STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION OF ACLIDINIUM BROMIDE AND FORMOTEROL FUMARATE BY RP-HPLC

[1] Dr. T N V S S Satyadev
[1]Department of chemistry, PB Siddhartha College of Arts and Science, Vijayawada, AP.

Abstract
A validated stability indicating RP-HPLC method for Aclidinium Bromide and Formoterol fumarate was developed by separating its degradation products on a C_{18} (150x4.6mm, 3.5µm) waters symmetry column using 1ml Tri Ethyl Amine of pH=7.0 adjusted with Ortho Phosphoric acid and acetonitrile in simple isocratic at a flow rate of 1.0 ml/min. The column effluents were monitored by a photodiode array detector set at 264nm. The method was validated in terms of specificity, linearity, accuracy, precision, detection limit, quantification limit and robustness. The proposed method is validated as per ICH Q2 (R1) guidelines.

***Index Terms*- Aclidinium Bromide and Formoterol fumarate, RP-HPLC**

INTRODUCTION

Aclidinium bromide is long- acting; inhaled muscarinic antagonist (LAMA) approved in the US on July 24, 2012 [1] as a maintenance treatment for chronic obstructive pulmonary disease (COPD) [2]. Evidence shows that it can improve quality of life and prevent hospitalization in those with COPD. However, it does not appear to affect the risk of death [3] or the frequency steroids [4, 5] are needed. It is unclear if it differs from the similar medication tiotropium or other commonly used medications from the class of LAMAs.

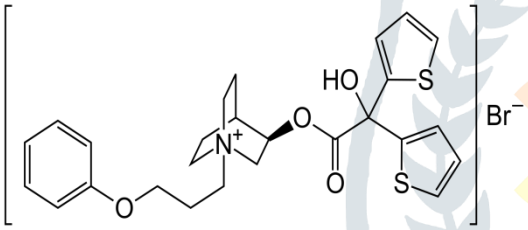


Fig. 1: Structure of Aclidinium bromide

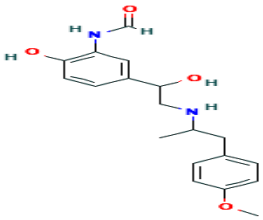


Fig.2: Structure of Formoterol fumarate

Formoterol, also known as formoterol is a long-acting $ß_2$ agonist (LABA) used as a bronchodilator [6, 7] in the management of asthma [8] and COPD. Formoterol has an extended duration of action (up to 12h) compared to short- acting $ß_2$ agonist [9] such as salbutamol (albuterol)[10], which are effective for 4h to 6h. LABA such as formoterol are used as "Symptom controllers" to supplement prophylactic corticosteroid [11, 12] therapy. A reliever short- acting $ß_2$ agonist (e.g. salbutamol) is still required, since LABAs are not recommended for the treatment of acute asthma [13, 14]. It was patented in 1972 and came into medical use in 1998. It is also marketed in the combination formulations budesonide/formoterol [15, 16] and mometasone/formoterol.

The literature survey has revealed that no article is published so far. The proposed method was simple economical and sensitive for the estimation of Aclidinium bromide and Formoterol fumarate.

MATERIALS AND REQUIREMENTS

Instrument:
HPLC, make: Waters alliance e-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

Reagents:
Acetonitrile (HPLC grade), Ortho Phosphoric acid (HPLC grade), Water (HPLC grade), Tri ethyl amine.

Mobile Phase Preparation:
Mobile Phase-A: 1 ml of Tri ethyl amine of pH= 7.0 adjusted with Ortho Phosphoric acid. Filter through 0.45µ filter paper.
Mobile Phase-B: Acetonitrile

Diluent Preparation: Mix Mobile Phase-A and Mobile phase-B in 50:50 v/v.

Optimization of mobile phase:

Different trials have done, different buffers and different mobile phases were used to develop the method. In all trials peaks are not separated properly. Finally for the proposed method all the peaks are separated and the entire suitability conditions are within the limit.

