METHOD DEVELOPMENT AND VALIDATION OF GILCOPYRROLATE AND FORMOTEROL FUMERATE BY USING RP HPLC

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Abstract:
*A new method was established for simultaneous estimation of Glycopyrronium and Formoterol by RP-HPLC method". "The chromatographic conditions were successfully developed for the separation of Glycopyrronium and Formoterol by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm".*

***KEY WORDS**: "Glycopyrronium, Formoterol, RP-HPLC, validation*

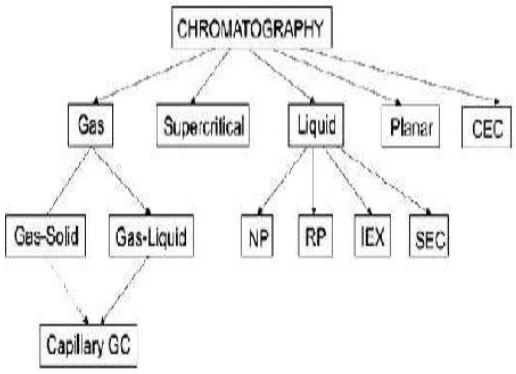
INTRODUCTION

Glycopyrrolate is a quaternary ammonium salt. Chemically, Glycopyrrolate is (RS)-[3(SR)- Hydroxy-1, 1-dimethylpyrrolidinium bromide] α-cyclopentylmandelate. The chemical formula is C19H28BrNO3 . The molecular weight is 398.33g/mol.1 Glycopyrrolate is a crystalline white powder. It is dissolvable in water and alcohol, and much insoluble in chloroform and ether.2 Glycopyrrolate, as other anticholinergic (antimuscarinic) drugs, impedes the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine yet require cholinergic innervation. Thus, it diminishes the volume and free acidity of gastric secretions and controls Submission Date: 18-09-2017 Revision Date: 08-11-2017; Accepted Date: 18-04-2018 excessive pharyngeal, tracheal, and bronchial secretions.3 Formoterol acts as a bronchodilator. It extends the airways of the lungs, so that it helps to inhale all the more effortlessly. It may even be utilized to forestall respiratory issues caused by exercise. It can also be utilized for long-term treatment of chronic obstructive pulmonary disease (COPD).4 Chemically, Formoterol is N-[2-Hydroxy5-[(1RS)-1-hydroxy-2-[[(1RS)-2(4-methoxyphenyl) -1-methylethyl]-amino] ethyl] phenyl] formamide (E)-2-butenedioate dihydrate. The chemical formula is C19H24N2 O4 . C4 H4 O4 .2H2 O. The molecular weight is 840.91g/mol.

Analytical chemistry 4
"Analytical chemistry is a scientific discipline used to and behaviour of matter" "The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts" "Quality control in manufacturing industries, the monitoring of clinical and environmental samples the assaying of geological specimens and the support of fundamental and applied research are the principal applications" "Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative quantitative and structural information.

Types of Chromatography"
"The mobile phase could be either a liquid or a gas and accordingly we can subdivide chromatography into Liquid

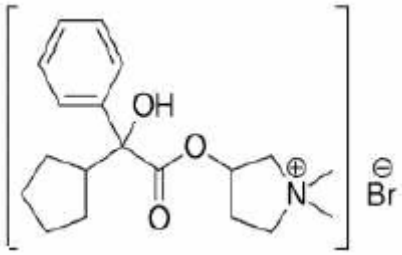
Chromatography LC or Gas Chromatography" "GC Apart from these methods there are two other modes that capillary forces Chromatography.



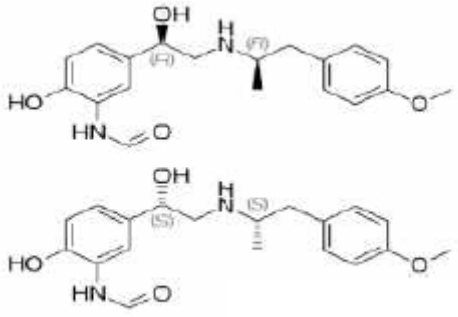
Normal phase chromatography"

