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A New Separation Technique for Method, Development and Validation of Aclidinium Bromide and Formoterol Fumarate in Its Pure and Pharmaceutical Dosage Form by Using Rp-HPLC

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Abstract

A new simple, precise, selective and accurate, a new method of development and validation using Rp-HPLC for the estimation of Aclidinium bromide and Formoterol Fumarate in its pure and pharmaceutical dosage form. Chromatogram was run through DIKMA Spursil, C18 segment (4.6×150 mm, 5µ0). Mobile phases contain 0.1% OPA: Acetonitrile (30:70) with the use of the 0.1% OPA buffer, stream rate of 1 ml/min and measured in 280 nm. The run time was found to be 1.965 mins and 3.826 mins and precision of Aclidinium and Formoterol was found to be 0.7, 0.2. The accuracy of Aclidinium and Formoterol were found to be 99.74 and 100.53. % recovery obtained in Aclidinium and Formoterol 99.74% and 100.53%. LOD, LOQ results were 3.03, 3.00 and 10.02, 10.00% of purity of Aclidinium and Formoterol was found to be 100.14% and 99.92%. The method exhibits precision of 0.4 and 0.2 for Aclidinium and Formoterol, which shows that the method is repeatable when performed on different days as well. The acceptance threshold of intermediate precision is RSD should not be more than 2.0%. The range of 98.0% to 102.0% should be the percentage recovery, which is the accuracy limit. Validation of the suggested methodology indicates that Dolutegravir has good accuracy.

Keywords: Rp-HPLC, aclidinium bromide, formoterol fumarate, UV spectrophotometer, validation, development

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INTRODUCTION

Aclidinium Bromide & Formoterol Fumarate is sold under the trade name Duaklir Pressair [1–6]. Combination of Aclidinium Bromide, a long-acting anticholinergic and Formoterol Fumarate, a long-acting beta2-agonist (400/12µg twice daily) achieves improvements in lung function greater than monotherapy in patients with chronic obstructive pulmonary disease (COPD) and is approved in the European Union as a maintenance treatment [7]. The effect of this combination on symptoms of COPD and exacerbations is less well established. We examined these outcomes in a pre-specified analysis of pooled data from two 24-week, double-blind and parallel-group, active- and placebo-controlled, multicenter, randomized Phase III studies (ACLIFORM and AUGMENT). A literature review revealed some methods of

analysis in inhalation and human serum by voltammetry, in urine by gas chromatography mass spectrometry, Rp- HPLC, UV spectroscopy for the estimation of formoterol alone and in combination and chromatographic method for the determination of aclidinium and formoterol in their dosage form [8–15]. The main aim of the study are to study the physiochemical properties of medication (ph, pKa, solvency and sub atomic weight), selection of chromatographic condition (versatile stage, segments, stream rate and so on), optimization of technique, study of framework reasonableness boundaries, validation of proposed technique and applying created technique to showcased definition (Figures 1, 2) (Table 1).

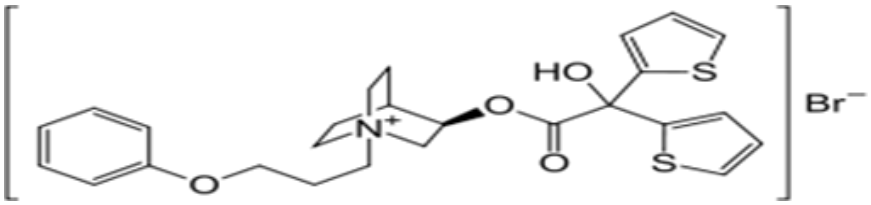


Figure 1. Aclidinium bromide.

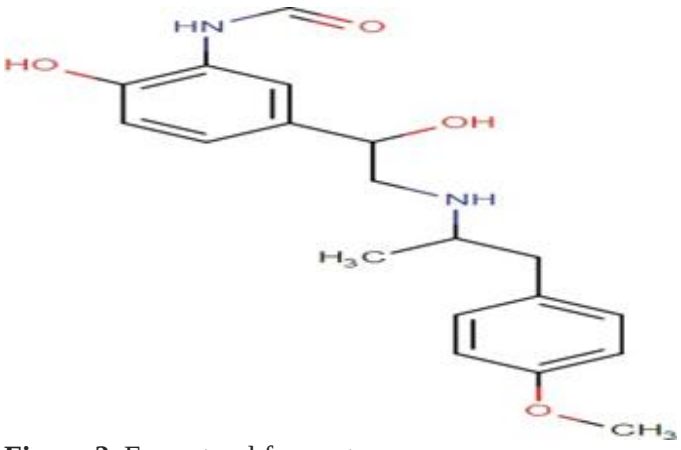


Figure 2. Formoterol fumarate.

Table 1. Properties of aclidinium bromide and formoterol fumarate.

Properties	**Aclidinium Bromide**	**Formoterol Fumarate**
Chemical name	(3R)-3-{[2-hydroxy-2,2-bis(thiophen-2-yl) acetyl]oxy}-1-(3-phenoxy propyl)-1-zabicyclo [2.2.2] octan-1-ium	N-[2-hydroxy-5-(1-hydroxy-2-{[1-(4-ethoxyphenyl)propan-2-yl]amino}ethyl)phenyl] formamide
Molecular formula	$C_{26}H_{30}NO_4S_2$	$C_{42}H_{56}N_4O_{14}$
Molecular weight	484.641gm/mol	840.9 gm/mol
Category	Adrenergic, Inhalants	Adrenergic Agents
Half life	2.4 minutes	10 hours
Solubility	Soluble in water; sparingly soluble in methanol R; practically insoluble in acetone R.	Slightly soluble in water
Mechanism of action	Aclidinium is a long-acting, competitive and reversible anticholinergic drug that is specific for the acetylcholine muscarinic receptors. It binds to all 5 muscarinic receptor subtypes to a similar affinity. Aclidinium's effects on the airways are mediated through the M3 receptor at the smooth muscle to cause bronchodilation	The pharmacologic effects of beta2-adrenoceptor agonist drugs, including formoterol, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes 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.

METHODOLOGY

Instruments Used

HPLC (WATERS software: Empower, 2695 separation module, 2487 UV detector), UV/Vis spectrophotometer (labindia UV 3000+), pH meter, Weighing machine, Pipette, Burette and Beakers.

Chemicals Used

Aclidinium Bromide, Formoterol fumarate (Supplied by Cipla Labs), KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Water, Ortho phosphoric acid.

Advanced Chromatographic Condition (Figure 3)

- Instrument used : Waters HPLC with auto sampler and UV indicator
- Temperature : Ambient (25ºC)
- Method of division : Isocratic mode
- Section : DIKMA Spursil, C18 segment (4.6 x 150 mm, 5 µ)
- Buffer : 0.1% OPA
- Mobile phase : 0.1% OPA: Acetonitrile (30:70)
- Stream rate : 1 ml for each min
- Wavelength : 280 nm
- Injection volume : 20 µL
- Run time : 10 min.

RESULT AND DISCUSSION

Chromatographic Conditions

- *Column* *:* DIKMA Spursil, C 18 column (4.6 x 150mm, 5µ)
- *Mobile phase ratio* *:* 0.1% OPA: Acetonitrile (30:70)
- *Detection wavelength :* 280 nm
- *Flow rate* *:* 1 ml/min
- *Injection volume* *:* 10 µl

Assay

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

Assay Results: (Aclidinium) = $\frac{2380851}{2372795.7} * \frac{340}{100} * \frac{1.0}{10} * \frac{100}{548} * \frac{10}{1.0} * \frac{528}{340} * \frac{99.8}{100} * 100 = 100.14\%$

Assay Results: (For Formoterol) = $\frac{96441.3}{96329.3} * \frac{12}{100} * \frac{1.0}{10} * \frac{100}{548} * \frac{10}{1.0} * \frac{548}{340} * \frac{99.8}{100} * 100 = 99.92\%$

Standard Solution Preparation

Aclidinium and Formoterol working doses should be precisely measured, transferred and diluted into a 100 ml clean, dry volumetric jar. Add around 70 mL of diluent and sonicate to completely breakdown the mixture and make the volume sufficient with the same dissolvable. (Stock configuration). Pipette 1.0 ml of the stock mixtures into a volumetric flagon with a 10 ml capacity and dilute with diluent to the proper strength. (340 g/ml and 12 g/ml, respectively) as shown in Figure 4(a).

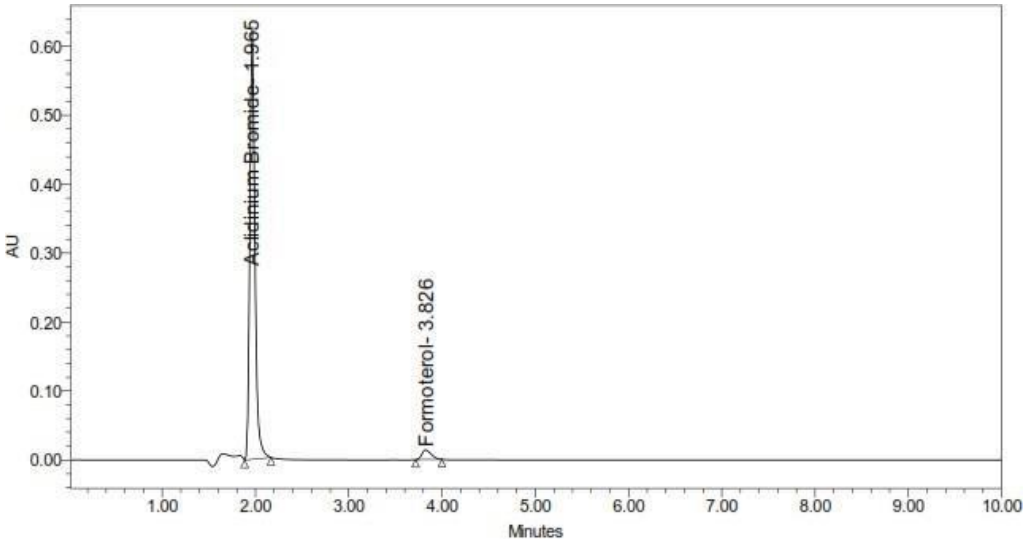


Figure 3. Chromatographic condition.

