IJBPAS, February, 2022, 11(2): 693-707

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

STABILITY INDICATING UPLC METHOD FOR ESTIMATION OF ACLIDINIUM BROMIDE AND FORMOTEROL FUMARATE IN DRY POWDER INHALER

SHYAMALA[*1], BALAYYAS[2], SANDESH JS[2], SWAPNA K[2], SANDEEP[2], SHARMA JVC[3]

1: Faculty, Department of Pharmaceutical Analysis, Joginpally B R Pharmacy College, Moinabad, T.S, 500075

2: Student, Joginpally B R Pharmacy College, Moinabad, T.S, 500075

3: Principal, Joginpally B R Pharmacy College, Moinabad, T.S, 500075

***Corresponding Author: Shyamala: E Mail: shyamala.mudavath@gmail.com**

Received 15th March 2021; Revised 16th April 2021; Accepted 1st June 2021; Available online 1st Feb. 2022

https://doi.org/10.31032/IJBPAS/2022/11.2.5886

ABSTRACT

Objective: The aim of this work is to develop and validate a simple, synchronous and novel UPLC method for stability indicating assay method to estimate Aclidinium bromide and formoterolfumarate in both bulk and its dosage form. **Materials and Methods**: This method is based on isocratic elution using HiberC_{18}(100x 2.1mm 1.8µ) column with phosphatebuffer : Acetonitrile (60:40) mobile phase . Detection was monitored at 240nm with flow rate 0.3ml/min. **Results**: The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. The retention times of Aclidinium and formoterol are 1.09 and 1.39, respectively. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range for Aclidinium 100-600µg/ml and formoterol 3-18µg/ml. **Conclusion**: No chromatographic interference from dry powder inhaler's excipients and degradants were found. The proposed method was successfully used for concurrent estimation of Aclidinium and formoterol in dry powder inhaler dosage form.

Keywords: stability indicating, UPLC, Aclidinium andformoterol

INTRODUCTION

Bronchodilators, including long-acting b2-agonists (LABAs) andlong-acting muscarinic antagonists (LAMAs), are mainstays in thetreatment of moderate to severe chronic obstructive pulmonarydisease (COPD) **[1]**. Aclidinium bromide is a LAMA that provides significant and persistent bronchodilation compared with placebo, and has been shown to reduce dyspnea and improve health-related quality of life in patients with COPD **[2-4]**. Formoterolfumarate, a LABA that improves bronchodilation with a rapid onset (<5 min) and a sustained duration of action (12 h), also improves dyspnea **[5, 6]**. The recent approval of a fixed-dose combination of aclidinium400 mg and formoterol 12mg for maintenance bronchodilator treatment to relieve symptoms in patients with COPD.

Aclidinium bromide is an anticholinergic **[7, 8]**. It is a synthetic, quaternary ammonium compound, chemically described as 1-azoniabicyclo[2.2.2]octane, 3-[(hydroxydi-2-thienylacetyl)oxy]-1-(3-phenoxypropyl)-, bromide, (3R)- **(Figure 1)**. It is a white powder with a molecular formula of $C_{26}H_{30}NO_4S_2Br$ and a molecular mass of 564.56 g/mol.

Formoterolfumarate, a racemate, is a selective beta2-adrenergic agonist and exhibits its bronchodilator activity through its long andselective sympathomimetic action on the bronchial smooth muscles **[9]**. Its chemical name is (±)-2- hydroxy-5-[(1RS)-1-hydroxy-2-[[(1RS)-2-(4-methoxyphenyl)-1methylethyl]-amino]ethyl]formanilidefumaratedihydrate.

It has a molecular weight of 840.91 g/moland its molecular formula is $(C_{19}H_{24}N_2O_4)2\bullet C_4H_4O_4\bullet 2H_2O$. Formoterolfumarate is a white to yellowish crystalline powder.

Literature survey revealed that several methods were developed for the determination of formoterol either alone **[10-13]** or in many other combinations **[14-18]**, and only one UV **[19]** spectroscopic method was developed for the assay of aclidinium and formoterol in their combined dosage form.It has been reported that no work has been carried out on stability indicating method. The present study describes an isocratic UPLC method, with a high sensitivity, precision and accuracy for determination of aclidinium and formoterol in a metered dose inhaler. The objective of the study is to outline the procedure and evidence whether the stability indicating assay method for the both drugs in marketed pressurized Inhaler by UPLC method is

suitable for its intended purpose to establish the quality with a consistent assay results.

MATERIALS AND METHODS:

Reagents:

Aclidinium and Formoterol were supplied by Spectrum Labs (Hyderabad). Acetonitrile, water (HPLC review, Merck) and the various reagents were acquired from Rankem. Dry powder inhaler, DUAKLIR (AstraZeneca) containing 400mg of Aclidinium and 12mg of Formoterol were used.

