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Validated RP-HPLC method for simultaneous estimation of aclidinium and formoterol in bulk drug and dosage form

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Abstract
A simple, Accurate, precise method was developed for the simultaneous estimation of the Aclidinium and formoterol in Pharmaceutical dosage form. Chromatogram was run through Standard Azilent 150 x 4.6 mm, 5µ. Mobile phase containing Buffer potassium dihydrogen phosphate: Acetonitrile taken in the ratio 65:35 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was potassium dihydrogen phosphate buffer. Retention time of Aclidinium and Formoterol were found to be 2.221 min and 2.801 min. %RSD of the Aclidinium and Formoterol were and found to be 0.9 and 1.2 respectively. %Recovery was obtained as 100.41% and 100.57% for Aclidinium and Formoterol respectively. LOD, LOQ values obtained from regression equations of Aclidinium and Formoterol were 0.33, 1.0 and 0.04, 0.12 respectively. 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.

Keywords: Aclidinium bromide, formoterol fumarate, validation, RP-HPLC, robustness

Introduction

Aclidinium Bromide: (3R)-3-{[hydroxy-2,2-bis(thiophen-2-yl)acetyl]oxy}-1-(3phenoxypropyl)-1-azabi-cyclo[2.2.2]octan-1-ylium bromide. The molecular formula of active substance is $C_{26}H_{30}BrNO_4S_2$ and its relative molecular mass is 564.6 g/mol. It is slightly soluble in water, soluble in methanol, very soluble in acetonitrile.

Aclidinium bromide inhalation powder is indicated for the long-term, maintenance treatment of bronchospasm associated with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema Aclidinium does not prolong the QTc interval or have significant effects on cardiac rhythm. Aclidinium structure is shown in the fig-1.

Structure

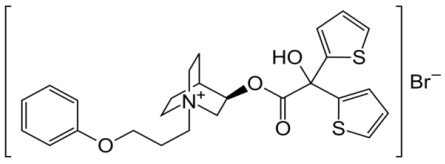

Fig 1: structure of Aclidinium bromide

Formoterol fumarate

(*E*)-but-2-enedioicacid;*N*-[2-hydroxy-5-[(1*R*)-1-hydroxy-2-[[(2*R*)-1-(4-methoxyphenyl)propan-2-yl]amino]ethyl]phenyl]formamide;hydrate. The molecular formula of Formoterol fumarate is $C_{42}H_{56}N_4O_{14}$ and its relative molecular formula is 840.924g/mol.

For use as long-term maintenance treatment of asthma. Also used for the prevention of exercise-induced bronchospasm, as well as long-term treatment of bronchospasm associated with COPD. Formoterol is a long-acting selective beta2-adrenergic receptor agonist (beta 2-agonist). Inhaled formoterol fumarate acts locally in the lung as a bronchodilator. To stimulation of intracellular adenyl cyclase, the enzyme that catalyses the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibits the release of pro-inflammatory mast-cell mediators such as histamine and leukotrienes.

Formoterol structure is shown in the fig-2.

Structure:

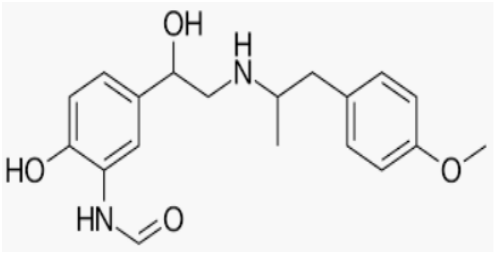

Fig 2: Structure of formoterol fumarate

•Literature review reveals that only two analytical methods had been carried on aclidinium bromide and formoterol fumarate drugs i.e., U.V [1], HPLC [2, 3, 4, 5].

Materials and methods

Chemicals and Reagents

Aclidinium and Formoterolpure drugs (API), were obtained from Pharma Spectrum Labs, Hyderabad. Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid are from Rankem, Avantor performance materials India Limited.

