry, [4] RP-HPLC is routinely employed to qualify drugs before their release, ensuring their purity and efficacy. [5] This methodology is particularly relevant for analyzing compounds like Vilanterol trifenatate and Fluticasone furoate.

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Vilanterol is a long-acting beta2-adrenergic agonist used in combination with other bronchodilators for managing chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. [6, 7] Fluticasone furoate, a corticosteroid, is indicated for the treatment of corticosteroid-responsive dermatoses, asthma, and COPD. [8, 9] The present work aims to develop a novel, simple, rapid, accurate, efficient, and reproducible RP-HPLC method, along with a spectroscopic method, for the simultaneous analysis of Vilanterol trifenatate and Fluticasone furoate. The developed method will be validated according to the guidelines established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), ensuring its robustness, precision, and applicability in routine pharmaceutical analysis.

METHODOLOGY

Method development for the simultaneous estimation of Fluticasone furoate and Vilanterol trifenatate by using RP-UPLC

Selection of wavelength

10 mg of Fluticasone furoate and Vilanterol trifenatate was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Fluticasone furoate and Vilanterol trifenatate. The isobestic point was taken as detection wavelength. [10]

Preparation of phosphate buffer

2.95 grams of KH2PO4and 5.45 grams of K2HPO4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 20 ml (30%) and 60 ml of methanol (HPLC grade-60%) and Acetonitrile (20%) degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Preparation of the Fluticasone furoate and Vilanterol trifenatate standard and sample solution

Standard Solution Preparation:

Accurately weigh and transfer 25 mg of Vilanterol trifenatate and 200 mg of Fluticasone furoate working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 25 mg of Vilanterol trifenatate and 200 mg of Fluticasone furoate sample into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 10µL of the standard, sample into the chromatographic system and measure the areas for Vilanterol trifenatate and Fluticasone furoate peaks and calculate the %Assay by using the formulae.

System suitability

Tailing factor for the peaks due to Fluticasone furoate and Vilanterol trifenatate in standard solution should not be more than 1.5. Theoretical plates for the Fluticasone furoate and Vilanterol trifenatate peaks in standard solution should not be less than 2000.

Analytical Method Validation

The method was validated for the following parameters: linearity, precision, accuracy, selectivity, robustness, limit of quantitation (LOQ), limit of detection (LOD) and system suitability.

RESULTS

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Fluticasone furoate and Vilanterol trifenatatewas obtained and the isobestic point of Fluticasone furoateand Vilanterol trifenatateshowed absorbance's maxima at 280nm.

Assay calculation for Fluticasone furoate and Vilanterol trifenatate

The assay study was performed for the Fluticasone furoate and Vilanterol trifenatate. Each three injections of sample and standard were injected into chromatographic system.

Validation Report

Specificity

The specificity test was performed for Fluticasone furoate and Vilanterol trifenatate. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity

The linearity study was performed for the concentration of 100ppmto500 ppm of Fluticasone furoate12.5 ppm to 62.5ppm of Vilanterol trifenatate level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The linearity study was performed for concentration range of 100 µg/ml-500µg/ml of Fluticasone furoate and 12.5µg/ml-62.5 µg/ml of Vilanterol trifenatate and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999) respectively.

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Fluticasone furoate and Vilanterol trifenatate. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. The % Recovery for each level should be between 98.0 to 102.0%

Precision

Repeatability

The precision study was performed for five injections of Fluticasone furoate and Vilanterol trifenatate. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD.

The precision study was performed for the %RSD of Fluticasone furoate and Vilanterol trifenatatewas found to be 0.4 and 0.2 (NMT 2)

Intermediate precision/Ruggedness

The intermediate precision was performed for %RSD of Fluticasone furoate and Vilanterol trifenatate was found to be 0.7 and 0.3 respectively (NMT 2)

Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Fluticasone furoate and Vilanterol trifenatate. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.03ml/min. The method is robust only in less flow condition.

