World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

DEVELOPMENT AND VALIDATION OF A ROBUST RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ACLIDINIUM BROMIDE AND FORMOTEROL FUMARATE IN PHARMACEUTICAL DOSAGE FORM

Shaik Fardeen*, C Rupasi Pratyusha, S. Shahidha Bee, Dr. MD Sultan Ali Basha

Department of Pharmaceutical Analysis, SAFA College of Pharmacy, B.Thandrapadu, Kurnool, Andhra Pradesh- 518007, Email: fardeenshaik432@gmail.com.

Received: 31-05-2025 / Revised Accepted: 02-06-2025 / Published: 05-06-2025

ABSTRACT:
A precise, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Aclidinium Bromide and Formoterol Fumarate in pharmaceutical tablet dosage forms. The chromatographic analysis was performed using a Kromasil C18 column (250 × 4.6 mm, 5 µm), with a mobile phase comprising 0.1% perchloric acid buffer and acetonitrile in a ratio of 45:55 v/v. The flow rate was maintained at 1.0 mL/min, and detection was carried out at 230 nm. The retention times were approximately 2.1 minutes for Aclidinium Bromide and 2.7 minutes for Formoterol Fumarate. The method was validated according to ICH Q2(R1) guidelines for parameters including linearity, precision, accuracy, sensitivity, robustness, and system suitability. The developed method proved to be simple, economical, and suitable for routine quality control analysis in pharmaceutical industries.

INTRODUCTION

Aclidinium bromide is a quaternary ammonium compound and a long-acting muscarinic antagonist (LAMA). Its chemical name is 3-[(2-hydroxy-2,2-dithiophen-2-ylacetoxy)phenylmethyl]-1-azabicyclo[2.2.2]octane bromide. It has a molecular formula of C26H30BrNO4S2 and a molecular weight of approximately 558.6 g/mol. It is poorly absorbed systemically due to its highly polar and charged nature, and it undergoes rapid hydrolysis in plasma. Aclidinium bromide acts by competitively inhibiting the muscarinic M3 receptors in the bronchial smooth muscle, leading to bronchodilation. It has a long duration of action and is primarily used in the management of chronic obstructive pulmonary disease (COPD). Due to its rapid systemic clearance and minimal systemic bioavailability, it has a favorable safety profile with reduced systemic anticholinergic effects.

Formoterol fumarate is a long-acting β2-adrenergic receptor agonist (LABA). Its chemical name is (±)-N-[2-hydroxy-5-[(1RS)-1-hydroxy-2-[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide fumarate dihydrate. The molecular formula is C42H60N4O10, and the molecular weight is approximately 840.96 g/mol (as fumarate dihydrate). It is a racemic mixture, typically formulated as a dry powder or inhalation solution. Formoterol fumarate acts by stimulating β2-adrenergic receptors in the bronchial smooth muscle, causing relaxation and prolonged bronchodilation. It has a rapid onset of action (within minutes) and a long duration (up to 12 hours), making it effective for both maintenance therapy in COPD and asthma, and for preventing exercise-induced bronchospasm. It is often used in combination with inhaled corticosteroids or LAMAs for enhanced control of respiratory symptoms.

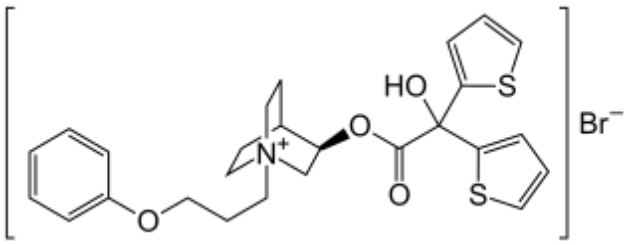

Fig.1. Chemical structure of Aclidinium bromide

Address for Correspondence: Shaik Fardeen, Department of Pharmaceutical Analysis, SAFA College of Pharmacy, B.Thandrapadu, Kurnool, Andhra Pradesh- 518007, Email: fardeenshaik432@gmail.com.

