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STABILITY INDICATING DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF FORMOTEROL AND ACLIDINIUM RP-HPLC METHOD

Syed Reehana Saleen*[1], P. Sreenivasa Prasanna[2] and K. Thejomoorthy[3]

[1]Department of Pharmaceutical analysis, M.L. College of Pharmacy, S. Konda-523101.
[2]Principal, M.L. College of Pharmacy, S.Konda-523101.
[3]Head, Department of Pharmaceutical analysis, M.L. College of Pharmacy, S. Konda-523101.

***Corresponding Author: Syed Reehana Saleen**

Department of Pharmaceutical analysis, M.L. College of Pharmacy, S. Konda-523101.

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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 Std BDS C8 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.01N 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 0.01N Kh2PO4 buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 230 nm. Retention time of Formoterol and Aclidinium were found to be 2.146 min and 2.698. %RSD of the Formoterol and Aclidinium were and found to be 0.3% and 0.6% respectively. %Recovery was obtained as 100.76% and 99.67% for Formoterol and Aclidinium respectively. LOD, LOQ values obtained from regression equations of Formoterol and Aclidinium were 0.05, 0.56 and 0.15, 1.69 respectively. Regression equation of Aclidinium is y = 13617x + 3371.1, and y = 5306.5x + 149.57 of Formoterol. Retention times were decreased and 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 is (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 C26H30BrNO4S2 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.

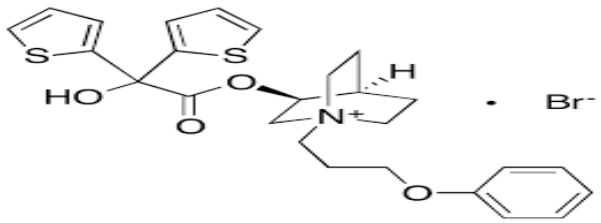

Figure1: structure of Aclidinium Bromide.

Formoterol fumarate. it is chemically called as (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 C42H56N4O14 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.

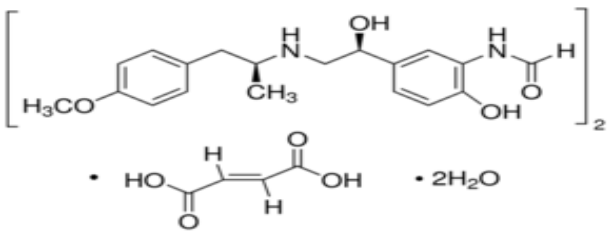

Figure2: structure of Formoterol fumarate.

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.

Methodology

Diluent: Based up on 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 100mg of Aclidinium, 3mg of Formoterol and transferred to 50ml and 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. (2000µg/ml of Aclidinium and 60µg/ml 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 stock solutions: The contents of nasal spray deliveried by 50 actuations (400mcg & 12mcgmcg each) were collected in 100 ml volumetric flask. Then 20ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 4000 & 120µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF) (4000µg/ml of Umeclidinium and 120µg/ml of Vilanterol)

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (200µg/ml of Aclidinium and 6 µg/ml of Formoterol)

Preparation of buffer

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

Buffer: 0.01N Potassium dihyrogen ortho phosphate

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 added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution

Validation[6-8]

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Aclidinium (200ppm) and Formoterol (6ppm) 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 100mg of Aclidinium, 3mg of Formoterol and transferred to 50ml and 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. (2000µg/ml of Aclidinium and 60µg/ml 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)

Linearity

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

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

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

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (200µg/ml of Aclidinium and 6µg/ml of Formoterol)

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

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (300µg/ml of Aclidinium and 9µg/ml of Formoterol)

Accuracy

Preparation of Standard stock solutions: Accurately weighed 100mg of Aclidinium, 3mg of Formoterol and transferred to 50ml and 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. (2000µg/ml of Aclidinium and 60µg/ml Formoterol)

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 Guide lines.