Equipment

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultra-sonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Aclidinium and Formoterol solutions.

Chromatography condition

The mobile phase consists of a mixture of Methanol: 0.1% OPA (O-phosphoric acid) (50:50 v/v) was filtered through 0.45µm air-soluble membrane filters before using use. The injection volume was 10 µL with a flow rate 10µl/minand detection wavelength 236 nm.

Preparation of Standard stock solutions: Accurately weighed, 100mg of Aclidinium and 3mg of Formoterol transferred to 50ml volumetric flask and 3/4th of diluents was added to this flask and sonicated for 10 minutes. Flask were made up with diluents and labelled as Standard stock solution. (200µg/ml of Aclidinium and 60µg/ml of Formoterol)

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. (200µg/ml of Aclidinium and 6µg/ml of Formoterol)

Preparation of Sample solutions: The contents of nasal spray delivered by 50 actuations (1.2&40 mcg each) were collected in 50 ml volumetric flask. Then 20ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 12&400µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45µm filters using (Millipore, Milford, PVDF)

5ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (200µg/ml of Aclidinium and 6µg/ml of Formoterol).

Preparation of buffer

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium di hydrogen 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 3.48 with dil. Orthophosphoric acid solution.

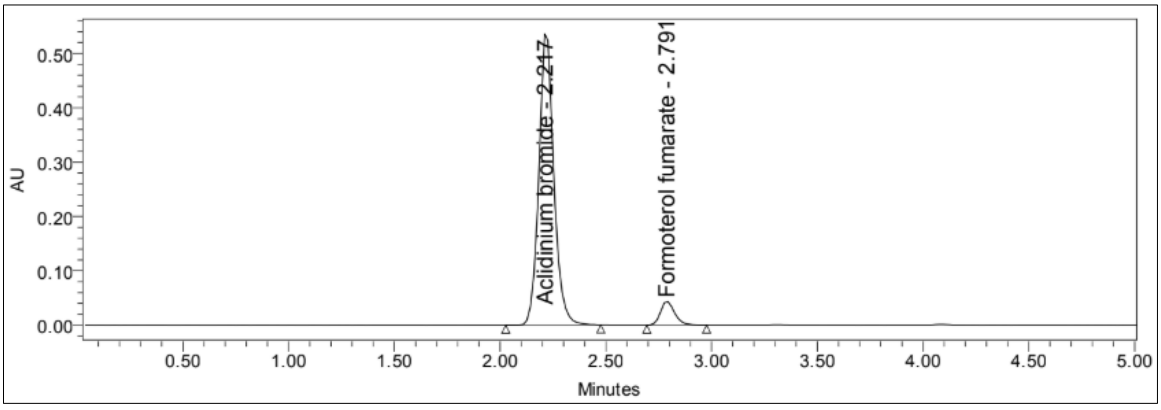

Fig 3: Standard chromatogram of aclidinium bromide and formoterol fumarate

Degradation Studies

Oxidation

To 1 ml of stock solution of Aclidinium and Formoterol, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 6µg/ml & 200µg/ml solution and 10µl were injected in to the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stock solution Aclidinium and Formoterol, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 6µg/ml & 200µg/ml solution and10µl solutions were injected in to the system and the chromatograms were record edto assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution Aclidinium and Formoterol, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 6µg/ml & 200µg/ml solution and 10µl 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 placedinovenat105°C for1h to study dry heat degradation. For HPL C study, the resultant solution was diluted to 6µg/ml & 200µg/ml solution and 10µl 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 60µg/ml & 2000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200Watts hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 6µg/ml & 200µg/ml solutions and 10µl 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 refluxingthedruginwaterfor1hr at temperature of 60º. For HPLC study, the resultant solution was diluted to 6µg/ml and 200µg/ml solution and 10µl were injected in to the system. The chromatograms were recorded to assess the stability of the sample.