Instrumentation

UPLC instrument used was of WATERS UPLC SYSTEM with Auto Injector and Acquity TUV detector. Software used is Empower 2.

Chromatographic conditions

The elution method was isocratic. The mobile phase is comprised of a mixture of (Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.8 with dil. Orthophosphoric acid solution). 0.01N KH_2PO_4buffer and acetonitrile (60:40 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter before using the same. A Hiber C_{18} (100x 2.1mm 1.8µ) column was utilized for determination. The rate of flow was 0.3 ml/min and also the column was operated at temperature ~30oC. The sample volume injected was 0.5µL. The column was saturated for not less than 30min with mobile phase flowing through the system prior to injection of the solutions. The wavelength of the UV detector was set at 240nm. A typical UPLC chromatogram of Aclidinium and Formoterol is presented in **Figure 1**.

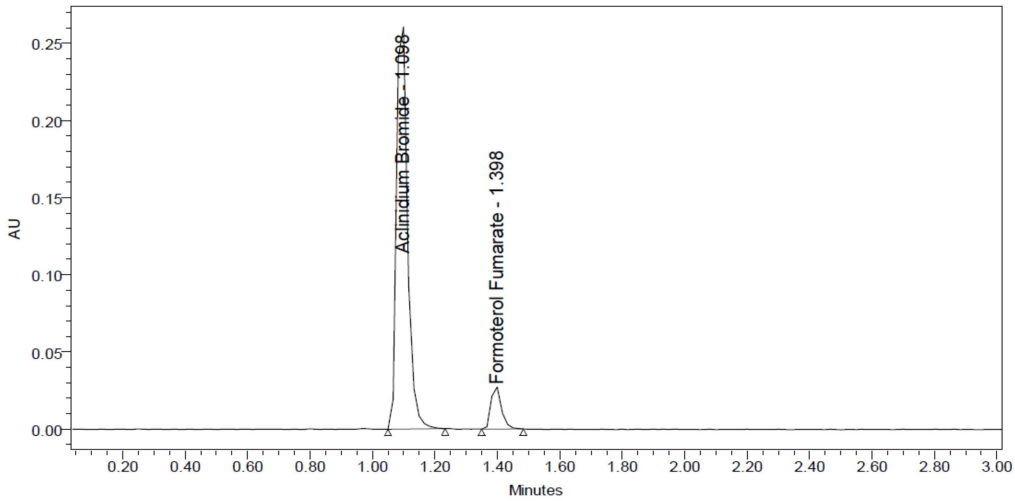


Figure 1: UPLC chromatogram of Standard Aclidinium and Formoterol

Standard Preparation:

Accurately Weighed and transferred 100mg of Aclidinium and 3mg of Formoterol working Standards into a 25ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. (4000ppm of Aclidinium and 120ppm of Formoterol).1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (400ppm of Aclidinium and 12ppm of Formoterol)

Preparation of Sample solutions: The contents of nasal spray delivered by 20 actuations (400&12µg each) were collected in 10 ml volumetric flask. Then 8ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 800 & 24µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (PVDF)

5ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (400ppm of Aclidinium and 12ppm of Formoterol)

Method Validation:

The developed method was validated as per ICH guidelines **[20]** for its linearity, accuracy, precision, robustness, and specificity, limit of detection and limit of quantification by using the below mentioned procedures. The validated parameters are provided in **Table 1**.

System suitability

The validation of system suitability and chromatographic parameters was performed such as tailing factor, asymmetry factor, and number of theoretical plates were calculated.

Linearity and Range

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample. The calibration curve showed good linearity in the range of 100 – 600 µg/ml and 3-18 µg/ml for Aclidinium and 12ppm of Formoterol (API) respectively with correlation coefficient (r2) of 0.999 for both drugs. A typical calibration curve for both drugs were given in **Figure 2, 3** and results in **Table 2**.

Precision

Precision Repeatability

System precision

Six standard solutions of the similar concentration (100%) were arranged and injected into the UPLC system as per test methodology. The outcomes were given in **Table 3.1 (A)**.

Method precision

Six sample solutions of the identical concentration (100%) were arranged and injected into the HPLC system as per test

strategy. The results were given in **Table 3.1(B)**.

Intermediate Precision

Day to Day variability

The study was conducted for two days according to test strategy. The six sample solutions of the identical concentration (100%) were prepared and injected into the HPLC system as per test methodology for Day-1 and Day-2. The results were given in **Table 3.2(A)**.

Accuracy

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in **Table 4(A) and (B)**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Aclidinium and formoterol were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD and LOQ of Aclidinium were observed to be 2.82µg/ml and 8.55µg/ml respectively. The LOD and LOQ of Formoterol were found to be 0.01µg/ml and 0.03µg/ml respectively. Results are given in **Table 5**.

