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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF GLYCOPYRROLATE IN BULK AND TABLET DOSAGE FORMS

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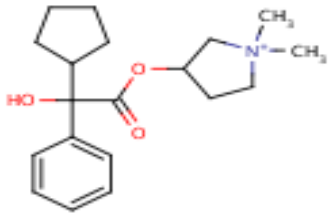
ABSTRACT

A simple, accurate and precise efficient reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for estimation of glycopyrrolate in bulk and its tablet dosage forms. Separation was done by using mobile phase consists of Mixture of buffer (sodium sulphate buffer), 1N Sulfuric acid, acetonitrile and methanol in the ratio of 1230:6:470:300 v/v/v/v. Chromatography separations were carried out on µ Bondapak C-18 column (300X3.9mn; 10µm) at a flow rate of 2.3 ml/min and UV detection at 222nm, column temperature at 30^{0}C and the retention time for glycopyrrolate is 4.2 minutes. Mixture of purified water and Acetonitrile (60:40 v/v) was used for the extraction of the drug from the dosage form. The linear dynamic response was found to be in the concentration of 20µg-80µg/ml. The correlation coefficient was found to be 0.999698. The percentage recovery of glycopyrrolate was found to be 99.08%. Proposed methods were found to be simple, accurate, precise and rapid and could be used for routine analysis. This condition is applied only for tablet dosage form. The statistical parameters and recovery studies were carried out and reported.

Keywords: Glycopyrrolate; Estimation; RP-HPLC; Validation; Tablets.

INTRODUCTION

Glycopyrrolate is a chemically 3-[(2-cyclopentyl-2-hydroxy-2-phenylacetyl) oxy]-1, 1-dimethylpyrrolidin-1-ium (fig.1). Glycopyrrolate is a synthetic anticholinergic agent with a quaternary ammonium structure. A muscarinic competitive antagonist used as an antispasmodic, in some disorders of the gastrointestinal tract, and to reduce salivation with some anesthetics.



MATERIALS AND METHODS

Chemicals and Reagents

Glycopyrrolate reference standard The HPLC grade solvents used were of E-Merck India. All other chemicals and reagents were of analytical reagent grade. HPLC grade water was prepared using Millipore purification system. Maxalt tablets containing 10 mg of Glycopyrrolate was used for analysis purpose.

Equipment

An Agilent liquid chromatography instrument equipped with a pump in isocratic mode. Analytical column used was µ Bondapak C_{18} (3.9x300mm), 10µm the system was connected with the help of chemistration software in a computer system for data collection and processing

Chromatographic conditions

Chromatographic separation was carried out at room temperature with Bondapak C_{18} (3.9x300mm), 10µm column with 3.5mc size. Mobile phase containing of 1230 volumes of Phosphate buffer, 6 volumes of 1N Sulphuric acid , 470 volumes of Acetonitrile and 300 volumes of Methanol. The ratio pH was found to be 6.5. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 5 minutes. The injection volume for standard and sample were 50 µL and eluted at a flow rate of 2.3 mL / min, the eluents were monitored at 222 nm fig.2.

Standard preparation

Accurately weigh and transfer about 50 mg of Glycopyrrolate into a 50 mL volumetric flask. Add about 30 ml of diluent sonicate for 10 minutes and dilute to the volume with diluent and mix. Pipette 2.0 ml of above solution into 50 ml volumetric flask and dilute to volume with diluent and mix (40 mcg/ml of Glycopyrrolate).

Then filter the solution through the 0.45 µm filter. This solution was injected into the column and the peak area and retention time was record as shown fig -3.

Sample preparation

Accurately weigh and powder 20 tablets. Accurately weigh and transfer a quantity of powdered tablets equivalent to 10 mg of Glycopyrrolate (about 1000 mg powder) into a 250 mL volumetric flask, add about 150 mL of diluent, and sonicate for 20 minutes, and cool to room temperature. Dilute to volume with diluent, mix and filter the solution through a 0.45µ membrane filter. (Duplicate preparation)

Analysis of formulation

Twenty tablets each containing 10mg of Glycopyrrolate were weighed and average weight was calculated. A quantity of fine powder equivalent to 10mg of Glycopyrrolate was weighed accurately, and transferred into 10ml volumetric flask, and made up to volume with mobile phase.

Further dilution of this sample stock solution in the linearity range were made using mobile phase and filtered through 0.45µ membrane filter. Then 50 µL solutions were injected into column and chromatogram was recorded. All the determinations were conducted in triplicate.

Method of Validation

The described method has been validated for the assay of Glycopyrrolate using following parameters-

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered and the retention was examined.

In all the deliberate varied chromatographic conditions a flow rate (0.2ml/min+-0.05), mobile phase composition at various ratios (1230:6:470:300 v/v/v/v) showed that the retention time of peak remains unaffected but for small changes (Table-1a, 1b).