Isocratic Programme:

Mobile phase-A and Mobile phase B = 50:50

Chromatographic conditions:

The chromatographic system was carried out in symmetry C_{18}, (150x4.6mm, 3.5µm) column. Flow rate was maintained at 1.0ml/min, injection volume is 10µl and sample and column temperatures are ambient. Wavelength detection is maintained at 264nm.

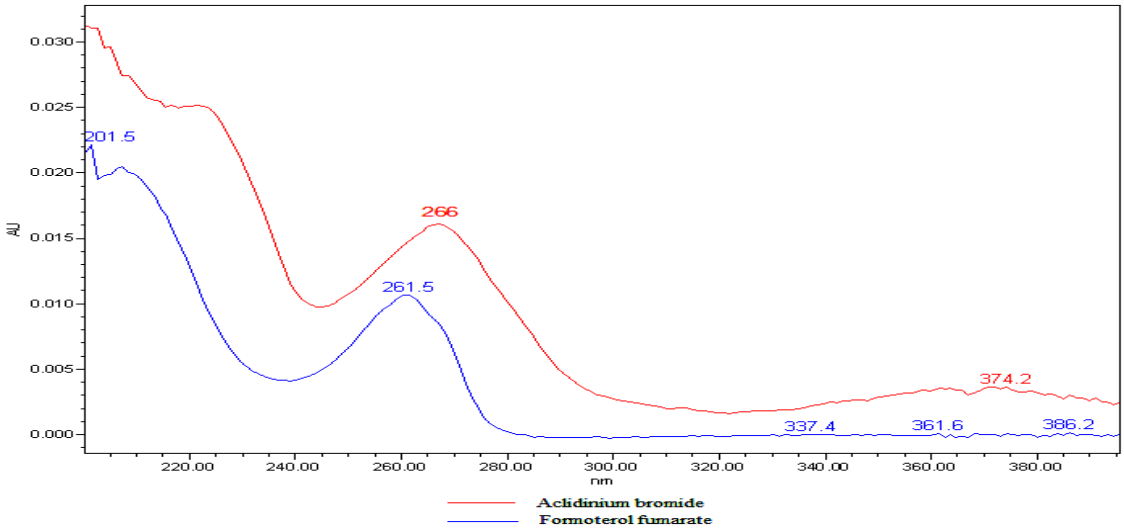


Fig. 3: PDA Spectrum

Standard Solution:

Weigh accurately 340mg of Aclidinium bromide and 12 mg of formoterol fumarate. These working standards were transferred into a 100ml volumetric flask; add 70ml of diluent sonicated for 20min to dissolve the contents make up to the mark with diluent. Further dilute 5ml of above solution to 50ml with diluent.

Sample Solution:

Transfer 340mg of Aclidinium bromide and 12mg of Formoterol fumarate equivalent weight of sample into a 100ml volumetric flask diluted to volume with diluent. Filter through 0.45µ nylon syringe filter.

RESULTS AND DISCUSSION

Validation of proposed method

The method was validated for parameters like system suitability, specificity, and linearity, LOD, LOQ, Precision, Accuracy, Robustness and Ruggedness as per ICH guidelines [17, 18].

System Suitability

The HPLC system was stabilized for 60min to get a stable baseline. Six replicate injections of standard solution were injected. The results are summarized below table 1.

Table 1: System Suitability data

System Suitability parameter	Acceptance criteria	Drug Name	
		Aclidinium bromide	Formoterol fumarate
% RSD	NMT 2.0	0.15	0.17
USP Tailing	NMT 2.0	1.05	1.08
USP Plate Count	NLT 3000	5369	6870

