Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

A Novel Stress Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Aclidinium and Formoterol in bulk and its Tablet Dosage Forms

Ismail Y[1], Haja Nazeer Ahamed[2*], D. Chinababu[3], Voleti Vijaya Kumar[4], N. Delhiraj[5] A. R. Vijaya Kumar[6] and Govindarao Yedalapalli[7]

[1*&2] Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamil Nadu, India.
[3] Department of Pharmacy, ITM University, NH-44, Gwalior, Madhya Pradesh, India.
[4&5]School of Pharmacy, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India.
[6]Faculty of Pharmacy, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chromepet, Tamil Nadu, India.
[7]Department of Pharmaceutical Analysis, St. Xaveer Institute of Pharmacy, Phirangipuram, Guntur Dist, Andhra Pradesh, India.

***Corresponding Author: Haja Nazeer Ahamed** Associate Professor,
*Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamil Nadu, India.

(Received: 07 October 2023 Revised: 12 November Accepted: 06 December)

KEYWORD

Aclidinum and Formoterol, , RP-HPLC, Ortho phosphoric acid buffer

ABSTRACT:

A simple reversed-phase high performance liquid chromatographic method was Developed for the simultaneous estimation of the Aclidinium and formoterol in bulk and its tablet dosage form. The method was developed on Dikma Spursil, C_{18} column (4.6 x 150mm, 5µ) particle size, wavelength was fixed at 280nm with photo diode array detection. The mobile phase was consisted mixture of Buffer: Acetonitrile (30: 70), pH 4.3 was adjusted with hydrochloric acid and flow of mobile phase through the column was maintained 1mL/min. The retention times of Aclidinum and Formoterol were found to be 3.04 and 4.45 min respectively. The method was statistically validated with concern of precision, linearity, range and robustness of method was found for Aclidinumand Formoterol. The above method was afforded excellent percentage recovery was found to be 99.87-100.22%& 99.68-100.17% for Aclidinum and Formoterol respectively. The Limit of detection & Limit of quantification were found 0.10,0.34µg/ml and 0.16, 0.53 µg/ml for Aclidinum and Formoterol. The forced degradation studies were performed. A new simple, selective, reproducible, rapid and accurate reversed phase liquid chromatographic method was developed and validated for the estimation of Aclidinum and Formoterol.

Introduction:

Aclidinium (Fig1) is a long-acting, reversible antagonist [1] at muscarinic receptors, with equal affinity to all five subtypes. Inhaled Formoterol works like other β2 agonists, which causes bronchodilation by relaxing the smooth muscle in the airway to treat asthma exacerbation. Formoterol (Fig 2) 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). Increases cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibits the release of pro-inflammatory mast-cell mediators such as histamine and leukotrienes [2,3].From literature review reported some methods of analysis in inhalation and human serum by voltammetry [4], in urine by gas chromatography & mass spectrometry [5], UV spectroscopy [6,7] for the estimation of formoterol either alone and in other combinations [8-12] and chromatographic methods were also developed for the determination of aclidinium and formoterol in their dosage forms [11,12]. Aclidinium bromide and Formoterol fumarate present in its pure form as well as formulation validated according to ICH Q2 (R1) guidelines [13].

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

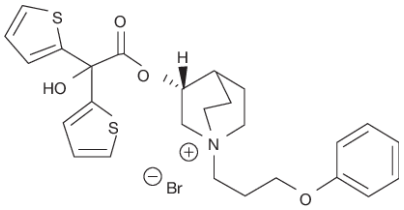

Figure:1 Structure of Aclidinum Bromide

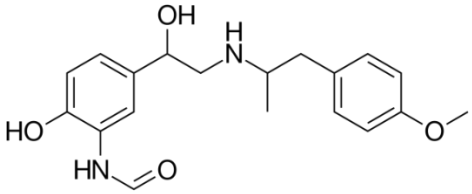

Figure:2 Structure of Formoterol

Materials and Methods

All the chemicals and reagents were of analytical grade. Water was double distilled and filtered with a membrane filter. Acetonitrile – Hihg Performance Liquid Chromatography grade (Merck, India), hydrochloric acid and ortho phosphoric acid (SD fine chem, India) were used to prepare mobile phase. Pharmaceutical grade standard drugs viz., Aclidinum and Formoterol were kindly gifted by Ajanta Pharma Limited, Mumbai, India.

