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Development and Validation of New RP-HPLC Method for the Quantification of Glycoprrolate and Formaterol in Bulk and Pharmaceutical Dosage Forms

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[1]Sahithi V, *[2]Sareesh Kankanala, [3]Santhosh Anasuri, [4]Sunil Kumar Chaitanya Padavala

[1]Department of Pharmaceutical Analysis, St. Pauls College of Pharmacy, Hyderabad, Telangana, India-501510

[2,3]Assistant Professor, St. Pauls College of Pharmacy, Hyderabad, Telangana, India 501510

[4]Professor, St. Pauls College of Pharmacy, Hyderabad, Telangana, India 501510

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ABSTRACT

A new, simple and accurate, precise RP-HPLC method was developed for simultaneous determination of Glycopyrrolate and Formoterol fumarate in bulk and in combined pharmaceutical dosage form. The separation of Glycopyrrolate and Formoterol fumarate was achieved within 8 minutes on an Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column using Methanol: Acetate Buffer pH-3.8 (24:76v/v) as the mobile phase. Detection was carried out using wavelength at 262nm. The method showed adequate sensitivity concerning linearity, accuracy and precision over the range 100-500µg/ml and 30-70µg/ml for Glycopyrrolate and Formoterol fumarate, respectively. Careful validation proved advantages of high sensitivity, accuracy, precision, selectivity, robust and suitability for quality control laboratories. The developed method was robust as the %RSD was within the range and without effecting system suitability parameters. The proposed method is suitable for simultaneous determination of Glycopyrrolate and Formoterol fumarate in bulk and pharmaceutical dosage form.

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INTRODUCTION

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. High performance liquid chromatography (HPLC) is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product.

The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase. Depending upon the partition behavior of different components, elution at different time takes place.

The sample compound with the greater affinity to the stationary layer will travel slower and for a shorter distance in comparison to compounds with less affinity which travel faster and for a longer distance. The High Performance Liquid Chromatography is more versatile than gas chromatography since (a) it is not limited to volatile and thermally stable samples, and (b) the choice of mobile and stationary phases is wider. [1-2]

HPLC has numerous advantages like

- ✓ Simultaneous Analysis
- ✓ High Resolution
- ✓ High Sensitivity
- ✓ Good repeatability
- ✓ Small sample size
- ✓ Moderate analysis condition.
- ✓ Easy to fractionate the sample and purify.

Classification of HPLC can be done as:

- ➢ Preparative HPLC and analytical HPLC (based on scale of operation)

- Affinity chromatography, adsorption chromatography, size exclusion chromatography, ion exchange chromatography, chiral phase chromatography (based on principle of separation)

- Gradient separation and isocratic separation, (based on elution technique)

- Normal phase chromatography and reverse phase chromatography (based on modes of operation).

- **A. Normal phase chromatography:**

In normal phase chromatography, mobile phase is non-polar and stationary phase is polar. Hence, the station phase retains the polar analyte. An increase in polarity of solute molecules increases the adsorption capacity leading to an increased elution time. Chemically modified silica (cyanopropyl, aminopropyl and diol) is used as a stationary phase in this chromatography. For example A typical column has an internal diameter of around 4.6 mm, and a length in the range of 150 to 250 mm. Polar compounds in the mixture that are passed through the column will stick longer to the polar silica than the non-polar compounds. Therefore, the non-polar ones will pass more quickly through the column.

Fig. No. 1: Diagram of HPLC Equipment

B. RP-HPLC (Reversed phase HPLC):

RP-HPLC has a non-polar stationary phase and polar or moderately polar mobile phase. RP-HPLC is based on the principle of hydrophobic interaction. In a mixture of components those analytes which are relatively less polar will be retained by the non-polar stationary phase longer than those which are relatively more polar. Therefore the most polar component will elute first. [3-5]

MATERIALS AND METHODS

Glycopyrrolate from Sura labs, Formoterol fumarate from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

HPLC Method development:

Trails

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Glycopyrrolate and Formoterol fumarate working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 3ml of Glycopyrrolate and 0.5ml of Formoterol fumarate from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines. [6-10]

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetate Buffer pH-3.8 in proportion 24:76 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Method validation

Preparation of mobile phase:

Accurately measured 240 ml (24%) of Methanol and 760 ml of Acetate Buffer (76%) and were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent. [11-15]

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model
Temperature	:	37ºC
Column	:	Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
Mobile phase	:	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Flow rate	:	1ml/min
Wavelength	:	262nm
Injection volume	:	10 µl
Run time	:	8 min