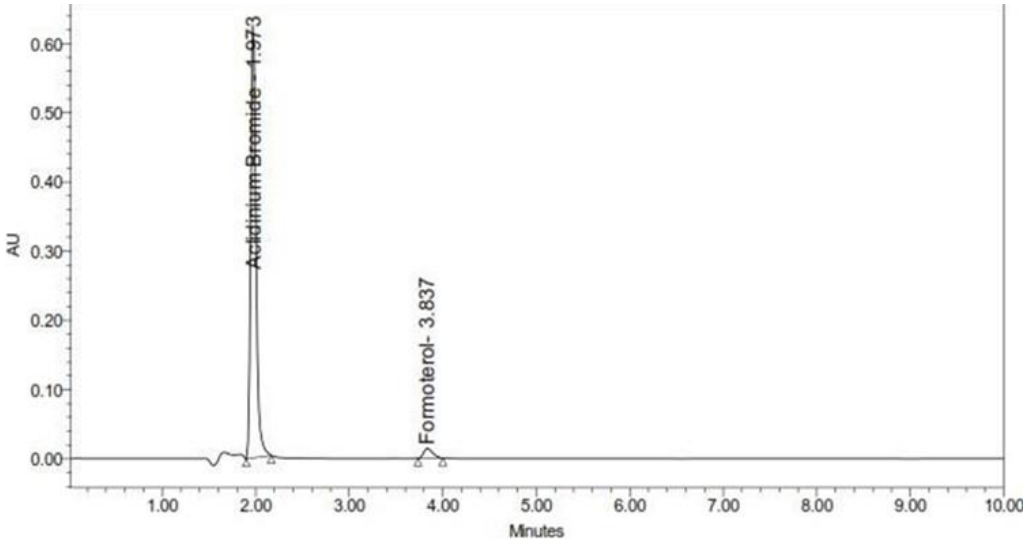


Figure 4(a). Chromatogram for standard.

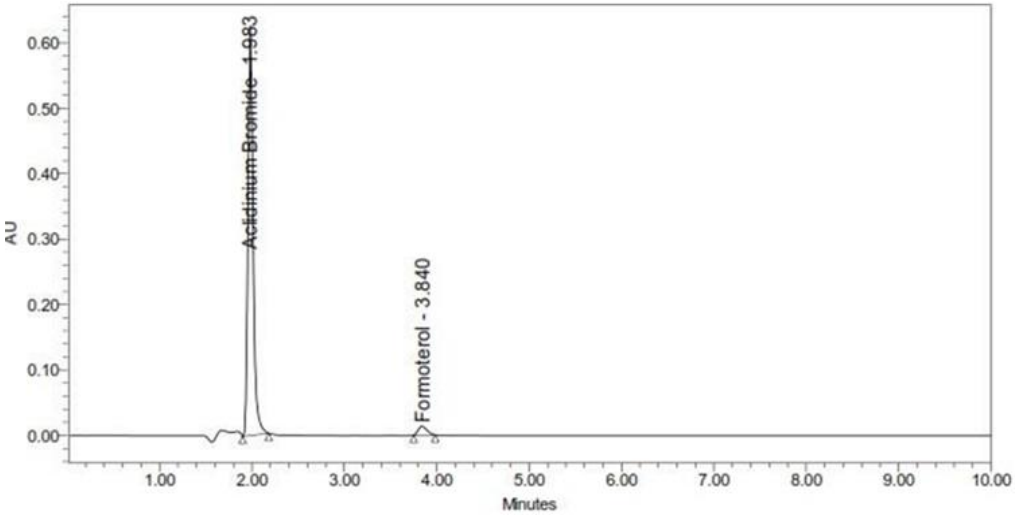


Figure 4(b). Chromatogram for sample.

Test Solution Preparation

Put a precise measurement of the inhalation powder–340 mg of Aclidinium and 12 mg of Formoterol–into a 100 ml clean, dry volumetric carafe. Add around 70 ml of diluent and sonicate to completely dissolve it and make the volume suitable for the same dissolvable. (Stock configuration) Pipette 1.0 ml of the stock mixtures into a volumetric flagon with a 10 ml capacity and dilute with diluent to the proper strength (340 g/ml and 12 g/ml, respectively) as shown in Figure 4b. Its system stability is shown in Figure 5 and Table 2.

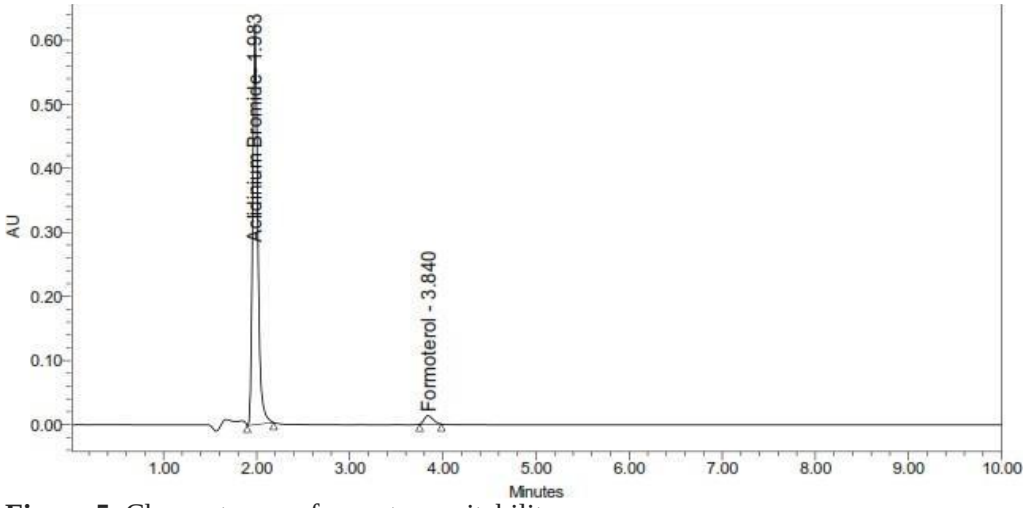


Figure 5. Chromatogram for system suitability.

Table 2. Results of system suitability parameter.

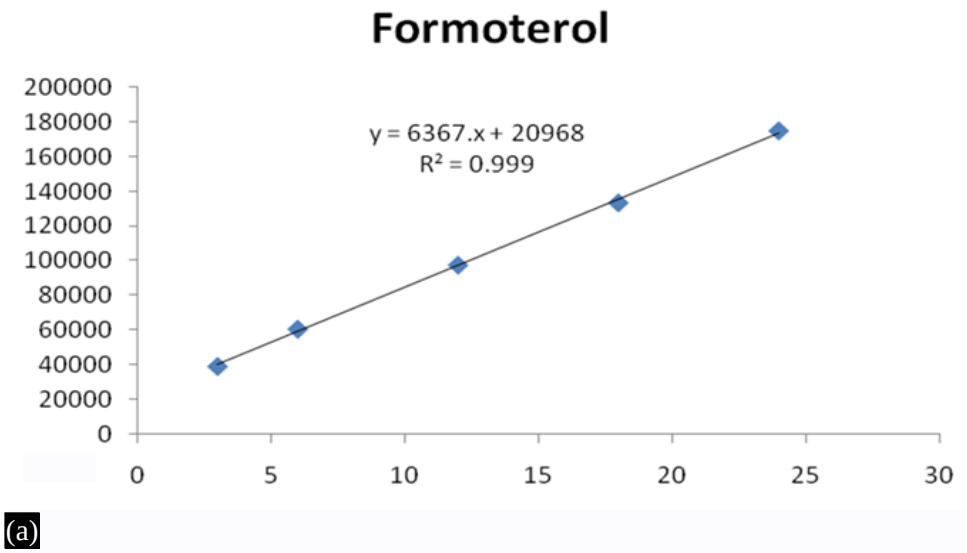
S.N.	Name	RT (min)	Area (µV sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Aclidinium	1.983	2397007	632448		1.22	5113.2
2	Formoterol	3.840	96218	13973	12.75	1.28	6945.14

Accuracy

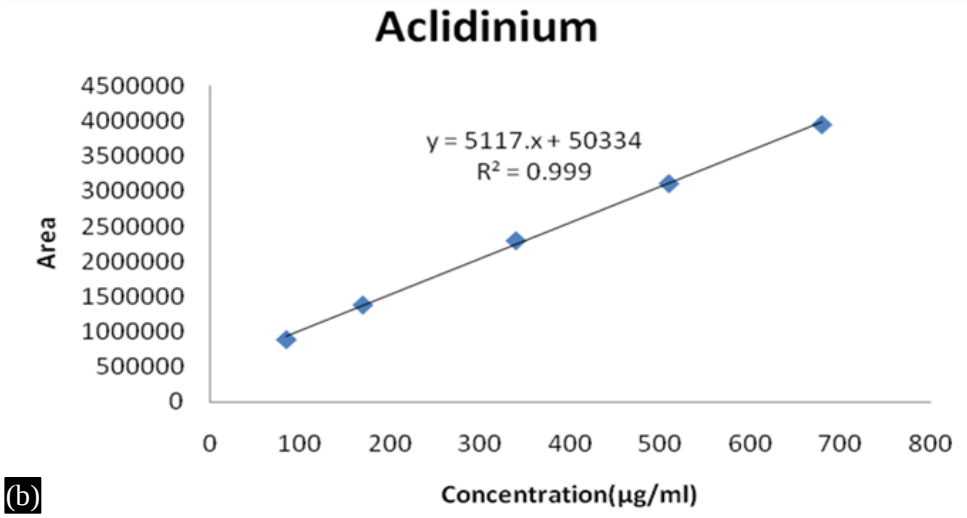
- *Readiness of stock arrangement:* In a 100 ml clean, dry volumetric jar, measure out and transfer 340 mg of Aclidinium and 12 mg of Formoterol as working standards. Add around 70 ml of diluent and sonicate to completely dissolve it and create volume sufficient with a similar dissolvable. (Stock configuration) Pipette 1.0 ml of the stock mixtures into a volumetric jar with a 10 ml capacity and dilute with diluent to the proper strength (Figures 6 and 7).
- *System:* The HPLC is used to estimate the territory for each of the six infusions of the standard arrangement. The %RSD for the area of six consecutive infusions was as close to zero as was possible (Tables 3–6). It is mentioned in Table 7 and Figure 8 a, b, c, d, e, f, g, h, i.
- *Acknowledgment criteria:* The results from the area of six standard infusions cannot have an RSD of more than 2%. The % RSD for the six recreated infusions' territory was viewed as internally as possible.
- *Explicitness:* The system is filled with blank and standard for specificity. No top is interfering in any way with how long scientific peaks are retained in memory.

