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A STUDY OF ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION FOR SIMULTANEOUS ESTIMATION OF GLYCOPYRROLATE AND FORMOTEROL FUMARATE IN BULK DRUG BY UHPLC METHOD

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Keywords:

Glycopyrrolate, Formoterol fumarate, UHPLC, HPLC, Forced degradation

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ABSTRACT: The present study was proposed to develop a simple, rapid, and economical UHPLC method was described for the method development, validation and forced degradation for the simultaneous estimation of glycopyrrolate and formoterol fumarate in bulk drug. The chromatographic conditions were observed in the column using Zorbax RXC18 (150 × 4.6 mm) 5 µm using a mobile phase composition of Water: Acetonitrile: Methanol (20:30:50 v/v) and pH was adjusted to 3.0 with dilute orthophosphoric acid. The flow rate was 1.0 ml/min, and the analytes were recorded at 279 nm. The Retention time (RT) was observed in 1.208 for Glycopyrrolate and 5.897 for Formoterol fumarate. A calibration curve was linear with a coefficient correlation between 0.999 to 1.0 over a concentration range of 20-60µg/ml for glycopyrrolate and formoterol fumarate. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.003,0.011µg/ml for Glycopyrrolate and 0.108,0.32µg/ml for formoterol fumarate. All the validation parameters were within the acceptance range according to ICH norms. The validation and forced degradation study was performed for the proposed method and applied successfully to simultaneously estimate glycopyrrolate and formoterol fumarate. This method was precise, accurate, robust and economical.

INTRODUCTION:

Ultra High-Performance Liquid Chromatography: Totally porous, spherical silica dominated the HPLC market from the 1980s to the 2000s. The average particle size continually decreased from 10 µm to 5 µm to 3 µm [1, 2]. The trend towards smaller particles is driven by improved performance in terms of speed, sensitivity, and resolution. Smaller particles, however, require higher operating pressures, and in 2004 instruments were commercialized that could utilize columns prepared with sub-2 µm particles. **Fig. 1** illustrates the chromatographic performance of four typical particle sizes used in HPLC.

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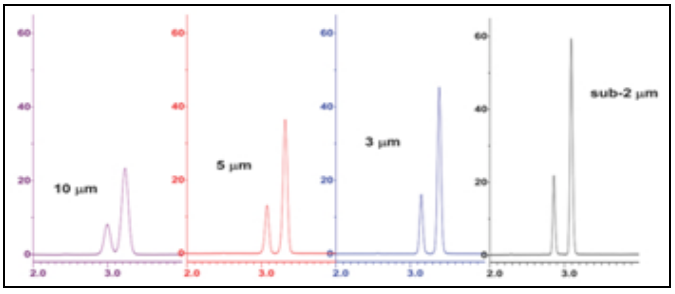


FIG. 1: CHROMATOGRAPHIC PERFORMANCE FOR FOUR COMMON PARTICLE SIZES

Principle: The UHPLC is based on the principle of use of stationary phase consisting of particles less than 2.5 µm (while HPLC columns are typically filled with particles of 3 to 5 µm). The underlying principles of this evolution are governed by the "Van Deemeter equation" which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency) [3].

$$H=A+ B/v+ Cv$$

Where; A, B , and C are constants; V is the velocity of the sample.

Analytical Method Development of Glycopyrrolate and Formoterol Fumarate: Glycopyrolate 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 398.33 g / mol. Glycopyrolate is a crystalline white powder. It is completely dissolvable in water and alcohol and much insoluble in chloroform and ether. Glycopyrrolate, as other anticholinergic (anti muscarinic) drugs, the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine it require cholinergic innervations [4]. Thus, it diminishes the volume and free acidity of gastric secretions and controls excessive pharyngeal, tracheal and bronchial secretions [5]. Formoterol acts as a bronchodilator. It extends the airways of the lungs, so that it helps to inhale all the more effortlessly.

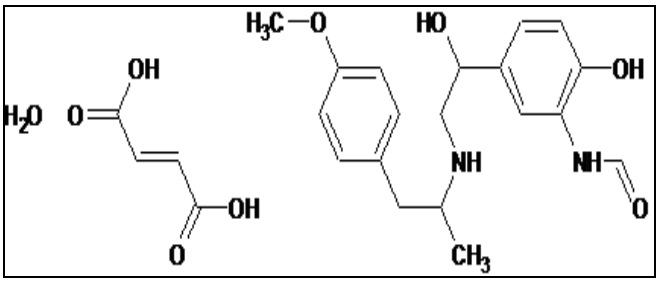


FIG. 2: STRUCTURE OF FORMOTEROL FUMARATE

It may even be utilized to forestall respiratory issues caused by exercise. It can also be utilized for the long-term treatment of chronic obstructive pulmonary disease (COPD) [6]. Chemically, Formoterol is N-[2-Hydroxy5-[(1RS)-1 - hydroxyl - 2 - [(1RS) - 2(4 - methoxyphenyl) - 1 - methylethyl] - amino] ethyl] phenyl] formamide (E) - 2 - butenedioatedihydrate. The chemical formula is $C_{19}H_{24}N_2$ O. $C_4H_4O_2H_2$ O. The molecular weight is 840.91 g / mol.