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 affected 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 Aclidinium, Formoterol, 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 Aclidinium and Formoterol, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies[9-10]

Oxidation

To 1 ml of stock solution of Aclidinium and Formoterol, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 200µg/ml&6µg/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 s solution Aclidinium and Formoterol, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 200µg/ml&6µg/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 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 200µg/ml&6µg/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 200µg/ml&6µg/ml solution and10µl 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 2000µg/ml&6µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 200µg/ml&6µg/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 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 200µg/ml&6µg/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 development: Method development was done by changing various, mobile phase ratios, buffers etc.

Optimized method:

Chromatographic conditions

Mobile phase : **6**5% 0.01N KH2PO4 (3.0PH): 35% Acetonitrile

Flow rate : 1 ml/min

Column : BDS C8 (4.6 x 150mm, 5µm)

Detector wave length : 230 nm

Column temperature : 30°C

Injection volume : 10µL

Run time : 5min

Diluent : Water and Acetonitrile in the ratio 50:50

RESULTS

Both peaks have good resolution, tailing Factor, theoretical plate count and resolution.

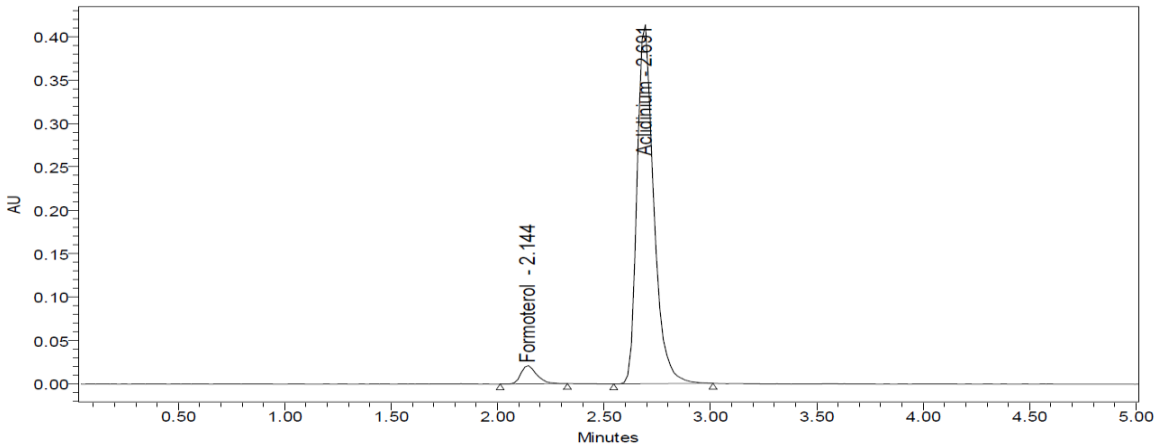

Fig 3- Optimized Chromatogram.

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

Table: 1 System suitability parameters for Aclidinium and Formoterol.

S no	Formoterol			Aclidinium			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.139	3941	1.24	2.688	6169	1.33	3.9
2	2.140	3903	1.25	2.692	6518	1.31	3.9
3	2.143	4321	1.19	2.692	6713	1.30	3.9
4	2.144	4216	1.19	2.694	6569	1.27	3.9
5	2.144	3866	1.22	2.694	6558	1.31	3.9
6	2.146	4240	1.24	2.698	5814	1.36	3.8