Robustness

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and Mobile phase ratio (± 5nm) were studied to determine the robustness of the method. The results were in favour of (% RSD < 2%) the developed UPLC method for the analysis of Aclidinium and Formoterol. The results are given in **Table 6**.

Assay

The assay and purity were computed for brand DUAKLIR (AstraZeneca) with label claim 400mg and 12mg. The observed value was evaluated by comparing with the standard value without impedance from the excipients utilized in the powder inhaler dosage form. The outcomes were given in **Table 7**.

Results of Stability studies

Oxidation:

To 1 ml of stock solution of Aclidinium and Formoterol 1 ml of 10% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For UPLC study, the resultant solution was diluted to obtain (400ppm & 12ppm) solution and

0.5µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stocks solution Aclidinium and Formoterol1 ml of 1N Hydrochloric acid was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain (400ppm & 12ppm) solution and 0.5µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Aclidinium and Formoterol 1 ml of 1N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain (400ppm & 12ppm) solution and 0.5µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6Hrs to study dry heat degradation. For UPLC study, the resultant solution was diluted to (400ppm & 12ppm) solution and 0.5µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the (4000ppm & 120ppm) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For UPLC study, the resultant solution was diluted to obtain (400ppm & 12ppm) solutions and 0.5µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For UPLC study, the resultant solution was diluted to (400ppm & 12ppm) solution and 0.5µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

The results of all the degradation studies were presented in **Table 8 & Figure 4 (A-E)**.

RESULTS

Table 1: System Suitability Parameters

Aclinidium Bromide					Formoterol Fumarate				
S:No	RT(min)	Area	USP Plate Count	Tailing	RT(min)	Area	USP Plate Count	Tailing	Resolution
1	1.094	906751	4721	1.20	1.396	63165	7608	1.22	4.6
2	1.096	894752	4732	1.20	1.398	63929	8231	1.25	4.7
3	1.097	894207	4130	1.22	1.399	64336	7755	1.20	4.7
4	1.097	901930	4846	1.10	1.400	64557	8432	1.19	4.7
5	1.097	883089	4785	1.12	1.400	63460	7651	1.23	4.8
6	1.098	892554	4849	1.09	1.400	64067	7853	1.22	4.8
Mean		895547				63919			
Std. Dev.		8159.2				525.8			
% RSD		0.9				0.8			

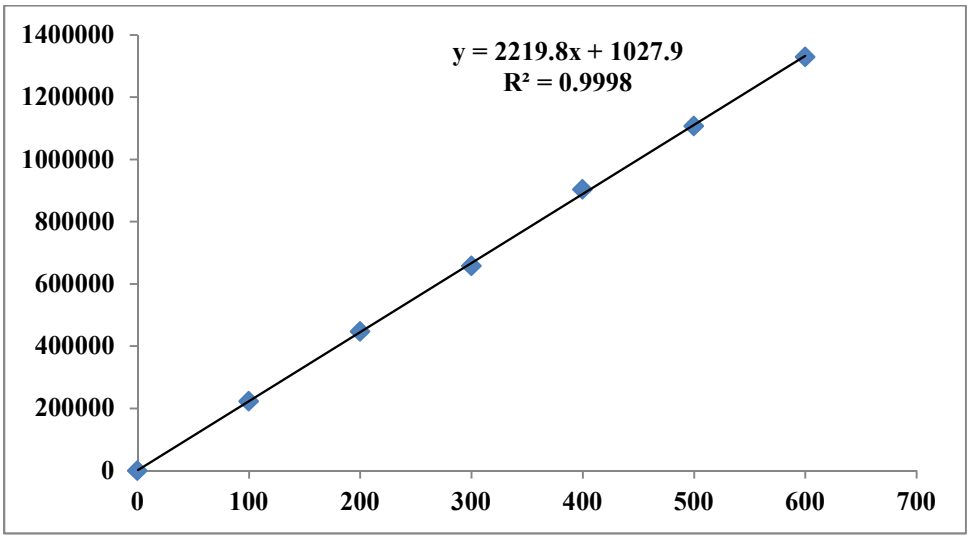


Figure 2: Graphical Representation of Linearity of Aclidinium in the range 100 µg/ml to 600 µg/ml

