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A Novel RP-HPLC Method for the Simultaneous Estimation of Aclidinium Bromide and Formoterol Fumarate in Bulk and Pharmaceutical Dosage Forms with Stability Studies

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Keywords: Formoterol, Aclidinium, Stability Studies, Validation, Robustness & RP-HPLC

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Formoterol and Aclidiniumin Pharmaceutical dosage form. Chromatogram was run through StdAzilent150 x 4.6 mm, 5µ. Mobile phase containing Buffer Kh2po4: 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 KH_2PO_4 buffer. Retention time of Formoterol and Aclidinium 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.

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

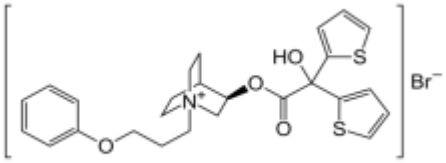

Fig 1: structure of Aclidinium bromide

Formoterol fumarate (E)-but-2-enedioicacid;N-[2-hydroxy-5-[(1R)-1-hydroxy-2-[[(2R)-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). An increased cyclic AMP level 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

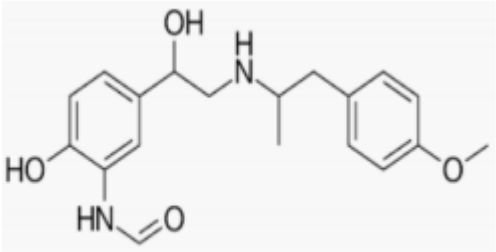

Fig 2: Structure of formoterol fumarate

Literature review of the selected combination of drugs reveals that only two analytical methods have been used to evaluate the selected combination of drugs (1-5). Here an attempt has been made to evaluate the selected combination of drugs i.e. Aclidinium Bromide and Formoterol fumarate by using a novel RP-HPLC method.

MATERIALS AND METHODS

Chemicals and Reagents

- Formoterol and Aclidinium pure drugs (API), Combination Formoterol fumarate and Aclidinium bromide inhaler (Duaklir®), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instruments:

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Autosampler 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 Formoterol and Aclidinium solutions.

Methods:

Diluent: Based upon the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

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

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

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

Preparation of buffer:

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

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen orthophosphate 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.

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of

Formoterol (6ppm) and Aclidinium (200ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

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

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

Preparation of Sample solutions: The contents of nasal spray delivered by 50 actuations (1.2&40 mcg each) were collected in 10 ml volumetric flask. Then 8ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 12&400 µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm 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. (6µg/ml of Formoterol and 200µg/ml of Aclidinium)

Linearity:

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

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (1.5µg/ml of Formoterol and 50µg/ml of Aclidinium)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (3µg/ml of Formoterol and 100µg/ml of Aclidinium)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (4.5µg/ml of Formoterol and 150µg/ml of Aclidinium)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (6.0µg/ml of Formoterol and 200µg/ml of Aclidinium)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (7.5µg/ml of Formoterol and 250µg/ml of Aclidinium)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (9.0µg/ml of Formoterol and 300µg/ml of Aclidinium)

Accuracy:

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

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and

temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines.

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

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Formoterol, Aclidinium, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Formoterol, Aclidinium, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies:

Oxidation:

To 1 ml of stock solution of Formoterol and Aclidinium, 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 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution Formoterol and Aclidinium, 1 ml 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 and 10 µl 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 Formoterol and Aclidinium, 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 10 µl 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 1 h to study dry heat degradation. For HPLC 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 200 Watt hours/m^2 in photostability chamber. For HPLC study, the resultant solution was diluted to obtain 6µg/ml & 200µg/ml solutions and 10 µl 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 1hr at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to 6µg/ml & 200µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

Method Validation: The validation of the Process carried out was validated as per ICH guidelines and the following parameters were reported as follows: (6-7)

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table No. 1: System Suitability of Aclidinium Bromide and Formoterol Fumarate

S. No.	Aclidinium			Formoterol			
Inj	RT(min)	USP late Count	Tailing	RT (min)	USP Plat 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