On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

Table 1: Sample and Standard Details

S.No	Samples	% Assay
1	Vilanterol trifenatate&Fluticasone furoate Tablets 25 mcg &200 mcg	100.19 % for Fluticasone Furoate
2	Vilanterol trifenatate&Fluticasone fur	100.45% for Vilanterol trifenatate

Table 2: Showing% RSD results for Fluticasone furoate &Vilanterol trifenatate

Injection	Area for Fluticasone furoate	Area for Vilanterol Trifenatate
Injection-1	448662	218753
Injection-2	446873	214829
Injection-3	446352	216426
Injection-4	447562	218452
Injection-5	447529	216468
Injection-6	446244	217567
Average	447203.7	217082.5
Standard Deviation	907.4	1468.9
%RSD	**0.2**	**0.7**

Table 3: Intermediate precision/Ruggedness of Vilanterol trifenatate and Fluticasone furoate

Injection	Area for Fluticasone furoate	Area for Vilanterol Trifenatate
Injection-1	448776	218573
Injection-2	445735	218562
Injection-3	447673	214652
Injection-4	448673	215354

Injection-5	445876	216454
Injection-6	448676	216457
Average	447568.2	216675.3
Standard Deviation	1424.2	1618.5
%RSD	**0.3**	**0.7**

Table 4: System suitability results for Fluticasone furoate

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.9	1.46	4626.92
2	1.0	1.46	4725.92
3	1.1	1.46	4865.39

Table 5: System suitability results for Vilanterol trifenatate

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.9	3.31	1.29	6132.29
2	1.0	3.18	1.29	6256.39
3	1.1	3.18	1.29	6352.29