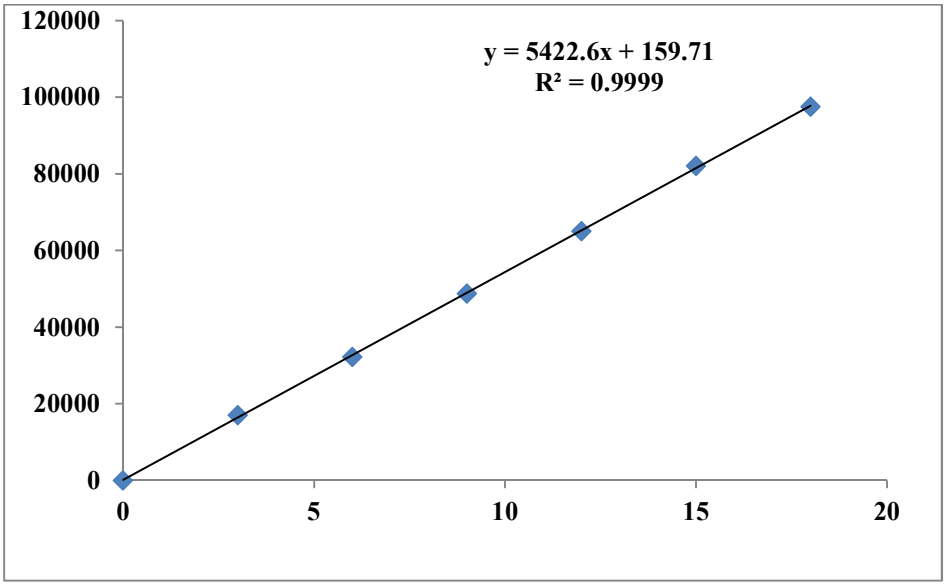
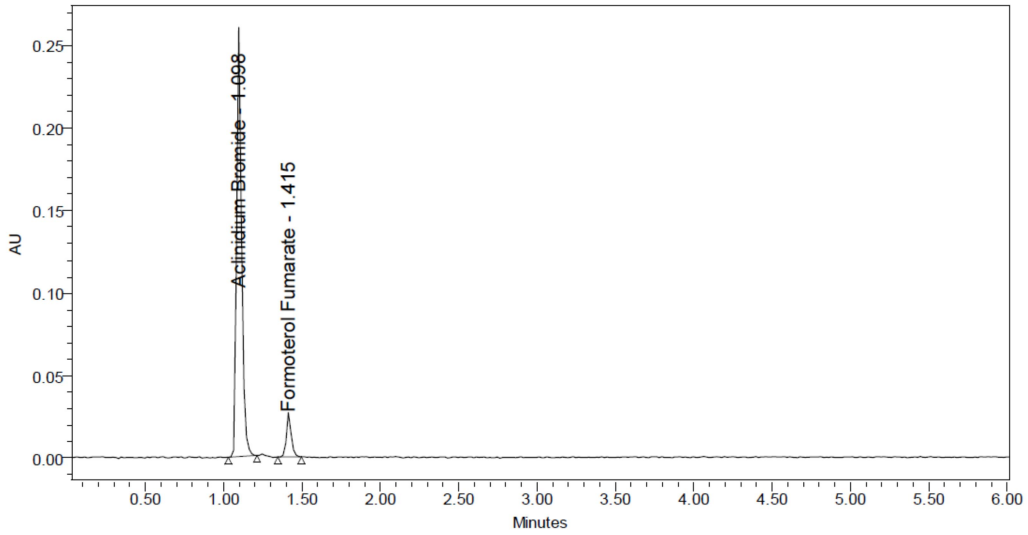
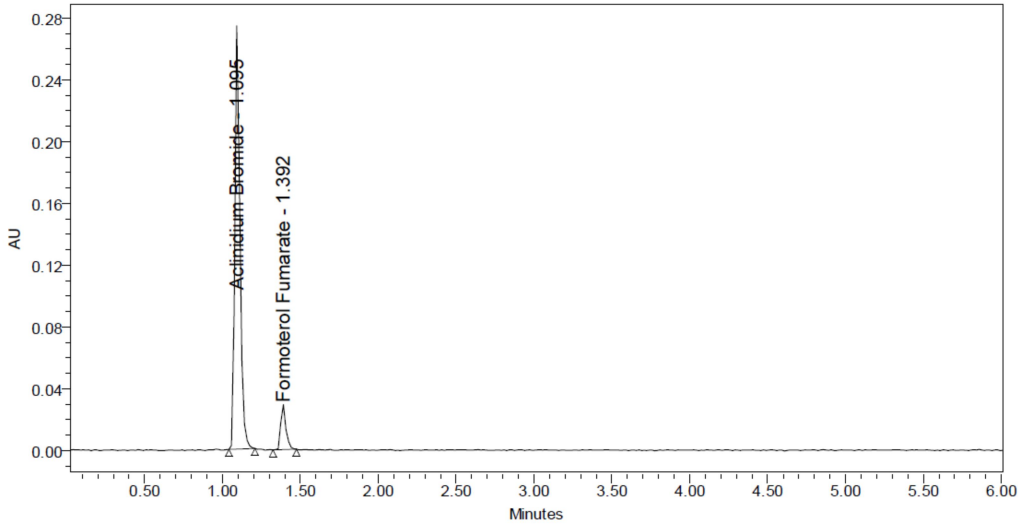