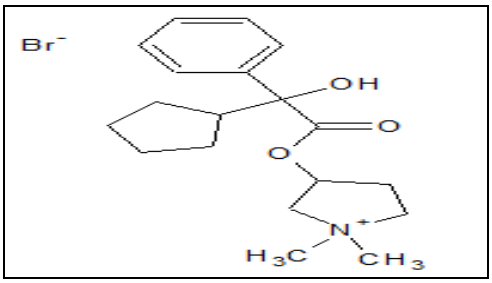


FIG. 3: STRUCTURE OF GLYCOPYRROLATE

The main aim of the work is to develop a simple, accurate, precise, and economical new RP-UHPLC method development, validation, and forced degradation for the simultaneous estimation of Glycopyrrolate and Formoterol fumarate in bulk drug. It includes the objective of the experimental work has been planned as to following the methods *i.e* Solubility determination of Glycopyrrolate and Formoterol fumarate in various solvents and buffers and to determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for UHPLC method development, Optimize the mobile phase and flow rates for proper resolution and retention times and Validate the developed method and forced degradation as per ICH guidelines.

Literature survey revealed that it has very few analytical methods had been reported for the simultaneous estimation of glycopyrrolate and formoterol fumarate by using UV-spectroscopy [7], RP-HPLC [8-15], gas chromatography by individually or simultaneously with other drugs. Form this survey; it confirms that there is no method has been reported for the simultaneous estimation of glycopyrrolate and formoterol fumarate in bulk drug by using UHPLC.

The present method has so many advantages like decrease the runtime and increase the sensitivity and reduce the time consuming and easy to handle, and simple preparation of the mobile phase and standard solutions with low cost of the solvents. All the parameters are satisfied with the ICH guidelines for validation of the simultaneous estimation of glycopyrrolate and formoterol fumarate in bulk drugs.

MATERIALS AND METHODS:

Instruments: The method has made up of Agilent infinity 1290 with having Zorbax RX C18 (150 × 4.5 mmID) 3 µm column with UV-Detector.

Chemical Solutions and Reagents: Glycopyrrolate and formoterol fumarate obtained from madras pharmaceuticals, Chennai, India and potassium hydrogen phosphate buffer analytical grade from Rankem and water, Acetonitrile, methanol from Merck chemicals private limited.

Preparation of Standard Solution: About 10 mg of Glycopyrrolate and 10 mg of Formoterol fumarate were weighed into a 50 mL volumetric flask, to this 50 mL of mobile phase was added, sonicated, and the volume was made up to mark with the mobile phase. 1ml was Pipetted out from the above stock solution and transferred into 10 ml volumetric flask and made up to 10 ml with the mobile phase.

Preparation of Orthophosphoric Acid Buffer pH: Buffer solution was made with dissolving 1ml of orthophosphoric acid in 1000 ml of water. Buffer was filtered through 0.45 µm filters to remove all fine particles and gases.

Mobile Phase Pomposition: Simultaneous estimation of glycopyrrolate and formoterol fumarate was carried out with different combinations of solvents like water: Acetonitrile: methanol.

Sample Solution: Crush 20 tablets, then weigh the quantity of powder equivalent to 50 mg of glycopyrrolate and 100 mg of formoterol fumaratein 100 mL volumetric flask and add 70 mL of mobile phase then sonicated for 30 min intermittent shaking after 30 min make up the volume with the mobile phase. Pipetted 5 mL of the clear solution in to 50 mL volumetric flask and make up the volume with mobile phase. Filter the solution through 0.45 µm filter paper.

Chromatographic Conditions: The method development for separation of glycopyrrolate and formoterol fumarate using different solutions finally optimized method was obtained with mobile phase water: Acetonitrile: methanol (20:30:50 v/v) pumped at flow rate is 1 ml / min. The separation of the peaks was scanned at 279 nm by observing of UV-Detector. Mobile phase is filtered with vacuum filtration by using 0.45 µm membrane filter.

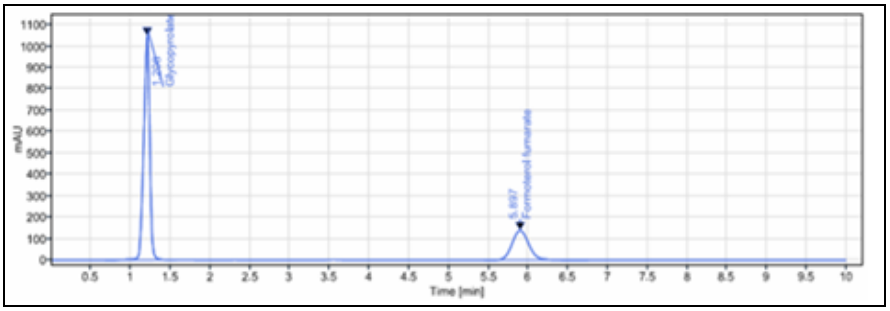
Method Validation: The developed method was validated according to ICH guidelines it followed by ICHQ2 (R1) include system suitability, linearity, precision, LOD, LOQ, accuracy, robustness [16].