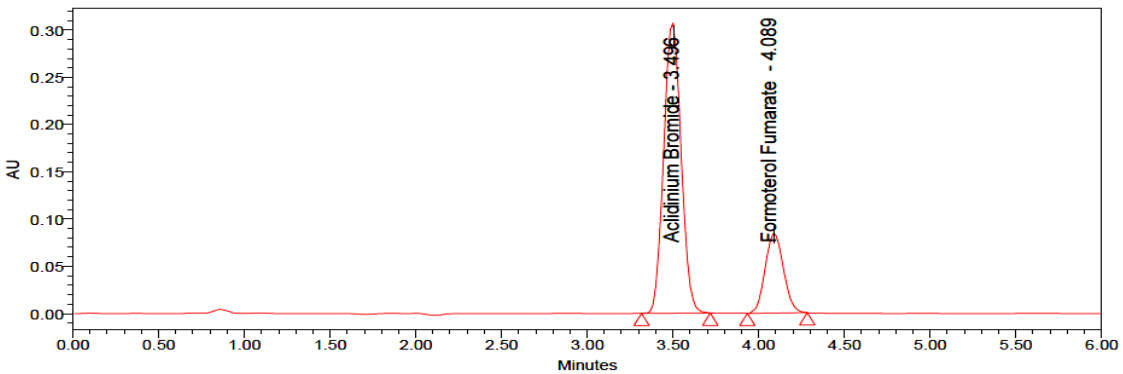


Fig. 4: Chromatogram for system suitability

Specificity

There is no interaction of peaks in blank and standard, sample, placebo chromatograms in the total runtime of chromatogram. Hence it proves that method is specific.

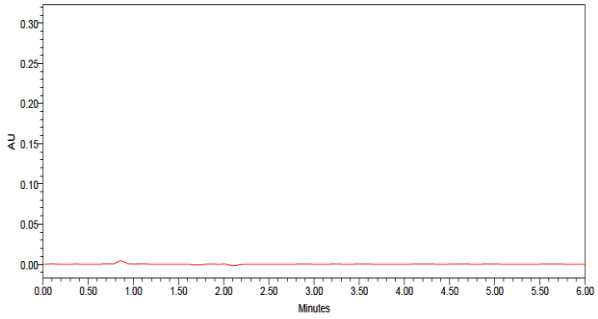


Fig. 5: Chromatogram for Blank

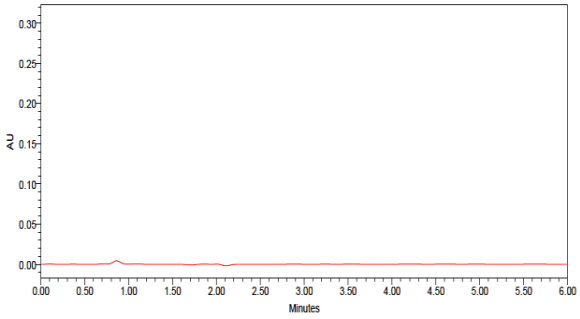
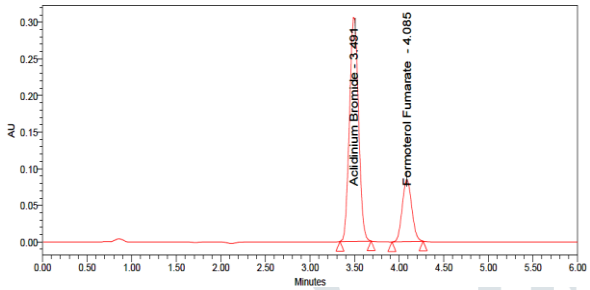


Fig. 6: Chromatogram for Placebo



Chromatogram of standard

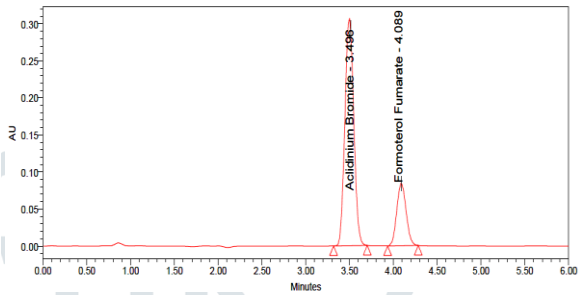


Fig. 8: Chromatogram for sample

Fig.7:

Linearity

The linearity was observed in the concentration range of 34-510µg/ml of Aclidinium bromide. The regression equation is Y= 6615x+28.873 and correlation coefficient was found to be 0.9996. Formoterol fumarate concentration range from 1.2-18µg/ml, regression equation is Y= 54702x+603.54 and correlation coefficient was found to be 0.9996.