Preparation of standard solution:

Accurately weigh and transfer 340 mg of Aclidinium and 12 mg of Formoterol working standards into a 100 ml clean dry volumetric flask add about 70 mL of Diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1.0 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (340 µg/ml&12 µg/ml respectively).

Preparation of sample solution:

Accurately weigh and transfer the Inhalation powder equivalent to 340 mg of Aclidinium and 12 mg of Formoterol sample into a 100 ml clean dry volumetric flask add about 70 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1.0 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Optimization of method

Method was optimized with different suitable parameters and results were shown in table 1

Table:1. Results for Optimization of method

Instrument used	Waters HPLC with auto sampler and UV detector.
Temperature	Ambient
Mode of separation	Isocratic mode
Column	DIKMA Spursil, C_{18} column (4.6 x 150mm, 5µ)
Mobile phase	Buffer: Acetonitrile (30: 70)
Flow rate	1 mL/min
Wavelength	280 nm
Injection volume	20 µL
Run time	20 min

Method validation

The method validation was performed according to International Council for Harmonization guidelines. The following method validation parameters resembling specificity, precision, accuracy, linearity, robustness, limit of detection and limit of quantification[8].

Specificity:

The specificity was studied by injecting the mobile phase (blank), standard and sample solution prepared as per the developed method and injected into the HPLC system and study the any interference with retention times of Aclidinum and Formoterol.

Acceptance criteria: No peaks eluted at retention times of Aclidinum and Formoterol

System suitability parameters:

To make certain critical parameters were met all system suitable requirements conducted on all the days. The chromatogram was eluted and showed symmetrical peaks, results showed in chromatograms 3, 4, 5 and tabulated as in table no 2.

