Research Article

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Method development and validation of simultaneous estimation of glycopyrrolate and formoterol fumarate in its bulk form by RP-HPLC

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ABSTRACT

An economic, uncomplicated, selective, detailed, and accurate RP-HPLC procedure for simultaneous quantitative determination of glycopyrrolate and formeterol fumarate in combined dosage forms was validated according to ICH guidelines. The method was developed using Shimadzu LC2010 CHT and Zorbax RX C18 (150x4.6mm ID) 3µm in isocratic mode, with mobile phase comprising of Water: Acetonitrile: Methanol (20:30:50) the flow rate was 1.0 ml/min and the detection was carried at a wavelength of 279nm. The retention time and percentage assay of purity for glycopyrrolate and formeterol fumarate was found to be 2.233 min and 3.224 min, 101.9% and 99.7% respectively. The method was successfully validated for accuracy, precision, ruggedness, linearity and range, specificity and robustness in accordance with ICH guidelines. The proposed method was found to be within the acceptance limits indicating that the method is accurate, specific and economical.

Keywords: Glycopyrrolate, Formeterol fumarate, Precision, Accuracy, Linearity, HPLC.

INTRODUCTION

Glycopyrrolate is a medication of the muscarinic anticholinergic group. Glycopyrrolate is a synthetic quaternary amine. The cation, which is the active moiety, is called glycopyrronium or glycopyrrolate. [1] In June 2018, it was approved by the FDA to treat excessive underarm sweating becoming the first drug developed specifically to reduce excessive sweating. [2]

In anesthesia, glycopyrronium injection can be used as a before surgery in order to reduce salivary, tracheobronchial, and pharyngeal secretions, as well as decreasing the acidity of gastric secretion. It is also used in conjunction with neostigmine, a neuromuscular blocking reversal agent, to prevent neostigmine's muscarinic effects such as bradycardia. It is also used to reduce excessive saliva (sialorrhea), [3, 4, 5] and Ménière's disease. [6] It decreases acid secretion in the stomach and so may be used for treating stomach ulcers, in combination with other medications. It has been used topically and orally to treat hyperhidrosis, in particular, gustatory hyperhidrosis. [7, 8] In inhalable form it is used to treat chronic obstructive pulmonary disease (COPD). Doses for inhalation are much lower than oral ones, so that swallowing a dose will not have an effect. [9, 10]

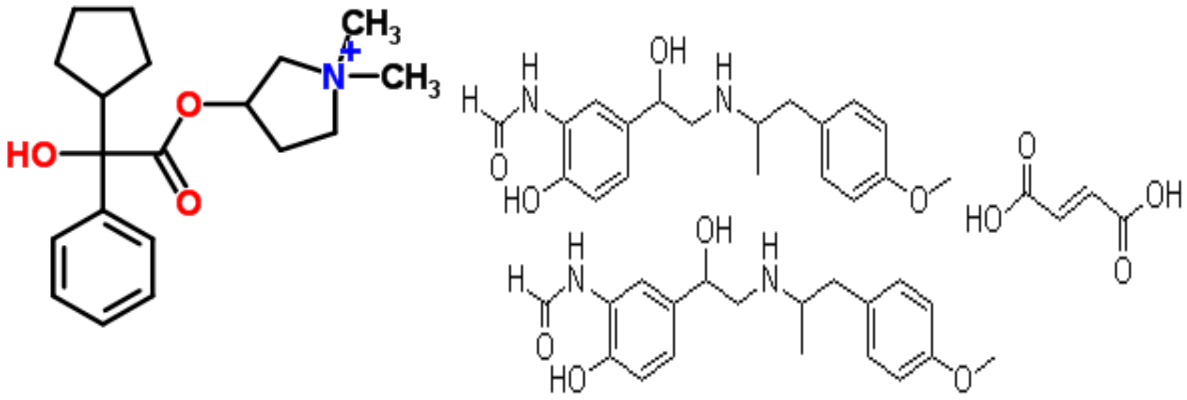

Figure 1: Structure of Glycopyrrolate and Formeterol fumarate

Formoterol or eformoterol is a long-acting β2 agonist (LABA) used as a Bronchodilator in the management of asthma and Chronic obstructive pulmonary disease. Formoterol has an extended duration of action (up to 12 h) compared to short-acting β2 agonists such as salbutamol (albuterol), which are effective for 4 h to 6 h. LABAs such as formoterol are used as "symptom controllers" to supplement prophylactic corticosteroid therapy. [11] Formoterol may also help stimulate mitochondrial biogenesis. Mitochondrial dysfunction is related to many degenerative diseases — particularly neurodegenerative disorders. [12] Preliminary research offers hope that formoterol may be a useful treatment in Down syndrome. [13]