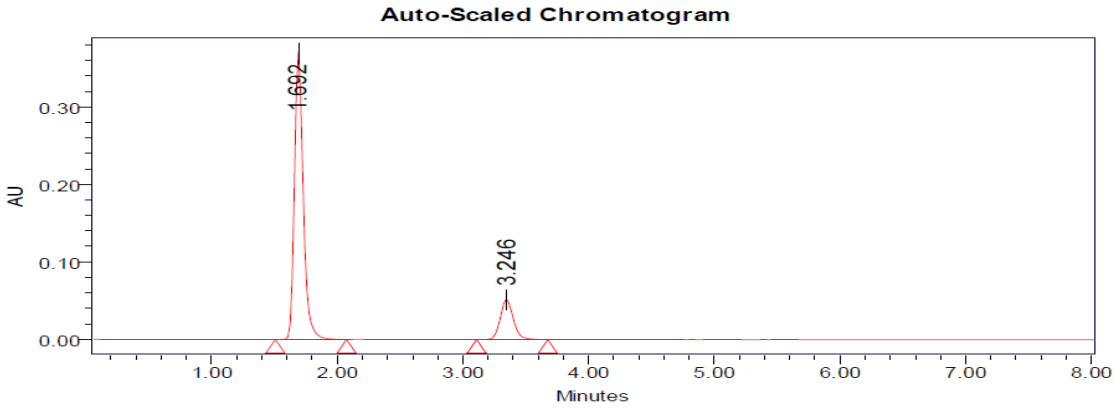

Figure No. 2: Optimized Chromatogram (Standard)

Table No. 1: Peak results for optimized Chromatogram (Standard)

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Glipizide	2.061	247392	58952	1.2	7243
2	Metformin	2.462	3530866	371748	1.1	3389

Observation: From the above experiment it was found that Glycopyrrolate and Formoterol fumarate can effectively be analyzed by using the RP-HPLC method with Mobile phase at a flow rate of 1 ml/min and detection wave length of 262nm. The retention time of Glycopyrrolate and Formoterol fumarate were found to be 1.692 and 3.246 minutes respectively.

Optimized Chromatogram (Sample)

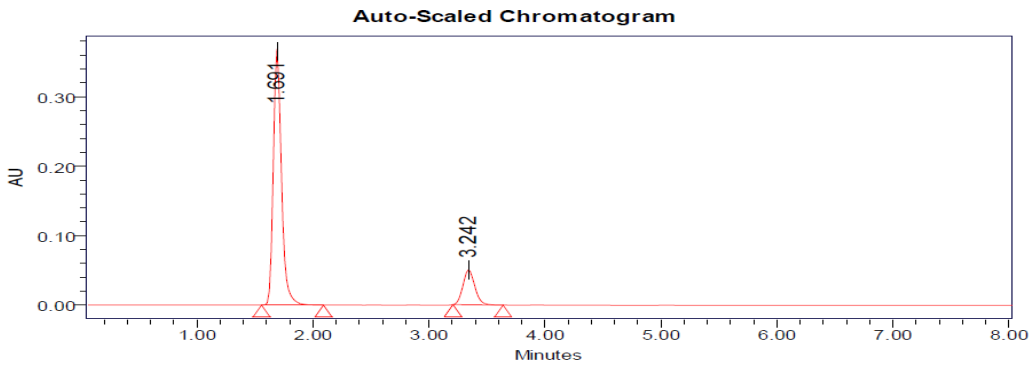

Figure No. 3: Optimized Chromatogram (Sample)

Table No. 2: Peak Results for Optimized Chromatogram (Sample)

S. No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Glycopyrrolate	1.691	1669558	7695	1.70
2	Formoterol fumarate	3.242	436589	6359	1.61

% ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

System Suitability Results:

1) Tailing factor obtained from the standard injection is 1.69.

2) Theoretical plates obtained from the standard injection are 7586.

Assay limits for Glycopyrrolate **and Formoterol fumarate is 98-102%.**

Table No. 3: Shown Assay Result

Label claim	% purity
Glycopyrrolate and Formoterol fumarate	99.86%

The assay limits for Glycopyrrolate and Formoterol fumarate was 98-102% and the results obtained for Glycopyrrolate and Formoterol fumarate was found to be 99.86%.

Linearity

Table No. 4: Chromatographic Data for Linearity Study Glycopyrrolate:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
I	100	585985
II	200	1182468
III	300	1768785
IV	400	2326852
V	500	2856874

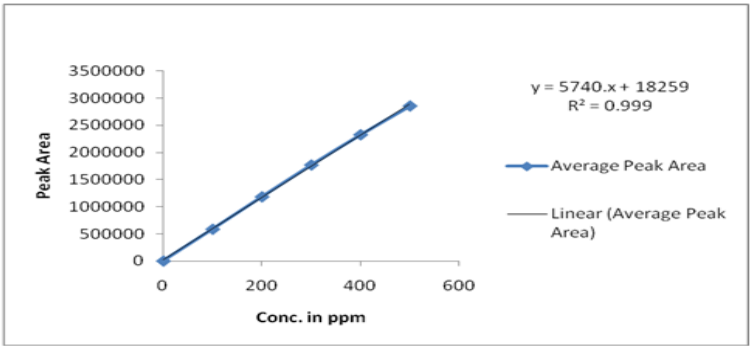

Fig. No. 4: Calibration Curve for Glycopyrrolate

Table No. 5: Linearity Observation of Formoterol fumarate

Concentration Level (%)	Concentration µg/ml	Average Peak Area
I	30	268764
II	40	356958
III	50	445631
IV	60	535186
V	70	624698