RESULTS:

Optimization of Chromatographic Conditions: A number of the eluting system was examined for optimization of the mobile phase for the separation of the drugs. Mixtures containing phosphate buffer and Acetonitrile and methanol were used as an eluting system based on drug polarity, the mixture of water, Acetonitrile, and methanol in the ratio of 20:30:50 v/v proved an efficient separation of the drugs with good peak shapes and retention time. The flow rate 1.0 ml/min, and the injection volume were 10 µl; the obtained peaks were scanned at 279 nm using UV-detector.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITION FOR THE SIMULTANEOUS ESTIMATION OF GLYCOPYRROLATE AND FORMOTEROL FUMARATE

Parameter	Condition
Column	Zorbax RX C18 (150x4.6mm ID)
Elution mode	5.0µm
Mobile phase	Isocratic
Flow rate	Water: Acetonitrile: Methanol
Detection	(20:30:50) %v/v/v
wavelength	1.0mL/min
Injection volume	279nm
Run time	10µL
	10min



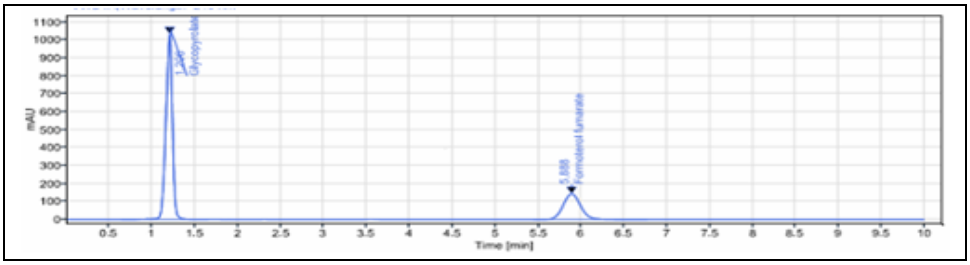
Name	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
Glycopyrolate	1.208	92229	6578	1.25
Formoterol Fumarate	5.987	57172	4566	1.09

FIG. 4: CHROMATOGRAM FOR OPTIMIZED METHOD

This gives retention times of 1.208 for glycol-pyrolate and 5.897 for formoterol fumarate with good efficiency, peak shape and good resolution. So, this method was considered and validated according to ICH guidelines respectively. Hence this method was finalized for the simultaneous estimation of glycopyrolate and formoterol fumarate shown in the **Table 1** and **Fig. 4**.

Method Validation:

System suitability:



Compound Name	Retention Time	Peak Area	Theoretical Plate Count	Tailing Factor	Resolution
Glycopyrolate	1.206	552979	9269	0.87	18.2
Formoterol fumarate	5.888	202657	32138	1.09	18.2

FIG. 5: CHROMATOGRAM OF SYSTEM SUITABILITY INJECTION

The developed method has been produced at theoretical plates above 2000 for glycopyrrolate and formoterol fumarate with a tailing factor less than 2, which ensures the suitability of the developed method. The results of the system suitability results were shown in **Fig. 5** and **Table 2**.

TABLE 2: SYSTEM SUITABILITY OF THE DEVELOPED METHOD [N=6]

Parameters	Glycopyrrolate	Formoterol Fumarate	Glycopyrrolate Acceptance Criteria
Retention time	1.206	5.888	-------
Theoritical plates	9269	32138	>2000
Tailing factor	0.87	1.09	<2
Resolution	18.2	18.2	>2

Linearity: Linearity of the calibration curve was obtained in the concentration ranges from 20-60µg/ml for glycopyrrolate and formoterol fumarate. The linear response of the drug was found to be in the selected concentration range. The correlation coefficients for glycopyrrolate and formoterol fumarate were found to be 0.999 and 0.999, respectively. The results of the linearity summarized in **Table 3, 4, 5,** and **Fig. 6.**

TABLE 3: LINEARITY DATA OF GLYCOPYRROLATE

S. no.	Concentration (µg/mL)	Peak Area
1	20	214975
2	30	305389
3	40	399899
4	50	481896
5	60	584269

TABLE 4: LINEARITY DATA OF FORMOTEROL FUMARATE

S. no.	Concentration (µg/mL)	Peak Area
1	20	127453
2	30	170442
3	40	215109
4	50	253818
5	60	296342

TABLE 5: REGRESSION EQUATION OF THE LIN-EARITY PLOTS AND REGRESSION COEFFICIENT

Drug Name	Concentration	Equation of Regression Line	R2 Value Range
Glycopyrolate	20-60	y = 91540x + 122607	0.999
Formoterol fumarate	20-60	y = 421.15x + 862.87	0.999

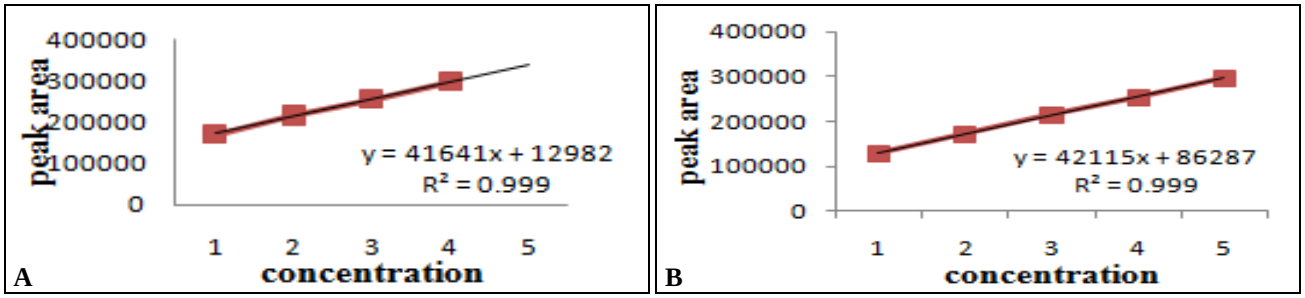
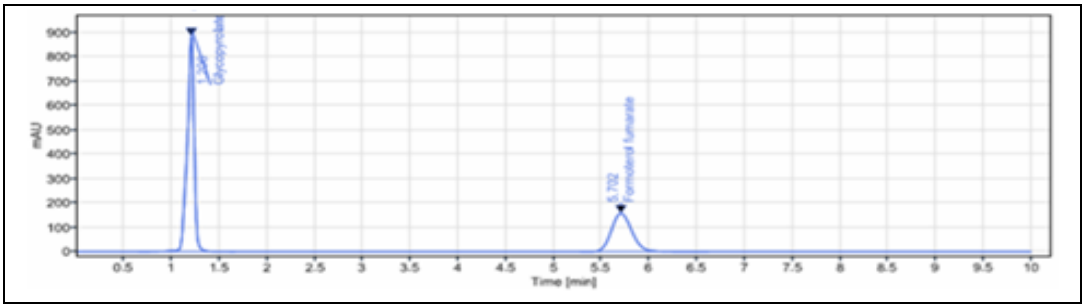