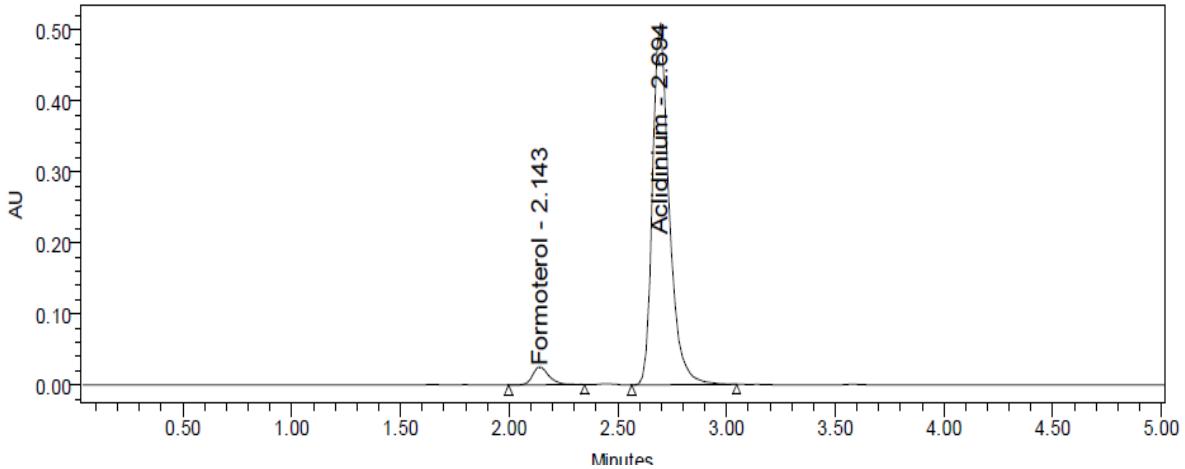

Fig 4 System suitability Chromatogram.

Validation
Specificity

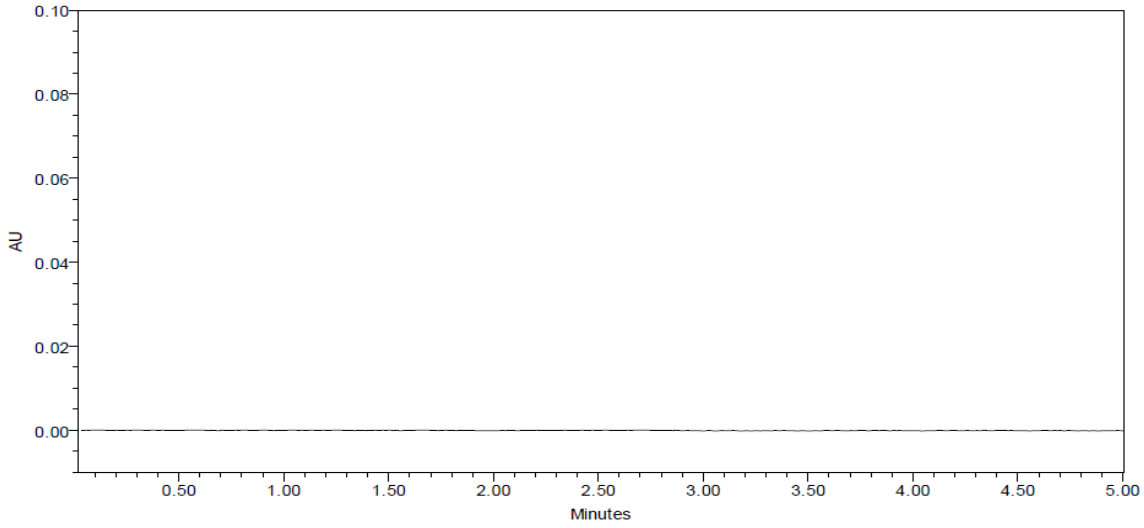

Figure 5. Chromatogram of blank.