MATERIALS AND METHOD

Glycopyrrolate and Formeterol fumarate gift samples obtained from pharma industry were used for the study. All the solvents and reagents used were of HPLC grade.

Equipment

Shimadzu LC2010 CHT for HPLC system was provided. The chromatographic analysis was performed using Zorbax RX C18 (150x4.6mm ID) 3µm as a stationary phase.

Chromatographic Conditions

Mobile phase was pumped at a flow rate of 1 mL/min using a mixture of Water: Acetonitrile: Methanol (20:30:50) %v/v/v in isocratic mode. The injection volume of 10 µL was given and the detection wavelength for glycopyrrolate and formeterol fumarate was set at 279 nm and the separation was achieved at 30°C.

Preparation of Standard Solution

About 90 mg of glycopyrrolate and 48mg of formoterol fumarate were weighed into a 100 ml volumetric flask, to this 70ml of mobile phase was added, sonicated and the volume was made up with the mobile phase. Pipette 5 ml of the clear solution in to 50 ml volumetric flask and make up volume with mobile phase. Filter the solution through 0.45µm filter paper. The chromatogram was recorded using the solution.

Preparation of sample solution

Crush more than 20 tablets then weigh a quantity of powder equivalent to 90 mg of glycopyrrolate and 48 mg of formoterol fumarate in 100 mL volumetric flask and add 70 mL of mobile phase then sonicated it for 30 min intermittent shaking after 30 min make up volume with mobile phase. Pipette out 5 mL of the clear solution in to 50 mL volumetric flask and make up volume with mobile phase. Filter the solution through 0.45µm filter paper. The resulting solution is used to record the chromatogram

RESULTS AND DISCUSSION

Table 1: Optimized chromatographic conditions

Mobile phase	Water : Acetonitrile : Methanol (20:30:50) %v/
Column	Zorbax RX C8 (150x4.6mm ID) 5.0µm
Flow rate	1.0mL/min
Column temperature	30°C
Sample temperature	15°C
Wavelength	279 nm
Injection volume	10µL
Run time	10 min