FIG. 6: GRAPH FOR LINEARITY DATA FOR GLYCOPYRROLATE [A] AND FORMOTEROL FUMARATE [B]

Accuracy: The accuracy of the method was determined by recovery studies. The recovery studies were carried out three times, and the percentage recovery and percentage mean recovery were calculated for drug and shown in **Fig.7** and **Table 6, 7.**



S. no.	Compound Name	Retention Time	Peak Area	Theoretical Plate Count	Tailing Factor
1	Glycopyrolate	1.206	453837	11729	0.84
2	Formoterol fumarate	5.702	235162	26936	1.14

FIG. 7: CHROMATOGRAM FOR ACCURACY -100%

The % mean recovery of the glycopyrrolate and formoterol fumarate should be not less than 98.0% and not more than 102%. The results of the recovery studies were described in **Table 6, 7**.

TABLE 6: ACCURACY DATA FOR GLYCOPYROLATE

Concentration (µg/ml)	Amount Present (µg/mL)	Amount Found (µg/mL)	% Recovery	% Mean Recovery
50%	90	45.39	99.1	
100%	90	90.45	99.5	99.7
150%	135	138.1	100.7	

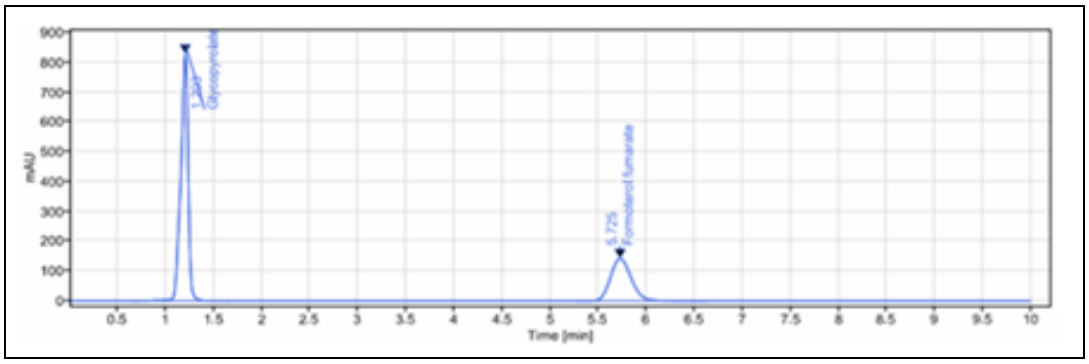
TABLE 7: ACCURACY DATA FOR FORMOTEROL FUMARATE

Concentration (µg/ml)	Amount Present (µg/mL)	Amount Found (µg/mL)	% Recovery	% Mean Recovery
50%	24	24.37	98.7	
100%	48	48.84	98.2	99.1
150%	72	71.77	100.4	

Precision: The developed method has shown in % RSD less than 2. It indicates that this method was precise is shown in **Fig. 8** and **Table 8**.

LOD and LOQ: Limit of detection and limit of quantification were quantified from the standard deviation of the y-intercepts and slope of the calibration curve of glycopyrrolate and formoterol fumarate. The LOD and LOQ were found to be 0.003 µg/ml, 0.011 µg/ml for glycopyrrolate and 0.108 µg/ml, 0.32 µg/ml for formoterol fumarate respectively. This data showed that the developed method could detect and quantify at lower concentration was highly sensitive.

Specificity: Specificity was carried out by evaluation of standard solution injections. The chromatogram of standard and spiking sample solution was compared, the correlation was good, there is no interference of excipients with drug was observed and shown in **Fig. 9, 10, 11**.



S. no.	Compound Name	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	Glycopyrolate	1.203	442161	10539	0.82
2	Formoterolfumarate	5.725	214189	26960	1.15

FIG. 8: CHROMATOGRAM FOR SYSTEM PRECISION

TABLE 8: PRECISION OF THE DEVELOPED METHOD OF GLYCOPYROLATE AND FORMOTEROL FUMARATE [N=6]

Drug	Concentration (µg/ml)	Peak Area	SD	%RSD
Glycopyrolate Formoterol fumarate	40	442161	18.32	0.004
	40	214189	0.87	0.040

N is number of determinations, SD is standard deviation, RSD is the relative standard deviation