Table 2: System suitability results of Aclidinum and Formoterol

S.No	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

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

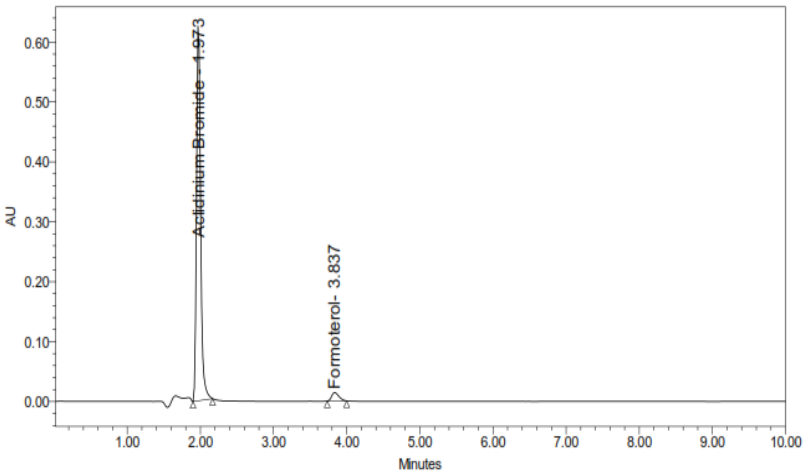

FIGURE:3. Standard chromatogram

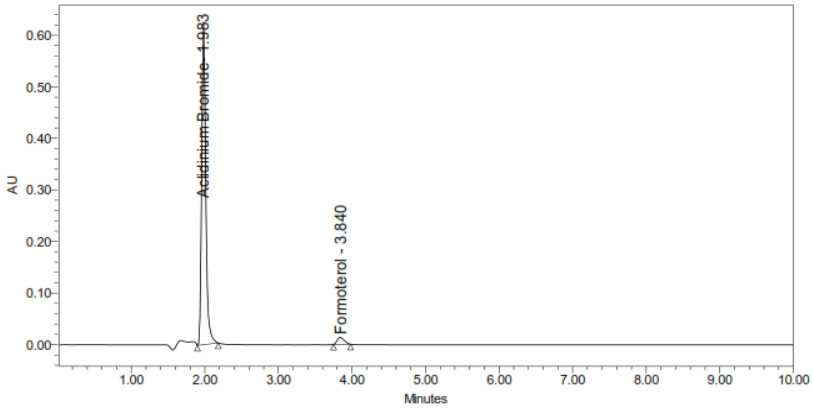

FIGURE: 4. Sample chromatogram

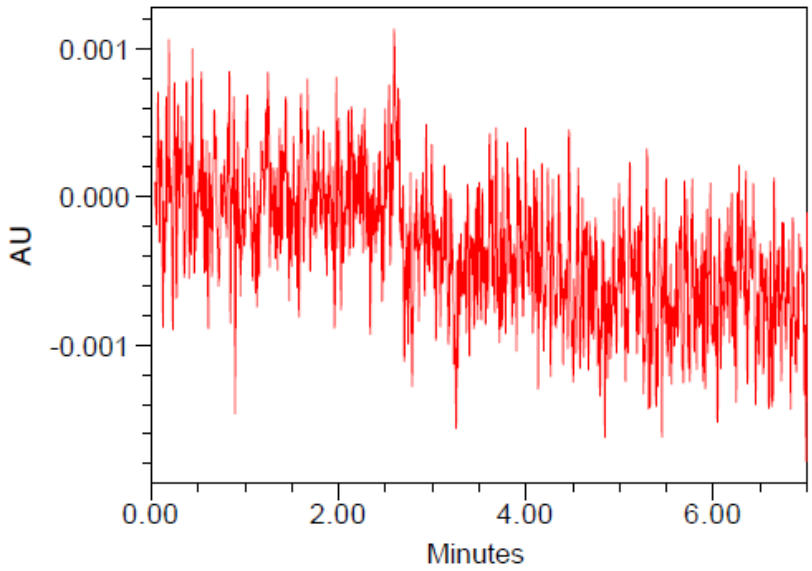

FIGURE:5. Blank chromatogram

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

Precision:
The precision was assessed through assay with respect to intermediate precision and intraday precision. The repeatability of the system was studied by injecting analyte with 6 replicate injections. The %RSD values varied from 0.7-0.2%. The results were showed good intra-day precision. The results were tabulated in table no 3. Acceptance criteria The % RSD should not more than 2.0

Table 3: Precision results of Aclidinum 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

Linearity:
The linearity of the method was obtained through calibration curve (peak area vs concentration).The pure solution was checked in the concentration range of 85µg/mL to 680 µg/mL of Aclidinium, 3 µg/ml to 24 µg/ml of Formoterol and chromatograms The calibration curve was showed linear over concentration range and R^2 values were found to be 0.999 for aclidinium and 1 for Formoterol ,that results indicated good linearity between peak area and concentration. The data of graphs were showed in figures6 & 7. Acceptance criteria: Correlation coefficient should be not less than 0.999.

