RESEARCH PAPER

Stability Indicating RP-HPLC Method Development and Validation for Estimation of Glycopyrrolate in Parentrals Dosage Form

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ABSTRACT

Anovel, accurate and precise RP-HPLC method for determination of Glycopyrrolate in parenteraldosage form has been developed and validated. Separation was achieved on Inertsil ODS-3, 5µm (250x4.6mm) column using Buffer (pH -3.0): ACN as mobile phase at a flow rate of 1.0 ml/min and UV detection at 222 nm. The developed method was applied for determination of assay of Glycopyrrolate drug in Parentrals 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 25- 150µg/ml for Glycopyrrolate. The mean recovery was found to be of 99.4%. 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 estimation of Glycopyrrolate in injections.

KEYWORDS: Glycopyrrolate, Reverse-phase, HPLC, Method Validation

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INTRODUCTION

A Stability-indicating assay method can be defined as "Validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and drug products are specific so that the content of active ingredients and degradation products can be accurately measured without interference" [1]. Generally forced degradation/stress testing is used to generate the samples for stability-indicating assay methods. Forced degradation/stress testing is definedas "the stability testing of drug substance and drug product under conditions exceeding those used for accelerated stability testing" [2]. Degradation can be achieved by exposing the drug, for extended periodof time, to extremes of pH (HCl or NaOH solutions of different strengths), at elevated temperature, to hydrogen peroxide at room temperature, to UV light, and to dry heat (in an oven) to achieve degradation to an extent of 5–20%. Generally, trial and error experimentation is used during these experiments. This trial and error approach is generally cost, labor, and time intensive and should be substituted with some systematic approach. From exhaustive literature, it was observed that experimental design in forced degradation experiments can be used to save cost and labor by avoiding trial and error experimentation [3].Glycopyrrolate is a quaternary ammonium anticholinergic agent. The quaternary ammonium moiety renders Glycopyrrolate highly ionized at physiological pH and it thus penetrates the blood brain and placental barriers poorly. Glycopyrronium Bromide has a more gradual onset and longer duration of action than atropine.

Name of the Active Substances: (Glycopyrrolate) Glycopyrrolate injection 0.2mg/ml, (Official in USP and EP) Category: Glycopyrrolate Injection is a synthetic anticholinergic agent. It is intended for intramuscular or intravenous administration. Each 1 mL contains 0.2 mg of Glycopyrrolate, water for injection, sodium chloride as a tonicity agent, and hydrochloric acid or sodium hydroxide as pHadjusters. It is preservative free. Glycopyrrolate binds competitively to the muscarinic acetylcholine receptor. Like other anticholinergic (antimuscarinic) agents, it inhibits the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine but lack cholinergic innervation. It is freely soluble in water, soluble in ethanol

(96%). [EP USP) Very slightly soluble in methylene chloride. [EP] Practically insoluble in chloroform and in ether. [USP] PH: Glycopyrrolate injection pH about 2-3 Pka: pka (Strongest Acidic): 11.53, pka (Strongest Basic): -4.3, Dispensed as: Rx

Trade Name: Nodapton, Tarodyn and Glycopyrronium bromide Protein Chemical Formula: C19H28BrNO3 Melting point: 193-194.5 C Molecular Weight: 398.33 Potential isomerism: Two asymmetric atoms of carbon are present in the molecule of Glycopyrrolate; therefore, four opticalisomers exist. Glycopyrrolate produced is represented by the (RS) (SR) pair or threo structure. IUPAC/Chemical name:Pyrrolidinium, 3-[(SR)-(cyclopentylhydroxyphenylacetyl) oxyl-1.1- dimethyl- [RS] bromide (RS)-13-(SR)-Hydroxy 1,1-dimethylpyrrolidinium bromide acyclopentylmandelate Form of Dosage form: Glycopyrrolate injection 0.2mg/mL (vials) Maximum daily dose:0.8mg/day BCS Classification: Class III (High solubility and Low permeability) Storage condition(API):Preserve at controlled room temperature at 15°C to 30°C in closed container Storage condition (DP): Preserve at controlled room temperature at 20°Cto25°C in closed container. Drug substance shelf life: Vendor given retest date after 5years Drug product shelf life: Based on the results, a shelf-life of 24 months has been set for the Inst ampoules, and a shelf-life of 18 months has been set for the 3ml ampoules. The storage instructions for both ampoule sizes are 'Do not store above 25°C and Keep the ampoules in the outer carton in order to protect from light.

Materials and Methods:

The following listed were used in carrying out the research work

Table-1 List of Instruments

S.NO	**INSTRUMENTNAME**	**MODEL**	**SOFTWARE**
1	HPLC	Waters2690PDAdetector	Empower2
2	HPLC	Shimadzu2010CHT-UVDetector	LCSOLUTION
S.NO	**Equipment's**	**Model**	**Company**
1	ElectronicBalance	CP225D	SARITORIUS
2	TopLoadbalance	GP5202	SARITORIUS
3	Ultra-Sonicator	-------	LIFECARE
4	Thermaloven	-------	NEWTRONIC
5	pH Meter	BENCHTOP	ORION3STAR
6	Filter Papermicrons 0.45	-------	MILLIPORE

Table-2 List of Chemicals

S.NO	**Chemicals/standards and reagents**	**Grade**	**Company**
1	Sodium di-Hydrogen Phosphate Monohydrate	ACS	Merck
2	Triethylamine	HPLC	Merck
3	Methanol	HPLC	Rankem
4	Acetonitrile	HPLC	Finar
5	OrthoPhosphoricacid	AR	Rankem

