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Development and Validation of Simultaneous Estimation of Glycopyrrolate and Formoterol Fumarate in its Bulk and Pharmaceutical Rota Caps Dosage form by using RP-UPLC

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Abstract
A new, simple, rapid, selective, precise, and accurate isocratic reverse-phase high-performance liquid Chromatography assay method has been developed for simultaneous estimation of Glycopyrrolate and Formoterol Fumarate tablet formulations. The separation was achieved by using column BEH C18 (2.6m x 50mm 1.8µm) in the mobile phase consisted of pH 2.5 triethyl amine buffer and Methanol in the ratio of 50:50 v/v. The flow rate was 0.2 mL/min, column oven temperature 25° C, the injection volume was 6 µL, and detection was performed at 250 nm using a photodiode array detector (PDA), Run time 6 minutes. The retention time of Glycopyrrolate and Formoterol Fumarate was noted to be 0.68 minutes and 1.05 minutes respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: *Liquid Chromatography; Glycopyrrolate, Formoterol Fumarate, combined dosage forms; Simultaneous estimation, Validation.*

I. Introduction

Glycopyrrolate is a quaternary ammonium salt. Chemically, Glycopyrrolate is (RS)-[3(SR)- Hydroxy-1, 1-dimethylpyrrolidinium bromide] α-cyclopentylmandelate. The chemical formula is $C_{19}H_{28}BrNO_3$. The molecular weight is 398.33g/mol [1]. Glycopyrrolate is a crystalline white powder. It is dissolvable in water and alcohol, and much insoluble in chloroform and ether [2].

Glycopyrrolate, like another anticholinergic (antimuscarinic) drug, impedes the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetyl-choline yet require cholinergic innervation. Thus, it diminishes the volume and free acidity of gastric secretions and controls excessive pharyngeal, tracheal, and bronchial secretions [3].

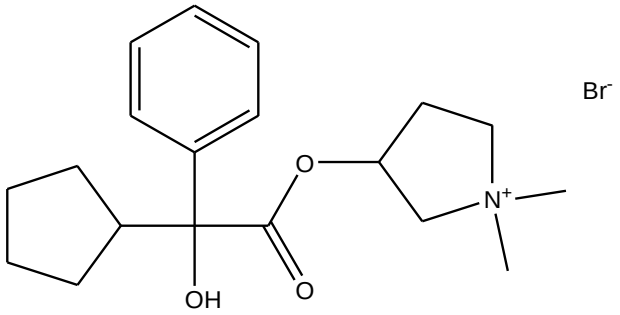

Fig.1.01: Structure of Glycopyrrolate

Formoterol acts as a bronchodilator. It extends the airways of the lungs so that it helps to inhale all the more effortlessly. It may even be utilized to forestall respiratory issues caused by exercise. It can also be utilized for long-term treatment of chronic obstructive pulmonary disease (COPD) [4]. Chemically, Formoterol is N-[2-Hydroxy- 5-[(1RS)-1-hydroxy-2-[[(1RS)-2(4-methoxyphenyl) -1-methylethyl]-amino] ethyl] phenyl] formamide (E)-2-butenedioate dihydrate. The chemical formula is $C_{19}H_{24}N_2O_4$. $C_4H_4O_4.2H_2O$. The molecular weight is 840.91g/mol.[1]