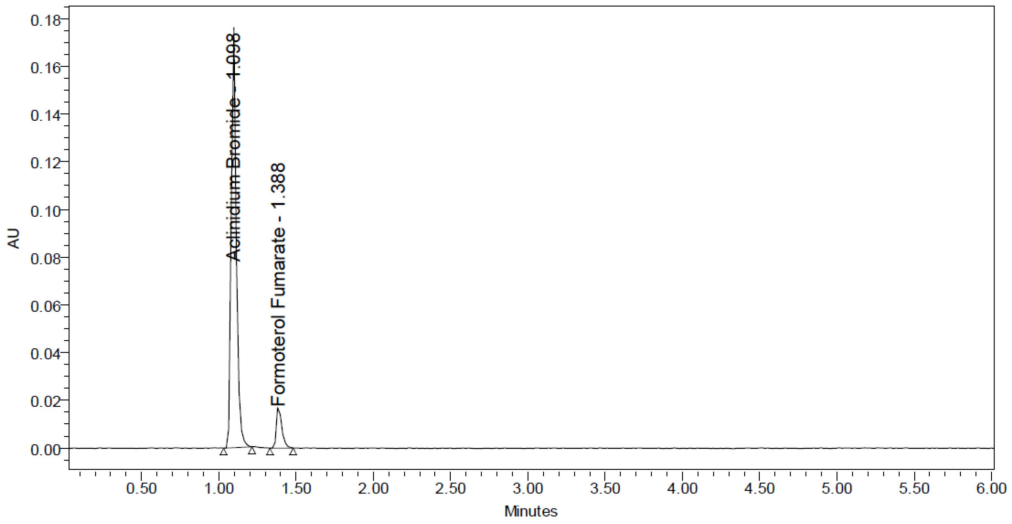
Figure 3: Graphical Representation of Linearity of Formoterol in the range 3 µg/ml to 18µg/ml