6	Water	TKA	Merck
7	Glycopyrrolate	NA	AMSA

RP-HPLC METHOD DEVELOPMENT Method development for the estimation of Glycopyrrolate in parenteral dosage form was initiated based on the method development guidelines and literature review. Several trails were conducted by varying the chromatographic parameters for optimization of method. Preparation of Buffer solution Preparation of mobile phase Preparation of standard stock solutions Preparation of standard working solution Preparation of Sample solution Preparation of Buffer solution: Dissolved about 5gm of sodium dihydrogen phosphate monohydrate and 1ml of trimethylamine in 1000ml of water and adjust pH to 3.0 (±0.05)with dilute orthophosphoric acid (OPA). Filtered the buffer solution through 0.45 µm membrane filter. Preparation of mobile phase: Mobile phase A: Dissolved about 5gm of sodium dihydrogen phosphate monohydrate and 1ml of trimethylamine in 1000ml of water and adjust pH to 3.0 (±0.05)with dilute ortho phosphoric acid (OPA). Filtered the buffer solution through 0.45 µm membrane filter Mobile phase B: Degassed mixture of Acetonitrile and methanol in the ratio of 60:40(%v/v). 600ml of Acetonitrile is measured and transferred into 1000ml beaker and added 400ml of methanol mixed well and sonicated for degass and the final degassed mixture is used as mobile phase

Buffer: Dissolved about 5gm of sodium dihydrogen phosphate monohydrate and 1ml of trimethylaminein 1000ml of water and adjust pH to 3.0 (±0.05)with dilute ortho phosphoric acid (OPA). Filtered the buffer solution through 0.45µm membrane filter. Mobile phase A dissolved about 5gm of sodium dihydrogen phosphate monohydrate and1ml of trimethylaminein 1000ml of water and adjust pH to 3.0 (±0.05)with dilute ortho phosphoric Acid (OPA). Filter the buffer solution through a 0.45 µm membrane filter.

Mobile Phase B: Prepare a degassed mixture of acetonitrile and methanol in the ratio of 60:40 (% v/v). Measure 600 mL of acetonitrile and transfer it into a 1000 mL beaker. Add 400 mL of methanol, mix well, and sonicate for degassing. The final degassed mixture is used as the mobile phase.

Preparation of standard stock solutions: 20 mg of Glycopyrrolate is weighed and transferred into clean and dry 20ml volumetric flask. To this 10ml diluent is added kept it for sonication diluted up to volume with diluent. Preparation of standard working solution: 1ml of stock solution is transferred into clean and dry 10ml volumetric flask and diluted up to volume with diluents and mixed well. (100ppm) Preparation of Sample solution: 1mg of sample is weighed and transferred in to 10ml volumetric flask and diluted up to volume with diluents and mixed well. (100ppm) Trail 1: Chromatographic conditions: Column Particulars: Microbond pack C-18 (300 × 3.9 mm, 10 µm) column Mobile phase (v/v %): Buffer (pH-3.0): ACN (70:30) Diluent: Mobile phase Flow rate (mL/ min): 2ml/min Wavelength: 222nm Injection volume: 20µL Run time: 25min

The Glycopyrrolate peak was eluted at 14.7min, peak tailing minimized by changing the column. But plate count was very less. Hence next trial was taken for better elution of Glycopyrrolate peak. Compare to previous trial the RT of Glycopyrrolate was reduced but the peak shape was not good. Tailing was observed and plate count was very less. Hence next trial was taken by changing of pump mode isocratic to gradient.On changing to gradient program Glycopyrrolate peak plate count was good, Rt of Glycopyrrolate reduced, the tailing of Glycopyrrolate peak was occurred, to minimize the tailing and to improve better resolution next trial was taken to change the gradient composition

Column particulars: Inertsil ODS (250×4.6mm,5µm) Column, Mobile phase(v/v%):Buffer(pH-3.0) (A): ACN (B), Diluent: Mobile phase (60:40), Flow rate (mL/ min): 1.0ml/min, Wavelength: 222nm Injection Volume: 20µL, Run Time: 20 min.

Glycopyrrolate and benzyl alcohol peak shape was good, both peaks were eluted and separated, peak tailing was minimized and resolution were also good. Hence this trial was considered as final.

Discussion: The standard solution was prepared as per test method and injected six times into the HPLC system. The system suitability parameters were evaluated as per the test method and found to be within the limits. Correlation co-efficient should be not less than 0.999. The correlation co-efficient is found to be within the limits (0.9995) Acceptance criteria- The % RSD for six sample preparations should not be more than 2.0% %RSD of six samples were found to be within the acceptable limits.

Specificity: Chromatograms of blank and placebo have not shown any peak at the retention time of analyte peak. The mean % recovery Glycopyrrolate at each level should be not less than 98% and not more than 102%. Calculated, mean % recovery at each level and the results were found to be within the acceptable limits. Purity threshold should be more than the purity angle. There should be no interference of the degraded peaks with that of main peak. Results complies acceptance criteria.

The assay test was performed with sample and %assay was found to be as 99.5%. It was found to be within the limits.

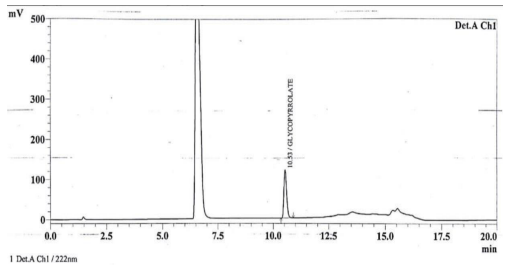

Figure-1 Optimized Chromatogram of sample

Forced Degradation: The specificity of the method was determined by checking the interference of any of the possible degradation products generated during the forced degradation study of the drugs.

Acid degradation: Subjected the test preparation to acid stress degradation by treating 1mg/ml of sample with 1 ml of 0.1N HCl. Heated it at 80°C for 2 hours and neutralized with 1 ml of 0.1N NaOH and diluted it as per the test procedure and injected into the HPLC system.

Alkali degradation: Subjected the test preparation to alkali stress degradation by treating 1mg/ml of the sample with 1 ml of 0.1N NaOH. Heated it at 80°C for 2 hours and neutralized with 1 ml of 0.1N HCland diluted it as per the test procedure and injected into the HPLC system.