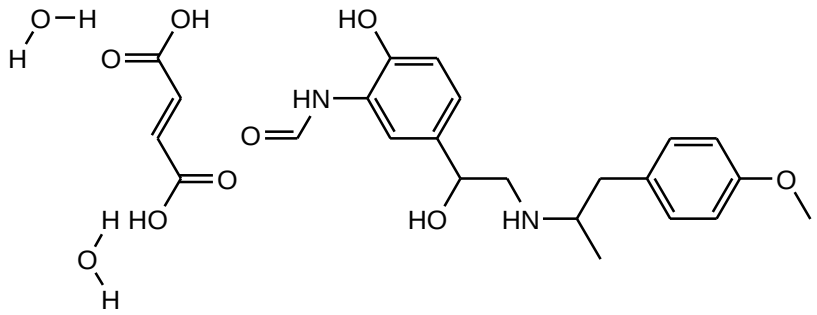

Fig.1.02: Structure of Formoterol Fumarate

There are various analytical methods reported in the literature for the assay of Glycopyrrolate and Formoterol separately and also with other drugs include spectrophotometry, HPLC, HPTLC, and other types. For only Glycopyrrolate, separate RP HPLC methods were available in bulk, tablet dosage forms [5], and for parenteral [3]. There is also a method for Glycopyrrolate alone in human plasma by liquid chromatography–electrospray ionization mass spectrometry [6] and liquid chromatography-Tandem mass spectrometry method for quantification of Glycopyrrolate in horse plasma [7]. There are various methods for determination for single Formoterol alone, like Automated and sensitive method for the determination of Formoterol in human plasma by high-performance liquid chromatography and electrochemical detection [8]. There are various RP-HPLC methods for the Simultaneous estimation of Formoterol Fumarate and Tiotropium Bromide [9], Formoterol Fumarate, and Budesonide in metered-dose inhaler formulation [10], a spectroscopic method for the simultaneous estimation of Mometasone Furoate and Formoterol Fumarate in Rotacaps [11]. chromatographic methods for the simultaneous determination of Mometasone furoate and Formoterol fumarate dihydrate in a combined dosage form [12] and Estimation of Formoterol Fumarate and Mometasone Furoate in Metered Dose Inhalation Form by High-Performance Liquid Chromatography [13], UV spectroscopic method for the determination of beclomethasone dipropionate and Formoterol fumarate in rotacap dosage form,4 Simultaneous spectroscopic determination of Formoterol fumarate and budesonide in their combined dosage form [14], RP-HPLC method for estimation of Formoterol fumarate and budesonide in pressurized meter dose inhaler form [15], Simultaneous Reversed-Phase HPLC Method for Formoterol Fumarate and Fluticasone Propionate in Metered-dose inhaler [16].

As per the literature survey, no reported method was available for the simultaneous determination of Glycopyrrolate and Formoterol. The present method was to build up a straightforward, minimal effort RP-UPLC technique for concurrent estimation of Glycopyrrolate and Formoterol in bulk and also in other dosage forms. The method was validated according to ICH guidelines [17].

II. Experimental

A. Chemicals and Reagents

Triethyl amine and Orthophosphoric acid (HPLC Grade) Water Milli-Q. All other chemicals of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

B. Instrumentation and Chromatographic Conditions

Instrumentation

Waters Acquity 2996 series U.V-Visible detector/2695 Separation Module, equipped with Empower 2 software, Bandelin ultrasonic bath, pH Meter (Adwa Model), Analytical Balance (Sartorius) were used in the present assay.

Preparation of 2.5 pH triethyl amine:

Take 1.0 ml of triethylamine transfer into 1000mL beaker dissolve and dilute to volume with 1000mL milli-Q water. Adjust the pH to 2.5 ± 0.05 with dilute orthophosphoric acid.

Preparation of mobile phase:

Transfer 500mL of pH 2.5 triethyl amine buffer and 500mL of Acetonitrile into 1000mL beaker mixed well.

Filter through 0.45µ membrane filter and degas.

Diluent Preparation:

The mobile phase used as a diluent

Standard preparation:

Accurately weighed and transferred 18 mg of Glycopyrrolate and 9.6mg of Formoterol Fumarate working standard into a 20 ml clean dry volumetric flask add about 14.0 mL of Diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 5.0 ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent.

Sample preparation:

Sample preparation of Glycopyrrolate and Formoterol fumarate rota caps were prepared in house preparation method an accurately weighed and taken the number of rota caps powder equivalent to 45 mg of Glycopyrrolate and 24mg Formoterol Fumarate sample into a 50 mL clean dry volumetric flask add about 25mL of Diluent and sonicated it up to 15mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through a 0.25-micron Injection filter. (Stock solution)

Further pipette 5ml of Glycopyrrolate and Formoterol Fumarate from the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Chromatographic conditions

BEH C18 (2.6m x 50mm 1.8µm) Column was used for analysis at 25°C column temperature. The mobile phase was pumped through the column at a flow rate of 0.2 mL/min. The sample injection volume was 6 µL. The photodiode array detector was set to a wavelength of 250nm for the detection and Chromatographic runtime was 6 minutes.