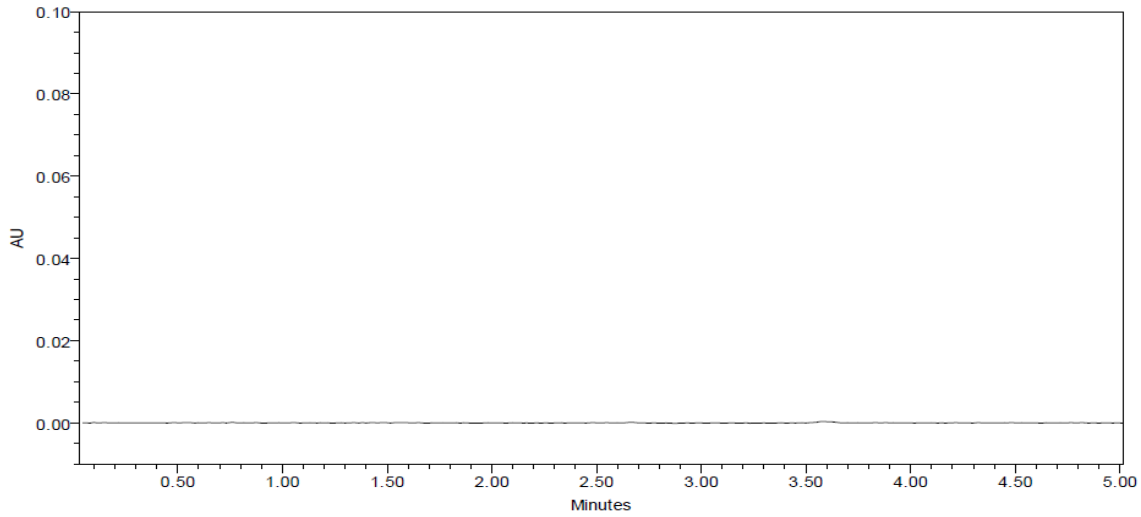

Figure No. 6 Chromatogram of placebo.

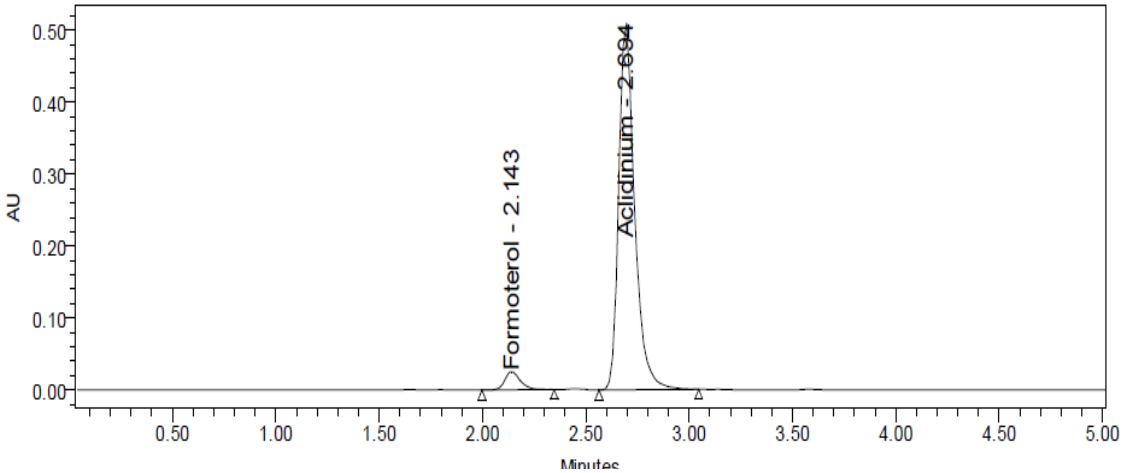

Fig 7 Typical Chromatogram.

Discussion: Retention times of Formoterol and Aclidinium were 2.143 min and 2.694 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Table 2 Linearity table for Aclidinium and Formoterol.