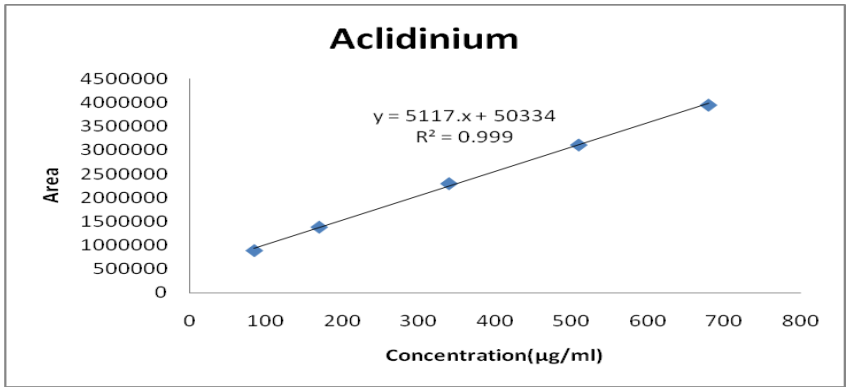

Figure:6 Linearity curve of Aclidinum Bromide

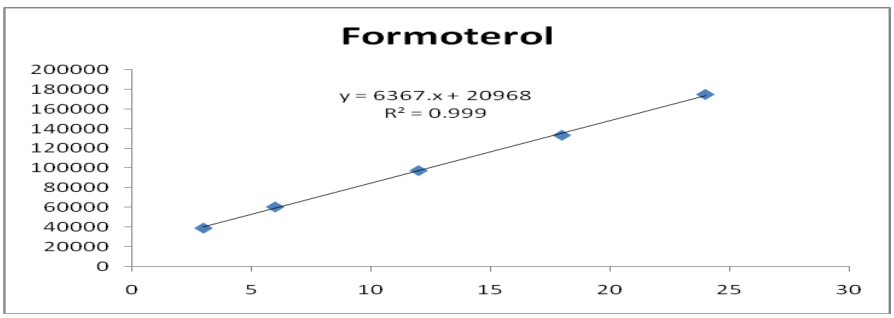

Figure:7 Linearity curve of Formoterol

Accuracy
The accuracy of the method was studied by spiking standard solution with analyzed sample solution at three concentration levels 50%, 100%, 150%. The recovery studies were performed under optimized conditions in replicate. The results were showed in table no 4. The accuracy should between 98%-102%. The % RSD value should not more than 2.0.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

Table 4: Accuracy results of Aclidinium and Formoterol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	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	

Table 5: The accuracy results for Formoterol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	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	

Limit of detection and Limit of quantification:

The detection limit and quantification limit were determined through signal to noise ratio 3:1 and 10:1 ratio. The limit of detection and limit of quantification were estimated 0.12µg/ml-0.43 µg/ml and 0.08µg/ml-0.027 µg/ml. The results were tabulated in table no 6 & 7 and figures shown in 7 & 8.

Table 6: Results of Limit of Detection& Limit of Detection

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

Table 7: Results of Limit of Detection & Limit of Quantification

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Aclidinium	62	621	10.02
Formoterol	62	620	10.00

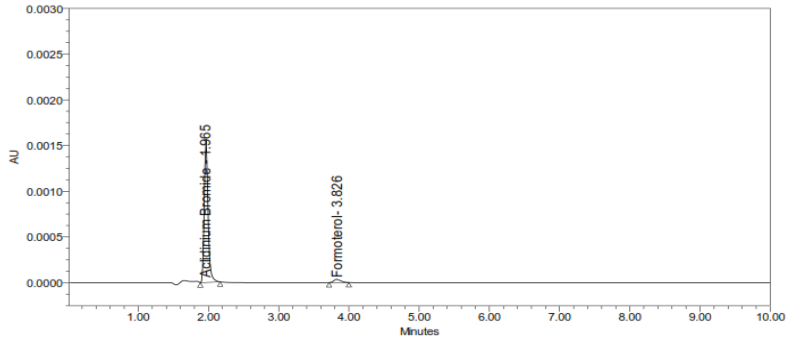

Figure: 8 LOD chromatogram of Aclidinum & Formoterol

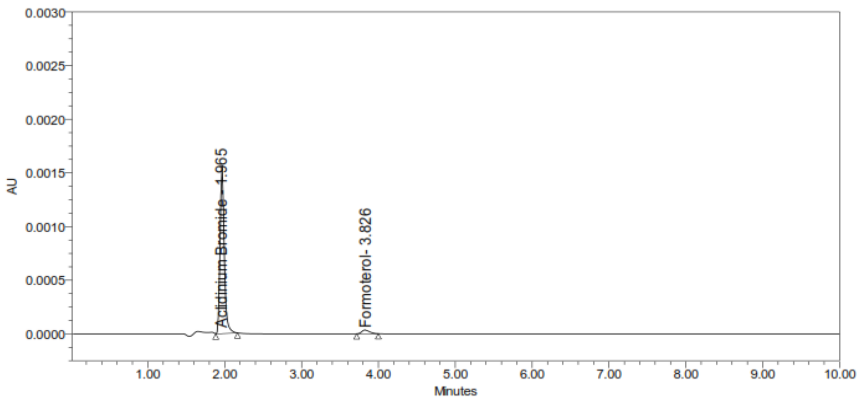

Figure: 9 LOQ chromatogram of Aclidinum & Formoterol

Robustness:

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

The method was unaffected with deliberate changes with respected to flow rate (± 2), temperature of column, (± 5ºC) mobile phase composition (± 3mL of organic phase) were performed at 100% test concentration. The method was robust to the above mentioned conditions. The results tabulated in table no 5. Acceptance criteria: The %RSD value should not more than 2.0.

i. Effect of flow rate

The effect of flow rate (Table 8) was studied with the variation of ± 0.2 mL/min from the normal conditions of the method. The mixture of both drugs containing solution analyzed by HPLC method as three independent samples.

ii. Effect of temperature

The effect of temperature was assessed with the variation of ±5.0 º C from the normal conditions of the method. The mixture of both drugs containing solution analyzed by HPLC method as three independent samples.

iii. Effect of wavelength

The effect of wavelength was evaluated with the variation of the wave length ±2.0 nm from the normal conditions of the method. The mixture of both drugs containing solution analyzed by HPLC method as three independent samples.

Table 8: Robustness results of Aclidinium and Formoterol

S. No	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

Table: 9 Results of Flow rate variation

S. No	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 Results of Organic Phase variation

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		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 System suitability results Organic Phase variation

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		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

Assay of Aclidinium and Formoterol in Solid dosage form:

Twenty tablets of marketed formulation (Mavyret) contained 40 mg of Aclidinium and 100 mg of Formoterol. Accurately weigh equivalent quantity of tablet powder of Aclidinium and Formoterol. The final concentration of was Aclidinium80µg/ml and Formoterol was 200µg/ml. chromatograms were shown in figures 9 & 10.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

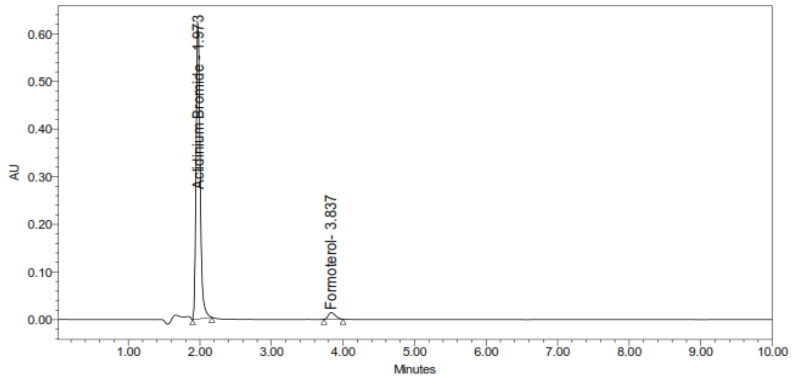

Figure: 10 Standard Chromatogram of Aclidinum and Formoterol

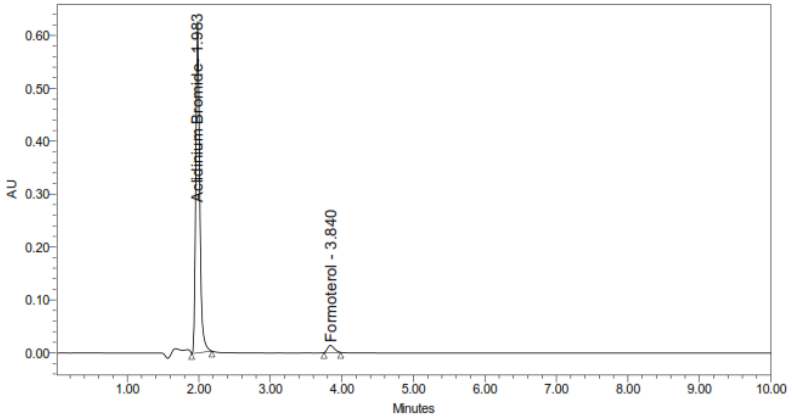

Figure: 11 Sample Chromatogram of Aclidinum and Formoterol

Force degradation studies:

The force degradation studies were performed on the Aclidinium and Formoterol. There was no interference of degradants and blank, the developed RP-HPLC method proves the capability of stability indicating method for the analysis of Aclidinium and Formoterol. Different stress indicating studies were conducted like acid(0.1 N HCl, refluxed for 1 H at 80ºC), Base (0.1 N NaOH refluxed for 4H at 80ºC), H_2O_2 (3% H_2O_2 Stored at room temperature for 2 H), hydrolytic at 80ºC and UV light (near UV ≥200 for 10 days)[Snyder LR et al].The degradation conditions were optimized to obtain target degradation between 10 to 30% as per ICH guidelines [ICH]. The results were summarized in table no 7. Figures 11-15 shows chromatograms of different stress degradation conditions.

Table no-18: 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

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

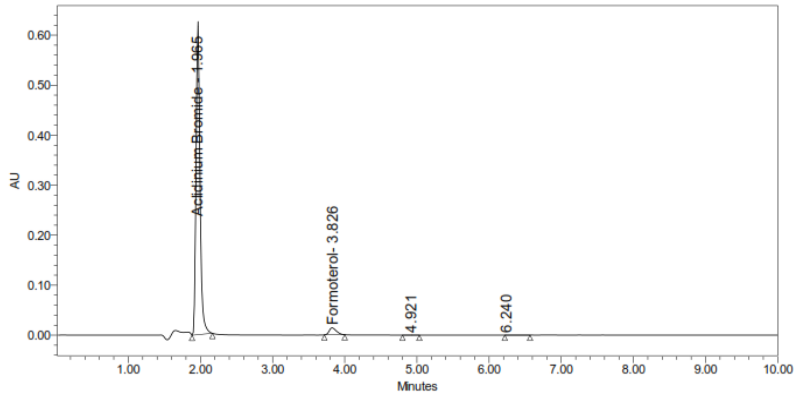

Figure 12: Chromatogram of Acid degradation

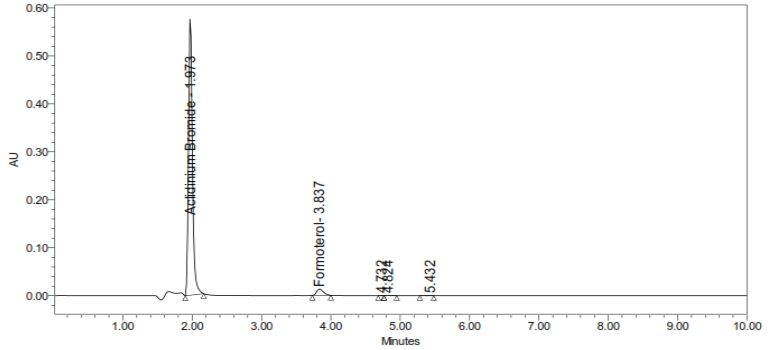

Figure 13: Chromatogram of Base degradation

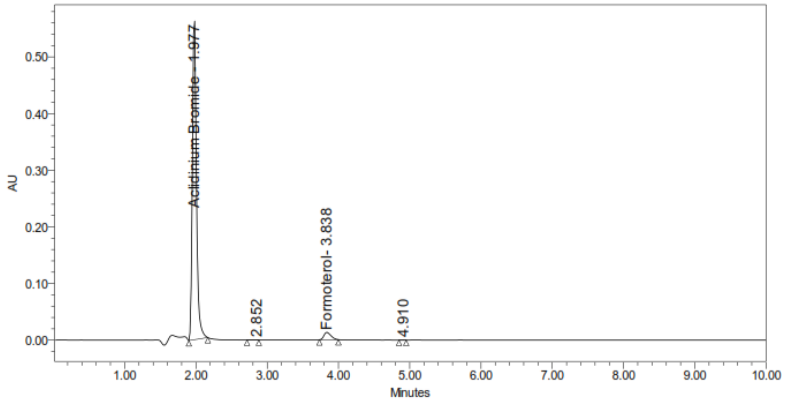

Figure 14: Chromatogram of Peroxide degradation

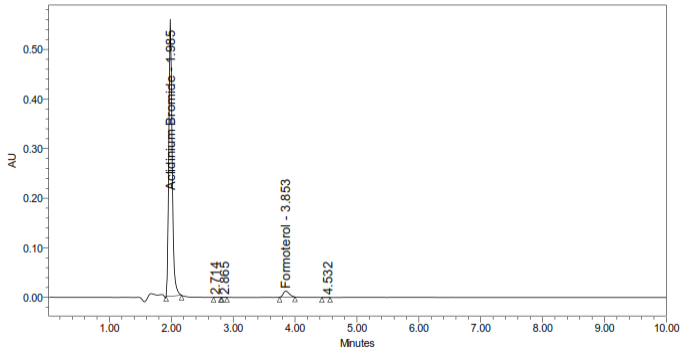

Figure 15: *Chromatogram of Thermal degradation*

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

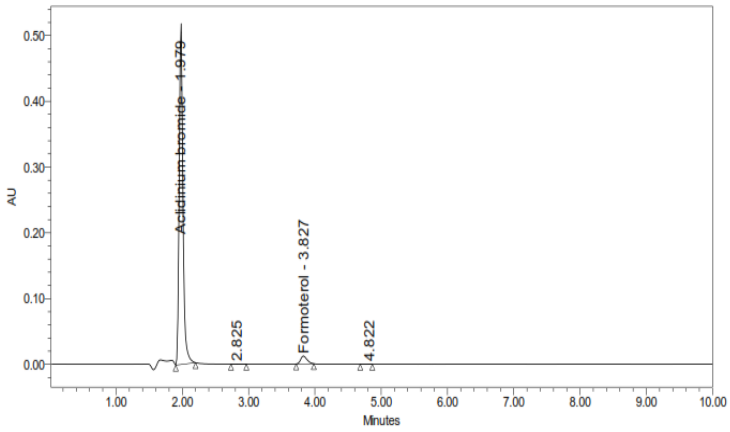

Figure 16: Chromatogram of Photo degradation

DISCUSSION:

A new method was established for simultaneous estimation of A clidinium and Formoterol by RP-HPL C method. The chromatographic conditions were successfully developed for the separation of A clidinium and Formoterol by using DIKMA SPURSIL C_{18}(150*4.6) 5umcolumn,flow ratewas1 ml/min, mobile phase ratio was OPA (Orthophosphoric Acid) (0.1%) (30:70%v/v) ACN (detection wave lengthwas280nm.The instrument used was WATERSHPL CAuto `Sampler, Separation module 2695, 2487 UV Detector with Empower-softwareversion-2. The