Table 3. Area of different concentration of aclidinium and formoterol.

S.N.	Aclidinium		Formoterol	
	Concentration (µg/ml)	*Area*	*Concentration (µg/ml)*	*Area*
1	85	893096	3	39224
2	170	1386386	6	60703
3	340	2301704	12	97583
4	510	3115267	18	133512
5	680	3954729	24	174942

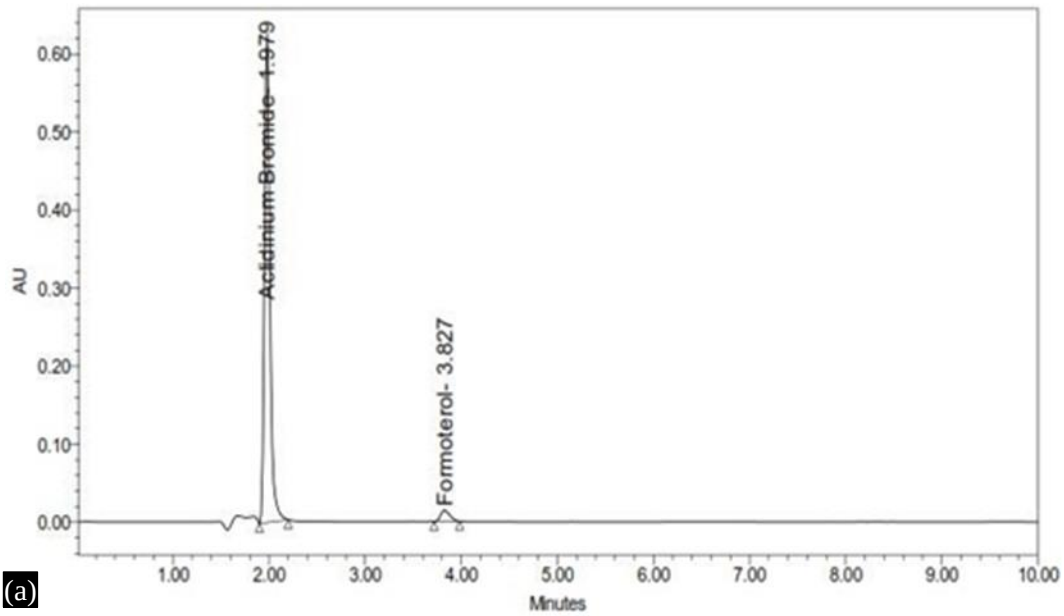


(a)

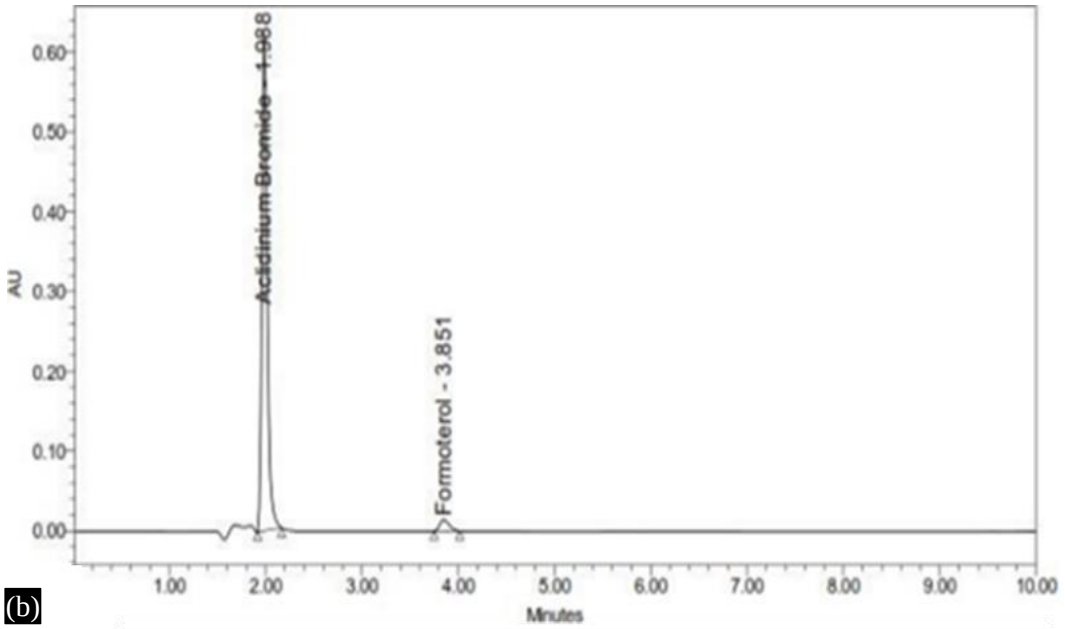


(b)

Figure 6. a and b show the calibration graph for formoterol and aclidinium.



(a)



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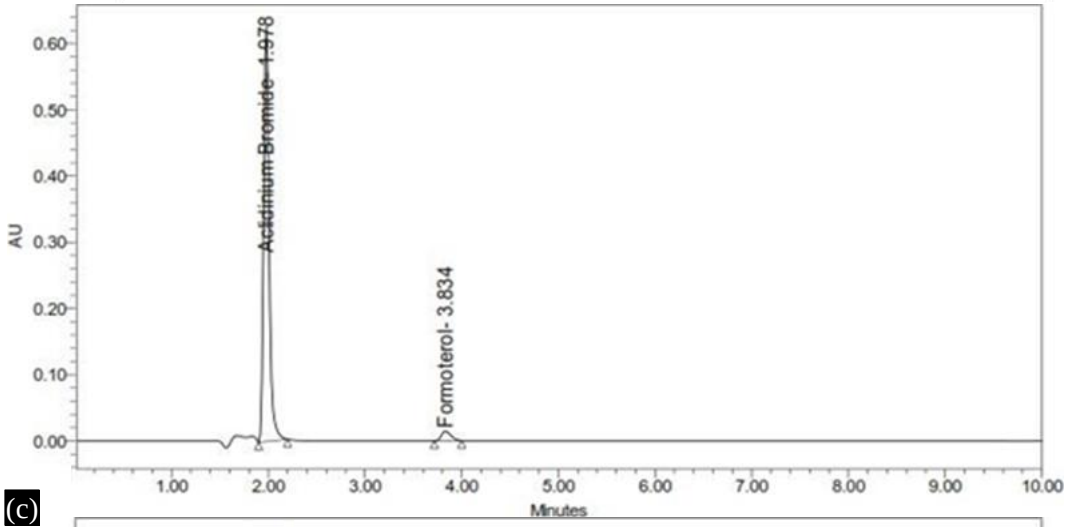

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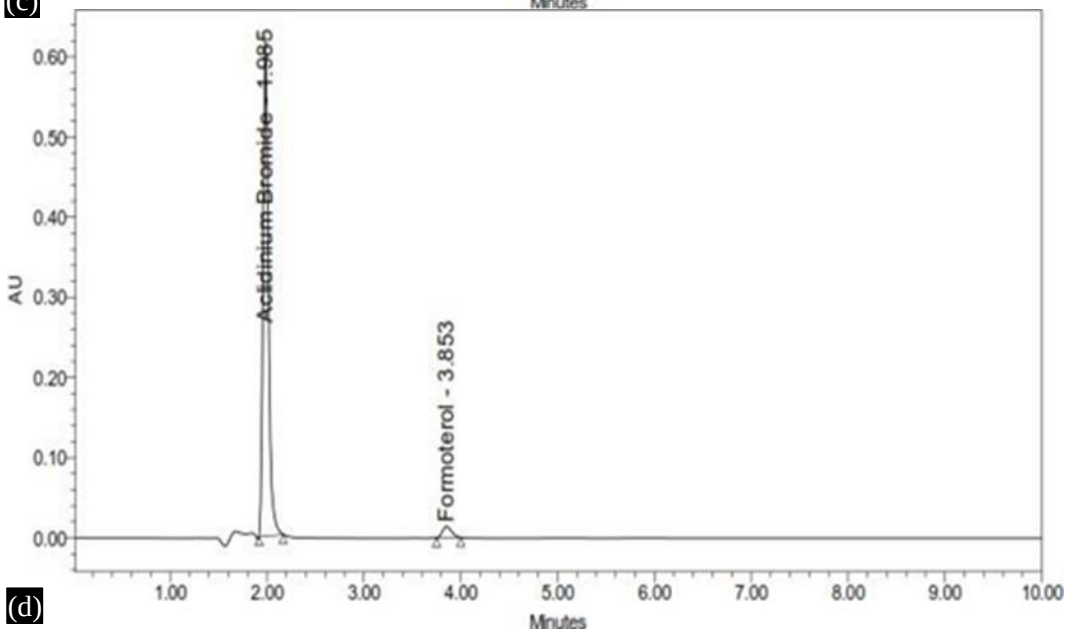


(d)