How to Cite this Article: Shaik Fardeen, DEVELOPMENT AND VALIDATION OF A ROBUST RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ACLIDINIUM BROMIDE AND FORMOTEROL FUMARATE IN PHARMACEUTICAL DOSAGE FORM, World J Pharm Sci 2025; 13(02): 159-163;https://doi.org/10.54037/WJPS.2022.100905

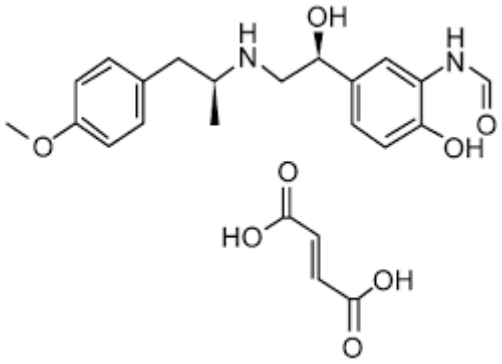

Fig.2. Chemical structure of Formoterol Fumarate

Given the increasing use of this combination, a validated analytical method for its simultaneous estimation in dosage forms is essential for regulatory compliance and routine quality testing. This study reports the development and validation of an RP-HPLC method in accordance with ICH Q2(R1) guidelines.

The aim of the present research was to develop a reliable RP-HPLC method for the simultaneous estimation of Aclidinium Bromide and Formoterol Fumarate in tablet dosage form, to validate the developed method as per ICH guidelines and to demonstrate the applicability of the method for routine quality control analysis in pharmaceutical industries.

Materials and Methods

Instrumentation:

The chromatographic system consisted of an HPLC instrument equipped with a UV detector and auto-sampler. Data acquisition was done using Empower software.

Preparation of Standard stock solutions: Accurately weighed 40mg of Aclidinium Bromide , 12mg of Formoterol Fumarate and transferred to 100ml individual volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (4000µg/ml of Aclidinium Bromide and 120µg/ml Formoterol Fumarate)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (400µg/ml of Aclidinium Bromide and 12µg/ml of Formoterol Fumarate)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (4000µg/ml of Aclidinium Bromide and 50µg/ml of Formoterol Fumarate)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (400µg/ml of Aclidinium Bromide and 12µg/ml of Formoterol Fumarate)

Method development:

A reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed using a Kromasil C18 column (250 × 4.6 mm, 5 µm) for the effective separation and quantification of the analytes. The mobile phase consisted of 0.1% perchloric acid buffer and acetonitrile in a 45:55 v/v ratio, which provided adequate resolution and peak symmetry. The flow rate was maintained at 1.0 mL/min, ensuring a suitable balance between analysis time and efficiency. The detection wavelength was set at 230 nm, which was found to be optimal for maximum absorbance of the analytes. The column was thermostated at 30°C to enhance reproducibility and minimize retention time fluctuations. A fixed injection volume of 10 µL was used for all analyses to maintain consistency. These optimized chromatographic conditions offered reliable and reproducible results suitable for routine quality control and analytical applications.

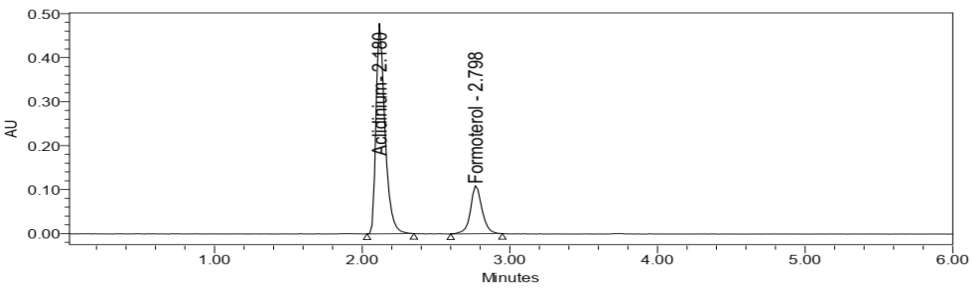

Fig 6.8 Optimized Chromatogram

System Suitability

System suitability was evaluated before analysis to ensure the performance of the chromatographic system. Retention times were found to be 2.1 min for Aclidinium Bromide and 2.7 min for Formoterol Fumarate. Theoretical plate numbers were within acceptable limits, and tailing factors were found to be less than 2, indicating acceptable peak symmetry and resolution.

Linearity

The method showed excellent linearity in the concentration range tested. The calibration curves were linear with regression equations:

- Aclidinium Bromide: y = 4751x + 4404.2
- Formoterol Fumarate: y = 23616x + 793.96

The correlation coefficients (R^2) for both drugs were greater than 0.999, confirming the linearity of the method.