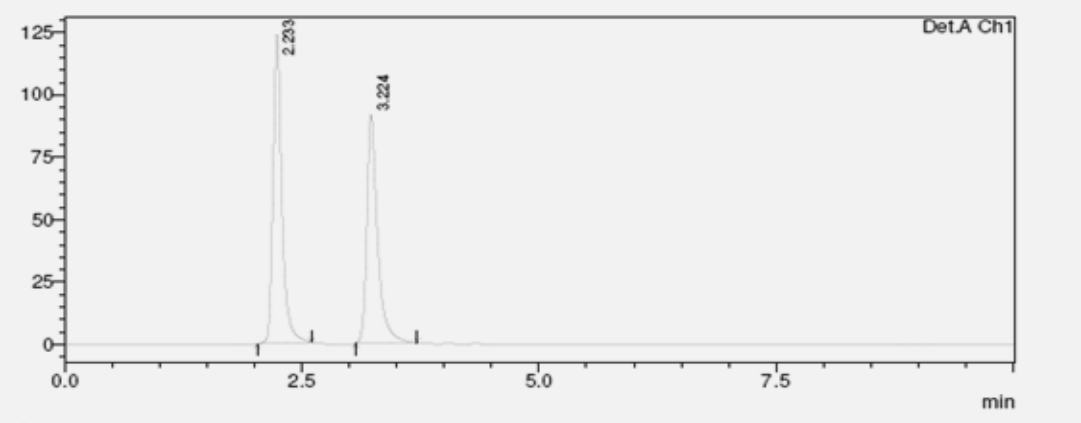

Figure 2: Typical Chromatogram of Glycopyrrolate and Formeterol fumarate

Assay

Table 2: Assay results

Glycopyrrolate			**Formoterol Fumarate**	
Standard Area	1	924225	1	577339
	2	916710	2	572957
	3	922097	3	570728
	4	921097	4	571728
	5	919838	5	569261
	6	919810	6	570393
	Average	920630	Average	572068
Sample area	1	906498	1	567626
	2	918201	2	564175
	3	922456	3	572334
	4	920306	4	573527
	5	923706	5	574574
	6	914952	6	553709
	Average	920793	Average	567658
Tablet average weight		205.5	Mg	205.5
Standard weight		91.2	Mg	48.2

Sample weight		204.5	Mg	204.5
Label amount		90	Mg	48
std. purity		99.8		99.6
Amount found in mg	mg	**91.67**	mg	**47.87**
Assay(%purity)		**101.9**		**99.7**

Validation of the HPLC Method: The proposed method was validated as per ICH guidelines.

Linearity and range

Linearity of detector response of assay method was found by injecting standard solutions with concentration ranging from 50 % to 150 % of the test concentration Peak area is measured and each level injected into the chromatographic system and the. A graph plotted of peak area versus concentration the correlation coefficient calculated. The results were shown in Table 3, 4 and Figure 2,3.

Table 3: Linearity of Glycopyrrolate

S.No	Concentration (µg/mL)	Area
1	45	483927
2	72	792394
3	90	998257
4	108	1196914
5	135	1437461

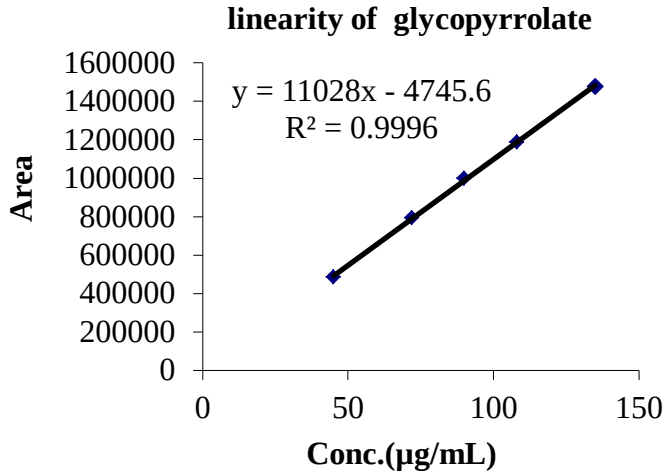

Figure 3: Linearity graph of Glycopyrrolate

Table 4: Linearity of Formoterol Fumarate

S.No	Concentration (µg/mL)	Area
1	24	294145
2	38.4	474171
3	48	615087
4	58.6	785915
5	72	1002772

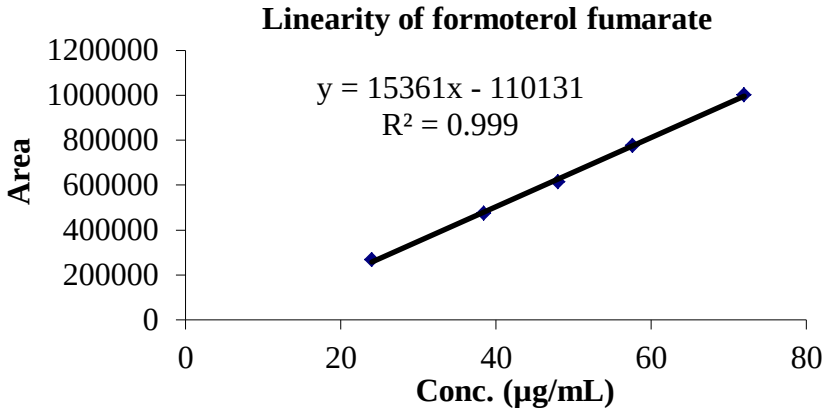

Figure 4: Linearity graph of Formoterol Fumarate

Accuracy

Recovery studies and accuracy of the method was determined. To the formulation which is a pre analyzed sample, at the level of 50%, 100%, 150% the reference standards of the drugs were added and the recovery studies were carried out three times. The results were shown in Table 5, 6.