III. Results and Discussion

Method development

To develop a suitable and robust LC method for the determination of Glycopyrrolate and Formoterol Fumarate, different mobile phases were employed to achieve the best separation and resolution. The method development was started with BEH C18 (2.6x100mm,1.8µm) with the following mobile phase Acetonitrile: Water (80:20). Detector wavelength 250 nm, column temperature 25° C, Injection volume 4 µL, and Flow rate 0.4 ml/min using. Peak shapes were not good, Due to asymmetry in peak and lesser retention time, and no elution of the second peak. So, another trial was made with a change in flow rate.

For the next trial, the mobile phase composition was changed from Acetonitrile: Water (80:20) to Acetonitrile: Water (80:20) remaining chromatographic conditions are the same. The peak shape was not good, Due to tailing in peaks and asymmetry.

For the next trial buffer was changed to pH 2.5 Triethyl amine buffer and acetonitrile (50:50 v/v). detector wavelength 250 nm, column temperature 25°C, injection volume 6 µL, and flow rate of 0.2 ml/min used. The retention time of Glycopyrrolate and Formoterol Fumarate was found to be 0.65 and 1.05 min acceptable. The chromatogram of Glycopyrrolate and Formoterol Fumarate standard using the proposed method is shown in **(Figure: 1.03.)** System suitability results of the method are presented in **Table-1.**

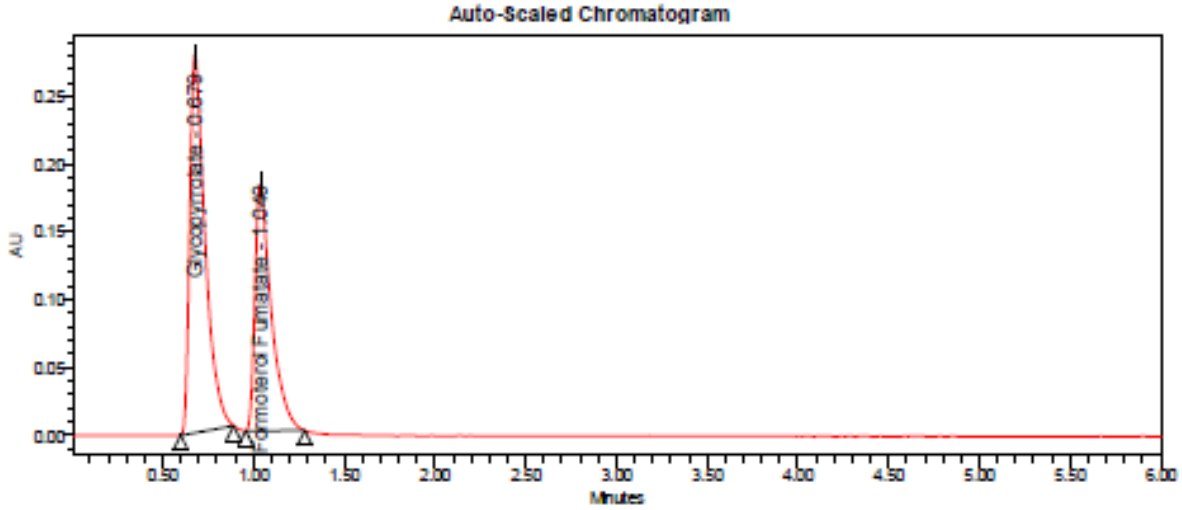

Figure: 1.03 A typical UPLC Chromatogram showing the peak of Glycopyrrolate and Formoterol Fumarate

Method validation

The developed RP-LC method extensively validated for assay of Glycopyrrolate and Formoterol Fumarate using the following Parameters.

Specificity

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo were injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution **(Figure:1.04)** showed no peaks at the retention time of Glycopyrrolate and Formoterol Fumarate peak. This indicates that the diluent solution used in sample preparation does not interfere in the estimation of Glycopyrrolate and Formoterol Fumarate in tablets. Similarly, a Chromatogram of Placebo solution **(Figure: 1.05)** showed no peaks at the retention time of Glycopyrrolate and Formoterol Fumarate peak. This indicates that the Placebo used in sample preparation does not interfere in the estimation of Glycopyrrolate and Formoterol Fumarate in Glycopyrrolate and Formoterol Fumarate tablets.