Aclidinium		Formoterol	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
50	703886	1.5	8186
100	1354997	3	16212
150	2029509	4.5	23901
200	2711219	6	32414
250	3450605	7.5	39429
300	4071497	9	48062

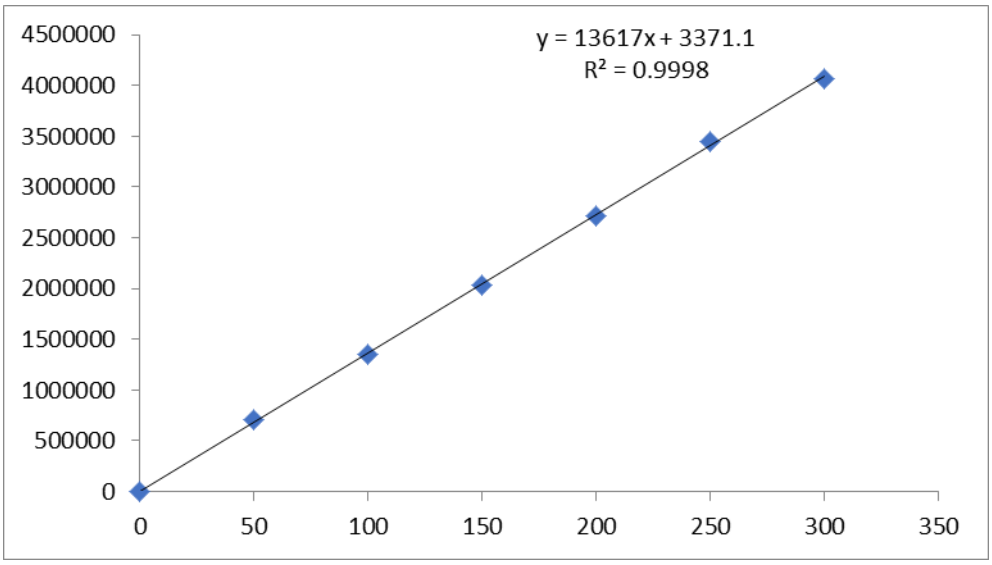

Fig No. 8 Calibration curve of Aclidinium.

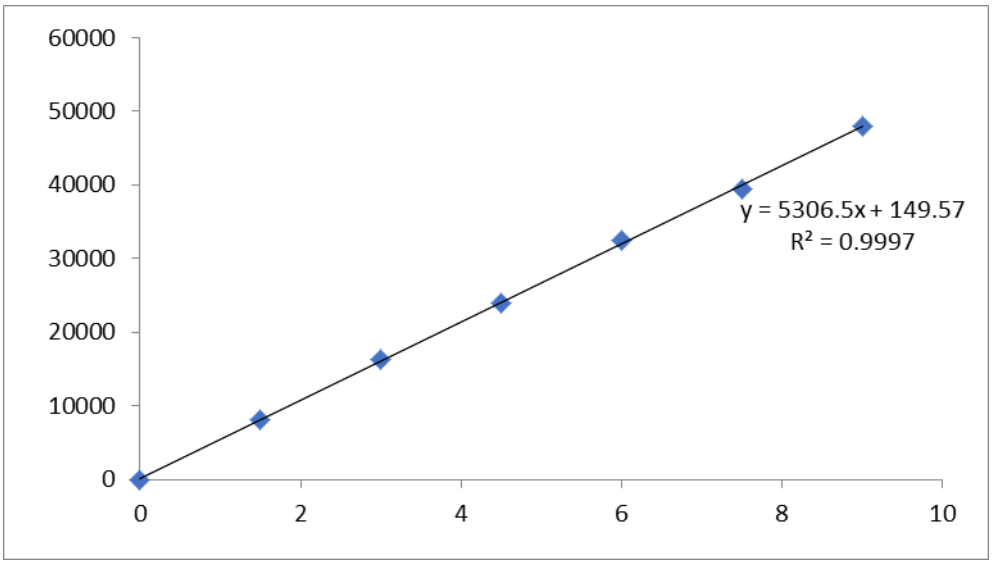

Fig No. 9 Calibration curve of Formoterol.

Precision

System Precision

Table 3 System precision table of Aclidinium and Formoterol.