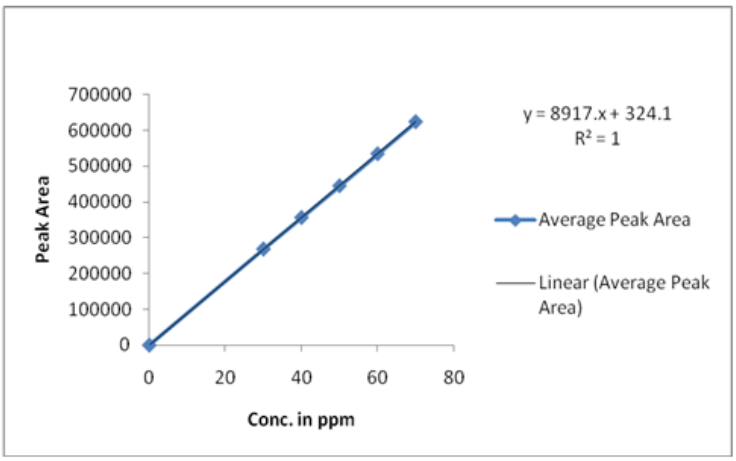

Fig. No. 5: Calibration Curve for Formoterol fumarate

Accuracy:

Table No. 6: Accuracy Observation of Glycopyrrolate

% Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	100.112%
100%	1743252	300	300.521	100.172	
150%	2609693	450	450.598	100.132	

Table No. 7: Accuracy Observation of Formoterol fumarate

% Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	100.16%
100%	445748.3	50	49.952	99.904%	
150%	670006.3	75	75.101	100.134%	

Acceptance Criteria:

The accuracy studies showed % recovery of the Glycopyrrolate 100.112%-and Formoterol fumarate 100.16%. The results obtained for Glycopyrrolate and Formoterol fumarate were found to be within the limits. Hence the method was found to be accurate.

System precision:

Table No. 8: Observation of System Precision

S. No	Sample Area 1	Sample Area 2
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515
Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
% RSD	**0.1702**	**0.043963**

The acceptance limits should be not more than 2% and the results were found to be within the acceptance limits.

Ruggedness:

Table No. 9: Observation of Ruggedness Day 1

S. No.	Sample Area 1	Sample Area 2
1	1665985	436598
2	1662598	436855
3	1668484	436598
4	1664598	436587
5	1663579	436741
6	1664587	432659
Mean	**1664972**	**436006.3**
Std. Dev.	**2060.327**	**1643.285**
% RSD	**0.123745**	**0.376895**

Acceptance Criteria:

- % RSD of five different sample solutions should not more than 2.

Table No. 10: Observation of Ruggedness Day 2

S. No.	Sample Area 1	Sample Area 2
1	1648598	415985
2	1642587	415267
3	1649852	415986
4	1648754	415265
5	1645289	415874
6	1647581	415632
Mean	**1647110**	**415668.2**
Std. Dev.	**2699.291**	**337.2106**
% RSD	**0.16388**	**0.081125**

Acceptance Criteria:

- % RSD of five different sample solutions should not more than 2.

System Suitability Parameters:

Table No. 11: Observation of system suitability parameters

S. No.	Parameter	Glycopyrrolate	Formoterol fumarate
1.	Retention Time (min)	1.688	3.282
2.	Theoretical Plates	7586	6235
3.	Tailing factor	1.69	1.58
4.	Area	1658768	426589
5.	Resolution	10.89	

The system suitability parameters were found to be within the specified limits for the proposed method.

Robustness

Table No. 12: System suitability results Glycopyrrolate

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	7269	1.61	1.868
Actual organic phase	55:45	7586	1.69	1.688
More organic phase	60:40	7496	1.64	1.675

Table No. 13: System suitability result Formoterol fumarate

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	6182	1.54	3.621
Actual organic phase	55:45	6235	1.58	3.282
More organic phase	60:40	6322	1.56	2.302

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Glycopyrrolate and Formoterol fumarate was done by RP-HPLC. The separation was optimized with mobile phase consists of Methanol: acetate buffer (pH-3.8) mixed in the ratio of 24:76%v/v. An Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column or equivalent chemically bonded to porous silica particles were used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Glycopyrrolate and Formoterol fumarate were found to be from 100-500µg/ml, 30-70µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Glycopyrrolate and Formoterol fumarate.LOD and LOQ were found to be within limits.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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