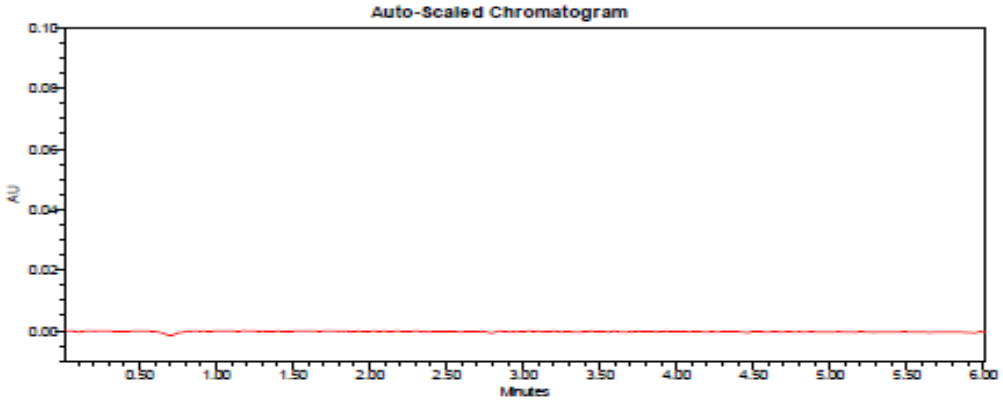

Figure: 1.04 A typical chromatogram of Blank

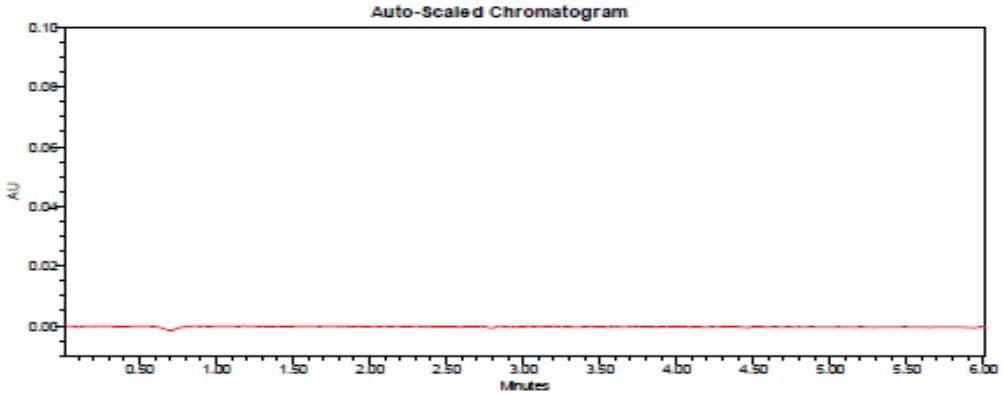

Figure: 1.05 A typical chromatogram of placebo

Table 1: System suitability parameters for Glycopyrrolate and Formoterol Fumarate by proposed method

Parameters	Glycopyrrolate	Formoterol Fumarate
USP Resolution	2.80	
Retention time (min)	0.68	1.05
No. of Theoretical plates	3017	2513
Tailing factor	1.39	1.48

Precision

The method precision study for six sample preparations in marketed samples showed an RSD of 0.19% for Glycopyrrolate. Similarly, the method precision study for six sample preparations in marketed samples showed an RSD of 0.13% for Formoterol Fumarate.