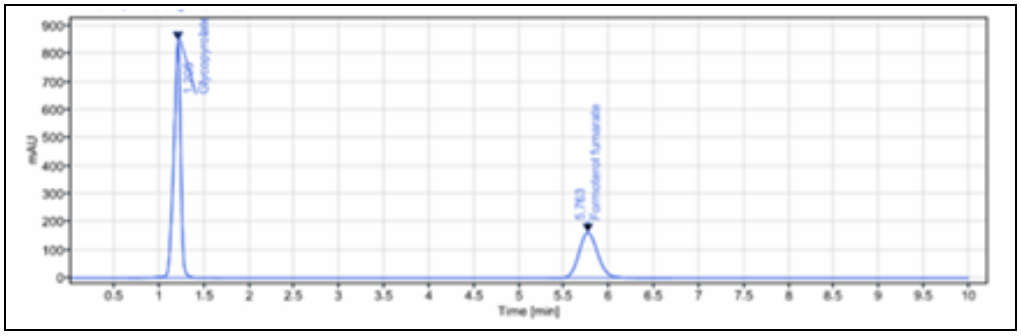


FIG. 9: SPECIFICITY CHROMATOGRAM OF STANDARD INJECTION

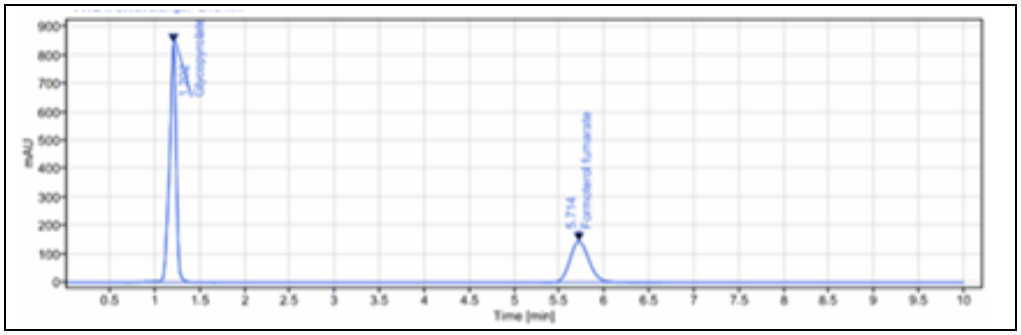


FIG. 10: SPECIFICITY CHROMATOGRAM OF SPIKED SAMPLE INJECTION

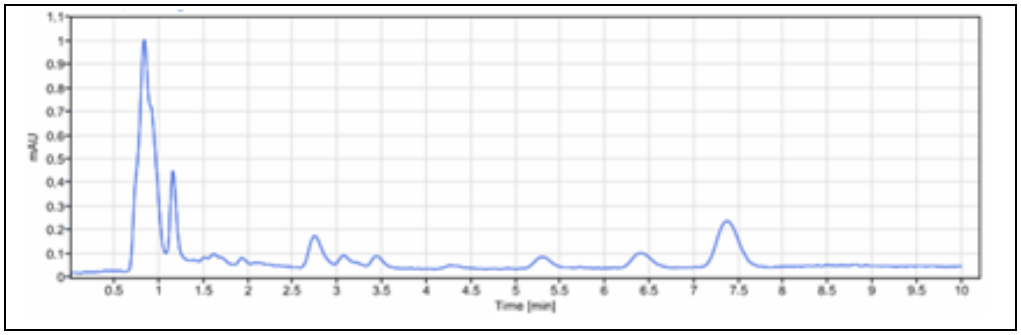


FIG. 11: SPECIFICITY OF PLACEBO INJECTION

Robustness: The Robustness of the proposed method was described. The results were obtained by the effect of variation in method parameters are summarized below.

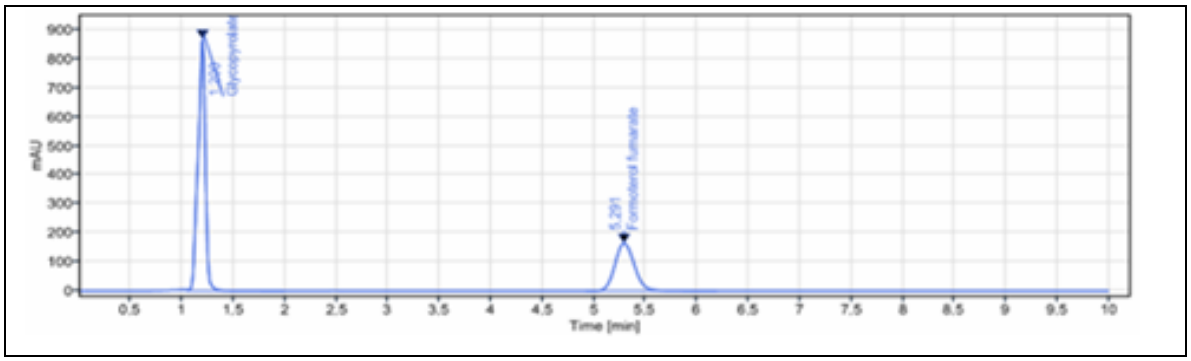
These results are satisfied with acceptance criteria (*i.e.* plate count is>2000 and tailing factor <2) and are mentioned in **Table 9** and **Fig. 14**.

TABLE 9: RESULTS FOR ROBUSTNESS OF GLYCOPYRROLATE AND FORMOTEROL FUMARATE

Chromatographic Changes		Plate Count		Tailing factor	
		Glycopyrrolate	Formoterol Fumarate	Glycopyrrolate	Formoterol Fumarate
Flow rate (mL/min)	0.8	15306	35978	0.85	1.20
	1.5	10107	32334	0.85	1.10
Wavelength (nm)	276	13825	37474	0.85	1.12
	278	14029	36929	0.83	1.14
	280	14245	37082	0.84	1.12
	281	13981	37410	0.82	1.14

Ruggedness: The ruggedness of the method was studied by determining the analyst to analyst variation by performing the method by two different analysts.