Aclinidium Bromide - 1.098
Formoterol Fumarate - 1.415
AU
Minutes

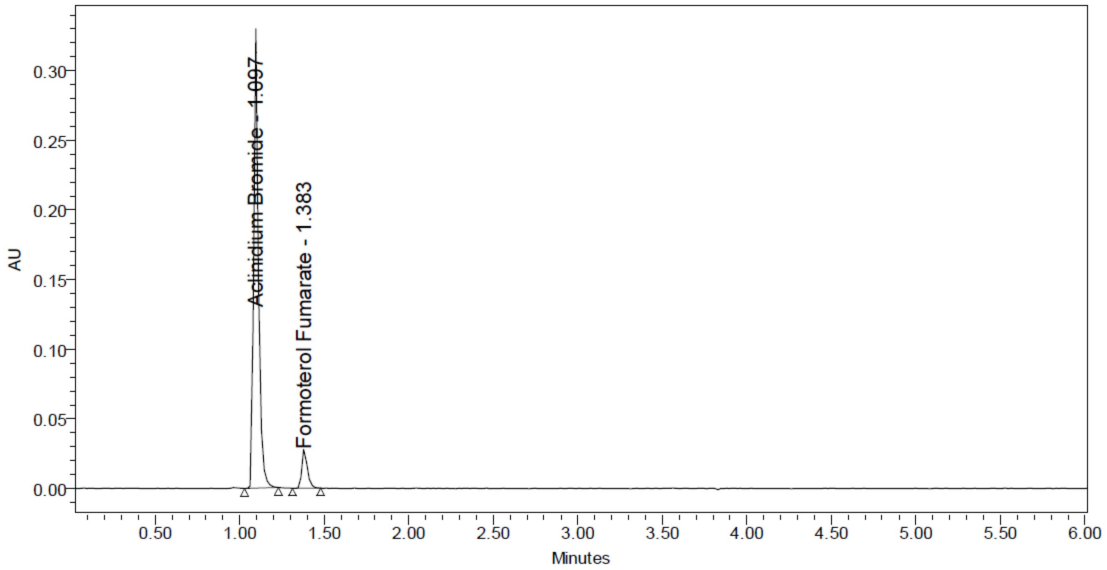
A


Aclinidium Bromide - 1.095
Formoterol Fumarate - 1.392
AU
Minutes

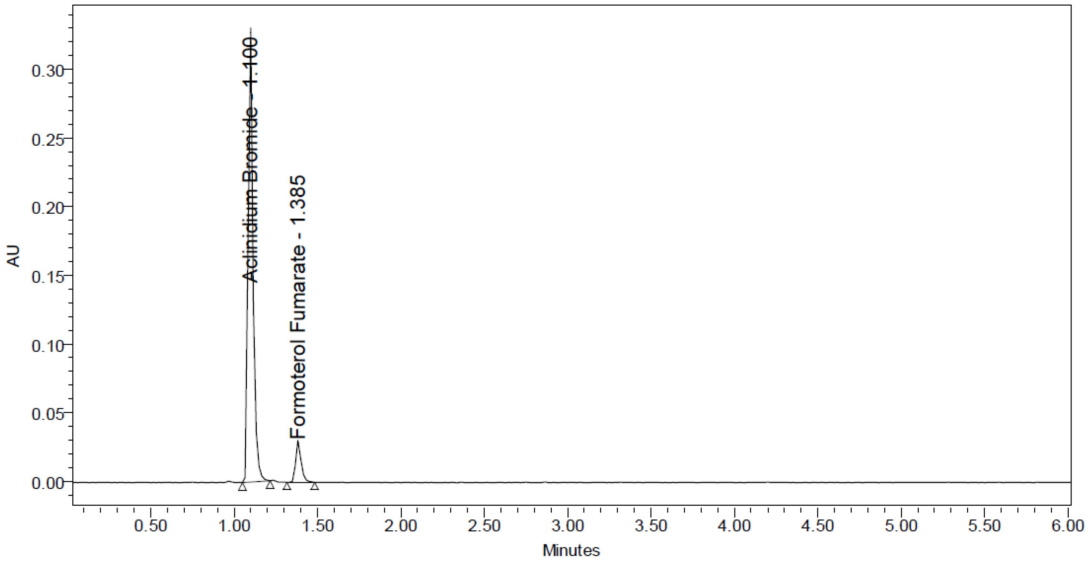
B


Aclinidium Bromide - 1.098
Formoterol Fumarate - 1.388
AU
Minutes

C



D



E

Figure 4: Representative chromatogram of Acid (A), Base (B), Peroxide C), UV (D), Hydrolysis (E) of Aclidinium and formoterol

Table 2: Linearity Data of Aclidinium and Formoterol

S. No.	Aclidinium		Formoterol	
Injection	Conc(µg/ml)	Average retention area	Conc(µg/ml)	Average retention area
	0	0	0	0
1	100	222850	3	17020
2	200	446953	6	32282
3	300	657748	9	48776
4	400	904009	12	65039
5	500	1107553	15	82091
6	600	1329658	18	97532
	y= 2219.x+1027 R^2=0.999		y= 5422.x+159.7 R^2=0.999	

Table 3.1(A): Precision (Repeatability) – System Precision Data of Aclidinium and Formoterol

Injection	Aclidinium	Formoterol
Injection-1	906751	63165
Injection-2	894752	63929
Injection-3	894207	64336
Injection-4	901930	64557
Injection-5	883089	63460
Injection-6	892554	64067
AVG	895547	63919
SD	8159.2	525.8
% RSD	0.9	0.8

***Average of six determination, y is the area under curve and x is the concentration, SD is the standard deviation, RSD is the relative standard deviation**

Table 3.1(B): Precision (Repeatability) – Method Precision Data of Aclidinium and Formoterol

Injection	Aclidinium	Formoterol
Injection-1	900003	64585
Injection-2	911064	63877
Injection-3	906414	63420
Injection-4	902273	63589
Injection-5	904676	63872
Injection-6	906607	64014
AVG	905173	64014
SD	3838.9	402.6
% RSD	0.4	0.6

***Average of six determination, SD is the standard deviation, RSD is the relative standard deviation**

Table 3.2: Intermediate Precision Data (Day To Day Variability) of Aclidinium and Formoterol.

S. No	Area of aclidinium	Area of formoterol
1.	869261	62589
2.	879797	62170
3.	878392	63263
4.	896398	63876
5.	884449	63317
6.	886745	63542
Mean	882507	63126
S.D	9104.1	631.4
%RSD	1.0	1.0

***SD is the standard deviation, RSD is the relative standard deviation**

Table 4 (A): Accuracy Data Aclidinium

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	50	201.3379901	100.67	100.09%
	50	202.8080216	101.40	
	50	198.2929247	99.15	
100%	100	401.9315007	100.48	
	100	401.6692204	100.42	
	100	402.2023434	100.55	
150%	150	600.2302839	100.04	
	150	593.1563767	98.86	
	150	595.6196485	99.27	

Table 4 (B): Accuracy Data of Formoterol

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	50	5.982902988	99.72	99.54%
	50	5.926835116	98.78	
	50	6.054832165	100.91	
100%	100	11.83535596	98.63	
	100	12.02753596	100.23	
	100	11.9338436	99.45	
150%	150	17.88478421	99.36	
	150	17.81469937	98.97	
	150	17.96482848	99.80	

Table 5: LOD and LOQ of Aclidinium and Formoterol.