S. No	Area of Aclidinium	Area of Formoterol
1.	2707963	33060
2.	2687608	32897
3.	2698068	32825
4.	2704211	33091
5.	2713822	33055
6.	2720966	32979
Mean	2705440	32985
S.D	11760.2	105.0
%RSD	0.4	0.3

Repeatability

Table 4 Repeatability table of Aclidinium and Formoterol.

S. No	Area of Aclidinium	Area of Formoterol
1.	2684570	33139
2.	2698428	32961
3.	2713561	33028
4.	2699919	33198
5.	2728520	33187
6.	2702075	33089
Mean	2704512	33100
S.D	14966.6	93.1
%RSD	0.6	0.3

Intermediate precision (Day_ Day Precision)

Table 5 Intermediate precision table of Aclidinium and Formoterol.

S. No	Area of Aclidinium	Area of Formoterol
1.	2197589	30395
2.	2194947	30240
3.	2192994	30371
4.	2203457	30408
5.	2197517	30363
6.	2175869	30458
Mean	2193729	30373
S.D	9432.8	73.1
%RSD	0.4	0.2

Accuracy

Table 6 Accuracy table of Aclidinium.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	100	98.89	98.89	99.67%
	100	99.93	99.93	
	100	100.24	100.24	
100%	200	199.32	99.66	
	200	202.59	101.29	
	200	199.52	99.76	
150%	300	297.31	99.10	
	300	296.81	98.94	
	300	297.67	99.22	

Table 7 Accuracy table of Formoterol.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	3	3.054	101.81	100.76%
	3	3.028	100.92	
	3	3.036	101.21	
100%	6	6.113	101.88	
	6	5.917	98.62	
	6	6.109	101.82	
150%	9	9.113	101.26	
	9	8.945	99.39	
	9	8.996	99.96	

Sensitivity

Table 8 Sensitivity table of Aclidinium and Formoterol.

Molecule	LOD	LOQ
Aclidinium	0.56	1.69
Formoterol	0.05	0.15

Robustness

Table 9 Robustness data for Aclidinium and Formoterol.

S.no	Condition	%RSD of Aclidinium	%RSD of Formoterol
1	Flow rate (-) 1.1ml/min	0.6	1.0
2	Flow rate (+) 1.3ml/min	0.3	0.4
3	Mobile phase (-) 70B:30A	0.3	0.2
4	Mobile phase (+) 60B:40A	1.0	1.4
5	Temperature (-) 25°C	0.5	0.4
6	Temperature (+) 35°C	0.5	0.8

Assay: (**Duakliar pressair)**, bearing the label claim Aclidinium 400mcg, Formoterol 12mcg. Assay was performed with the above formulation. Average % Assay for Aclidinium and Formoterol obtained was 99.77 and 100.15% respectively

Table 0 Assay Data of Aclidinium.

S.no	Standard Area	Sample area	% Assay
1	2707963	2684570	99.03
2	2687608	2698428	99.54
3	2698068	2713561	100.10
4	2704211	2699919	99.60
5	2713822	2728520	100.65
6	2720966	2702075	99.68
Avg	2705440	2704512	99.77
Stdev	11760.2	14966.6	0.55
%RSD	0.4	0.6	0.55

.

Table 11 Assay Data of Formoterol.

S.no	Standard Area	Sample area	% Assay
1	33060	33139	100.27
2	32897	32961	99.73
3	32825	33028	99.93
4	33091	33198	100.45
5	33055	33187	100.41
6	32979	33089	100.12
Avg	32985	33100	100.15
Stdev	105.0	93.1	0.3
%RSD	0.3	0.3	0.3