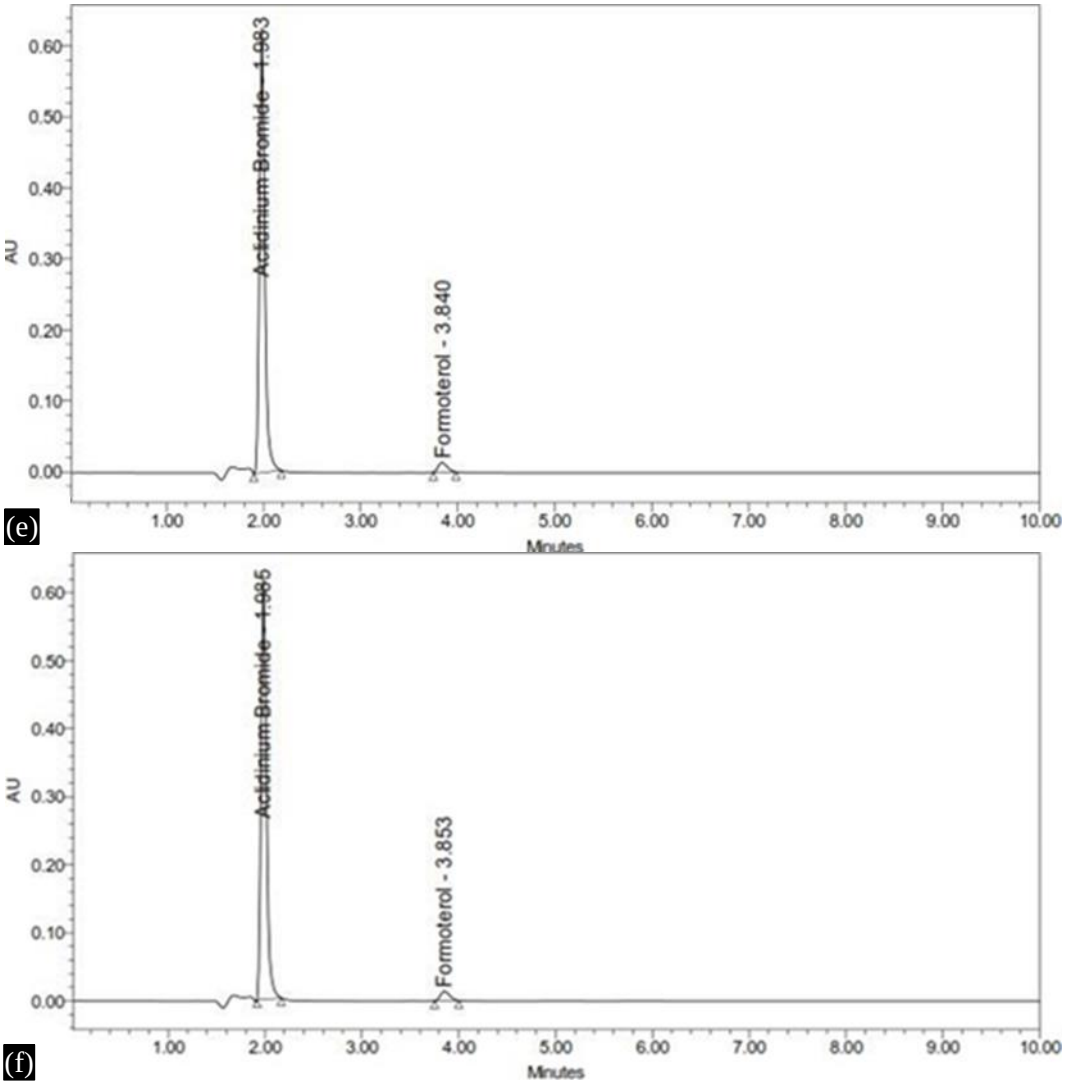
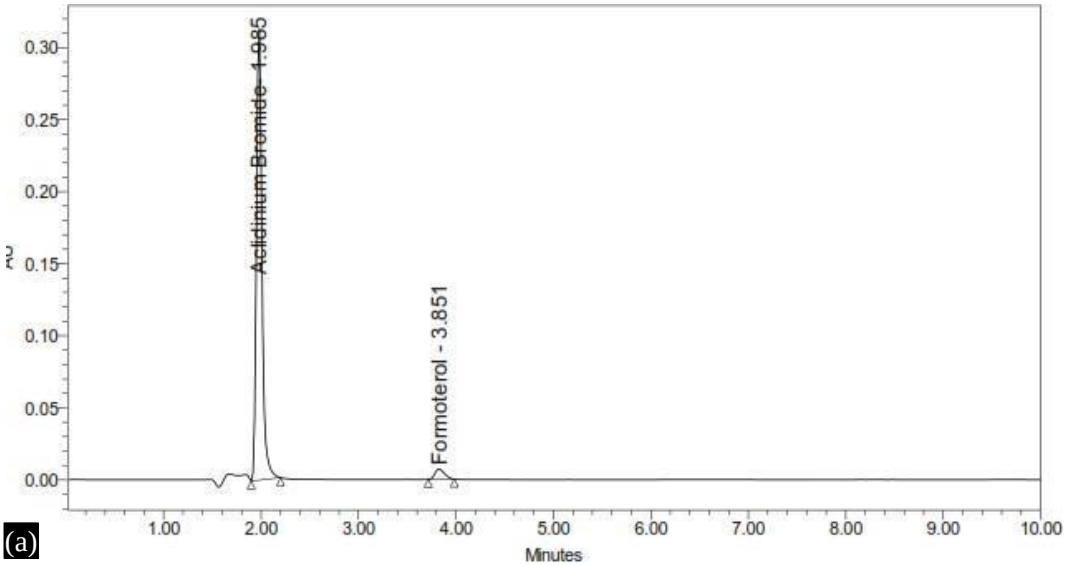
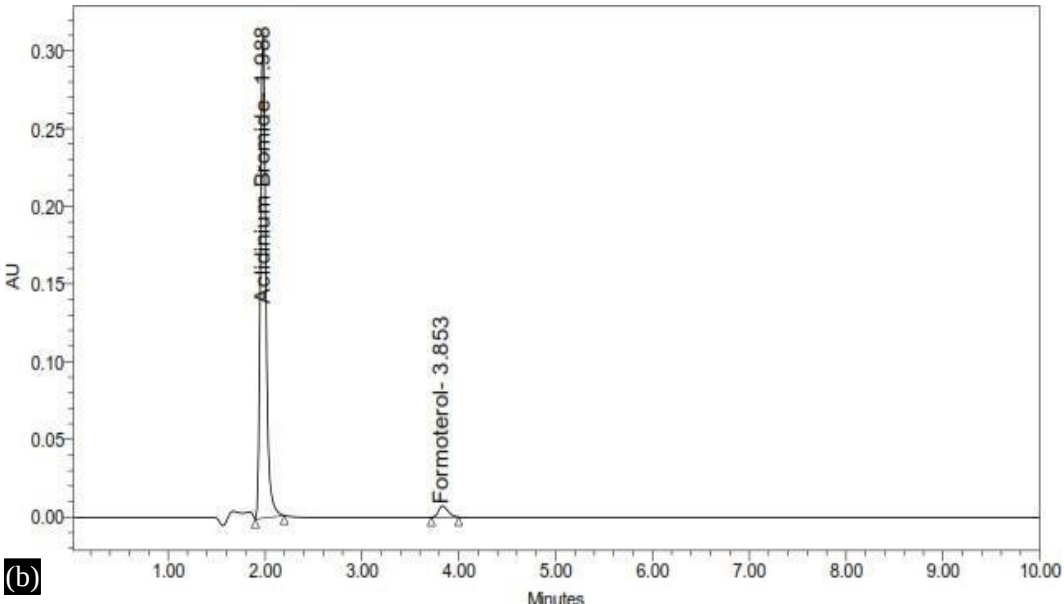
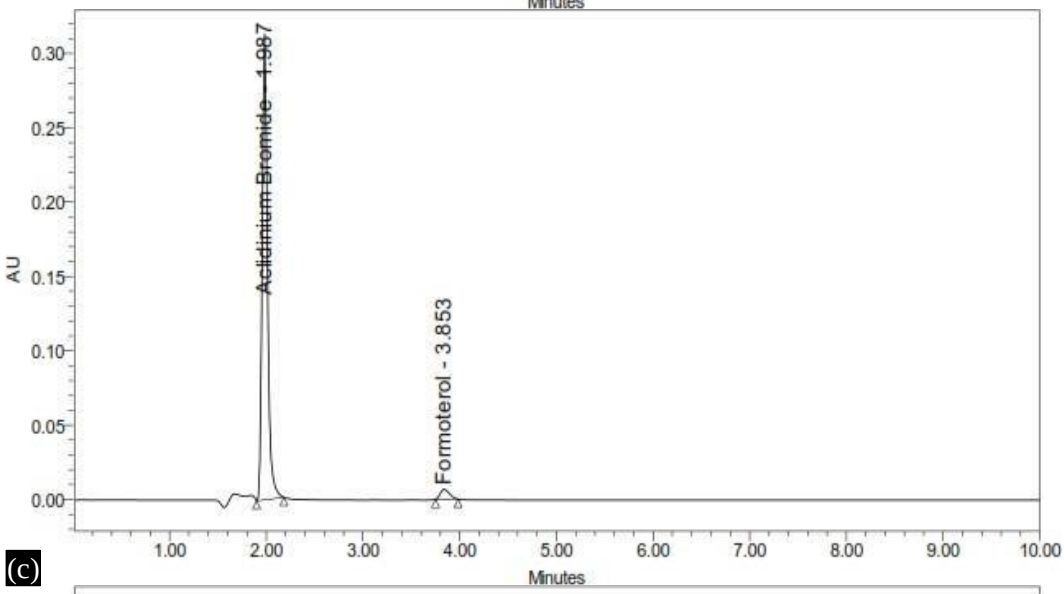


Figure 7. a = Chromatogram for precision -1, b = Chromatogram for precision -2, c = Chromatogram for precision -3, d = Chromatogram for precision -4, e = Chromatogram for precision -5 and f = Chromatogram for precision -6.