Results and discussion

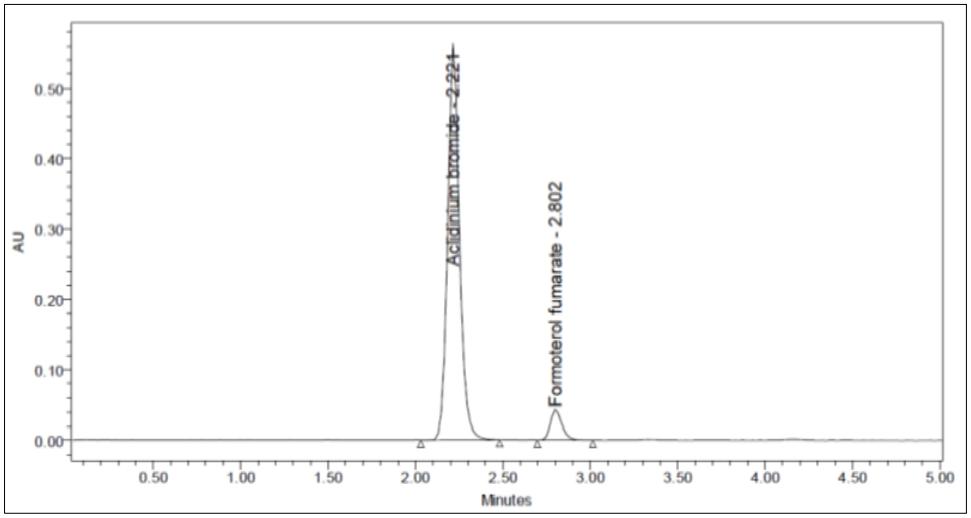

Fig 4: Estimation of aclidinium and formoterol fumarate in pharmaceutical dosage form

Method Validation [6, 7]

The described method has been validated for the assay of aclidinium and formoterol fumarate by using following parameters

Accuracy

The accuracy of the method was determined by recovery experiments. Placebo was spiked with known quantities of standard drugs at levels of 50% to 150% of Abel claim. The recovery studies were carried out 3 times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in table-1, 2.

Table 1: Accuracytable of the aclidinium.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	3	3.048	101.58	100.57%
	3	2.995	99.83	
	3	3.018	100.61	
100%	6	5.998	99.96	
	6	5.913	98.56	
	6	6.085	101.42	
150%	9	9.082	100.92	
	9	9.115	101.28	
	9	9.088	100.98	

Table 2: Accuracy table of the formoterol.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	100	101.04	101.04	100.41%
	100	100.09	100.09	
	100	99.36	99.36	
100%	200	197.08	98.54	
	200	202.66	101.33	
	200	201.68	100.84	
150%	300	305.60	101.87	
	300	303.54	101.18	
	300	298.37	99.46	

System suitability studies.

The system suitability test was carried out on freshly prepared stock solution of acalabrutinib to check various parameters such as column efficiency, tailing factor and theoretical are presented in below table3.The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within the table 3.

Table 3: System suitability studies

S no	Aclidinium			Formoterol			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.217	4882	1.12	2.791	8271	1.17	4.5
2	2.219	5052	1.13	2.796	8604	1.24	4.6
3	2.221	5471	1.16	2.801	8665	1.21	4.7
4	2.224	5651	1.18	2.806	8407	1.21	4.8
5	2.226	6108	1.19	2.810	8647	1.22	4.8
6	2.227	5644	1.14	2.810	8479	1.20	4.8

LOD and LOQ

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LODis the smallest concentration of the analyse that give the measurable response. The LOD for aclidinium bromide and formoterol fumarate was found to be 1.0 and 0.12 respectively.

LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified [signal to noise ratio of 10]. The LOQ was 0.33 and0.04 for aclidinium bromide and formoterol fumarate.