From this observation, the % RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged **Table 10.**



S. no.	Compound Name	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	Glycopyrrolate	1.200	434825	13825	0.85
2	Formoterolfumarate	5.291	214355	37474	1.12

FIG. 12: CHROMATOGRAM OF ANALYST 01 STANDARD PREPARATION

TABLE 10: RESULTS FOR RUGGEDNESS

Glycopyrolate	% Assay	Formoterol fumarate	% Assay
Analyst – 1	101.44	Analyst – 1	100.82
Analyst – 2	99.72	Analyst – 2	98.75
Analyst – 3	100.02	Analyst – 3	99.89
% RSD	0.24	% RSD	0.36

Assay: The assay was estimated by injecting the prepared concentration of tablet formulation into UHPLC system.

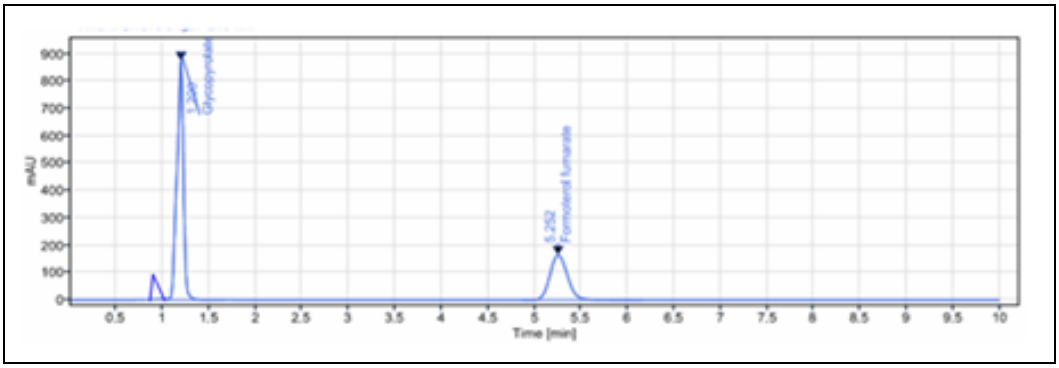
Assay results were calculated by comparing the peak area of tablet formulation with peak area of the standard solution.

TABLE 11: ASSAY DATA FOR THE FORMULATION FOR GLYCOPYROLATE AND FORMOTEROL FUMARATE (N=5)

Formulation	Labeled Amount (mg)	% assay	% RSD
(Bevespi Aerosphere)	Glycopyrolate – 100 mg	99.81	0.24
	Formoterol fumarate-50 mg	99.60	0.36

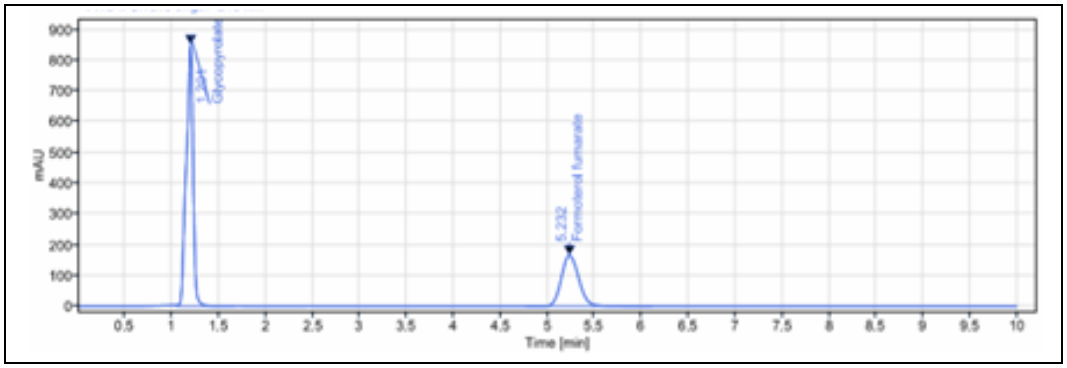
The % assay of glycopyrrolate and formoterol fumarate was found to be 99.81% and 99.60%, respectively. The percentage assay of both drugs was found to be more than 99.5%. Hence, the method was successfully applied for estimation of glycopyrrolate and formoterol fumarate in the bulk and pharmaceutical dosage form. The results of assay were described in **Table 11**.

Forced Degradation: Degradation studies were carried out with acid, base, peroxide, thermal, UV and water. It was observed that the response of peak area and retention time of glycopyrrolate and formoterol fumarate nearly same as obtained results. Degradation was found in peroxide and thermal conditions because extra peaks were observed and no degradation was found in UV, acid, base, water. Because they were no extra peaks. The observed data showed in **Table 12, 13,** and **Fig. 15-19**.