Molecule	LOD	LOQ
Aclidinium	2.82	8.55
Formoterol	0.01	0.03

*LOD- Limit of detection, LOQ- Limit of Quantitation

Table 6: Robustness data of Aclidinium and Formoterol

S. No.	Condition	%RSD of Aclidinium	%RSD of Formoterol
1	Flow rate (-) 0.27ml/min	1.2	1.0
2	Flow rate (+) 0.33ml/min	1.0	1.3
3	Mobile phase (-) 60B:40A	0.1	1.0
4	Mobile phase (+) 50B:50A	0.6	0.3
5	Temperature (-) 25°C	1.3	1.4
6	Temperature (+) 35°C	1.1	1.4

Table 7: Assay data of Aclidinium and Formoterol

	Aclidinium			Formoterol		
S. No	Standard Area	Sample area	% Assay	Standard Area	Sample area	% Assay
1	906751	900003	100.30	63165	64585	100.84
2	894752	911064	101.53	63929	63877	99.73
3	894207	906414	101.01	64336	63420	99.02
4	901930	902273	100.55	64557	63589	99.28
5	883089	904676	100.82	63460	63872	99.73
6	892554	906607	101.03	64067	64014	99.95
Avg	895547	905173	100.87	63919	64014	99.76
Std. dev	8159.2	3838.9	0.428	525.8	402.6	0.629
%RSD	0.9	0.4	0.4	0.8	0.6	0.6

*Average of six determinations, Std.dev is the standard deviation,RSD is the relative standard deviation

Table 8: Results of Degradation Studies

Type of degradation	Aclidinium			Formoterol		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	826293	92.08	7.92	60349	94.23	5.77
Base	833755	92.91	7.09	61332	95.76	4.24
Peroxide	823435	91.76	8.24	61246	95.63	4.37
Thermal	866486	96.56	3.44	63090	98.51	1.49
Uv	882250	98.32	1.68	63836	99.67	0.33
Water	892184	99.43	0.57	63955	99.86	0.14

DISCUSSION

The primary target in developing this stability indicating UPLC method is to achieve the resolution between Aclidinium and Formoterol and its degradation products. Active pharmaceutical ingredients of Aclidinium bromide and formoterolfumarate in bulk form are soluble in water and Acetonitrile. Different mobile phase compositionswere tried to separate the in mixed Aclidinium bromide and formoterolfumarate standard. Different mobile phases like water: methanol (50:50), Acetonitrile: methanol (50:50), KH_2PO_4 with orthophosphoricacid:acetonitrile (60:40) are tried in the method development with different columns like C_{18} and C_8. Adequate resolution is achieved with KH_2PO_4 with perchloricacid:acetonitrile (60:40), pH 3.8) with C_{18} column and this solventsystem was found to be most suitable for method development and validation. The detection was carried out at 240 nm in the proposedmethod, at this wavelength both the molecules shows maximum absorbance. It was found that the retention times were 1.098 min for Aclidinium and 1.398 min for Formoterol. In the present developed UPLC method, the standard and sample preparation involves simple and rapid extraction procedure and requires less time. A good linear relationship was observed for Aclidinium and formoterol in the concentration range of 100-600 and 3-18 µg/mL and linear regression equation being y= 2219.x+1027 and y= 5422.x+159.7, respectively. Linear regression least square fit, slope (m), intercept (b), standard deviation, residual sum of squares and correlation coefficient data obtained from the measurements are in the limits.The assay results and recovery studies shows good extraction and recovery from 50to 150 % of test concentration. Various stress stability studies were carried out like hydrolysis, oxidation, dry heat and photolytic degradation. Forced degradation study showed the method is highly specific and no degradation products were eluted at retention time of drugs.

CONCLUSION

A selective, sensitive and concurrent stability indicating UPLC method has been validated for the analysis of Aclidinium and formoterolin bulk drug and dry powder inhaler dosage form. Based on peak purity results, obtained from the analysis of stability indicating studying samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of both drugs, indicated that the developed method is specific for the estimation of

Aclidinium and formoterolin presence of degradation products. Further the proposed UPLC method has excellent precision, sensitivity and reproducibility. Even though no attempt has been made to identify the degraded products, proposed method can be used as stability indicating method for assay of Aclidinium and formoterolin commercial formulations.

CONFLICT OF INTEREST

There is no conflict of interest declared by authors.

REFERENCES

[1] James F. Donohue, Weily Soong, Xiao Wu, Pomy Shrestha, Alejhandra Lei, Long-term safety of aclidinium bromide/ formoterol fumarate fixed dose combination: Results of a randomized 1-year trial in patientswith COPD, Respiratory Medicine.2016; 116: 41-48.

[2] P.W. Jones, D. Singh, E.D. Bateman, A. Agusti, R. Lamarca, G. de Miquel. Efficacy and safety of twice-daily aclidinium bromide in COPD patients: the ATTAIN study. The European Respiratory Journal. 2012; 40(4): 830-836.