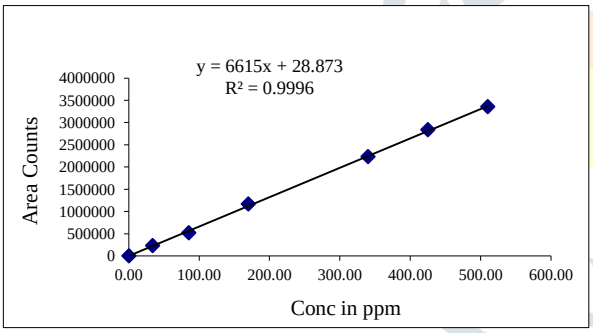


Fig.9: Linearity plot for Aclidinium Bromide

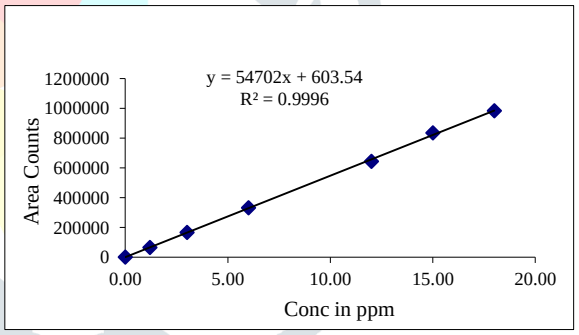


Fig.10: Linearity plot for Formoterol fumarate

Accuracy

Injecting samples in triplicate at 50%, 100% and 150% of the target concentration. The recovery results should be NLT 95% and NMT 105%.

Table 2: Accuracy results of Aclidinium Bromide

S. No.	% Level	% Recovery	Avg. % Recovery
1	50	99.6	99.5
2		99.5	
3		99.4	
4	100	99.6	99.9
5		100.3	
6		99.7	
7	150	100.0	99.9
8		100.2	
9		99.4	

Table 3: Accuracy results of Formoterol Fumarate

S. No.	% Level	% Recovery	Avg. % Recovery
1	50	100.5	100.2
2		100.2	
3		99.8	
4	100	100.1	99.8
5		99.7	
6		99.6	
7	150	99.8	99.5
8		99.2	
9		99.4	