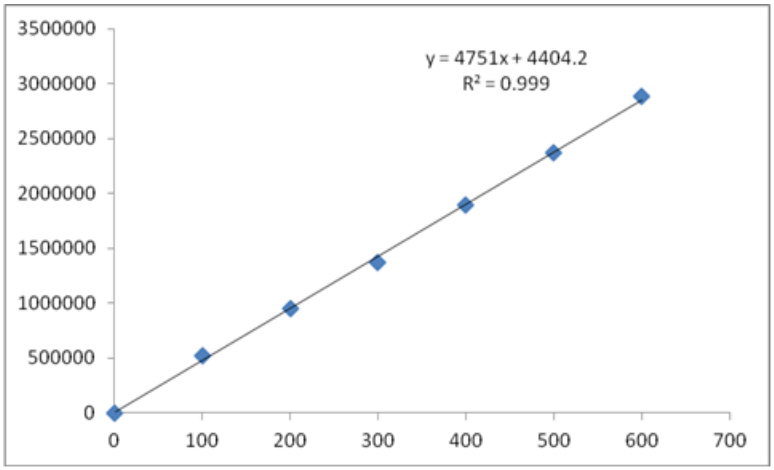

Fig No. 4 Calibration curve of Aclidinium Bromide

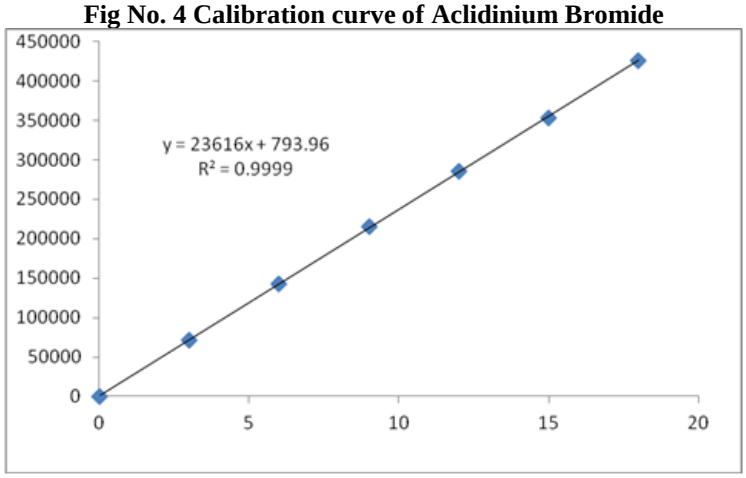

Fig No. 5 Calibration curve of Formoterol Fumarate

Precision

Precision was assessed through repeatability (intra-day) and intermediate precision (inter-day). The %RSD values were:

- Aclidinium Bromide: 0.8%
- Formoterol Fumarate: 1.0%

These results indicate that the method is precise and produces reproducible results under the same and varying conditions.

Accuracy

Accuracy was determined by recovery studies at three concentration levels (50%, 100%, and 150%). The recovery results were:

- Aclidinium Bromide: 100.44%
- Formoterol Fumarate: 100.87%

These values fall within the acceptable recovery range of 98–102%, demonstrating the method's accuracy.

Table 1. Accuracy table of Formoterol Fumarate

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	6	6.148235	102.47	100.87 %
	6	6.098546	101.64	
	6	6.132456	102.21	
100%	12	11.91705	99.31	
	12	11.87683	98.97	
	12	11.87817	98.98	
150%	18	18.18186	101.01	
	18	18.21206	101.18	
	18	18.36755	102.04	

Sensitivity

Sensitivity was evaluated by determining the LOD and LOQ based on standard deviation of response and slope:

- Aclidinium Bromide: LOD = 1.23 µg/mL, LOQ = 3.68 µg/mL
- Formoterol Fumarate: LOD = 0.08 µg/mL, LOQ = 0.24 µg/mL

These values indicate that the method is sensitive and suitable for the detection of low concentrations of both analytes.

Robustness

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (25:75A), mobile phase plus (35:65A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay of Marketed Formulations

Assay was performed with the formulation Duaklir. Average % Assay for Aclidinium Bromide and Formoterol Fumarate obtained was 99.48 and 99.70% respectively

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Aclidinium Bromide and Formoterol Fumarate in Tablet dosage form. Retention time of Aclidinium Bromide and Formoterol Fumarate were found to be 2.1 min and 2.7 min. %RSD of the Aclidinium Bromide and Formoterol Fumarate were and found to be 0.8 and 1.0 respectively. %Recovery was obtained as 100.44% and 100.87% for Aclidinium Bromide and Formoterol Fumarate respectively. LOD, LOQ values obtained from regression equations of Aclidinium Bromide and Formoterol Fumarate were 1.23, 0.08 and 3.68, 0.24respectively. Regression equation of Aclidinium Bromide is y = 4751x + 4404.2, and y = 23616x + 793.96 of Formoterol Fumarate . Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Acknowledgments

I would like thank our principal sir and management of SAFA College of Pharmacy for providing this opportunity.