[3] E.M. Kerwin, A.D. D'Urzo, A.F. Gelb, H. Lakkis, E. Garcia Gil, C.F. Caracta.Efficacy and safety of a 12-week treatment with twice-daily aclidinium bromidein COPD patients (ACCORD COPD I). Chronic Obstructive Pulmonary Disease.212; 9(2): 90-101.

[4] S.I. Rennard, P.D. Scanlon, G.T. Ferguson, L. Rekeda, B.T. Maurer, E. Garcia Gil. ACCORD COPD II: a randomized clinical trial to evaluate the 12-weekefficacy and safety of twice-daily aclidinium bromide in chronic obstructive pulmonary disease patients. Clinical Drug Investigation. 2013; 33(12): 893-904.

[5] W.E. Berger, J.A. Nadel. Efficacy and safety of formoterol for the treatment ofchronic obstructive pulmonary disease. Respiratory Medicine. 2008; 102(2):173-188.

[6] P. Steiropoulos, A. Tzouvelekis, D. Bouros, Formoterol in the management ofchronic obstructive pulmonary disease. International Journal of Chronic Obstructive Pulmonary Disease. 2008; 3(2): 205-215.

[7] Aclidinium Bromide, 2017, https://pubchem.ncbi.nlm.nih.gov/compound/Aclidinium_bromide

[8] M. Pisano and N. Mazzola. Aclidinium bromide inhalation

powder (Tudorza): a long-acting anticholinergic for the management of chronic obstructive pulmonary disease. Pharmacy and 6erapeutics.2013; 38(7): 393–396.

[9] Formoterol,https://pubchem.ncbi.nlm.nih.gov/compound/formoterol, 2017.

[10] B. Demircigil, S. ¨ Ozkan, ¨ O. Coruh, and S. Yılmaz. Electrochemical behavior of formoterolfumarate and its determination in capsules for inhalation and human serumusing differential-pulse and square-wave voltammetry. Electro analysis. 2002; 14, (2): 122–127.

[11] H. Kamimura, H. Sasaki, S. Higuchi, and Y. Shiobara. Quantitative determination of the β-adrenoceptor stimulant formoterol in urine by gas chromatography mass spectrometry.Journal of Chromatography B: Biomedical Sciences and Applications. 1982; 229 (2); 337–345.

[12] M. Gousuddin, S. A. Raju, and M. S. Sultanuddin. Developmentand validation of spectrophotometric methods forestimation of formoterol bulk drug and its pharmaceutical dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3: 300–309.

[13] S. T. Hassib, A. A. El-Zaher, and M. A. Fouad. Validated stability-indicating derivative and derivative ratio methods forthe determination of some drugs used to alleviate respiratorytract disorders and their degradation products. Drug Testing and Analysis. 2011; 3(5) 306–318.

[14] R. I. El-Bagary, M. A. Fouad, A. Manal, and E. H. Tolba. Forced degradation of mometasonefuroate and development of two RP-HPLC methods for its determination with formoterolfumarate or salicylic acid. Arabian Journal of Chemistry. 2016; 9(3)493–505.

[15] K. H. Assi, W. Tarsin, and H. Chrystyn. High performance liquid chromatography assay method for simultaneous quantitation of formoterol and budesonide in Symbicort Turbuhaler. Journal of Pharmaceutical and Biomedical Analysis. 2006; 41(1); 325–328.

[16] V. K. Parmar, H. N. Patel, and B. K. Patel. Sensitive, and robust methods, for simultaneous determination of beclomethasone dipropionate and formoterol-

fumarate dehydrate in rotacaps. Journal of Chromatographic Science. 2014; 52(10), 1255–1266.

[17] M. Rizk, M. Sultan, N. Talaat, and N. Youssef. A validated TLC—densitometric method for the simultaneous determination of formoterolfumarate and budesonide in pressurized metered-dose inhaler. JPC- Journal of Planar Chromatography -Modern TLC. 2017; 30 (1): 63–67.

[18] H. A. Merey, S. S. El-Mosallamy, N. Y. Hassan, and B. A. El-Zeany. Validated chromatographic methods for the simultaneous determination of Mometasone furoate and Formoterol fumaratedihydrate in a combined dosage form. Bulletin of Faculty of Pharmacy, Cairo University. 2016; 54(1): 99–106.

[19] Khalid A. M. Attia, Nasr M. El-Abasawi, Ahmed El-Olemy, and Ahmed Serag, Different Spectro-photometric Methods Manipulating RatioSpectra Applied for the Analysis of Aclidinium in Duaklir®Genuair® Inhalation Powder, Hindawi. Journal of Spectroscopy. 2018; 2018(7): 1-6.

[20] ICH harmonized tripartite guideline; validation of Analytical procedures: Text and Methodology Q2 (R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2005. 11-12.