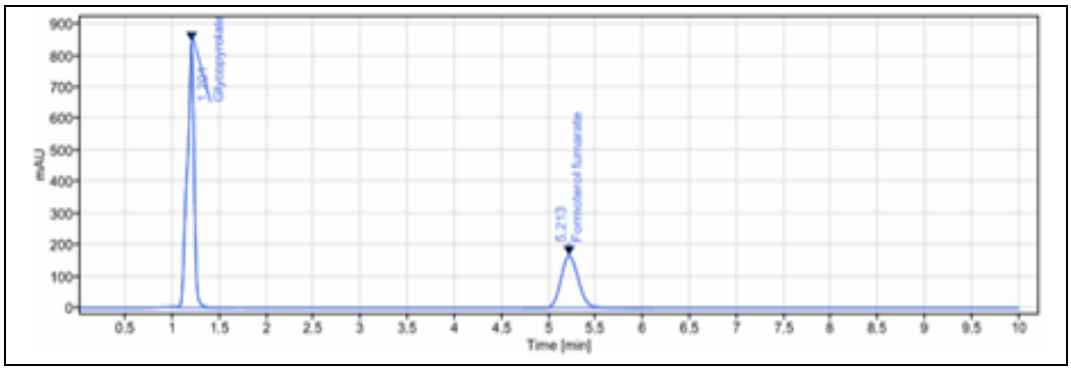
S. no.	Name	Retention time	Peak Area	Theoretical Plates	Tailing Factor
1	Glycopyrrolate	1.200	432609	14245	0.84
2	Formoterol Fumarate	5.252	214060	37082	1012

FIG. 13: CHROMATOGRAM FOR PEROXIDE DEGRADATION



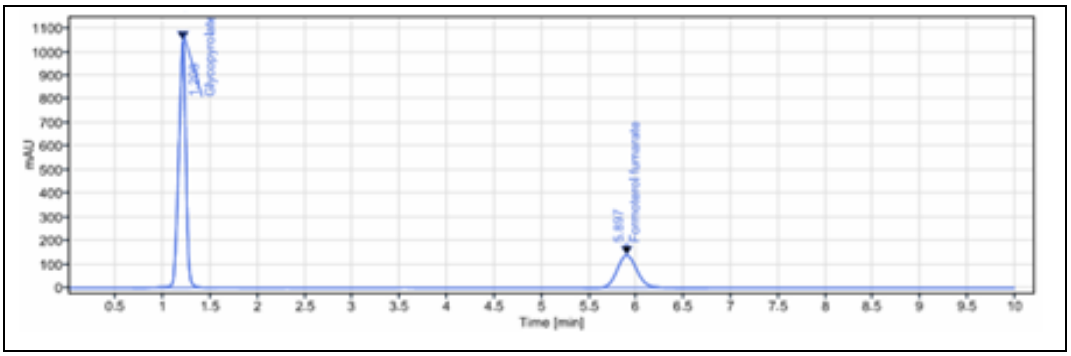
S. no.	Name	Retention time	Peak Area	Theoretical Plates	Tailing Factor
1	Glycopyrrolate	1.201	432664	13981	0.82
2	Formoterol Fumarate	5.2322	213919	37410	1.14

FIG. 14: CHROMATOGRAM FOR PHOTOLYTIC DEGRADATION



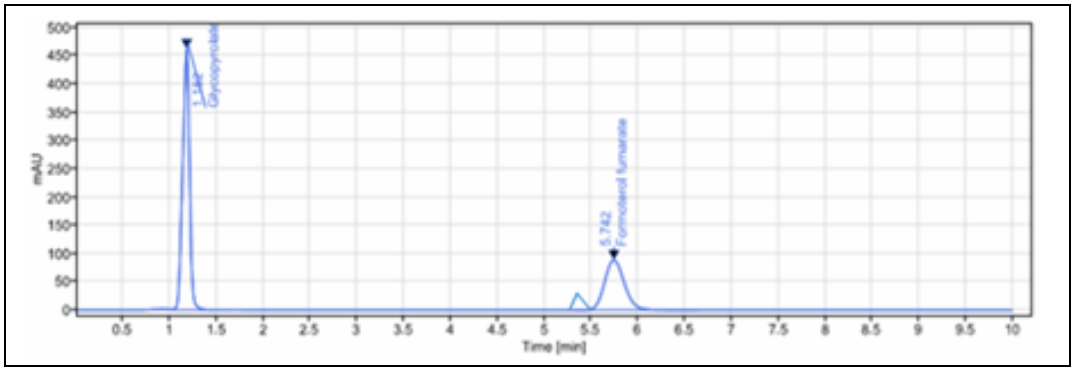
S. no.	Name	Retention time	Peak Area	Theoretical Plates	Tailing Factor
1	Glycopyrrolate	1.201	432464	14696	0.81
2	Formoterol Fumarate	5.213	213773	37164	1.13

FIG. 15: CHROMATOGRAM FOR ACIDIC DEGRADATION



S. no.	Name	Retention time	Peak Area	Theoretical Plates	Tailing Factor
1	Glycopyrrolate	1.208	551243	9471	0.89
2	Formoterol Fumarate	5.897	202695	30089	1.10

FIG. 16: CHROMATOGRAM FOR ALKALINE DEGRADATION



S. no.	Name	Retention time	Peak Area	Theoretical Plates	Tailing Factor
1	Glycopyrrolate	1.182	238258	8946	0.8
2	Formoterol Fumarate	5.742	130985	28122	1.14

FIG. 17: CHROMATOGRAM FOR THERMAL DEGRADATION

TABLE 12: RESULTS OF GLYCOPYRROLATE

Method	Std Area	Degradation Area	% Obtained	% Degraded
Peroxide	920630	432609	98.256	2.644
Photolytic	920630	432664	96.255	0.645
Acidic	920630	432464	98.254	0.646
Alkaline	920630	551243	99.254	0.646
Thermal	920630	238258	97.255	1.645

TABLE 13: RESULTS FOR FORMOTEROL FUMARATE

Method	Std Area	Degradation Area	% Obtained	% Degraded
Peroxide	572068	214060	98.856	1.044
Photolytic	572068	213919	97.854	0.046
Acidic	572068	213773	98.853	0.047
Alkaline	572068	202695	99.854	0.046
Thermal	572068	130985	99.856	2.044

DISCUSSION: Stability indicating UHPLC method is a simple, rapid, precise, accurate method for analyzing each component in a mixture. The previous study had reported in the literature survey. In this UHPLC method, we used UV-detector to prove the selectivity of the method. The method was validated according to the ICH guidelines on validation of analytical procedures and stability testing of new drug substance and products.