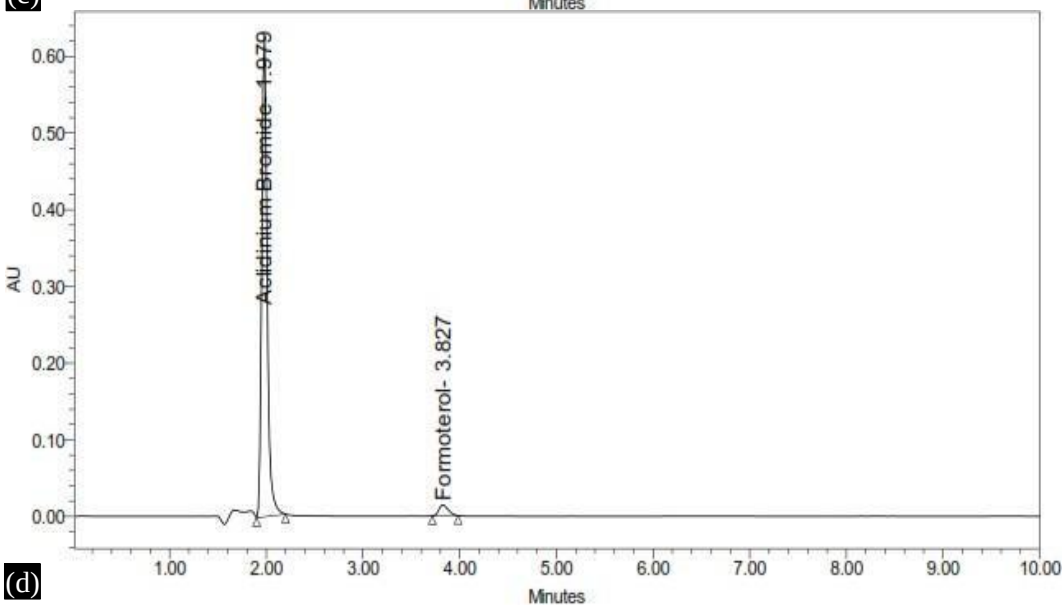




(b)



(c)



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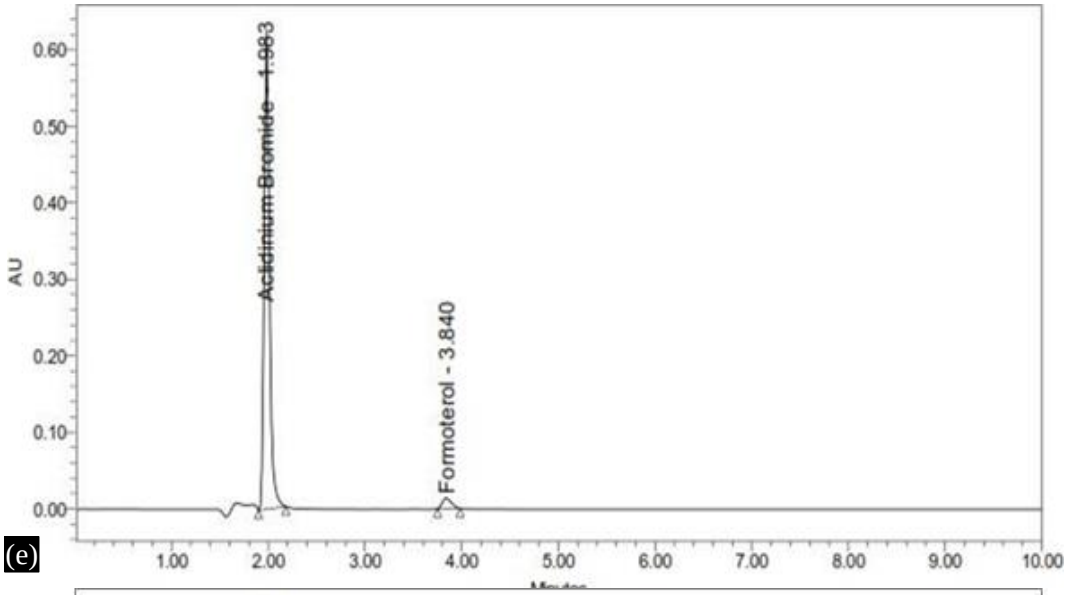

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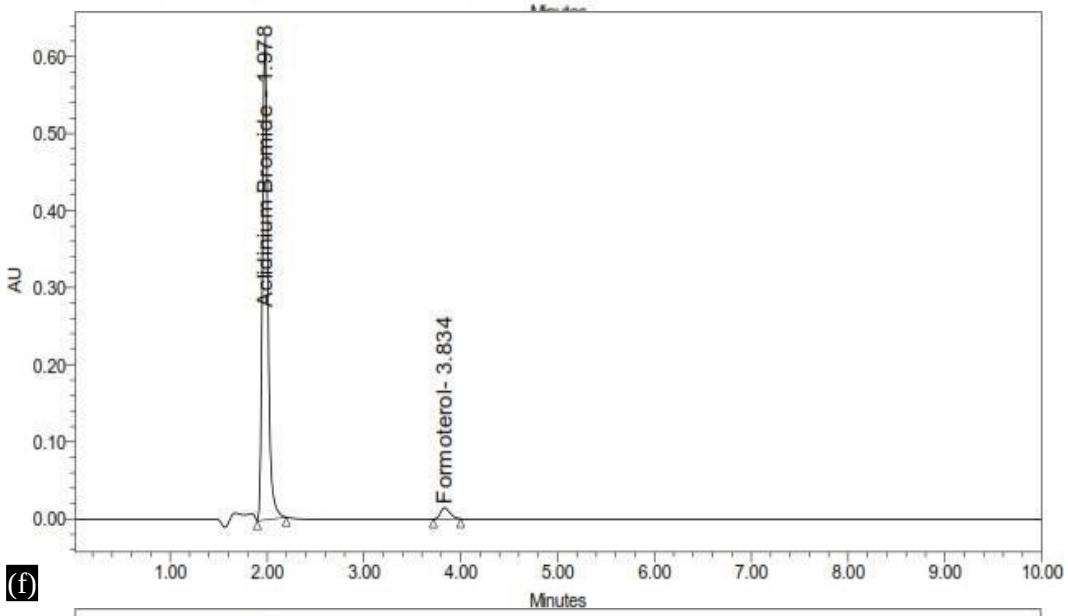
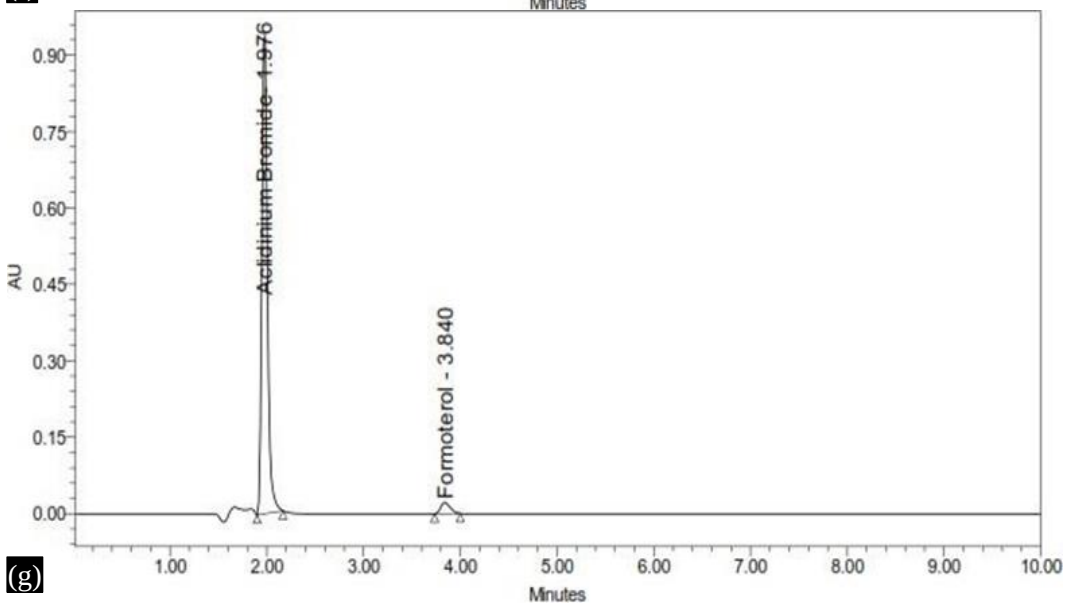


(f)



(g)