Figure: 1 Spectrum showing overlapping spectrum of Fluticasone

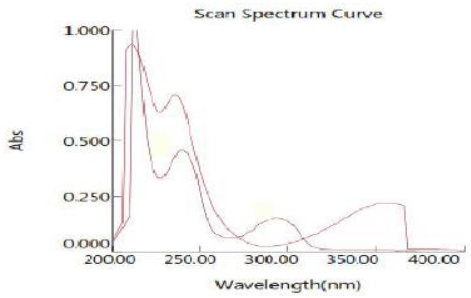


Figure: 2 Chromatogram of Fluticasone furoate and Vilanterol trifenatate

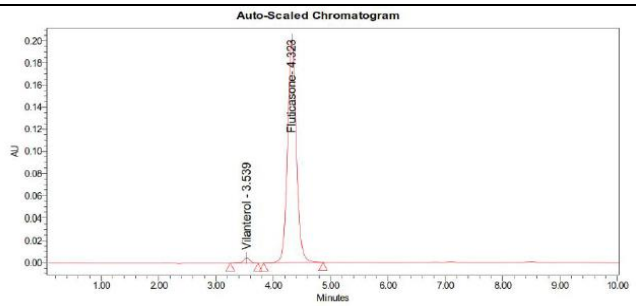


Figure: 3 Chromatogram showing assay of sample injection-1, 2, 3

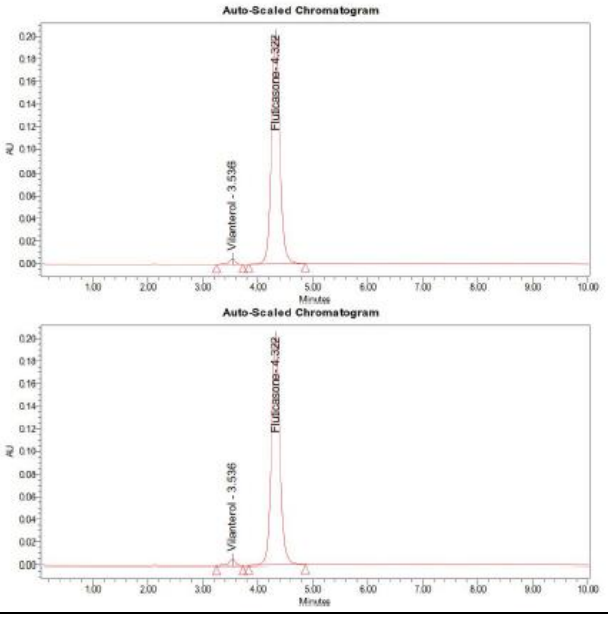


Figure: 4 Chromatogram showing standard injection

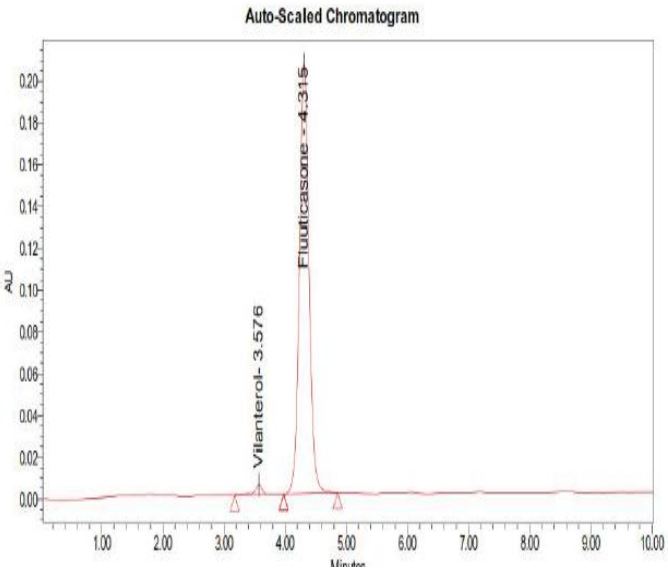


Figure: 5 Chromatogram showing sample injection

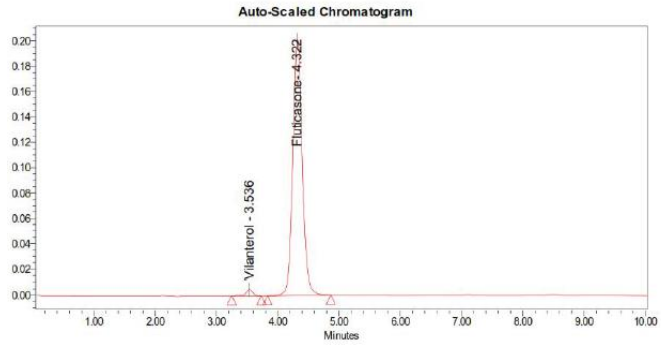


Figure: 6 calibration graph for Vilanterol trifenatate

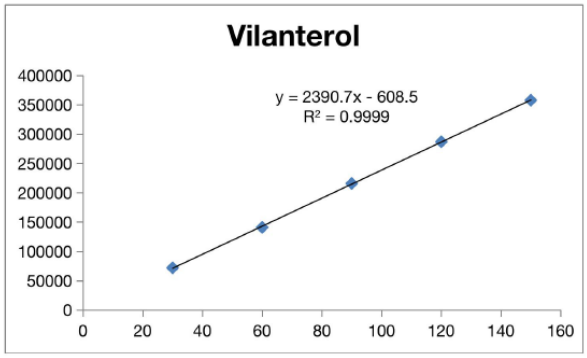
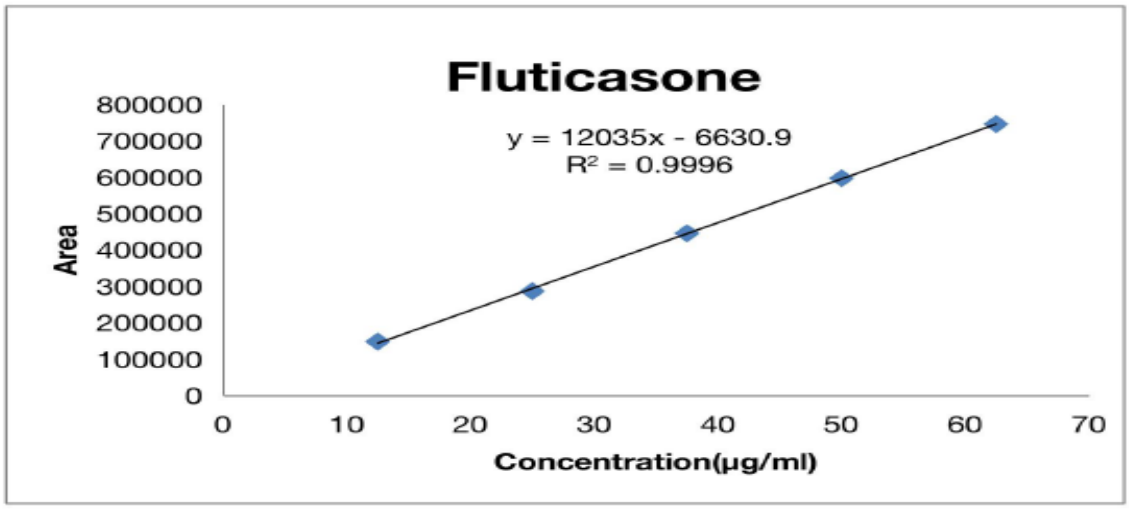


Figure: 7 calibration graph for Fluticasone furoate.