Linearity

The linearity of detector response is established by plotting a graph of concentration versus area of Glycopyrrolate standard and determined the correlation coefficient.

A series of solutions of Glycopyrrolate standard solutions in the concentration range from about LOQ level (50%) to about 150% of the target concentration were prepared and injected into the HPLC system. The detector response was found to be linear from LOQ level (50%) to150% of target concentration for Glycopyrrolate standard with a correlation coefficient values greater than 0.999 (table-2 & fig.4).

System suitability

Accurately weighed and transferred about 50 mg of Glycopyrrolate working standard in to the 50 mL volumetric flask. Add bout 30 ml of diluent, sonicated for 10 minutes and diluted to volume with diluent. Transfer 3 ml of the above solution into a 50ml volumetric flask and diluted to volume with diluent.

Then transferred the 5ml of the above solution into a volumetric flask and diluted to volume with diluent. Inject 100 µL of standard preparation for six times into the chromatograph and recorded the system suitability parameters as per procedure the results are given (Table-3).

Accuracy

Method was checked for the accuracy of the analytes covering the range of both Assay and Content Uniformity, % recovery of both analytes has been tabulated in (Table-4).

Precision

The precision of the analytical method was studied by analysis of multiple (six) sampling of homogeneous sample. The precision expressed as % RSD. The %RSD was found to be 0.50% in the results of precision and the assay range of the six sample preparation is 101.4 to 102.8. (Table-5).

RESULTS AND DISCUSSION

The Glycopyrrolate peak in the sample was identified by comparing with the Glycopyrrolate standard and the retention time was found to be 4.2 minutes. The estimation of Glycopyrrolate tablets was carried out by RP-HPLC using mobile phase having a composition of 1230 volumes of phosphate buffer, 6 volumes of 1N sulpuricacid 470 volumes of Acetonitrile, 300 volumes methanol.

The pH was found to be 6.5.then finally filtered using 0.45µ membrane filter and degassed in sonicator for 10 minutes. The column used was Bondapak C_{18} (3.9x300mm), 10µm column with 3.5mc size. Flow rate of mobile phase was 2.3 mL / minute; system suitability parameters such as RSD for six replicate injections were found to be less than 2%, theoretical plates 4232 and tailing factor 1.2. The quantitative estimation was carried out on tablet by taking the same concentration as for standard solution and assay results found to be 99.23% the acceptance criteria of system suitability was RSD should not be more than 2% and the method show system suitability 0.70% which shows that method was repeatable.

The acceptance criteria of method repeatability was RSD should not be more than 2.0% and the method show method repeatability 0.50% which shows that the method was precise. The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 50 to 150 µg/ml of target concentration for Glycopyrrolate standard with a correlation coefficient value is greater than 0.999698. The correlation coefficient of (r^2) = 1, which shows that the method was capable of producing good response in UV-dictator. The accuracy limits is the % recovery should be in the range of 99.0% to 100.2%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy.

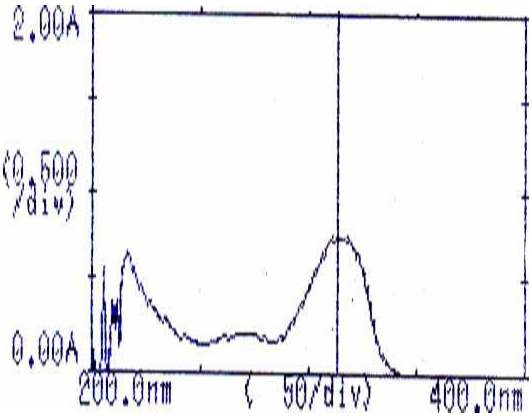


Fig. 2: Absorption Spectrum of Glycopyrrolate

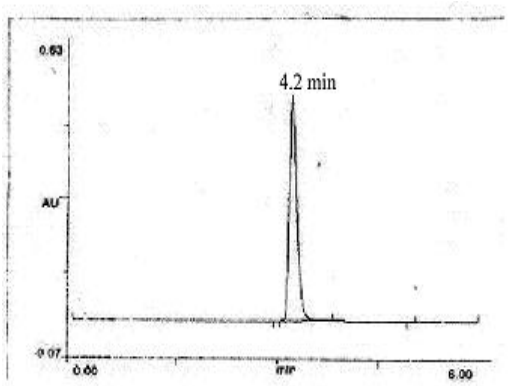


Fig. 3: Typical Chromatogram of Glycopyrrolate Retention Time of Glycopyrrolate was 4.2min