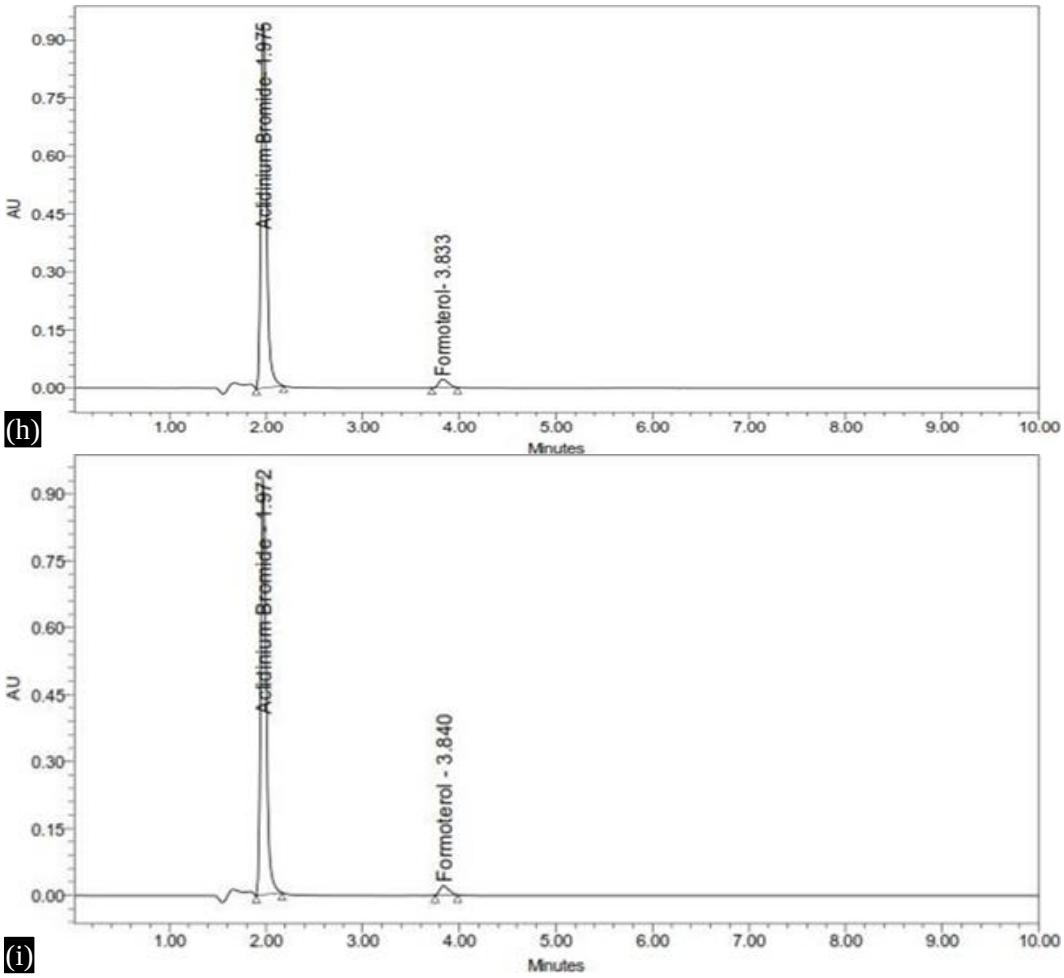


Figure 8. a = Chromatogram for accuracy 50%-1, b = Chromatogram for accuracy 50%-2, c = Chromatogram for accuracy 50%-3, d = Chromatogram for accuracy 100%-1, e = Chromatogram for accuracy 100%-2, f = Chromatogram for accuracy 100%-3, g = Chromatogram for accuracy 150%-1, h = Chromatogram for accuracy 150%-2, i = Chromatogram for accuracy 150%-3.

Table 4. Analytical performance parameters of aclidinium and formoterol.

Parameters	**Aclidinium**	**Formoterol**
Slope (m)	5177	6367
Intercept (c)	50334	20968
Correlation coefficient (R^2)	0.999	0.999

Table 5. Results of precision for aclidinium and formoterol.

Injection	**Area for Aclidinium**	**Area for Formoterol**
Injection-1	2373684	96855
Injection-2	2345262	96785
Injection-3	2364533	96564
Injection-4	2398744	96432
Injection-5	2376766	96243
Injection-6	2364758	96443
Average	2370624.5	96553.7
Standard Deviation	17621.4	231.5
%RSD	0.7	0.2

Table 6. Results of intermediate precision for aclidinium and formoterol.

Injection	Area for Aclidinium	Area for Formoterol
Injection-1	2364544	96753
Injection-2	2375755	96554
Injection-3	2364746	96656
Injection-4	2387744	96242
Injection-5	2365757	96533
Injection-6	2365757	96454
Average	2370717.2	96532.0
Standard deviation	9362.3	176.0
%RSD	0.4	0.2

Table 7. The accuracy results for aclidinium and formoterol.

%Concentration (At Specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50% Aclidinium	1194061.3	170	170.06	100.44	99.74
100%	2365150.7	340	338.23	99.48	
150%	3540917.7	510	506.37	99.29	
50% Formoterol	48659.3	6	6.05	100.82	100.53
100%	96624.3	12	12.01	100.11	
150%	145727.7	18	18.12	100.65	

Precision / Ruggedness

To assess the middle of the road exactness (otherwise called Ruggedness) of the strategy, Precision was performed on various days.

- *Readiness of stock arrangement:* Precisely gauge and move 340 mg of Aclidinium and 12 mg of Formoterol working standard into a 100 ml clean dry volumetric flagon. Include around 70 mL of diluent and sonicate to break it up totally and make volume sufficient with a similar dissolvable. (Stock arrangement). Further pipette 1.0 ml of the above stock arrangements into a 10 ml volumetric cup and weaken sufficiently with diluent.
- *Method:* The standard arrangements arranged in the exactness was infused a few days ago, for multiple times and estimated the region for every one of the six infusions in HPLC. The %RSD for the territory of six recreation infusions were seen as inside as possible as mentioned in Table 6.
- *Acknowledgment criteria:* The % RSD for the region of six standard infusions results ought not to be over 2%.
- *Explicitness:* For Specificity Blank and Standard are infused into system. There is no interference of any top in clear with the retention time of the scientific pinnacles**.**

Precision

- *Readiness of standard stock arrangement:* In a 100 ml clean, dry volumetric flagon, measure out and transfer 340 mg of Aclidinium and 12 mg of Formoterol as working standards. Add around 70 mL of diluent and sonicate to completely breakdown the mixture and make enough volume with the same dissolvable. Further pipette 1.0 ml of the above stock mixtures should be added to a 10 ml volumetric cup and sufficiently diluted with diluent.
- *Arrangement sample arrangements:* Considering the focus of the objective Assay, the half arrangement is as follows: Aclidinium and Formoterol working standards should be precisely measured, moved and added to a 100 ml clean, dry volumetric cup along with around 7 mL of diluent. The mixture should then be sonicated to completely break it up and make the volume necessary with the same dissolvable. (Stock configuration) Pipette 1.0 ml of the aforementioned stock mixtures into a 10 ml volumetric carafe and dilute with diluent to the proper strength.

- *To prepare a 100% arrangement (concerning the fixation of the objective of the assay):* Measure out and transfer 340 mg of Aclidinium and 12 mg of Formoterol working standards into a 100 ml clean, dry volumetric carafe. Add about 70 mL of diluent and sonicate to completely break them up and make the volume necessary with the same dissolvable. (Stock configuration) Pipette 1.0 ml of the previously mentioned stock mixtures into a volumetric jar with a 10 ml capacity and dilute with diluent to the proper strength.
- *To prepare a 150% arrangement (regarding the objective of the assay):* Accurately measure and transfer 510 mg of the Aclidinium working standard and 18 mg of the Formoterol working standard into a 100 ml clean, dry volumetric jar. Add about 70 ml of the diluent and sonicate to completely break it up and make volume sufficient with the same dissolvable.

Implement the standard arrangement along with the accuracy arrangements of 50%, 100% and 150%. Calculate the amount detected and the amount included for Aclidinium and Formoterol and then determine the mean and individual recovery values as mentioned in Figure 7(a–f) and Table 5.

Criteria for acknowledgement: Every level's % Recovery should fall between 98.0 and 102.

Linearity

Stock Arrangement Readiness

In a 100 ml clean, dry volumetric flagon, measure out and transfer 340 mg of Aclidinium and 12 mg of Formoterol as working standards. Add around 70 ml of diluent and sonicate to completely break it up and make the volume necessary with a similar dissolvable. (Stock configuration).

- *Arrangement of Level I:* 0.25 ml of the above stock arrangements was diluted with diluent to sufficient strength by adding 10 ml of volumetric cup.
- *Level II arrangement:* 0.5 ml of the stock arrangements were added to a volumetric cup containing 10 ml of diluent to make them sufficiently weak.
- *Arrangement of Level III:* 10 ml of volumetric cup was taken in by 1.0 ml of the aforesaid stock arrangements, which was sufficiently diluted with diluent.
- *Arrangement of Level IV:* 10 ml of volumetric cup was added to 1.5 ml of the aforesaid stock arrangements, which was then sufficiently weakened with diluent.
- *Readiness of Level V:* Using diluent, weaken the above stock arrangement by 2.0 ml in 10 ml of volumetric jar.

The plan is to measure the pinnacle zone and incorporate each level into the chromatographic structure. Determine the association coefficient by plotting a diagram of pinnacle territory vs focus (on the X-hub fixation and the Y-pivot Peak region).

- *Acknowledgment criteria:* Correlation coefficient ought to be at the very least 0.999 as shown in Figure 6(a) and (b) and Table 3 and Table 4.

Identification of Limit

Bracketing Point of Detection: (for Aclidinium and Formoterol)

Readiness of 0.10 µg/ml arrangement: In a 100 ml clean, dry volumetric flagon, measure out and transfer 340 mg of the Aclidinium working standard. Add around 70 mL of the diluent and sonicate to completely break it up and make the volume sufficient with the same dissolvable. (Stock configuration) Pipette 1.0 ml of the above stock mixtures into a volumetric jar with a 10 ml capacity and dilute with diluent to the proper strength. Pipette another 0.3 milliliters of the aforementioned stock mixture into a 10-milliliter volumetric container, weakening it just enough with the diluent. Pipette 0.1 ml of the above stock mixture into a volumetric jar with a 10 ml capacity and dilute it with diluent to the proper strength.as mentioned Table 8.

Cut of Quantification

Arrangement of 0.34 g/ml Planning: (for Aclidinium and Formoterol)

In a 100 ml clean, dry volumetric jar, measure out and transfer 340 mg of the Aclidinium working standard. Add around 70 ml of the diluent and sonicate to completely break it up and make the

volume suitable for the same dissolvable, (Stock configuration) as mentioned in Figure 9 and Table 9. Its system stability is visible in Table 10(a–b) and Table 11(a–b).

Table 8. Result of LOD of aclidinium and formoterol.

Drug name	Baseline Noise (µV)	Signal Obtained (µV)	S/N ratio
Aclidinium	62	188	3.03
Formoterol	62	186	3.00

Table 9. Results of LOQ.