Linearity and range

Linearity was studied by preparing standard solution at six different concentration levels. The linearity range was found to be Formoterol (1.5-9.0µg/ml) and Aclidinium (50-300µg/ml) 20µl of each solution was injected into chromatograph. Peak area was recorded for all the chromatogram. Calibration curve was constructed by plotting peak area [y axis] against amount of the drug in µg /ml [x axis]. Peak area of linearity range and the parameters were calculated and presented in table 4. The linearity curve of aclidinium bromide and formoterol fumarate was shown in the fig: 5, 6.

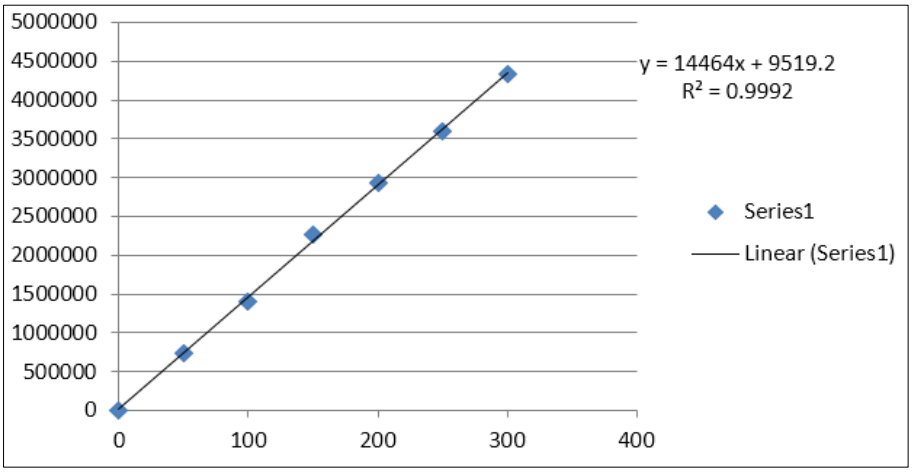

Fig 5: The linearity curve of the aclidinium concentration VS peak area

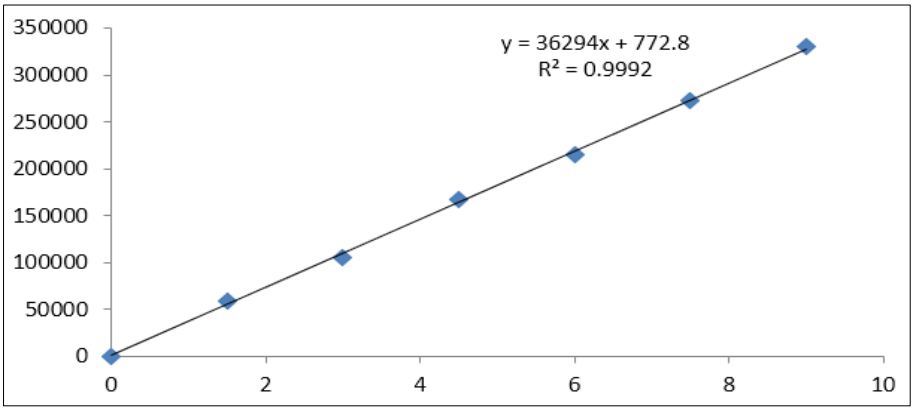

Fig 6: The linearity curve of the formoterolfumarate concentration VS peak area

Table 4: Results of the linearity curve

Aclidinium		Formoterol	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
50	731696	1.5	59199
100	1400610	3	105260
150	2265402	4.5	167729
200	2924817	6	214643
250	3605627	7.5	272138
300	4325971	9	329689

System precision: The system precision of the method was established by six replicate injections of the standard solution containing aclidinium bromide and formoterol fumarate. The percentage RSD were Calculated and presented in table 5. From the data obtained, the developed RP-HPLCmethod was found to precise.