Peroxide Degradation: Subjected the test preparation to peroxide stress degradation by treating 1 mg /ml of the sample with 1ml of 3% H2O2. After that heat it at 80°C for 2hrs and dilute it as per the test procedure and injected into the HPLC system. Thermal degradation: Subjected the test preparation to thermal stress degradation by heating the sample at 80°C for 5days, diluted it as per the test procedure and injected into the HPLC system.

A validated stability-indicating RP-HPLC method has been developed for Estimation of Glycopyrrolate in parenteral dosage form. The results obtained by the stress degradation conditions of the drug show that the method is specific and stability-indicating. The method was found to be simple, accurate, precise and sensitive. The method was successfully applied for the determination of Glycopyrrolate in injectable dosage form. In the future, this method may be applied for routine analysis drug in Injectable formulations.

Results and Discussion

Optimized HPLC Method:

Column particulars: Inertsil ODS (250 × 4.6 mm, 5 µm) column, Mobile phase (v/v %): Buffer (pH - 3.0) (A): ACN (B), Diluent: Mobile phase (60:40), Flow rate: 1.0 ml/min, Wavelength: 222 nm, Injection volume: 20 µL, Run time: 20.0 min.

TABLE-4 Data of Optimized Method

PeakName	Area	RT	USPTailing	USPPlateCount
Glycopyrrolate	1034401	10.53	1.3	31715
Benzyl alcohol	9666661	6.55	1.5	4408

Discussion:

Glycopyrrolate and benzyl alcohol peak shape was good, both peaks were eluted and separated, peak tailing was minimized and resolution were also good. Hence this trial was considered as final.

SYSTEM SUITABILITY:

Weighed accurately about 20 mg of standard drug and transferred into a clean and dry 20 ml volumetric flask and dissolved them by adding 3/4th volume of diluent and sonicated for 5 minutes. Make up to the volume with diluent. From the above stock solution, 1 ml was pipette out into a 10 ml volumetric flask and then made up to the final volume diluent. The standard solution was six times into the HPLC system. The system suitability parameters were evaluated as per the test method and found to be within the limits.

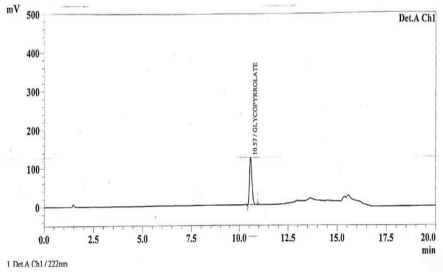

Figure-6 Chromatogram of standard

LINEARITY:

The linearity of an analytical procedure is its ability (within given range) to obtain test resultswhichare directlyproportionaltotheconcentration(amount)of Analyteinthe sample

Linearity was performed by diluting standard stock solution. Stock solution contains 1mg/ml ofGlycopyrrolate. From the stock solution aliquots of 0.5, 1, 1.5, 2, 2.5, 3ml diluted to 20ml withdiluent,

such that the final concentration of Glycopyrrolate was in the range of 25 to 150µg/ml. 20µlofeachsamplewasinjectedintoHPLCinduplicate foreachconcentrationleveland

Table-5 Data of system suitability

Injection No	Retention Time(min)	Area	USP Plate count
1	10.54	1047924	32428
2	10.54	1048854	32910
3	10.51	1049442	30992
4	10.54	1045485	31189
5	10.51	1048425	32433
6	10.51	1047264	32458

Discussion: The stan dard solut ion was prepared as per test method and injected six times into the HPLC system. The system suitability parameters were evaluated as per the test method and found to be within the limits.

Table-6 System suitability parameters of Glycopyrrolate

System suitability parameters	**Results**	**Acceptance criteria**
%RSD for area count of six replicate Injections of standard	0.134	Notmorethan2.0
Tailing factor for Glycopyrrolate Peak	1.3	Notmorethan2.0
Theoretical plates for Glycopyrrolate Peak	32428	Not less than2000

SPECIFICITY:

Specificity is the ability to asses unequivocally the analyte in the presence of components which may be expected to present. Typically this might be impurities, degradants, matrix.

Chromatograms of blank and placebo have not shown any peak at the retention time of analyte peak.