retention times were found to be 1.965 minsand3.826 mins. The% purity of A clidinium and Formoterol was found to be 100.14% and99.92% respectively.The system suitability parameters for Aclidinium and Formoterol such as theoretical plates and tailing factor were found tobe 5117.04,1.2 and6445.83,1.30the resolution was found to be12.38. The estimation of Aclidiniumand Form oterolwas doneby RP-HPLC. The Linearity of Aclidinium and Form oterol was found to belinear with a correlation coefficient of0.999 and 0.999 with concentration range of 85µg/ml to 680µg/ml and 3µg/ml to 24µg/ml respectively, whichshows that the method iscapable of producing good sensitivity and Linearity. The acceptance criteria of precisionis % RSDshouldbenotmorethan2.0%andthe method show precision0.7 and 0.2for Aclidinium and Formoterol which shows that the method is precise. The acceptanc e criteria of intermediate precision is% RSD should be not more than 2.0% and the method show precision0.4 and0.2 for Aclidinium and Formoterol which shows that the method is repea table when performed in different days also. The accuracy limitis the percentage recovery should be in the range of 98.0%- 102.0%. The to talreco very was found to be 99.74%and100.53%forAclidinium and Formoterol. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Aclidinium was found to be 3.03 and 10.02 and LOD and LOQ for Formoterol was found to be 3.00 and 10.00.The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions. The Forced Degradation also performed for the both drugs under stress conditions like Acid Base, Peroxide, Photo and Thermal conditions. The Developed method was also can be use for the stability studies of Aclidinium and Formoterol. Hence the Proposed method was used for the routine analysis of Aclidinium and Formoterol in its pure and Dosage form by using RP-HPLC. The proposed method was validated according to ICH Q2 Guidelines.