Table 2: Method Precision studies for Glycopyrrolate and Formoterol Fumarate by the proposed method

S.No	%Assay of Glycopyrrolate	%Assay of Formoterol Fumarate
1	100.06	100.03
2	100.16	100.03
3	100.23	99.79
4	99.79	99.83
5	99.98	99.96
6	99.73	100.11
Avg. assay	**99.99**	**99.95**
%RSD	**0.19**	**0.13**

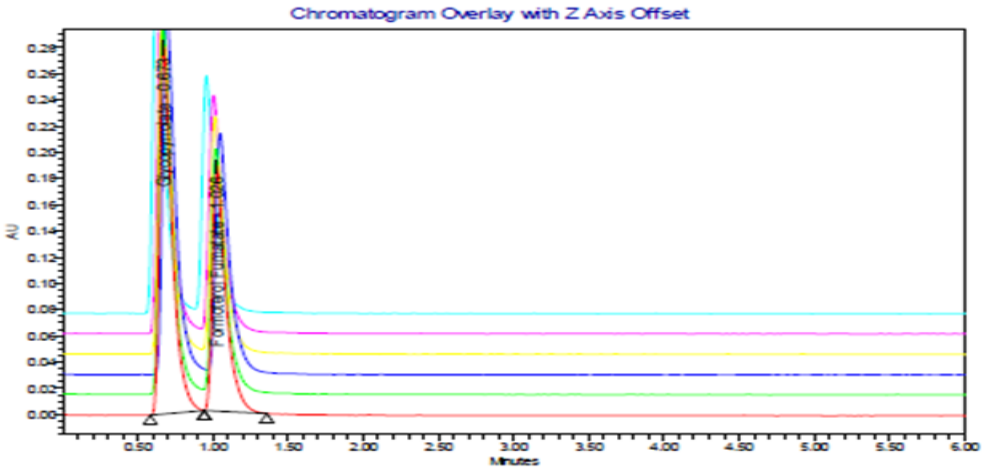

Figure: 1.06 A typical overlay chromatogram of method precision

Accuracy

A series of solutions were prepared by spiking the placebo and API in the range of about 50% to 150% of test concentration in triplicate and injected into the UPLC system and analyzed as per the test method. The percentage recoveries with found in the range of 99.85 to 100.70 for Glycopyrrolate and the percentage recoveries with found in the range of 100.30 to 101.11 for Formoterol Fumarate. From the data obtained given in **Table-:3 and Table-:4,** the method was found to be accurate.

Table 3: Recovery studies for Glycopyrrolate by the proposed method

Spiked level	Amount added (ppm)	Amount found (ppm)	%Recovery	%Mean recovery
50%	4.5	4.47	99.33	99.85
	4.5	4.52	100.44	
	4.5	4.49	99.78	
100%	9.0	9.08	100.89	100.70
	9.0	9.04	100.44	
	9.0	9.07	100.78	
150%	13.5	13.54	100.30	100.58
	13.5	13.59	100.67	
	13.5	13.51	100.07	

Table 4: Recovery studies for Formoterol Fumarate by the proposed method

Spiked level	Amount added (ppm)	Amount found (ppm)	%Recovery	%Mean recovery
50%	2.4	2.44	101.67	101.11
	2.4	2.41	100.42	
	2.4	2.43	101.25	
100%	4.8	4.79	99.79	100.35
	4.8	4.81	100.21	
	4.8	4.85	101.04	
150%	7.2	7.18	99.72	100.30
	7.2	7.21	100.14	
	7.2	7.23	100.42	

The linearity of detector response

The standard curve was obtained in the concentration range of 22.5-135.0 µg/ml for Glycopyrrolate and 12-72 µg/ml for Formoterol fumarate. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of the standard curve were calculated and given in **Figure: 1.07** For Glycopyrrolate and **Figure: 1.08** for Formoterol fumarate to demonstrate the linearity of the proposed method. From the data obtained which is given in **Table-5** For Glycopyrrolate and **Table-6** for Formoterol fumarate, the method was found to be linear within the proposed range.

Table 5: Linearity studies for Glycopyrrolate by the proposed method

Linearity Level	Concentration (ppm)	Average area	Statistical Analysis	
25	22.5	334272	Slope	14615
50	45.0	626084		
75	67.5	960544	Y-intercept	-13931
100	90.0	1315691	Correlation Coefficient R^2	0.9991
125	112.5	1611356		
150	135.0	1973885		