"Normal phase HPLC NP HPLC was the first kind of HPLC chemistry used and separate analytes based on polarity" "This method uses a polar stationary phase fairly polar in nature" "The polar analyte associates with and is retained by the polar stationary phase" "Absorption strengths increase with increase in analyte polarity and the interaction between the polar analyte and the polar stationary phase increases the elution time" "The interaction strength not only depends on the functional groups in the analyte molecule but also on stearic factors and structural isomers is often resolved from one another" "Use of more polar solvents decrease the retention time of the analyte while more hydrophobic solvents tend to increase retention times Particularly polar solvents in a mixture tend to deactivate the column by occupying the stationary phase surface. The most simplified way of explaining the cycle of operation without taking into account the compressibility of the solvents is as follows" "From the moment when the outlet valve of cylinder a closes and its entrance valve open the piston in A moving backwards sucks the eluent through the inlet check valve and the chamber fills Meanwhile cylinder B is open and its piston moves forward to force the mobile phase towards the injector and the column" "The volume displaced by piston B is half of that available in the chamber of piston" "A With chamber A full the entrance valve of a closes and the corresponding outlet valve opens". "Piston a now advances and pushes out the contents of the chamber Half of this volume is expelled directly towards the piston B retracts" "A pulse absorber is located between the two cylinders diagram courtesy of Agilent Technologies.

DRUG PROFILE
GLYCOPYRRONIUM



IUPAC Name :
(3S)-3-{[(2R)-2-cyclopentyl-2-hydroxy-2-phenylacetyl]oxy}-1,1-dimethylpyrrolidin-1-ium
Chemical formula : C19H28NO3
Molecular weight : 318.436
Category : "Adjuvants, Anesthesia,Alimentary Tract and Metabolism,Amines"
BRAND NAME : *Glycolate*
FORMOTEROL



IUPAC Name :
N-[2-hydroxy-5-(1-hydroxy-2-{[1-(4-methoxyphenyl)propan-2-yl]amino}ethyl)phenyl]formamide
Chemical formula : C19H24N2O4
Molecular weight : 344.4049
Category : "Adrenergic Agents,Adrenergic Agonists"

BRAND NAME : Foracort

LITERATURE REVIEW

1) **Sanagapati Manasa et** al.; "In the gift study, 2 analytical ways were developed for the estimation of Glycopyrronium in API. technique A": "RPHPLC technique, technique B: ultraviolet light spectroscopical technique". "In technique A, the drug showed dimensionality within the vary of 25-150µg/ml with a coefficient of correlation (r2) of zero.999, wherever as in technique B, the dimensionality 1-5µg/ml with a coefficient of correlation of (r2) zero.999".

2) **Subhashini Edla et al .;** "Formoterol and Glycopyrronium square measure prescribed for the treatment of patients with polygenic disease". "This study describes a speedy, simple, precise and correct RP-HPLC methodology for synchronal estimation of Formoterol and Glycopyrronium in their combined pill indefinite quantity kind". "The Oyster BDS RPC18 Column (150mm x four.6mm, five µm) column with associate degree isocratic mixture of Methanol: Acetonitrile: Water in 30:60:10 (v/v), at a rate of one.0 ml/min and actinic ray detection at 228 nm".

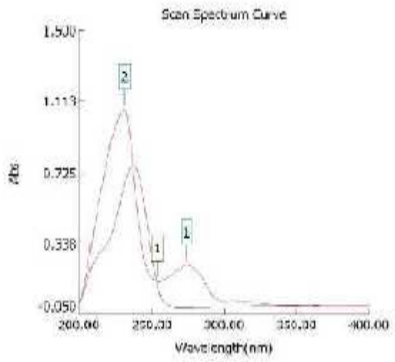
3) **Patil Sudarshan S. et al.;** "Simple spectrophotometric technique has been developed for cooccurring estimation of Glycopyrronium and Formoterol in combined dose kind". "The technique the tactic the strategy utilized as a solvent. the 2 wavelengths 229.5 nm and 237 nm" "were designated for estimation of Glycopyrronium and Formoterol severally". "Dimensionality was ascertained within the concentration vary of 3-15 µg/ml and 2-10 µg/ml for Glycopyrronium and Formoterol severally".

4) **Shammi Goyal et al.;** "A rapid, easy and sensitive RP-HPLC technique was developed and valid for the determination of Glycopyrronium in bulk drug and pharmaceutical formulation". "Optimum separation was achieved by victimisation one hundred fifty metric linear unit Ã— four.6 metric linear unit C18 column (3 Î¼m) with a mix of acetonitrile (ACN): water within the quantitative relation of six0:40 at a rate of flow of one ml/min". "The detection was meted out at 276 nm".