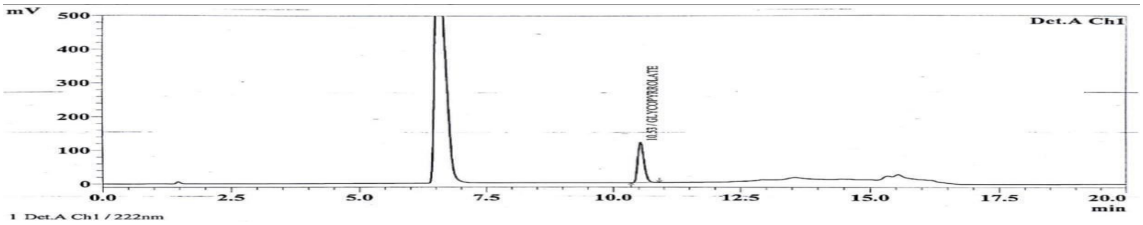

Figure-2 Chromatogram of Control Sample

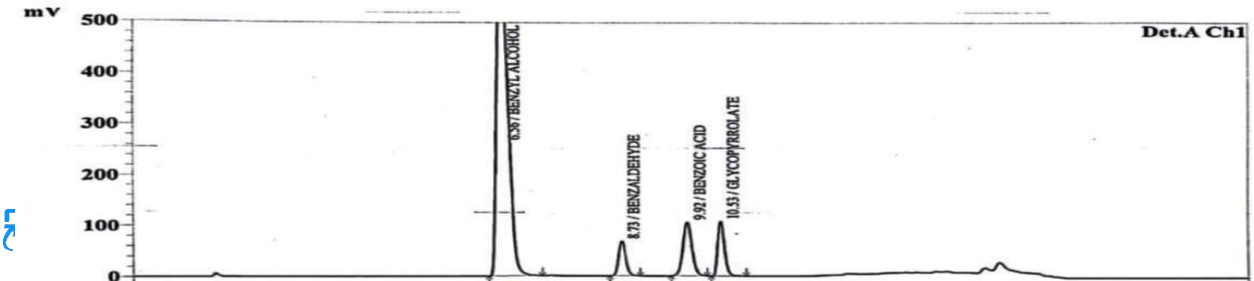

Figure-3ChromatogramofSpikedSample

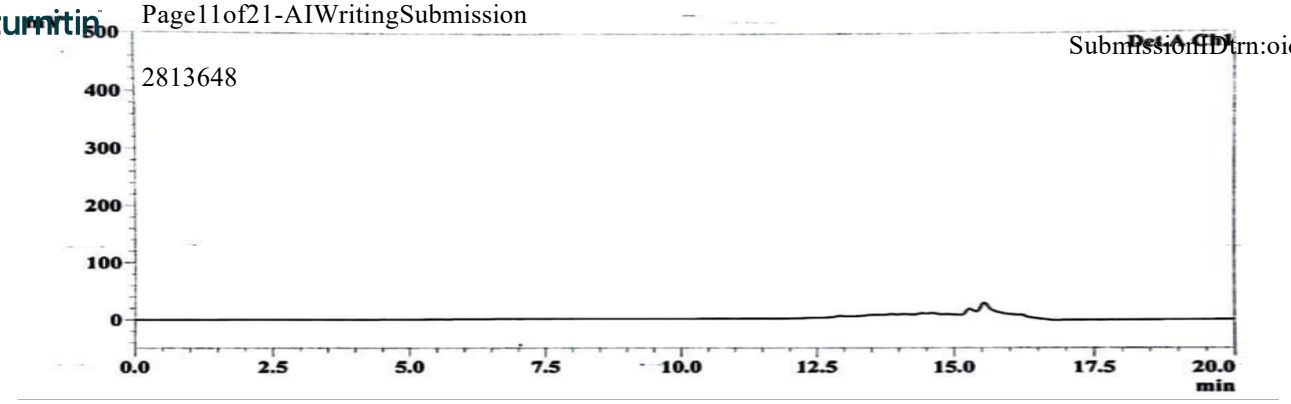

Figure-4 Chromatogram of blank

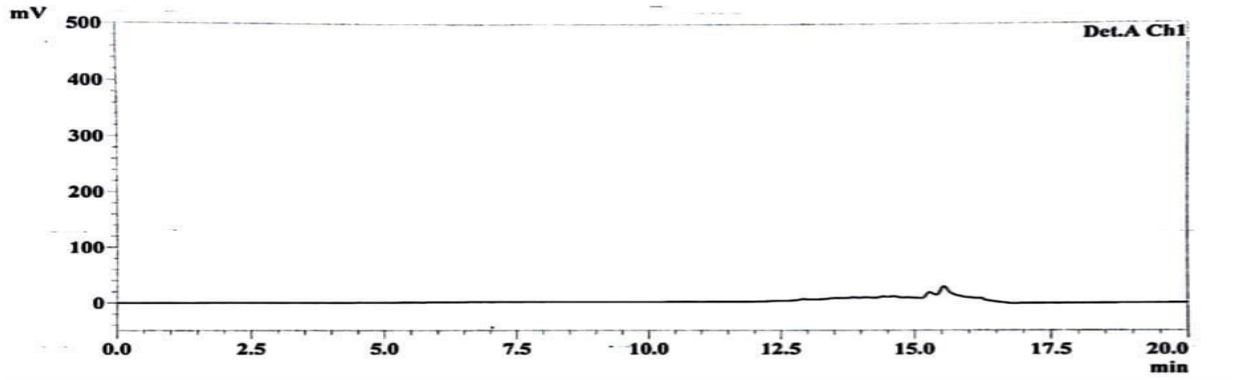

Figure-5 Chromatogram of placebo

Calibration curve was constructed by plotting the peak area versus the drug concentration.

Preparation of Glycopyrrolate standard stock solution: About 10 mg of standard Glycopyrrolate was weighed and transferred into a 10 ml volumetric flask, and dissolved it by adding 3/4th volume of diluent (Acetonitrile and Buffer) and sonicated for 5 minutes. Make up to the volume with diluent.

PREPARATION OF WORKING STANDARD SOLUTION:

From the above standard stock solution of Glycopyrrolate, further dilutions were prepared to make solutions of different concentrations ranging from 25-150 µg/ml by using diluent.

Table-7 Linearity data

%Linearity level	Concentration (ppm)	PeakArea
25	25ppm	258614
50	51ppm	517227
75%	76ppm	775842
100%	102ppm	1034456
125%	127ppm	1294071
150%	153ppm	1579037

