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RESEARCH ARTICLE

Analytical Method Validation of HPLC Method for Assay of Anticholinergic Drug in Parenteral Formulation

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ABSTRACT

A novel, accurate and precise HPLC method for determination of assay of Anti-cholinergic drug has been validated. Separation was achieved on Inertsil ODS-3, 3µm using Buffer:ACN:Methanol as mobile phase at a flow rate of 1.2 ml/min and UV detection at 222 nm. The developed method was applied for determination of assay of Anticholinergic drug in Parenteral formulation and the method was validated with respect to Specificity, Precision, Linearity, Accuracy, Robustness and analytical solution stability. The method was linear over the range of 60-140 µg/ml for Glycopyrrolate. The mean recovery was found to be in the range of 99.12-99.73 %. The percentage of relative standard deviation was found to be less than critical value. The method was found to be accurate, precise and selective for simultaneous estimation of Glycopyrrolate in injections.

KEYWORDS

Glycopyrrolate, Reverse-phase HPLC, Method Validation

INTRODUCTION

Glycopyrrolate is (3[(cyclopentylhydroxyphenyl-acetyl)oxy]-1,1-dimethyl pyrrolidinium bromide with molecular formula, $C_{19}H_{28}BrNO_3$; it is an Anticholinergic drug, used primarily for reducing secretions in the mouth, throat, airway and stomach before surgery. It is used before and during surgery to block certain reflexes and to protect against certain side effects of some medicines. Glycopyrrolate is an anti-muscarinic agent and inhibit the action of acetylcholine on structure innervated by postganglionic cholinergic nerves and on smooth muscle, cardiac muscle, the sinoarterial node, the atrioventricular node, exocrine glands and to a limited degree, in the autonomic ganglia. Thus, it diminishes the volume and free acidity of gastric secretion and controls excessive pharyngeal, tracheal, and bronchial secretions.

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MATERIALS AND METHODS

Material Used

Material Name	Manufacturer	Purity
1. Glycopyrrolate-standard	As per USP standard	100%
2. Glycopyrrolate inj.0.2mg/ml	Jubilant Generics Limited, Noida	NA

Chemicals and Reagents Used

Reagents	Manufacturer	Grade
Sulphuric acid	Fisher Scientific	ExcelaR Grade

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Sodium sulfate anhydrous	Fisher Scientific	ExcelaR Grade
Sodium 1-Hexane sulfonate monohydrate	Spectrochem	AR Grade
Methanol	SDFCL	HPLC Grade
Acetonitrile	SDFCL	HPLC Grade
Sodium Hydroxide	Fisher Scientific	ExcelaR Grade
Hydrogen Peroxide	Merck	Emparta Grade
Hydrochloric acid	Fisher Scientific	ExcelaR Grade
Milli- Q water	Millipore Ltd.	HPLC Grade

Instrument Used

Instrument	Manufacturer
HPLC Instrument	Agilent 1200 series with G1311A Quaternary pump and G1314B Variable Wavelength detector equipped with open lab software
HPLC Instrument	Waters 2695 seperations module with 2487 Dual wavelength Detector equipped with Empower Chromatographic Software
HPLC Instrument	Waters 2695 seperations module with 2996 Photodiode Array Detector equipped with Empower Chromatographic Software
Analytical Balance	XP 205 from Mettler Toledo
Microbalance	UMX2 from Mettler Toledo
Autopipette	100- 1000 µL (Eppendorf)
Ultrasonic Bath	Power Sonic 420
Photo stability Chamber	Thermo lab
Vacuum Oven	530 from Thermolab
Water bath	Kumar Precision bath
Thermo-hygrometer	J412- CTH from CE

Procedure

Preparation of 1N Sulphuric Acid Solution

Add about 27.5 ml of conc. Sulphuric acid to 1000 ml with water and mix well.