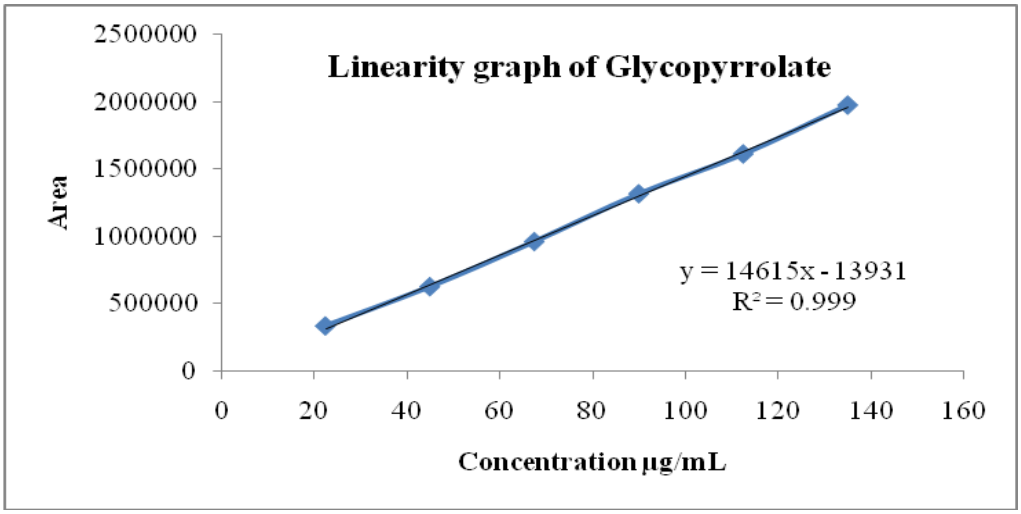

Figure: 1.07 Calibration curve for Glycopyrrolate

Table 6: Linearity studies for Formoterol Fumarate by the proposed method

Linearity Level	Concentration (ppm)	Average area	Statistical Analysis	
25	12	229292	Slope	19558
50	24	438380		
75	36	665410	Y-intercept	-24234
100	48	899822	Correlation Coefficient R2	0.9990
125	60	1155104		
150	72	1395268		