Drug Name	Baseline Noise (µV)	Signal Obtained (µV)	S/N Ratio
Aclidinium	62	621	10.02
Formoterol	62	620	10.00

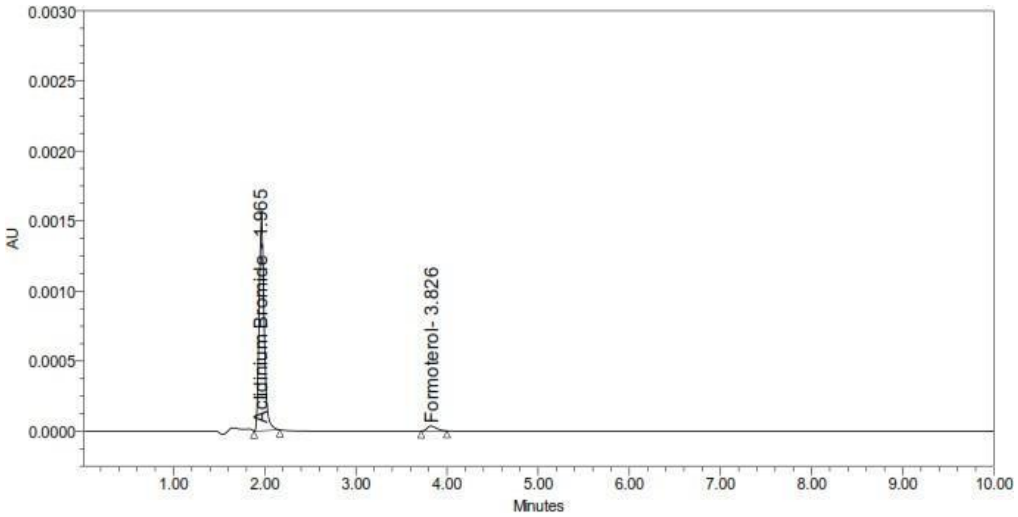


Figure 9. Chromatogram of aclidinium, formoterol showing LOQ.

Table 10(a). System suitability results for aclidinium of variation in flow.

S.N.	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.9	13.95	1.29	6618.78
2	1.0	12.38	1.30	6445.83
3	1.1	10.67	1.29	6454.23

Table 10(b). System suitability results for formoterol of variation in flow.

S.N.	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.9	1.26	5372.86
2	1.0	1.21	5114.07
3	1.1	1.26	5624.39

Degradation Studies: (as Mentioned in Table 12)

- *Acid degradation experiments***:** 1 ml of a stock solution of Formoterol and Aclidinium was treated with 1 ml of 2N HCl and refluxed at 60º C for 30 minutes. To determine the stability of sample D, the resulting solution was diluted to a concentration of 6 g/ml and 200 g/ml and 10 l were injected into the HPLC system. Chromatograms were then recorded as shown in Figure 10(a).
- *Alkali degradation studies:* 1 ml of a stock solution of Formoterol and Aclidinium was treated with 1 ml of 2N NaOH and refluxed at 60ºC for 30 minutes. The solution obtained was diluted to

obtain 6 g/ml and 200 g/ml solution and 10 l were injected into the system. The chromatograms were recorded to evaluate the stability of the sample as shown in Figure 10(b).

- *Oxidation studies:* 1 ml of 20% H_2O_2 was added separately to 1 ml of the stock solution of Formoterol and Aclidinium. The solution was maintained at 60ºC for 30 minutes. To determine the sample's stability, the resulting solution was diluted to a concentration of 6 g/ml and 200 g/ ml and 10 l were injected into the HPLC system. Chromatograms were then recorded as shown in Figure 10(c).
- *Dry heat degradation studies:* The original drug solution was heated at 105°C for one hour. The solution obtained was diluted to obtain 6 g/ml and 200 g/ml solution and 10 l were injected into the system. The chromatograms were recorded to evaluate the stability of the sample as shown in Figure 10(d).
- *Photo stability studies:* The drug was exposed to 60 g/ml and 200 g/ml solution to UV light by keeping the breaker in a UV chamber for 24 hours or 200 watts per square meter for photo stability. 10 l were then injected into the system and the chromatograms were recorded as shown in Figure 10(e).

Table 11(a). System suitability results for aclidinium (The Organic composition in the Mobile phase was varied from ±10%.).

S.N.	Change in Organic Composition in the Mobile Phase	System Suitability Results (A)	
		USP Plate Count	***USP Tailing***
1	10% less	1.26	5346.86
2	*Actual	1.21	5114.07
3	10% more	1.26	5465.98

Table 11(b). System suitability results for formoterol (The Organic composition in the Mobile phase was varied from ±10%.).

S.N.	Change in Organic Composition in the Mobile Phase	System Suitability Results (B)		
		USP Resolution	***USP Tailing***	***USP Plate Count***
1	10% less	14.01	1.29	6345.79
2	*Actual	12.38	1.30	6445.83
3	10% more	10.12	1.29	6623.23

Table 12. Degradation results for aclidinium and formoterol.

	Aclidinium		Formoterol	
Sample Name	**Area**	**% Degraded**	**Area**	**% Degraded**
Standard	2372796		96329.3	
Acid	2253633	5.02	94635	1.76
Base	2308497	2.71	93751	2.68
Peroxide	2295738	3.25	93167	3.28
Thermal	2197431	7.39	91563	4.95
Photo	2165656	8.73	92363	4.12

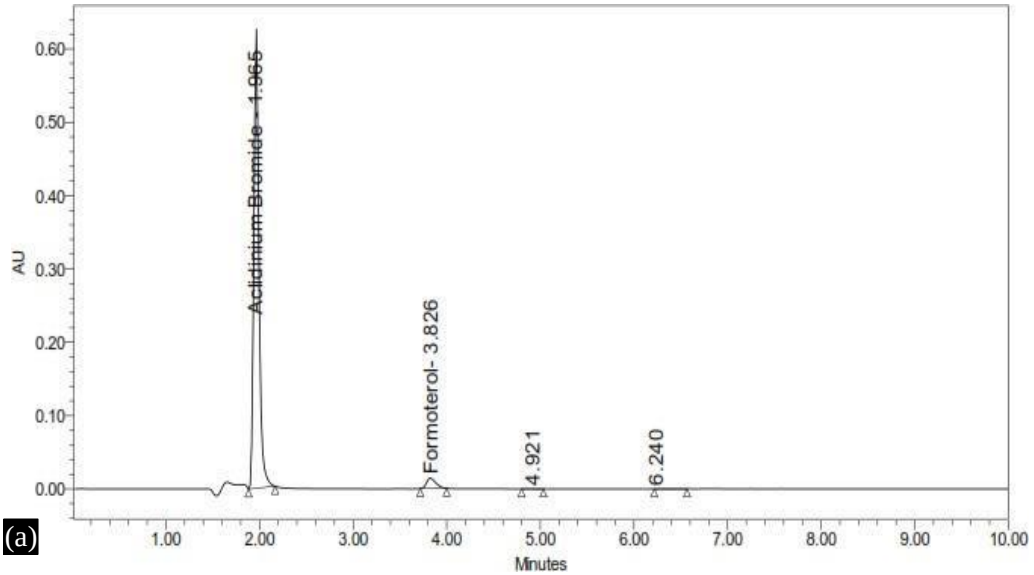
System Suitability

Validation Parameters

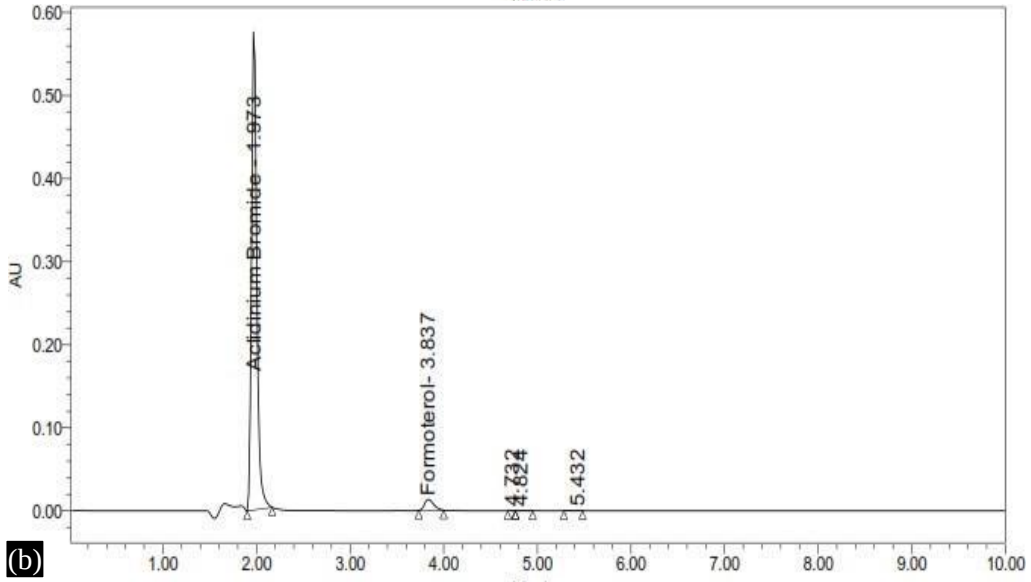
Linearity: The linearity range was found to lie from 85 µg/ml to 680 µg/ml of Aclidinium, 3 µg/ml to 24 µg/ml of Formoterol and chromatograms are shown below.

Precision

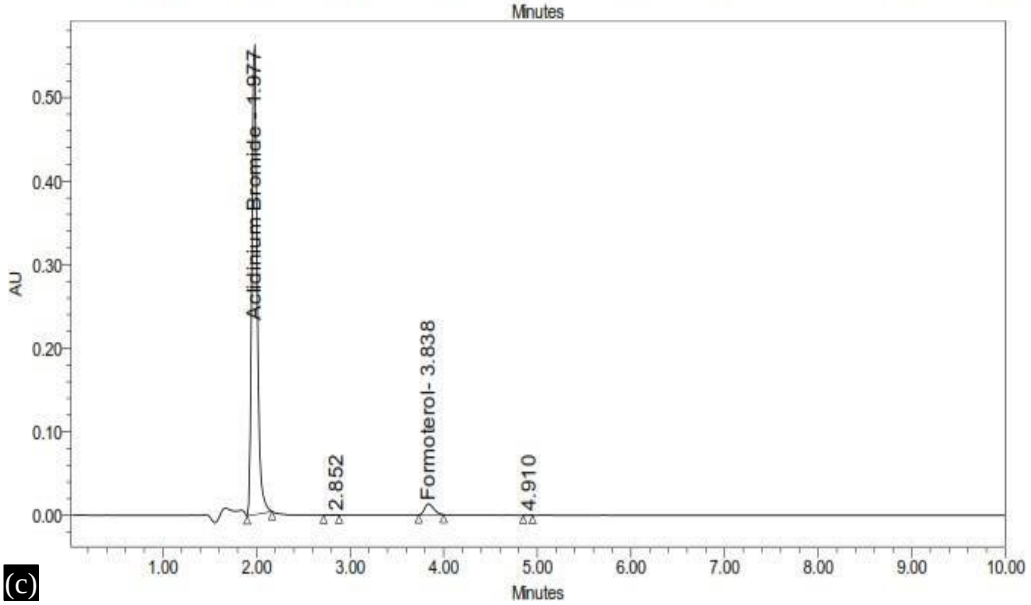
Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.