DISCUSSION

The study successfully developed and validated a new RP-HPLC method for the simultaneous estimation of Vilanterol trifenatate and Fluticasone furoate. The method utilized a Waters BEH C18 column (4.6×150mm, 5µm) with a flow rate of 1.0 ml/min and a mobile phase ratio of 60:20:20 methanol:acetonitrile buffer at pH 7. Detection was performed at 280 nm using a WATERS HPLC Auto Sampler, Acquity module, photo diode array detector 2996, and Empower-software version-2. Retention times for Vilanterol trifenatate and Fluticasone furoate were 3.539 mins and 4.232 mins, respectively. The method demonstrated excellent system suitability parameters, with theoretical plates and tailing factors for Vilanterol trifenatate and Fluticasone furoate being 993, 1.23 and 5775, 1.12, respectively. The resolution was 10.18, indicating a clear separation of the two compounds. Validation of the method according to ICH guidelines (ICH, Q2 (R1)) confirmed its linearity, precision, accuracy, and robustness. The linearity range for Vilanterol trifenatate was 50µg-250µg, and for Fluticasone furoate was 5µg-25µg, with correlation coefficients (r2) of 0.999 for both. The percentage recovery for Vilanterol trifenatate and Fluticasone furoate was 99.56% and 99.48%, respectively. The %RSD for repeatability was 0.1 and 1.4, and for intermediate precision, analyst 1 had %RSDs of 0.5 and 0.6, while analyst 2 had %RSDs of 0.8 and 0.3, respectively. LOD values were 0.39 µg/ml and 0.7 µg/ml, and LOQ values were 1.18 µg/ml and 2.12 µg/ml for Vilanterol trifenatate and Fluticasone furoate, respectively. Force degradation studies including acid, base, peroxide, thermal, and photolytic degradation demonstrated the method's suitability for stability studies of the selected drugs. These results indicate that the developed RP-HPLC method is robust and reliable for routine analysis of Vilanterol trifenatate and Fluticasone furoate in API and pharmaceutical dosage forms.

CONCLUSION

The newly developed RP-HPLC method for the simultaneous estimation of Vilanterol trifenatate and Fluticasone furoate was successfully validated according

to ICH guidelines. The method demonstrated high precision, accuracy, linearity, and robustness, making it suitable for routine quality control and stability studies of these drugs in pharmaceutical formulations. The method's reliability and efficiency confirm its applicability for the intended analytical purposes.

REFERENCE

1. Beckett A.H and StenlakeJ.B, *et al.* text book of pharmaceutical chemistry 4th Edn,- part 2 CBS publishers and Distriburots, New Delhi,1998, 278,30
2. Stinder Ahuja, Stephen Scypinski, *et al*. Hand book of modern pharmaceutical Analysis, 3, 1
3. Douglas Skoog A., James Hollar F. and Timothy Nieman, *et al.* A Principles of Instrumental Analysis. 5th ed., Thomson Learning Inc., *Singapore*, 1998, 110, 300.
4. Willard H.H,MerrittL.L,Dean J, *et al*, A. and settle F.A:Instrumental Methods of analysis,7th Edn,CBS Publishers and Distributors, New Delhi 1988, 436-439.
5. Patel, D., Namdev, K. K., Verma, K., Gururani, R., Tiwari, A., Kumar, P., Dewangan, R. P., Wabaidur, S. M., Sharma, S., & Dwivedi, J. *et al,* HPLCUV and spectrofluorimetric methods for simultaneous estimation of fluticasone furoate and vilanterol in rabbit plasma: A pharmacokinetic study. Journal of Chromatography B: Analytical Technologies in the *Biomedical and Life Sciences*, 1132, 2019, [121842].
6. Kondeti. Haritha Pavani *et al,* Stability Indicating Method Development and Validation For The Estimation Of Fluticasone Furoate And Vilanterol Trifenatate By RP-UPLC., *Indo Am. J. P. Sci*, 2021, 08(02).
7. Diviti Sai Sindhu, S. Shoba Rani. Stability indicating RP-HPLC method development andvalidation for the simultaneous estimation of vilanterol and umeclidinium bromide in bulk and pharmaceuticaldosage forms. *World J Pharm Sci*.10(01), 2022, 128- 135
8. Masimukku And Chintala, Development and Validation Of Spectrophotometric Methods For Simultaneous Estimation Of Vilanterol And Fluticasone Furoate In Pharmaceutical Formulations, *Asian J Pharm Clin Res*, 10(4), 2017, 302-305.
9. Kelleher DL, Mehta RS, Jean-Francois BM, Preece AF, Blowers J, Crater GD, Thomas P. *et al,* Safety, tolerability, pharmacodynamics and pharmacokinetics of umeclidinium and vilanterol alone and in combination: a randomized crossover trial. *PLoS One.* 7(12), 2012, e50716.
10. Malerba M, Nardin M, Santini G, Mores N, Radaeli A, Montuschi P. *et al,* Singleinhaler triple therapy utilizing the once-daily combination of fluticasone furoate, umeclidinium and vilanterol in the management of COPD: the current evidence base and future prospects. *Ther Adv Respir Dis.* 2018.