5**) Arayne MS et al.; "**The reversed-phase superior liquid natural action (RP-HPLC) technique has been developed to quantify Formoterol in material and pharmaceutical formulations exploitation C(18) analytical reverse-phase column". "Glycopyrronium was used as an interior commonplace". "Mobile section consisted of methanol-water (30:70 v/v), pumped-up at a flow of zero.5 ml/min at close temperature and therefore the retention time was concerning four.4 min with symmetrical peaks". "was detected by ultraviolet absorbance at 233 nm"

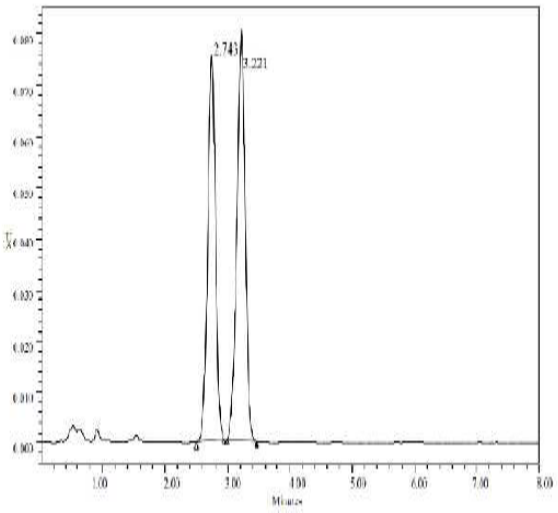
RESULTS AND DISCUSSION

WAVELENGTH DETECTION": "The detection wavelength was elect by dissolving the drug in mobile part to induce a amount. "The ensuing". "The overlay spectrum of Glycopyrronium and Formoterol Formoterol showed absorbance's maxima at 255nm". "

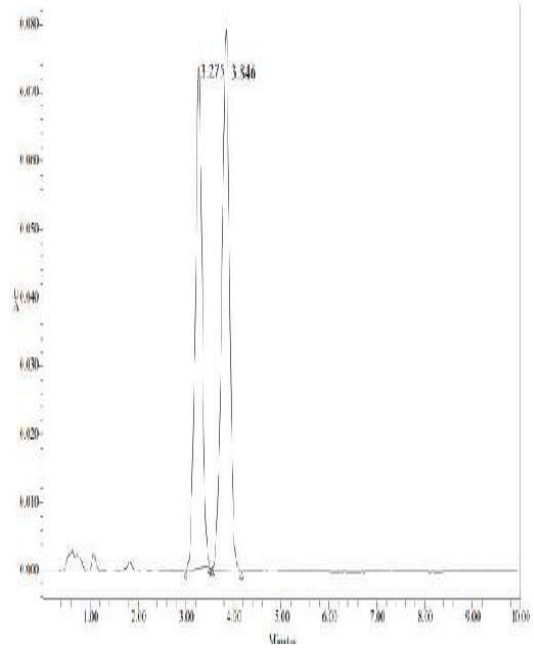


Overlay spectrum of Glycopyrronium and Formoterol

The Organic composition within the Mobile section was varied from seventieth to hr". "customary answer three hundred µg/ml of Formoterol& 3µg/ml of Glycopyrronium was ready and analyzed mistreatment the numerous Mobile section composition beside the particular mobile section composition within the technique".



Chromatogram for Robustness more organic



Chromatogram for Robustness less organic

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	USP Resolution
1	Glycopyr	3.275	740841	73795	2818.9	1.1	
2	Formoterol	3.846	903365	78845	3107.7	1.0	1.9

Details of Robustness les organic

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	2818	1.1
2	Actual	3125	1.1
3	10% More	2707	1.1

"System suitability results for Formoterol (Mobile phase)"

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	3107	1.0
2	Actual	3526	1.0
3	10% More	3001	1.0

System suitability results for Glycopyrronium (Mobile phase)"

SUMMARY AND CONCLUSION

"The developed RP-HPLC method is accurate precise and reliable for the analysis of Glycopyrronium and Formoterol in pharmaceutical formulations".

References:

1. "Sanagapati Manasa et al "Method" "Development" "and" "Validation" "of" "Formoterol" "in" "API" "By" "RPHPLC" "and" "UV-Spectroscopy"".

2. "Subhashini Edla et al "New" "Analytical" "Method" "Development" "and" "Validation" "For"The "Simultaneous" "Estimation" "of" Glycopyrronium" "and" "Glibenclamide" "in" "Bulk" "and" "Tablet""Dosage" "Form" "Using" "Rp-Hplc""

3. "Patil Sudarshan S. et al "Development" "and" "Validation" "of" "Analytical" "Method" "For" "Simultaneous" "Estimation" "of" "Formoterol" "and" "Glycopyrronium" "Hcl" "in" "Bulk" "and" "Tablets" "Using" "UV" – "Visible" "Spectroscopy".

4. "Shammi Goyal et al "Development" "and" "Validation" "of" "RP-HPLC" "Method" "For" "Estimation" "of" "Glipizide" " in" "Bulk" "Drug" "and" "Pharmaceutical" "Formulation"
5. "Arayne MS et al "Development" "and" "Validation" "of" "RP-HPLC" "Method" "For" "the" "Analysis" "of" "Glycopyrronium".