(a)



(b)



(c)

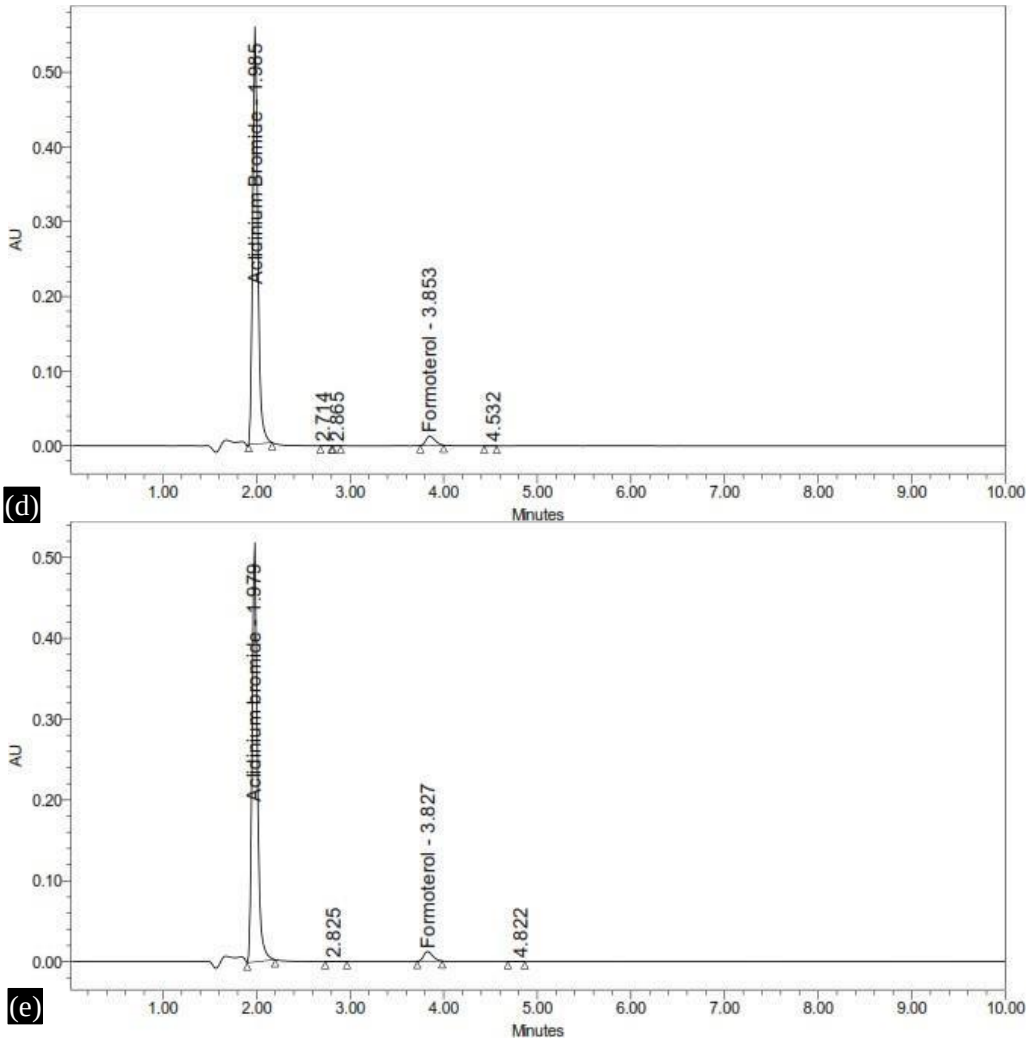


Figure 10. a = Chromatogram showing acid degradation, b = Chromatogram showing base degradation, c = Chromatogram showing peroxide degradation, d = Chromatogram showing thermal degradation and e = Chromatogram showing photo degradation.

Limit of Quantification for Formoterol and Rilpivirin

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

CONCLUSION

Aclidinium and Formoterol have been measured simultaneously using a new RP-HPLC technique. Using the DIKMA SPURSIL C18(150 * 4.6) 5um column, flow rate was 1 ml/min, mobile phase ratio was OPA (Orthophosphoric Acid) (0.1%) (30:70% v/v) ACN and detection wavelength was 280 nm, the chromatographic conditions were effectively created to separate Aclidinium and Formoterol. The apparatus used was the WATERS HPLC Auto 'Sampler, Separation module 2695, 2487 UV Detector and Empower-software version 2. It turns out that the running times were 1.965 and 3.826 minutes. Aclidinium and Formoterol were found at purity levels of 100.14% and 99.92%, respectively. Aclidinium and Formoterol's system suitability characteristics, such as theoretical plates and tailing factors, were discovered to be 5117.04, 1.2 and 6445.83, 1.30, respectively, with a resolution of 12.38. Aclidinium and Formoterol were estimated to be using RP-HPLC.

With concentration ranges of 85 g/ml to 680 g/ml and 3 g/ml to 24 g/ml, respectively and linearity coefficients of 0.999 and 0.999, respectively, the method may produce results with high sensitivity.

RSD should not exceed 2.0% according to the accepted precision criteria and the method's precision values of 0.7 and 0.2 for Aclidinium and Formoterol respectively, demonstrate the method's accuracy. The method exhibits precision of 0.4 and 0.2 for Aclidinium and Formoterol, which shows that the method is repeatable when performed on different days as well. The acceptance threshold of intermediate precision is RSD should be no more than 2.0%. The range of 98.0% to 102.0% should be the percentage recovery, which is the accuracy limit. For Aclidinium and Formoterol, the overall recovery was determined to be 99.74% and 100.53%, respectively. Validation of the suggested methodology indicates that Dolutegravir has good accuracy.

Consent and Ethical Approval

It is not applicable.

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Conflict of Interests

Declared none.

REFERENCES

1. Devadasu Ch, Bharani V. Development and Validation of RP-HPLC Method for Analysis of Aclidinium Bromide and Formoterol Fumarate in Pharmaceuticals. Asian J Pharm Anal. 2021; 11(2):63-9. Doi: 10.52711/2231-5675.2021.00012.
2. Ravi Chikke Gowda. Simultaneous RP-HPLC Method For Determination Of Impurities In Gowda RC. Simultaneous RP-HPLC method for determination of impurities in formoterol fumarate and aclidinium bromide in pharmaceutical dosage forms. Int J Chem Pharm Anal. 2016 Apr 1;3(3).
3. Akapo OS, Muhammad A, et al. Validation of a RP-HPLC method for the assay of formoterol and its related substances in formoterol fumarate dihydrate drug substance. J Pharm Biomed Anal. 2003;33(5):935–45.
4. Shyamala S, Sai Kala G, Sravani A, Anusha T, Srikanth K. Validated RP-HPLC method for simultaneous estimation of aclidinium and formoterol in bulk drug and dosage form. The Pharma Innovation J. 2020; 9(4): 242–247.
5. El-Bagary RI, Fouad MA, Manal A, Tolba EH. Forced degradation of mometasone furoate and development of two RP-HPLC methods for its determination with formoterol fumarate on salicylic acid. Arabian J Chem. 2016; 9(3): 493–505.
6. halid AM, Attia Nasr M, El-Abasawi, El-Olemy A, Serag A. Different spectrophotometric methods manipulating ratio spectra applied for the analysis of aclidinium in Duaklir® Genuair® inhalation powder. Hindawi J Spectrosc. 2018;2018:5625602. doi:10.1155/2018/5625602.
7. Hassib ST, El-Zaher AA, Fouad MA. Validated stability-indicating derivative and derivative ratio methods for the determination of some drugs used to alleviate respiratory tract disorders and their degradation products. Drug Test Anal. 2011; 3(5): 306–318.
8. Singh K. HPLC method for estimation of drug release of sofosbuvir in pharmaceutical formulations. World J Pharm Sci. 2017; 6(8):2249–2258.
9. Rani JS. A new RP HPLC method development and validation for simultaneous estimation of sofosbuvir and velpatasir in pharmaceutical dosage form. Int J Eng Technol Sci & Res. 2017;4(11):145–52.
10. Mastanamma SK. Development and validation of stability indicating RP HPLC method for simultaneous estimation of sofosbuvir and ledipasvir in bulk and their combined dosage form. Future J Pharm Sci. 2018;4(2):116–23. doi:10.1016/j.fjps.2017.11.003.
11. International Conference on Harmonization. Q2 (R1), harmonised tripartite guidelines, validation of analytical procedures: text and methodology. Geneva, Switzerland; 2005. p. 11–2.

12. Muthyala NI, Naresh B. Stability indicating RP-HPLC method development and validation for the quantitative estimation of chlorthalidone in API and tablet dosage form. Int J Adv Res Med Pharmaceut Sci. 2019;4(10):32–41.
13. Ahire ED, Sharma N, Gupta PC, Khairnar S, Surana K, Ahire B, Kshirsagar S. Developing formulations of prebiotics and probiotics. In: Kesharwani RK, Mohan Rao TJ, Keservani RK, editors. Prebiotics and probiotics in disease regulation and management. Scrivener Publishing LLC; 2022. p. 271–90.
14. Jadhav SR, Alhat HP, Joshi SV. Development of new RP HPLC method for the simultaneous estimation of lopinavir and ritonavir in API and in tablet dosage form. Asian J Res Chem. 2013;6:555–9.
15. Yeola CA, Sonawane VN, Sonawane VN, Surana KR, Patil DM, Sonawane DD. Development and validation of simple UV-spectrophotometric method for estimation of diclofenac sodium. Asian J Pharm Anal. 2023;13:183–9.