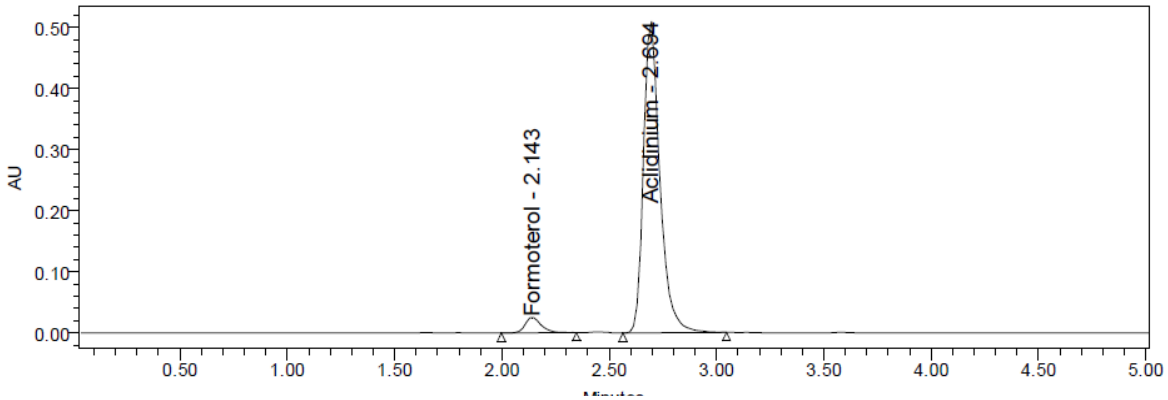

Fig 10 Chromatogram of working standard solution.

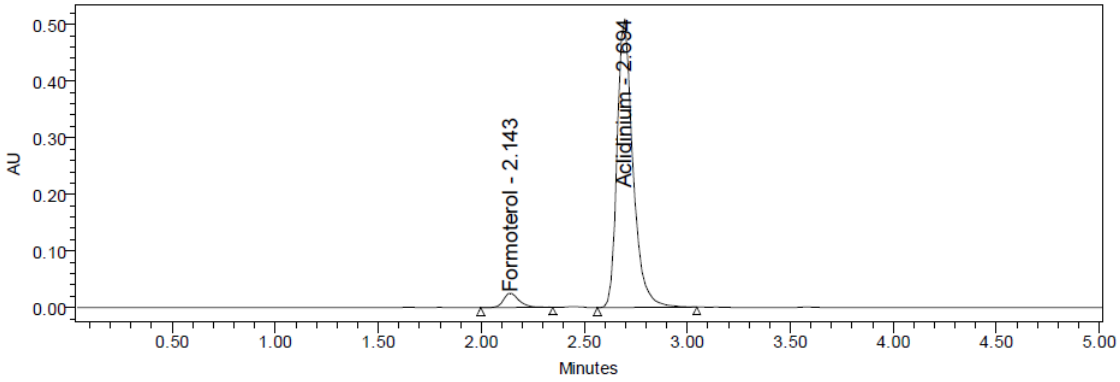

Fig No. 11 Chromatogram of marketed sample solution.

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table 12 Degradation Data of Aclidinium.

S.NO	Degradation Condition	Aclidinium			Formoterol		
		% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.14	0.141	0.275	5.01	0.346	0.432
2	Alkali	5.95	0.144	0.275	5.45	0.312	0.462
3	Oxidation	4.36	0.129	0.284	4.49	0.265	0.316
4	Thermal	4.74	0.156	0.284	2.60	0.287	0.452
5	UV	1.64	0.156	0.279	1.63	0.269	0.462
6	Water	0.86	0.120	0.275	0.68	0.288	0.491

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Formoterol and Aclidinium in Pharmaceutical dosage form. Temperature was maintained at 30°C. Optimized wavelength selected was 230 nm. Retention time of Formoterol and Aclidinium were found to be 2.146 min and 2.698. %RSD of the Formoterol and Aclidinium were and found to be 0.3% and 0.6% respectively. %Recovery was obtained as 100.76% and 99.67% for Formoterol and Aclidinium respectively. LOD, LOQ values obtained from regression equations of Formoterol and Aclidinium were 0.05, 0.56 and 0.15, 1.69 respectively. Regression equation of Aclidinium is y = 13617x + 3371.1, and y = 5306.5x + 149.57 of Formoterol. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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