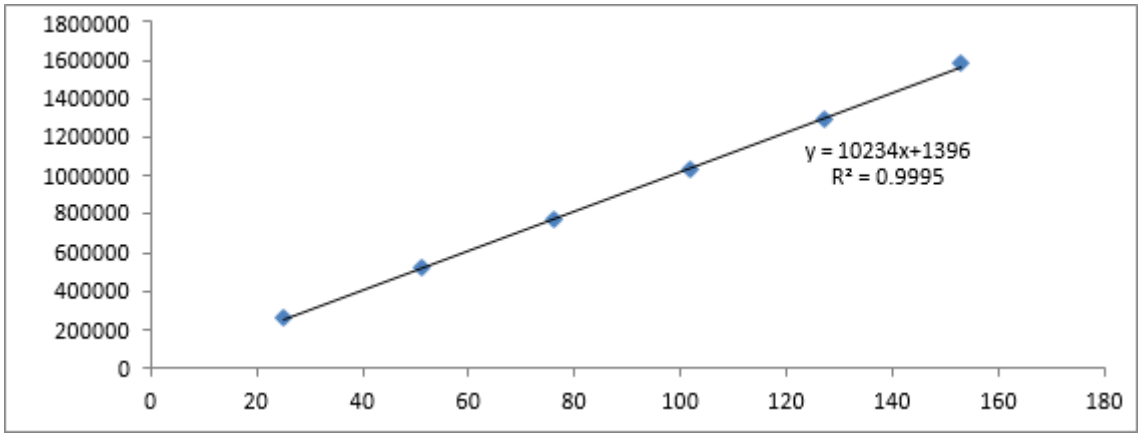

Figure-7 Calibration Curve of Glycopyrrolate

Discussion: Correlation co-efficient should be not less than 0.999 .The correlation co-efficient is found to be within the limits. (0.9995)

PRECISION:

The precision of an analytical method is a measure of the random error and is defined as theagreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) orrelative standard deviation (RSD) of the replicate measurement

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%RSD=Standarddeviationmean×100

To evaluate the precision for assay method, six samples were prepared and analyzed as per the test method. Acceptance criteria - The %RSD for six sample preparations should not be more than 2.0%. %RSD of six samples were found to be within the acceptable limits.

Table-8 Precision Data

SampleNo	AreaofSample
1	1041474
2	1040767
3	1038603
4	1047805
5	1041651
6	1034562
Mean	1040810
SD	4335.62
%RSD	0.42

Discussion: Acceptance criteria - The %RSD for six sample preparations should not be more than 2.0%. %RSD of six samples were found to be within the acceptable limits.

ACCURACY:
Accuracy is the closeness of agreement between the conventional true value and the value found. A series of solutions were prepared in triplicate by spiking Glycopyrrolate to placebo in the range of 50% to 150% of test concentration and injected into HPLC system and analyzed as per the test method. Sample solutions prepared were injected three times into the chromatographic system and recorded the chromatograms.

Table-9AccuracyData

Conc.Lev el (%)	**Vol.of stock sol(ml)**	**Amountsp iked(µg)**	**Average Area**	**AmountReco very(µg)**	**% Recovery**	**Average % Recovery**
50	1	51.4	527186	52.00	100.7	
50	1	51.4	528208	52.10	100.9	
50	1	51.4	527185	52.09	100.8	
100	2	102.8	1030876	100.41	98.3	99.4
100	2	102.8	1031607	101.43	98.5	
100	2	102.8	1043786	101.46	98.8	
150	3	154.2	1559683	153.83	99.3	

150	3	154.2	1560261	153.83	99.3
150	3	154.2	1559683	153.83	99.3

Discussion: The mean % recovery Glycopyrrolate at each level should be not less than 98% and not more than 102%. Calculated, mean % recovery at each level and the results were found to be within the acceptable limits.

SENSITIVITY:

Table10SensitivityData

Molecule	LOD(ppm)	LOQ(ppm)
Glycopyrrolate	2.87	4

ROBUSTNESS

Table11RobustnessData

Variations	Retentiontime	TailingFactor	USPPlatecount
Standard	10.51	1.3	31725
Wavelengthat220nm	10.49	1.3	30596
Wavelengthat224nm	10.52	1.3	32698
Flowrateat0.8ml/min	10.54	1.3	29648
Flowrateat1.2ml/min	10.48	1.3	34568