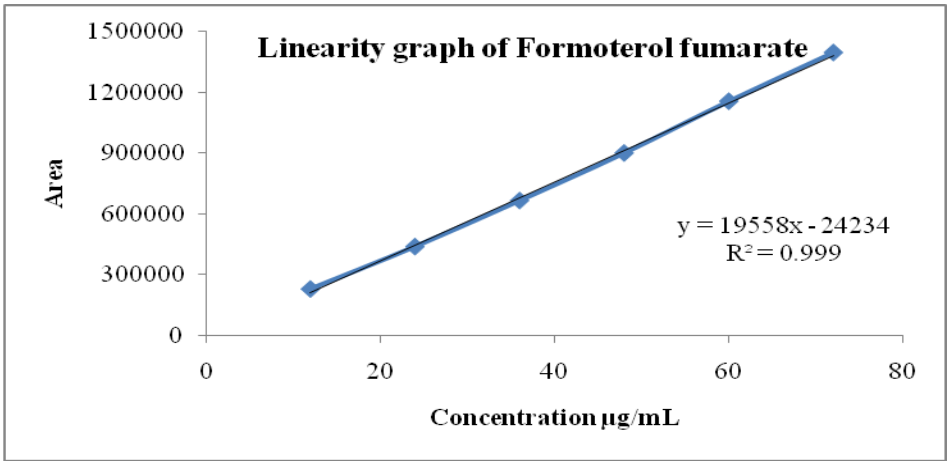

Figure: 1.08 Calibration curve for Formoterol Fumarate

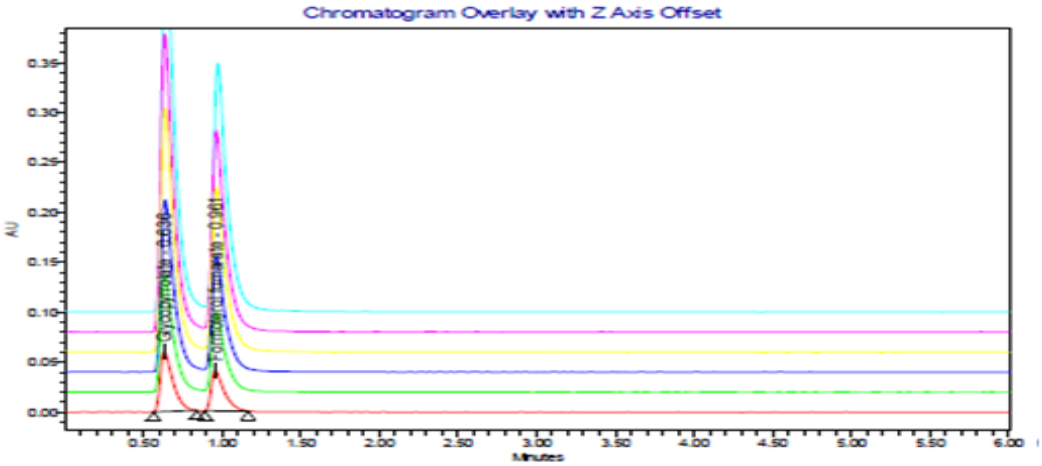

Figure: 1.09 A typical overlay chromatogram of Linearity

IV. Conclusion

An RP-UPLC method for simultaneous estimation of Glycopyrrolate and Formoterol fumarate was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 22.5-135.0 µg/ml for Glycopyrrolate and 12-72 µg/ml for Formoterol fumarate. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switchover of chromatographic conditions, equilibration of a column, and post-column flushing that is typically associated when different formulations and their drug substances are analyzed. We have developed a fast, simple, and reliable analytical method for the determination of Glycopyrrolate and Formoterol fumarate in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Glycopyrrolate and Formoterol fumarate. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with good accuracy and precision. It allows reliably the analysis of Glycopyrrolate and Formoterol fumarate in bulk, its different pharmaceutical dosage forms.

Acknowledgments

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V. References

[1] Bevespi Aerosphere™ (glycopyrrolate and formoterol fumigate) Prescribing information, AstraZeneca 2016.

[2] https://www.drugs.com/pro/glycopyrrolate-injection.html

[3] Mishra S, Arora V (2016). International Journal for Pharmaceutical Research Scholars.;5(4):101-4.

[4] Shah PD, Koradia S. (2014) World Journal of Pharmacy and Pharmaceutical Sciences.;3(5):611-23.

[5] Pabba KG, Akula G (2011). Asian Journal of Pharmaceutical and Clinical Research.;4(4):37-40.

[6] Stormea ML, t'Kindt RS, Goeteyn W, Reyntjens K, Van Bocxlaer JF (2008). Journal of Chromatogr. B.;876(1):24-30.

[7] Rumpler MJ, Sams RA, Colahan P (2011). Validation of liquid chromatography-tandem mass spectrometry method for quantification of Glycopyrrolate in horse plasma Journal of Analytical Toxicology.;35(9):656-64.

[8] Campestrini J, Lecaillon JB, Godbillon J (1997). Journal of Chromatogr. B.;704(1-2):221-9.

[9] Srinivasu K, Rao JV, Raju NA, Mukkanti K (2010). Asian Journal of Chemistry.;22(5):3943-8.

[10] Nanasaheb RK, Ashok PP, Javed AM, Govind ND (2014). World Journal of Pharmaceutical Research.;3(6):1386-99.

[11] Zanwar AS, Sen DB, Ruikar DB, Seth AK (2014). Indo American Journal of Pharmaceutical Research.;4(12):5928-33.

[12] Merey HA, El-Mosallamy SS, Hassan NY, El-Zeany BA (2016). Bulletin of Faculty of Pharmacy, Cairo University.;54(1):99-106.

[13] Srinivasarao K, Gorule V, Venkata RCh, Venkata KA (2012). Journal of Analytical and Bioanalytical Techniques.;3(7):1-4.

[14] Prasad AV (2006). Indian Journal of Chemical Technology.;13:81-3.

[15] Pai N, Patil SS (2013). Der Pharmacia Sinica.;4(4):15-25.

[16] Malik K, Kumar D, Tomar V, Kaskhedikar S, Soni L (2011). Der Pharmacia Sinica.;2(6):77-84.

[17] ICH harmonized tripartite guideline; validation of Analytical procedures: Text and Methodology Q2 (R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2005. 11-12.