CONCLUSION:

The developed RP-HPLC method was simple, sensitive, specific, accurate and precise, stability indicating simultaneous estimation of Aclidinium and Formoterol in tablet dosage form. The developed method showed excellent resolution between Aclidinium and Formoterol. The method was effectively validated in terms of system suitability, precision, linearity, range, accuracy, LOD, LOQ and robustness and stress indicating studies in agreement with ICH guidelines. Hence the method is routinely used for estimation and quality control & stability indicating samples of combined market formulation ofAclidinium and Formoterol.

ACKNOWLEDGEMENT:

The authors are very much thankful to the Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, India for providing essential services to carry out the study.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 3051-3060 | ISSN:2251-6727

REFERENCES:

1. Jones P. Aclidinium bromide twice daily for the treatment of chronic obstructive pulmonary disease: a review. *Advances in therapy*. 2013 30 (4): 354-68.
2. Gowda R. Simultaneous RP-HPLC method for determination of impurities in formoterol fumarate and aclidinium bromide in pharmaceutical dosage forms. *International Journal of Chemical and Pharmaceutical Analysis.* 2016 (3)3: 1005.
3. 12. 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 Journal*. 2020 9 (4): 242-247.
4. Demircigil B, Yılmaz S. Electrochemical behavior of formoterol fumarate and its determination in capsules for inhalation and human serum using differential-pulse and square-wave volatmmetry. *Electroanalysis*. 2002 14 (2): 122–127.
5. Kamimura H, Sasaki H, Higuchi S, Shiobara Y. Quantitative determination of the β-adrenoceptor stimulant formoterol in urine by gas chromatography mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1982 229 (2): 337–345.
6. Gousuddin M, Raju SA, Sultanuddin MS. Development and validation of spectrophotometric methods for estimation of formoterol bulk drug and its pharmaceutical dosage forms. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2011 3: 300–309.
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 Testing and Analysis*. 2011 3 (5): 306–318.
8. 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 Journal of Chemistry*. 2016 9 (3): 493–505.
9. Assi KH, Tarsin W, Chrystyn H. High performance liquid chromatography assay method for simultaneous quantitation of formoterol and budesonide in Symbicort Turbuhaler. *Journal of Pharmaceutical and Biomedical Analysis*. 2006 41 (1): 325–328.
10. Parmar VK, Patel HN, Patel BK. Sensitive, and robust methods, for simultaneous determination of beclomethasone dipropionate and formoterol fumarate dihydrate in rotacaps. *Journal of Chromatographic Science*. 2014 52 (10): 1255–1266.
11. Rizk M, Sultan M, Talaat N, Youssef N. A validated TLC—densitometric method for the simultaneous determination of formoterol fumarate and budesonide in pressurized metered-dose inhaler. *JPC-Journal of Planar Chromatography-Modern TLC*. 2017 30 (1): 63–67.
12. Merey HA, El-Mosallamy SS, Hassan NY, El-Zeany BA. Validated chromatographic methods for the simultaneous determination of Mometasone furoate and Formoterol fumarate dihydrate in a combined dosage form. Bulletin of Faculty of Pharmacy, *Cairo University*.2016 54(1): 99–106.
13. International Conference on Harmonization, Q2 (R1), Harmonised tripartite guidelines, Validation of analytical procedures: text and methodology, Geneva, November, 2005.