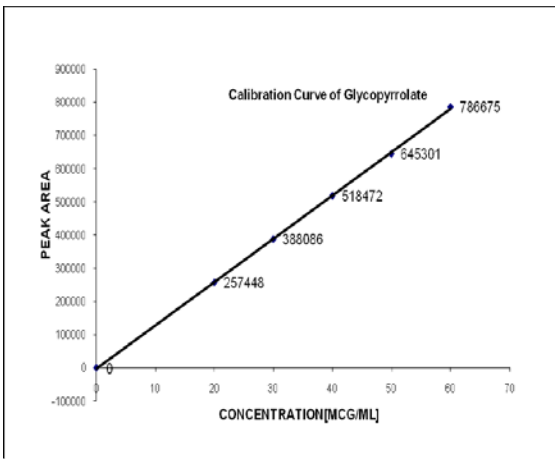


Fig. 4: Calibration graph of Glycopyrrolate

Table 1a: Influence on Flow Variation

S No.	System suitability	Low flow (2.1 mL)	High flow (2.5 mL)	Acceptance criteria
1	%RSD of Peak area	0.1	0.2	NMT 2.0
2	Tailing factor	1.2	1.1	NMT 2.0

Table 1b: Influence on variation of mobile phase Composition Organic phase Acetonitrile Variation

S No.	System suitability	Low organic phase (-5%)	High organic phase (+5%)	Acceptance criteria
1	%RSD of Peak area	0.1	0.1	NMT 2.0
2	Tailing factor	1.1	1.1	NMT 2.0

Table 2: Linearity

% Level	Concentration (mcg/mL) Glycopyrrolate	Peak Area (Average)
50	20.2	257448
75	30.2	388086
100	40.3	518472
125	50.4	645301
150	60.5	786675
Correlation coefficient (r^2)		**0.9996**

Table 3: System suitability

System suitability parameters	Results	Limits
%RSD of peak areas	0.7	NMT 2.0
Tailing factor (USP)	1.2	NMT 2.0

Table 4: Accuracy

Sample No.	Concentration Level	mg added	mg found (recovered)	% Recovery
1	50 %	5.0	5.0	100.1
2		5.0	5.0	100.2
3		5.0	5.0	99.9
Average				100.1
4	100%	10.0	10.0	100.0
5		10.1	10.0	99.0
6		9.9	9.9	100.0
Average				99.7
7	150%	14.9	14.9	100.0
8		14.8	14.7	99.3
9		14.9	14.9	100.0
Average				99.8
10	300 %	29.7	29.5	99.3
11		29.6	29.5	99.7
12		29.2	29.1	99.7
Average				99.5
Average				99.8

Table 5: Precision

Sample No	Glycopyrrolate Tablets USP 1 mg	
	Day 1 /Analyst 1/ Inst. 1 % of assay	Day 2 /Analyst 2/ Inst. 2 % of assay
01	102.8	99.5
02	101.8	99.7
03	101.7	99.4
04	102.4	99.5
05	102.3	99.6
06	101.4	99.4
Average	102.1	99.5
% RSD	0.5	0.1
Difference (%)	2.6	

Acceptance Criteria

The difference between the average assay results obtained by both analysts should not be greater than 3.0%

CONCLUSION

Based on the above validation data, it is evident that the HPLC method documented in the protocol for the determination of % assay for Glycopyrrolate in Glycopyrrolate Tablets USP 1 mg is treated as a validated method. Hence it is recommended to be use for the determination of % assay of Glycopyrrolate in Glycopyrrolate tablets USP 1 mg in routine testing and release and stability samples testing. Based on the chromatographic data and corresponding purity threshold and purity angle values, it is clear that the peaks obtained under various stress conditions are well separated and active analyte passed peak purity test. The analyte peak is completely degraded with base treatment. Peak data presented is acceptable and the HPLC analytical method given in the protocol is stability indicating method for the assay of Glycopyrrolate Tablets USP 1 mg.

REFERENCES

1. http\\Wikipedia.org/wiki/ Glycopyrrolate.
2. www.rxlist.com
3. www.drugbank.com
4. Willard HY, Merritt LL, Dean JA, Settle FA. Instrumental Methods of Analysis. 7th Ed. New Delhi: CBS Publishers and Distributors; p. 436-439.
5. Snyder LR, Loyd K, Glajch JL, Kirland JJ. Practical HPLC Method Development. 2nd ed. USA: A Wiley- Inter-science Publication; 1997. p.121, 234-265,705.
6. Sethi PD. HPLC -High Performance Liquid Chromatography: Quantitative analysis Pharmaceutical Formulations. 1st Ed. New Delhi: CBS Publishers and Distributors; 2001. p. 3-5.
7. Indian Pharmacopoeia, Volume-II. Government of India. Ministry of health and Family welfare New Delhi, The controller of publication 2007; p.1278-1279.
8. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. part II, New Delhi: CBS Publisher and Distributor; 1997. P. P. 158-164.277.
9. U.S.P. Asian Edition, Rockville: United Pharmacopoeial Convention Inc; 2005. p. 2386-2389.
10. ICH, Q2R1 Validation of Analytical Procedures: Text and Methodology; International Conference on Harmonization, Geneva; 1996.
11. ICH. Q1A (R2), Stability testing of new drug substances and products, International conference on Harmonization. Geneva, 2003.