FORCEDDEGRADATION

Table8DataofDegradationStudies

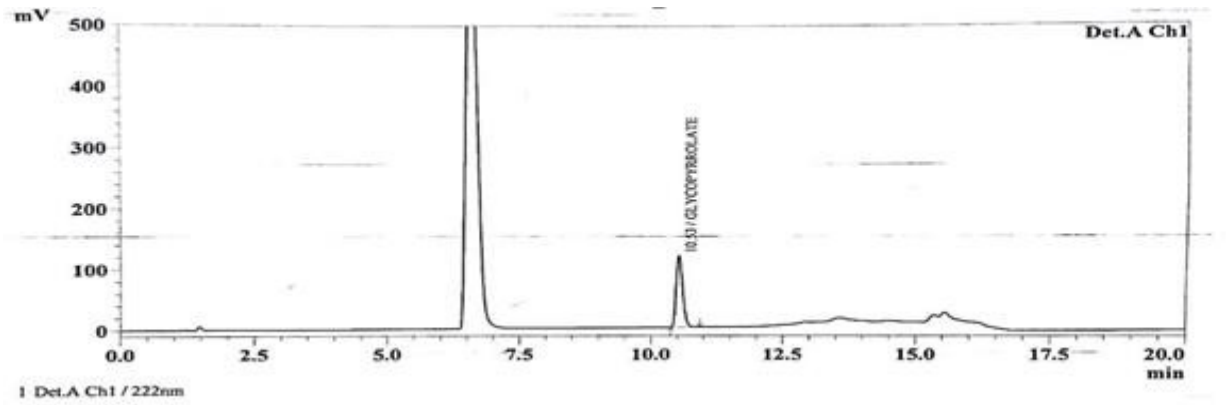

Figure-8ChromatogramofAciddegraded Sample

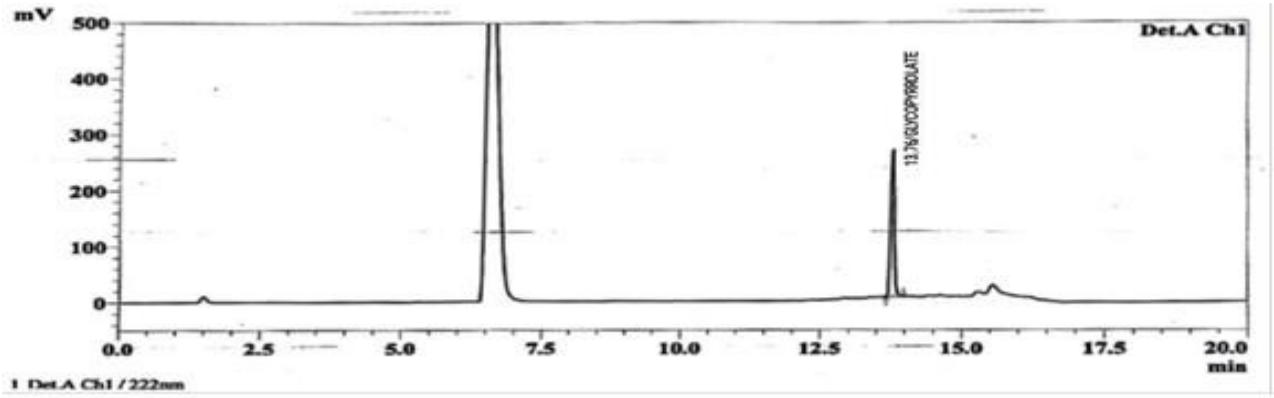

Figure-9ChromatogramofBasedegradedSample

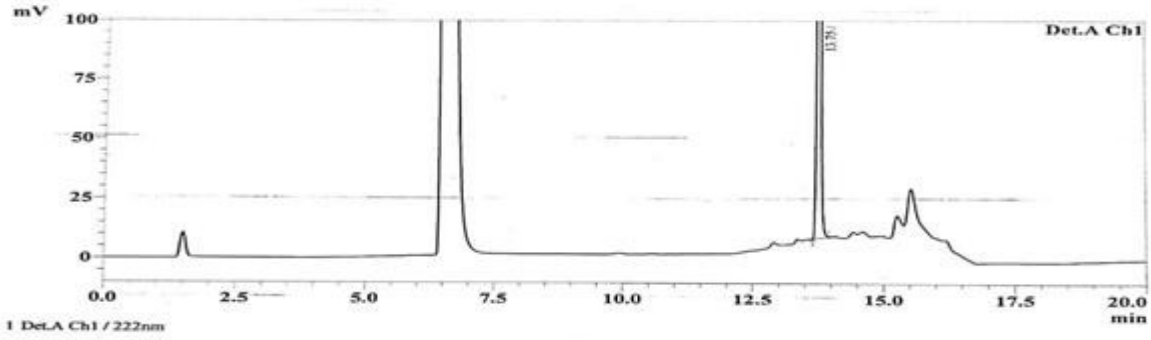

Figure-10Chromatogramof Peroxidedegraded Sample

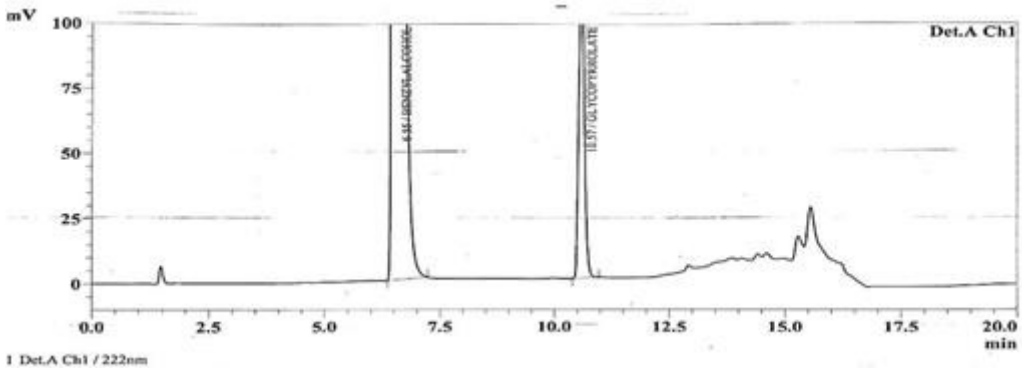

Figure-11ChromatogramofThermaldegradedSample Table-12 Data of degradation studies

Condition	Area	Purity Angle	PurityThreshold	%Degradation	Observation
Unstressed	1039256	0.095	0.125	NA	Complies
AcidDegradation	976848	0.087	0.239	0.96	Complies
AlkaliDegradation	982468	0.131	0.242	0.93	Complies

PeroxideDegradation	906748	0.166	0.243	0.33	Complies
ThermalDegradation	995987	0.090	0.299	0.19	Complies

Discussion: Purity threshold should be more than the purity angle. There should be no interference of the degraded peaks with that of main peak. Results complies acceptance criteria.

ASSAY

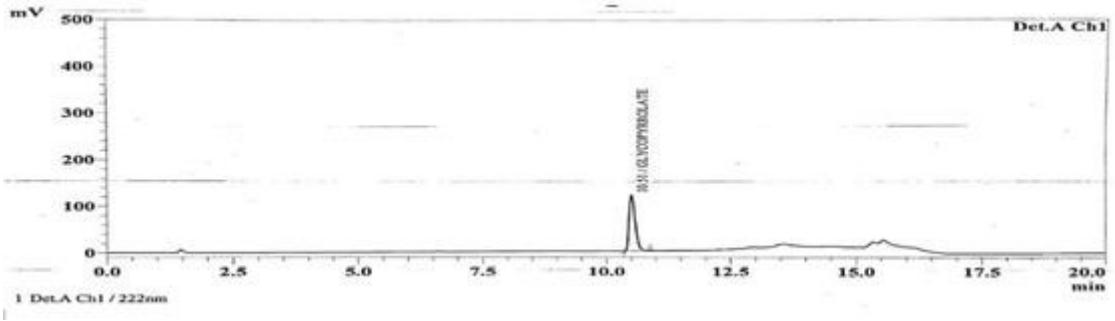

Figure-12Chromatogram of Standard (100ppm)