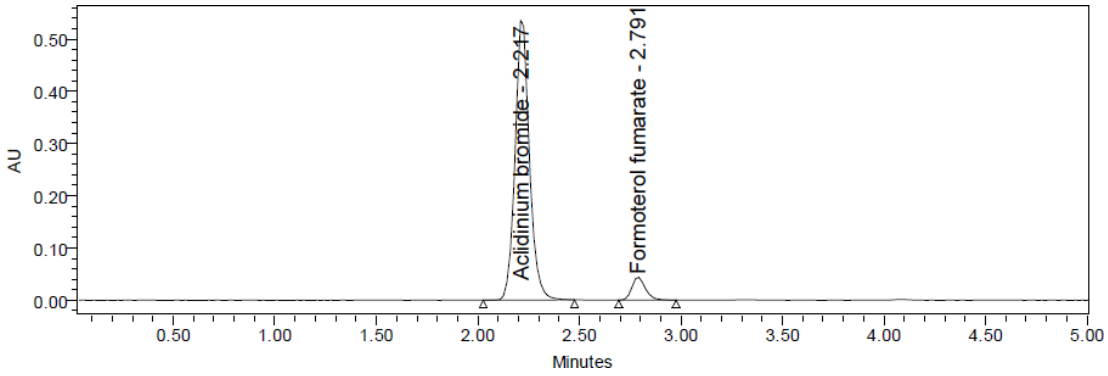

Figure No. 3: System suitability Chromatogram

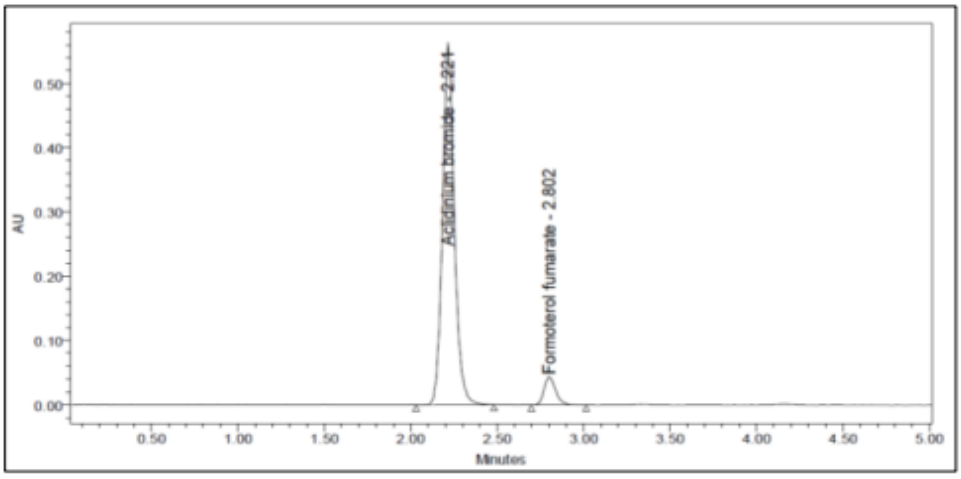

Fig 4: Estimation of aclidinium and formoterol fumarate in Pharmaceutical Dosage form

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 as follows:

Table No. 2: Accuracy table of Formoterol

% 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 No. 3: Accuracy table of Aclidinium

% 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	

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 LOD is 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 and 0.04 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 fig: 5, 6.

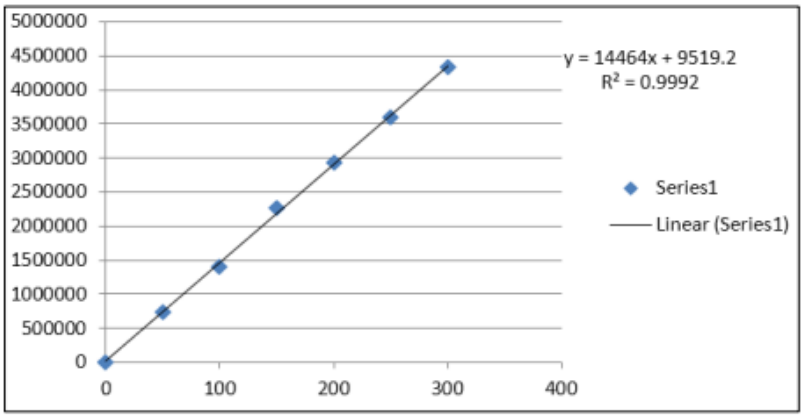

Fig 5: The linearity curve of the aclinidium concentration Vs peak area

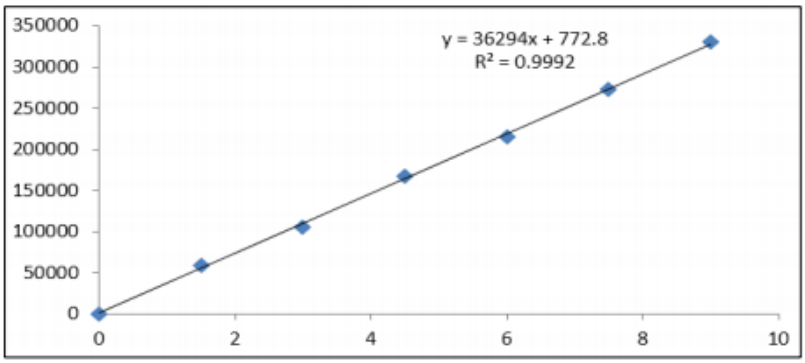

Fig 6: The linearity curve of the formeterol fumarate Vs peak area

Table No. 4: Linearity table for Formoterol and Aclidinium

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

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 was calculated and presented in table 5. From the data obtained, the developed RP-HPLC method was found to precise.

Table No. 5: System precision table of Formoterol and Aclidinium

S. No	Area of Formoterol	Area of Aclidinium
1.	214077	2889507
2.	218121	2857964
3.	217917	2810628
4.	212452	2895749
5.	211704	2879868
6.	212883	2844254
Mean	214526	2862995
S.D	2813.9	321846
%RSD	1.3	1.1

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 No. 6: Robustness data for Formoterol and Aclidinium

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 No. 7: Assay Data of 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

Table No. 8: Assay Data of 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

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

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REFERENCES

1. Khalid A M, Attia Nasr M, El-Abasawi, Ahmed ElOlemy, AhmedSerag. 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.

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