In order to develop a UHPLC method for estimation of glycopyrrolate and formoterol fumarate, different buffer ratios and flow rates were applied. Water: Acetonitrile: Methanol (20:30:50) v/v as mobile phase and discovery C18 column was selected. Separation of glycopyrrolate 1.208 min and formoterol fumarate is 5.897 min was detected by the wavelength of 279 nm. In this method UV-detector is able to identify the glycopyrrolate and

formoterol fumarate and degradation products. Method was validated. The results of validation parameters had shown in compliance of ICH guidelines. The range of linearity had good correlation with concentration and peak area. The correlation coefficients for glycopyrrolate and formoterol fumarate were 0.999 and 0.999, respectively. Which indicates that the concentration range was highly linear. In the assay the amount of both drugs recovered was found to be 99.81% and 99.60%, respectively.

Hence, the stability-indicating assay method was found to be appropriate for the analysis of the drug. The separation of degradation peak for degradants products was observed under peroxide and thermal hydrolysis.

CONCLUSION: A simple and sensitive stability-indicating UHPLC method was developed for simultaneous estimation of glycopyrrolate and formoterol fumarate. It concludes that all the parameters are within limits and meet the acceptance criteria of ICH guidelines for method validation. The proposed method was simple, accurate, specific precise, robust, and economical. Hence this method is validated and can be used for routine and stable sample analysis.

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REFERENCES:

1. Knox JX and Saleem M: High performance liquid chromatography. J chromatographic Sci 1969; 7: 614-22.
2. Kirkland J, Langlois TJ and Destefano JJ: Fused-Core Particles for HPLC Columns. Americ Lab 2007; 39: 18-21.
3. Susmitha GA and Rajitha G: Development and validation of stability indicating UPLC method for simultaneous estimation of sofosbuvir and velpatasir in tablet dosage form. Int J Pharmaceutical Sci and Res 2018; 9: 4764-69.
4. Kishor Kumar E and Sundaram RS: Synchronous estimation of glycopyrolate and formoterol in bulk and pharmaceutical dosage form by RP-HPLC Method. Indian J Pharm Edu and Res 2018; 52: 47-55.
5. Mishra S and Arora V: Analytical method validation of hplc method for assay of anti cholinergic drug in parentral formulation. Int J Pharm Res Scholars 2016; 5: 101-104.
6. Aarti Zanwar S, Dhanya B, Ashim Kumar S and Seth AK: Simultaneous estimation of mometastone furoate and formoterol fumarate by HPLC Method in Rota caps. Int J Pharmacy and Pharmaceutical Sci 2019; 11: 12-16.
7. Shah PD and Koradia S: Simultaneous determination of beclomethasone dipropionate and formoterol fumarate in rota caps dosage form using two different spectro photometric methods. WJPPS 2014; 3: 611-23.
8. Kusum M, Davinder Kumar, Vivek T, Satish K and Soni L: Simultaneous quantitative determination of formoterol fumarate and fluticanasone propionate by validated Reversed- Phase HPLC method dose inhaler. Der Pharmacia Sinica 2011; 2: 77-84.
9. Gosuddin M, Appala Raju S, Sultanuddin and Manjunath S: Development and validation of spectro photometric methods for estimation of formoterol bulk drug and its pharmaceutical dosage forms. Int J Pharmacy and Pharm Sci 2011; 3: 306-09.
10. Nandini P and Suhas SP: Development and validation of RP-HPLC method for estimation of formoterol fumarate and budesonide in pressurised meter dose inhaler form. Der Pharmacia Sinica 2013; 4: 15-25.
11. Swetha A and Kuber RB: A novel stability-indicating reverse phase liquid chromatographic method for the simultaneous estimation of metformin and teneligliptin in pure and pharmaceutical formulation. Int J App Pharm 2018; 10: 274-80.
12. Ashwini B, Shymala M and Sharma JVC: Analytical method validation report for essay of glycopyrolate and formoterol fumarate by RP-HPLC. Pharma Res Lib 2018; 6: 01-08.
13. Srinivasa Rao K, Ninayk G, Venkata Reddaiah CH and Krishna VA: Validated method development for estimation for formoterol fumarate and mometastone furoate in metered dose inhalation form by high performance liquid chromatography. J Anal Bioanal Techniques 2012; 3: 2-4.
14. Srinivasu K, Venkateswara Rao J, Appala Raju N and Mukkanti K: Simultaneous RP-HPLC Method for the Estimation of formoterol fumarate and tiotropium bromide in pharmaceutical dosage forms. Asian J Pharm Chem 2010; 22: 3943-48.
15. Kuber RB and Sravanthi PSK: Analytical method development and validation of ceftazimide pentahydrate and tazobactum sodium by RP-HPLC method in bulk and dosage forms. Research J of Pharmceutical, Biological and Chemical Sci 2017; 8: 2447-57.
16. ICH Guidelines, Q2 (R1): validation of analytical procedures: text and methodology. 1995.

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