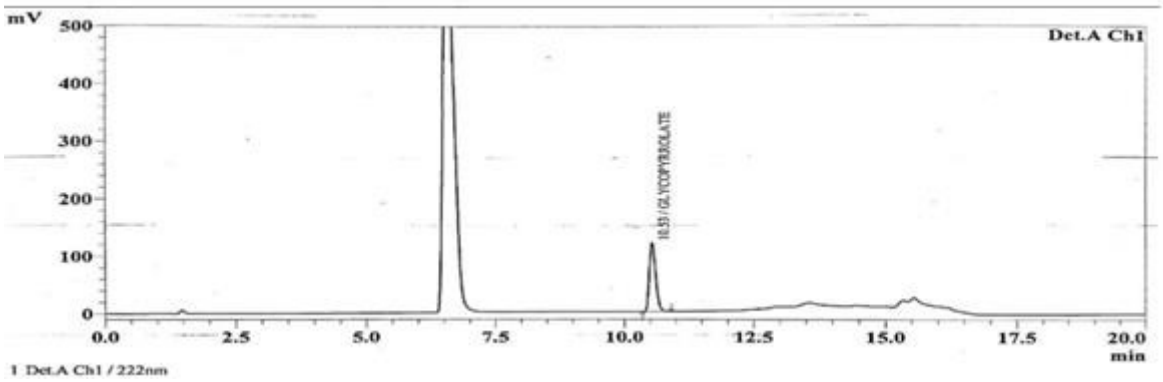

Figure-13Chromatogram ofSample (100ppm)

Table-13DataofAssay

DRUG	LABELCLAIM	AMOUNTPRESENT	%ASSAY
Glycopyrrolate	**0.2mg**	**0.199**	**99.5%**

Discussion: The assay test was performed with sample and % assay was found to be as 99.5%. It was found to be within the limits.

Limits: 90-110%

SUMMARY

A simple, sensitive, precise and specific Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for estimation of Glycopyrrolate in parenteral dosage form. The developed method was validated

according to ICH guidelines. The linear response was observed in the range. The proposed method had adequate specificity for estimation of Glycopyrrolate in parenteral dosage form. The percentage recoveries were found to be within limits of acceptance criteria between the ranges of 95–105%. Precision was found to be within limits and method was found to be robust. The results of assay showed good agreement with label claim. Summary of validation parameters is shown in Table: 10.1.

TABLE-14Summaryofvalidation parameters

Parameters	**Glycopyrrolate**	**LIMIT**
LinearityRange(ppm)	25-150ppm	R^2>0.999
RegressioncoefficientR^2	0.9995	
Assay	99.5%	90-110%
SystemSuitability %RSD Tailing factorTheoreticalplates	0.134 1.3 32428	NMT 2 NMT 2 NLT2000
Precision%RSD	0.42	NMT2.0%
Specificity	Specific	Nointerferenceofanypeak
Accuracy	99.4%	98-104%
LOD	2.87ppm	NMT3
LOQ	4ppm	NMT10

Conclusion:

A validated stability-indicating RP-HPLC method has been developed for Estimation of Glycopyrrolate in parenteral dosage form. The results obtained by the stress degradation conditions of the drug show that the method is specific and stability-indicating. The method was found to be simple, accurate, precise and sensitive. The method was successfully applied for the determination of Glycopyrrolate in injectable dosage form. In the future, this method may be applied for routine analysis drug in Injectable formulations.

BIBLIOGRAPHY

1. BakshiM.,SinghS.Developmentofvalidatedstability-indicatingassaymethods—criticalreview. Journal of Pharmaceutical and Biomedical Analysis.2002;28(6):1011–1040.doi:10.1016/s0731-7085(02)00047-x.

2. SinghS.,BakshiM.GuidanceonConductofStressTestingtoDetermineInherentStability ofDrugs.PharmaceuticalTechnology;2000.

3. SonawaneS.,GideP.Applicationofexperimentaldesignfortheoptimizationofforceddegradation and development of a validated stability-indicating LC method for luliconazole in bulkandcreamformulation.ArabianJournalofChemistry.2012 doi:10.1016/j.arabjc.2012.03.019.

4. Chauhan A, hartiMittu B, Chauhan P (2015) Analytical Method Development and Validation: AConcise Review.JAnalBioanalTech6:233.doi:10.4172/2155-9872.1000233:

5. Lindholm. J, Development and Validation of HPLC Method for Analytical and Preparativepurpose. ActaUniversitatisUpsaliensis,pg.13-14,(2004).

6. Remingtonn'sTheSciencesandPractiseofPharmacy,20thEdition(2000).

7. https://www.slideshare.net/pyundale/partition-chromatographyfinal.

8. http://shodhganga.inflibnet.ac.in/bitstream/10603/110204/8/08_part%20a%20section

%20%201.pdf.

9. https://www.slideshare.net/VishnuReddy85/introduction-and-principle-of-glc-hplc.

10. Gerber F, Krummen M, Potgeter H, Roth A, Siffrin C, Spoendlin C. Practical aspects of fastreversed-phase high-performance liquid chromatography using 3 μm particle packed columns andmonolithic columns in pharmaceutical development and production working under current goodmanufacturingpractice.JournalofChromatographyA.2004May21;1036(2):127-33.

11. Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg725-760.

12. Dr.S.RaviShankar,TextbookofPharmaceuticalanalysis,Fourthedition,Pg13.1-13.2.

13. DavidG.Watson.PharmaceuticalAnalysis, AtextbookforPharmacystudentsandPharmaceuticalChemists.HarcourtPublishersLimited;2ndEd.,Pg221-232.

14. Remingtonn'sTheSciencesandPractiseofPharmacy, 20thEdition(2000).

15. https://teaching.shu.ac.uk/hwb/chemistry/tutorials/chrom/chrom1.htm

16. Dorsey, J.G., Cooper, W.T. Retention mechanisms of bonded-phase liquid chromatography.Anal.Chem.66,857A-867A,1994.

17. Physicalchemistryofmacromolecules.Tanford,C.Wiley,London.1961.

18. http://www.scribd.com/doc/9508765/Physical-Properties-of-Drug.