Table 5: System precision results

S. No	Area of Aclidinium	Area of formoterol
1.	2889507	214077
2.	2857964	218121
3.	2810628	217917
4.	2895749	212452
5.	2879868	211704
6.	2844254	212883
Mean	2862995	214526
S.D	32184.6	2813.6
%RSD	1.1	1.3

Robustness

Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms. The results of robustness were presented in the table-6.

Table 6: Method robustness of aclidinium and formoterol in the dosage forms

S.no	Condition	%RSD of Aclidinium	%RSD of Formoterol
1	Flow rate (-) 0.9ml/min	1.1	1.3
2	Flow rate (+) 1.1ml/min	0.7	0.6
3	Mobile phase (-) 70B:30A	0.4	0.6
4	Mobile phase (+) 60B:40A	0.4	0.3
5	Temperature (-) 25°C	0.9	1.1
6	Temperature (+) 35°C	0.8	0.9

Assay of the marketed formulation

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated assay data was given in the table-7, 8.

Table 7: Assay data of the aclidinium.

S.no	Standard Area	Sample area	% Assay
1	2889507	2839292	98.97
2	2857964	2853166	99.46
3	2810628	2873212	100.16
4	2895749	2902067	101.16
5	2879868	2902180	101.17
6	2844254	2863168	99.81
Avg	2862995	2872181	100.12
Stdev	32184.6	25763.0	0.898
%RSD	1.1	0.9	0.9

Table 8: Assay data of the formoterol

S.no	Standard Area	Sample area	% Assay
1	214077	215133	100.08
2	218121	218649	101.72
3	217917	218666	101.73
4	212452	217256	101.07
5	211704	213065	99.12
6	212883	213131	99.15
Avg	214526	215983	100.48
Stdev	2813.9	2580.6	1.201
%RSD	1.3	1.2	1.2

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Aclidinium and Formoterol in bulk and dosage form. Retention time of Aclidinium and Formoterol were found to be 2.221 min and 2.802 min. %RSD of the Aclidinium and Formoterol were and found to be 0.9 and 1.2 respectively. %Recovery was obtained as 100.41% and 100.57% for Aclidinium and Formoterol respectively. LOD, LOQ values obtained from regression equations of Aclidinium and Formoterol were 0.33, 1.0 and 0.04, 0.12 respectively. Regression equation of Formoterol is y =36294x + 772.8, and y = 14464x + 9519 of Aclidinium. 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.

References

1. Khalid AM, Attia Nasr M, El-Abasawi, Ahmed El-Olemy, Ahmed Serag. Different Spectrophotometric Methods Manipulating Ratio Spectra Applied for the Analysis of Aclidinium in Duaklir® Genuair® Inhalation Powder. Hindawi Journal of Spectroscopy, 2018.
2. Ravi Chikke Gowda. Simultaneous RP-HPLC Method For Determination Of Impurities In Formoterol Fumarate And Aclidinium Bromide In Pharmaceutical Dosage Forms, Chemistry Published, 2016.
3. Srinivasu K, Venkateswara Rao J *et al*. Simultaneous RP-HPLC Method for The Estimation of Formoterol fumarate And Tiotropium Bromide In Pharmaceutical Dosage Forms, Asian Journal Of Chemistry. 2010; 22(5):3943-3948.
4. Rakshit Kanubhai Trivedi *et al*. A Rapid, Stability-Indicating RP-HPLC Method for The Simultaneous Determination of Formoterol Fumarate, Tiotropium Bromide, And Ciclesonide In A Pulmonary Drug Product, Sci Pharm. 2012; 80:591-603.
5. Samuel Akapo O, Muhammad Asif *et al*. Validation of A RP-HPLC Method For The Assay Of Formoterol And Its Related Substances In Formoterol Fumarate Dihydrate Drug Substance. Journal of Pharmaceutical and Biomedical Analysis. 2003; 33(5):935-945.
6. 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.
7. ICH. Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA, Geneva, 1996.