Preparation of Buffer

Dissolve about 1.5 g of sodium sulfate anhydrous and 300mg of sodium 1-hexanesulfonate monohydrate in 1000 ml of water. Add 5ml of 1N Sulphuric acid and mix well. Filter the solution through 0.45 µ nylon membrane filter.

Preparation of Mobile Phase A

Use degassed buffer solution as mobile phase A.

Preparation of Mobile Phase B

Use acetonitrile as mobile phase B

Preparation of Mobile Phase C

Use methanol as mobile phase C

Preparation of Diluents

Prepare a suitable quantity of a mixture of buffer solution, acetonitrile and methanol in the ratio of 70:20:10, mix well and degas.

Preparation of Standard Solution

Accurately weigh and transfer about 50mg of Glycopyrrolate working standard/ USP

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Glycopyrrolate RS to a 50ml volumetric flask. Add about 30 ml of diluent and sonicate to dissolve. Make up the volume with diluents and mix. Dilute 5ml of this solution to 50ml with diluent and mix well.

Preparation of Sample Solution

Carefully mix the content of not less than two vials and dilute 5ml to 10 ml with diluent and mix.

RESULT AND DISCUSSION

A simple, precise, accurate, rapid, economical analytical method for estimation of Glycopyrrolate is developed by using RP-HPLC method. The developed method is validated as per ICH guidelines. The developed method can be used for the analysis of routine quality control test. In the present work the RP-HPLC method for the estimation of Glycopyrrolate injection has been validated. The proposed method is simple, precise and accurate and do not suffer from any interferences due to common excipients. The newly developed methods can be used in pharmaceutical industry for routine assay estimation of Glycopyrrolate injection. The optimized chromatographic conditions and validation parameters are given below:

Table 1: Optimised Chromatographic Condition

Parameter Optimised	**Optimised condition**
Instrument(HPLC)	Waters 2695 UV Detector
Column	Inertsil ODS-3,3µm
Mode	Gradient
Mobile phase	Buffer:ACN:Methanol
Column oven Temperature(°C)	40°C
Auto sampler Temperature(°C)	25°C
Flow rate	1.2 ml/min
Detector	UV Detector
Temperature	Ambient room temperature
Detection wavelength	222nm
Injection volume	50 µL
Retention time(RT)	11 min
Run time	25 min

Table 2: Percent Assay observed of Glycopyrrolate

S. No.	**STD Area**	**Sample**	**Sample Area**
1	2309799	INJ-01	2319971
2	2317600	INJ-02	2306675
3	2318545	**Mean**	**2313323**
4	2315608	**Purity Angle**	**0.189**
5	2315652	**Purity Threshold**	**0.378**
Mean	**2317314**	**% Assay in Glycopyrrolate injection=99.0%**	
S.D.	**5502.9**		
% RSD	**0.24**		

Table 3: Results of different parameters

Parameter		**Result**
Specificity		Specific
Linearity	Regression equation y=mx+c	24579x+17489
	Intercept	17489
	Correlation coefficient	0.999

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Accuracy	Level 1	99.73 %
	Level 2	99.67 %
	Level 3	99.12 %
Precision	System Precision	0.20
	Method Precision	0.12
	Intermediate Precision	0.65
Robustness (% RSD)		< 2
Solution stability		53 h at 25°C.

CONCLUSION

A simple, precise, accurate, rapid, economical analytical method for estimation of Glycopyrrolate form has been validated by using RP-HPLC method. The developed method is validated as per ICH guidelines. The validated method can be used for the analysis of routine quality control sample. The proposed method shows good agreement with all validation parameters. The optimized method is precise, accurate and robust and so it can be applied as stability indicating for estimation of Glycopyrrolate injection. In the Specificity there is no interference from diluent and blank with main peak. In the accuracy % recovery is 99.01% and % RSD is 0.30 it meets criteria according to ICH Guidelines. In the study linearity and range also observed, in which we observed the linear relation between the concentration and the result.

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