19. ChardeMS,WelankiwarASandKumarJ.Methoddevelopmentbyliquidchromatographywithvalidation. InternationalJournalofPharmaceuticalChemistry.2014;4(2):57-61.

20. Ranjitsingh.HPLCmethoddevelopmentandvalidation.JPharmEducRes2013;4(1):26-33.

21. SabirAM,MolonyM,ParminderSB.HPLC MethodDevelopmentandvalidation:A Review.InternationalresearchJournalofpharmacy. 2013;4(4):39-46.

22. XiangY,LiuY,LeeML.Ultrahighpressureliquidchromatographyusingelevatedtemperature.Journal ofChromatography.2006;1104(1):198-202.

23. MalviyaR,BansalV,PaloP,andSharmaPK.HighPerformanceLiquidChromatography: AShortReview.JournalofGlobalPharmaTechnology.2010;2(5):22-23.

24. PratapB.etal.ImportanceofRP-HPLCinAnalyticalmethoddevelopment:Areview .Internationaljournalofnoveltrendsinpharmaceutical sciences 2013;3(1):15-23.

25. Lindholm J. Development and Validation of HPLC method for Analytical and PreparativePurpose.Acta Universities UpsaliensisUppsala.2004;13-14.

26. SethiPD.Introduction–HighPerformanceLiquidChromatography,1stedn,CBS Publishers,New Delhi.2001;1-28.

27. Santhosh G, Nagasowjanya G, Ajitha A, Uma Maheswara Rao Y. HPLC method developmentand validation: an overview. International Journal of Pharmaceutical

Research & Analysis. 2014;4(2):274-280.

28. Kayode J, Adebayo. Effective HPLC method development. Journal of Health, Medicine andNursing.2015;12:123-133.

29. Gad S. Pharmaceutical manufacturing handbook of regulations and quality. John wiley andsons;2006

30. https://pharmaceuticalupdates.com/2021/05/03/system-suitability-in-hplc-analysis/

31. Mr.GorheSG1,Miss.PawarGR2AReviewon HighPerformanceLiquid Chromatography,InternationalJournalofAdvance ScientificResearchandEngineeringTrends||Volume3

36. T.G. Venkateshwaran, D.T. King and James, T. Stewart HPLC Determination of Ondansetron- Atropine and Ondansetron-Glycopyrrolate Mixtures in 0.9% Sodium Chloride Injection, Journal ofliquidchromatographyvolume18,1995-Issue 13.

37. M.L.Stormea,R.S.t'Kindta,W.Goeteyna,K. Reyntjensb,J.F.VanBocxlaera, QuantitativedeterminationofGlycopyrrolateinhumanplasmabyliquid,chromatography–electrosprayionizationmassspectrometry:Theuseofavolatileion-pairingagentduringbothliquid–liquidextractionandliquidchromatography,Journal ofChromatographyB,876(2008)

38. M.J. Rumpler*, R.A. Sams, andP. Colahan , Validation of a Liquid Chromatography–TandemMass Spectrometry Method forQuantification of Glycopyrrolatein Horse Plasma ,Journal ofAnalyticalToxicology,Vol.35,November/December2011

39. MukkalaPrasada Reddy, ObireddySreekanth Reddy, MarataChinnaSubbaraoSubha*,AReversePhaseStabilityIndicatingHPLCMethodDevelopmentforEsti mationAssayofBenzyl Alcohol and Glycopyrrolate in Glycopyrrolate Injection, InternationalJournal of PharmaceuticalInvestigation,Vol12,Issue 1,Jan-Mar,2022

40. KusumaJogi[1,2,]MandavaVenkataBasavesw ||Issue1 ||January2018||ISSN

32. http://www.regulatoryinstitute.com/blog/approach-for-forceddegradation-study.

33. http://www.sciencedirect.com/science/article/pii/S2095177913001007.

34. M. C. Allwood and J. H. Plane, "The Wavelength-Dependent Degradation of Vitamin 'A'ExposedtoUltravioletRadiation".

35. Kumaraswamygandla et al RP-HPLC method development and validation for estimation ofGlycopyrrolateinbulkandtabletdosage formsAsian Journal of Pharmaceutical andClinicalResearchVol.4,Issue4,2011ISSN-0974-2441 araRao[2]andRudrarajuRameshraju[1],*,Developmentan dvalidationofStabilityindicatingRP-HPLCmethodfortheestimationofGlycopyrrolateandNeostigmineinbulkandtabletdosageform,Internati onalJournalofScientific&EngineeringResearch, Volume 7,Issue12,December-2016ISSN 2229-5518.

41. GajulaVani*,K.Thejomoorthy,P.Sreenivasa Prasanna,AvalidatedstabilityindicatingRP-HPLCmethoddevelopmentandvalidationofforsimultaneousestimationofindacaterolandGlycopyrrolat einpharmaceuticaldosageform,InternationalJournalofPharmaceuticalDrugAnalysis,Vol:9,Issue:2,20 21;71-79

42. BattulaSreenivasaRao1,MandapatiVaraprasadReddy*andBhatrajuSreenivasaRao,Combined quantification of Formoterolfumarate and Glycopyrrolate by stability indicating RP-HPLCmethodwithphotodiodearraydetector,EuropeanJournalofBiomedicalandPharmaceuticalscience sISSN2349-8870Volume:5Issue:2418-424Year:2018

43. MishraS*,AroraV,AnalyticalMethodValidationof HPLCMethodforAssayofAnticholinergic

44. DruginParenteralFormulation,InternationalJournalforPharmaceuticalResearchScholars (IJPRS)ISSNNo:2277–7873V-5,I-4, 2016.

45. https://www.rxlist.com/glyrx-pf-drug.htm#description