REFERANCES

1. B.k Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication , Meerut, (2007)
2. Lindholm.J, Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis, pg . 13-14, (2004).
3. Rashmin, An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences, Vol.2 , Issue 2, Pg 191-196 (2012).
4. Malvia R, Bansal V , Pal O.P and Sharma P.K. A Review of High Performance Liquid Chromatography. Journal of Global Pharma technology (2010)
5. Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760.
6. Ashok Kumar, Lalith Kishore, navpreet Kaur , Anroop Nair. Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutica Sciencia, Vol 2, Issue 3, Jul-Sep (2012)
7. Kaushal.C, Srivatsava.B, A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, Vol.2, Issue 2, 519-545, (2010)
8. Vibha Gupta, Ajay Deep Kumar Jain, N.S.Gill, Kapil, Development and Validation of HPLC method. International Research Journal of Pharmaeutical and Applied Sciences, Vol 2, Issue 4, Jul-Aug (2012)
9. Hokanson GC. A life cycle approach to the validation of analytical methods during Pharmaceutical Product Development. Part 1: The Initial Validation Process. Pharm Tech (1994) 92-100

10. Green JM. A Practicle guide to analytical method validation, Anal Chem (1996) 305A-309A
11. ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA , Geneva , (1996)
12. Ewelina rutkowska, Karolina paj k and Krzysztof J"ewiak* Lipophilicity – Methods of determination and its role in medicinal chemistry Acta Poloniae Pharmaceutica n Drug Research, Vol. 70 No.1 pp. 3n18, (2013).
13. Kulkarni R, More S. Capillary electrophoresis method for analysis of aclidinium bromide. J Chromatogr A. 2019;1605:360363.
14. Singh P, Kaur J. Ion-pair RP-HPLC method for simultaneous estimation of aclidinium and formoterol. Asian J Pharm Clin Res. 2020;13(2):85-90.
15. George A, Mathew A. Derivative UV-spectrophotometric estimation of aclidinium bromide. Pharma Chem. 2021;13(3):101-5.
16. Hassan H, Omar M. A green HPLC method for aclidinium bromide analysis. Green Chem Lett Rev. 2018;11(1):78-84.
17. Rao M, Deshpande D. Fluorimetric determination of aclidinium bromide in biological matrices. Luminescence. 2019;34(3):343-9.
18. Kumar A, Dey S, Jain A. Development and validation of RP-HPLC method for simultaneous estimation of Formoterol Fumarate and Tiotropium Bromide in inhalation dosage form. J Chem Pharm Res. 2014;6(7):140-145.
19. Shinde VM, Kulkarni MV, Patil SV. UV spectrophotometric method for estimation of Formoterol Fumarate in bulk drug. Int J Pharm Sci Res. 2016;7(5):2121-2124.
20. Ghorpade DA, Kharat AR, Kulkarni SS. Stability-indicating HPLC method for determination of Formoterol Fumarate in pharmaceutical dosage form. Int J Pharm Sci Rev Res. 2017;44(1):136-140.
21. Rathod AS, Jadhav RT, Chaudhari SP. Development and validation of HPTLC method for estimation of Formoterol Fumarate in single and combined dosage forms. Asian J Pharm Clin Res. 2018;11(7):218-221.
22. Tiwari G, Tiwari R, Rai AK. A green RP-HPLC method for estimation of Formoterol Fumarate in pharmaceutical dosage form. Int J Green Pharm. 2019;13(2):125-129.
23. Srivastava B, Pandey P, Singh S. Development and validation of a UPLC method for determination of Formoterol Fumarate in metered dose inhaler. J Appl Pharm Sci. 2020;10(5):101-105.
24. Waghmare AV, Kalyankar TM, Pande VV. Derivative spectrophotometric estimation of Formoterol Fumarate in pharmaceutical formulations. Int J Pharm Pharm Sci. 2016;8(12):135-138.