Table 5: Results for Recovery of Glycopyrrolate

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery*	% Mean Recovery
50%	45	45.39	100.9	101.0
100%	90	90.45	100.5	
150%	135	137.15	101.6	

***** Mean of three observations

Table 6: Results for Recovery of Formoterol Fumarate.

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery *	% Mean Recovery
50%	24	24.37	101.6	
100%	48	48.84	101.7	101.0
150%	72	71.77	99.7	

***** Mean of three observations

Precision

Sample preparations of Glycopyrrolate and Formeterol fumarate were prepared as per the method and injected 6 times into the column. And the relative standard deviation of assay results was calculated. The results were shown in Table 7.

Table 7: Results for Method precision of Glycopyrrolate and Formeterol fumarate

Glycopyrrolate			Formoterol Fumarate		
S.No.	Rt	Area	S.No.	Rt	Area
1	2.412	911802	1	3.203	569631
2	2.419	920511	2	3.213	570588

3	2.410	914027	3	3.202	562599
4	2.410	914048	4	3.201	570765
5	2.411	918485	5	3.202	572187
6	2.413	913152	6	3.204	568400
Avg	2.413	915338	**Avg**	3.204	569028
Stdev	0.003	3386	**Stdev**	0.004	3392
%RSD	**0.1**	**0.4**	**%RSD**	**0.1**	**0.6**

Intermediate Precision/Ruggedness

Precision was performed on a different day by using different columns of same dimensions and method evaluated. The area was measured in HPLC for the five times injected standard solution. The results were shown in Table 8.

Table 8: Results for Ruggedness

Glycopyrrolate	%Assay	Formoterol Fumarate	%Assay
Analyst 01	101.45	Analyst 01	100.85
Analyst 02	99.75	Analyst 02	98.76
%RSD	0.26	%RSD	0.35

Robustness

Chromatographic conditions variation

The prepared solution is injected at different variable conditions like Temperature and wavelength as per test method. System suitability parameters were compared with that of method precision. The results were shown in Table 9.

Table 9: Result of Robustness study

Chromatographic changes		Theoretical Plates		Tailing factor		Resolution
		Glycopyrrolate	Formoterol Fumarate	Glycopyrrolate	Formoterol Fumarate	Between Glycopyrrolate & Formoterol Fumarate
Flow rate (mL/min)	0.8	29016	37616	1.23	1.20	4.71
	1.2	22390	29410	1.25	1.20	4.75
Wavelength(nm)	277	2992	37610	1.27	1.21	4.81
	281	22455	29421	1.25	1.25	4.75

Limit of Detection

The LOD for this method was found to be 0.60µg/ml (Formoterol Fumarate) and 1.13µg/ml (Glycopyrrolate)

Results of Limit of Detection (LOD)

$$LOD = \frac{3.3\sigma}{S}$$

= (3.3)*(2809.16)/15361

= 0.60µg/ml (Formoterol Fumarate)

=(3.3)* (3780.19)/11028

=1.13µg/ml (Glycopyrrolate)

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantification

The LOQ for this method was found to be 1.82µg/ml (Formoterol Fumarate) and 3.42µg/ml (Glycopyrrolate)

Results of Limit of Quantification (LOQ)

$$LOQ = \frac{10\sigma}{S}$$

= (10)*(2809.16)/15361
= 1.82µg/ml (Formoterol Fumarate)
= (10)* (3780.19)/11028

=3.42µg/ml (Glycopyrrolate)
Where
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

The LOQ for this method was found to be 0.83µg/ml (Telmisartan) and 2.60µg/ml (Hydrochlorothiazide).

CONCLUSION

From the above it can be concluded that all validation parameters such as precision, accuracy, linearity and Ruggedness met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed RP-HPLC method is simple, rapid, accurate, and precise and can be applied for routine analysis of glycopyrrolate and formeterol fumarate in bulk forms.

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