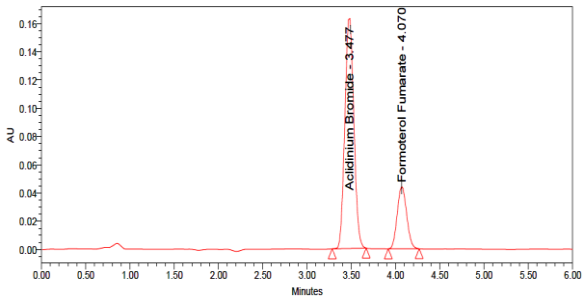


Fig. 11: Chromatogram for Accuracy 50%

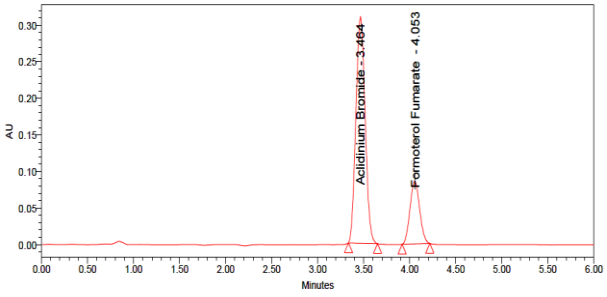


Fig. 12: Chromatogram for Accuracy 100%

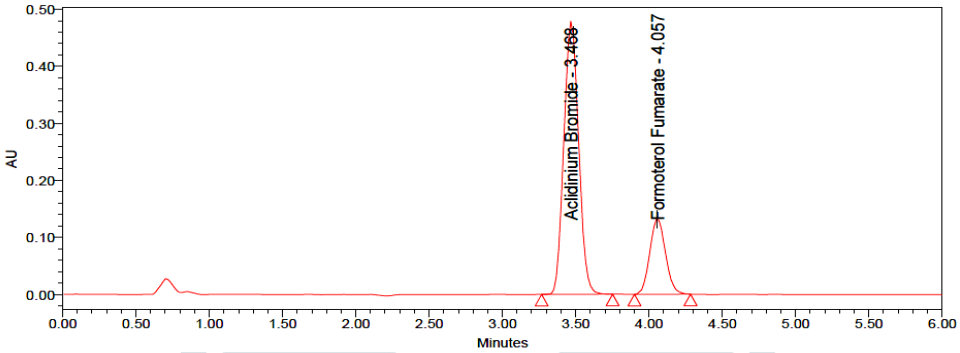


Fig. 13: Chromatogram for Accuracy 150%

Precision

Method Precision

Method Precision was investigated by the analysis of six separately prepared samples of the same batch. From these six separate samples solution was injected and the peak areas obtained used to calculate mean and percentage RSD values.

Intermediate Precision

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage of RSD was below 2% exhibits the ruggedness of the developed method. The results are given in table 4.

Table 4: Method Precision and Intermediate Precision results

Analyte	Amount present	Intra-day Precision	Inter-day Precision
		% RSD	
Aclidinium Bromide	340	0.39	0.06
Formoterol Fumarate	12	0.24	0.27

LOD and LOQ

LOD and LOQ were separately determined by calibration curve method [19]. LOD and LOQ of the compound were determined by injecting progressively lower concentrations of standard solutions using developed RP-HPLC method. The LOD concentrations for Aclidinium bromide and Formoterol fumarate and their s/n values are 0.34, 0.012µg/ml and 7, 4. The LOQ concentrations for Aclidinium bromide and formoterol fumarate and their s/n values are 3.4, 0.12µg/ml and 27, 24.

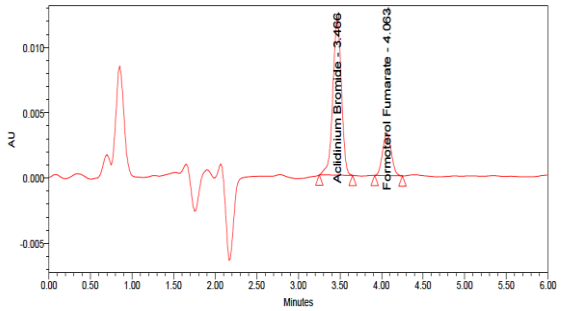


Fig. 14: Chromatogram for LOD

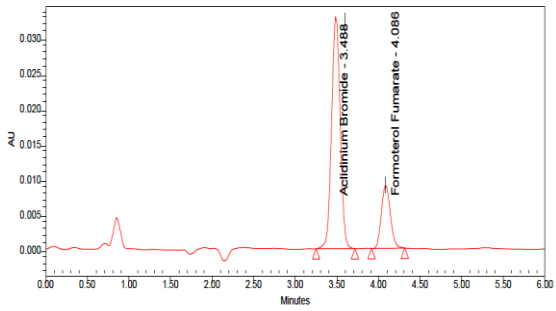


Fig. 15: Chromatogram for LOQ

Robustness

Robustness of the method was found to be %RSD should be less than 2%. Slightly variations were done in the optimized method parameters like flow rate (±20%), Organic content in mobile phase (±10%). The results are given in table 5.

Table 5: Robustness results

Drug Name	Flow Plus	Flow Minus	Organic Plus	Organic Minus
	% RSD			
Aclidinium Bromide	0.07	0.31	0.33	0.09
Formoterol fumarate	0.19	0.38	0.46	0.61

Stability

The stability of Aclidinium bromide and Formoterol fumarate in solution was determined by sample solution stability initial to 24h at different time intervals at room temperature. There is no significant deviation of purity.

Table 6: Results of solution stability

Stability	% Lable claim Aclidinium Bromide	% Deviation Aclidinium Bromide	% Lable claim Formoterol Fumarate	% Deviation Formoterol Fumarate
Initial	99.8	0.00	101.8	0.00
6h	99.5	0.00	101.7	-0.10
12h	99.	0.40	102.1	0.29
24h	99.6	-0.20	101.8	0.00

CONCLUSION

All the factors lead to the conclusion that the proposed method is simple, specific, accurate, precise and reproducible. Statistical analysis proves that the method is suitable for the analysis of Aclidinium bromide and Formoterol fumarate.

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