INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

SIMULTANEOUS ESTIMATION OF GLYCOPYRROLATE AND FORMOTEROL FUMARATEIN PHARMACEUTICAL DOSAGE FORM BY RP-UPLC

M.Suresh Babu*, B.A.S.S.S.Swaroop, B.Dileep, S.Jayasri, K.Rajan, CH.V.Deepthi

Department of Pharmaceutical Analysis, JITS College of Pharmacy, Kalgampudi, Andhra Pradesh, India.

ABSTRACT

A new precise, accurate, rapid method has been developed for the simultaneous estimation of Glycopyrrolate and Formoterol fumaratein pharmaceutical dosage form by RP-UPLC. The optimum wavelength for the determination of Glycopyrrolate and Formoterol fumaratewas selected at 279 nm on the basis of isobestic point. Various trials were performed with different mobile phases in different ratios, but finally Water: Acetonitrile : Methanol (20:30:50) %v/v/v was selected as good peak symmetry and resolution between the peaks was observed. TheRetention time of Glycopyrrolate and Formoterol fumaratewere found to be 1.208 & 5.934 min respectively. The Retention times for both the drugs were considerably less compared to the Retention time obtained for the drugs in the other mobile phase. The different analytical performance parameters such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 50-120 µg/mLFor Glycopyrrolate and50-120 µg/mL forFormoterol fumarate. From linearity the correlation coefficient R^2 value was found to be 0.9996for Glycopyrrolateand 0.999 for Formoterol fumarate. The proposed UPLC method was also validated for system suitability, system precision and method precision.

KEY WORDS: Glycopyrrolate, Formoterol fumaratein, RP-UPLC, pharmaceutical dosage form

Author for correspondence
M.Suresh Babu,
Department of Pharmaceutical Analysis,
JITS College of Pharmacy, Kalgampudi,
Andhra Pradesh, India.
Email id: sureshbabu3377@gmail.com

INTRODUCTION

Chromatography is a non-destructive procedure for resolving a multi-component mixture of traces, minor or constituents in to individual fractions.It is a method of separating a mixture of components in to individual components through a porous medium under the influence of solvent.For many years, researchers have looked at “fast LC” as a way to speed up analyses. The need for speed, the availability of affordable and easy to use mass spectrometers. Smaller columns and faster flow rates (amongst other parameters) have been used. Elevated temperature, having the dual advantages of lowering viscosity, and increasing mass transfer by increasing the diffusivity of the analytes, has also been investigated. However, using conventional particle sizes and pressures, limitations are soon reached and compromises must be made, sacrificing resolution.HPLC technology simply doesn’t have the capability to take full advantages of sub-2µm particles. UPLC can be regarded as new

invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography. UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that operates at high pressure than that used in HPLC & in this system uses fine particles(less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time. According to the van Deemter equation, as the particle size decreases to less than2.5 µm, there is a significant gain in efficiency, while the efficiency does not diminish at increased flow rates or linearvelocitiesTherefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed Ultra Performance Liquid Chromatography,orUPLC . The technology takes full advantage of chromatographic principles to run separationsUsing columns packed with smaller particles(less than2.5µm) and/or higher flow rates for increased speed, this gives superior resolution and sensitivity.

Formoterol is a long-acting (12 hours) beta2-agonist used in the management of asthma and/or chronic obstructive pulmonary disease (COPD). Inhaled formoterol works like other beta2-agonists, causing bronchodilatation through relaxation of the smooth muscle in the airway so as to treat the exacerbation of asthma. The pharmacologic effects of beta2-adrenoceptor agonist drugs, including formoterol, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibits the release of pro-inflammatory mast-cell mediators such as histamine and leukotrienes. Formoterol also inhibits histamine-induced plasma albumin extravasation in anesthetized guinea pigs and inhibits allergen-induced eosinophil influx in dogs with airway hyper-responsiveness. The relevance of these in vitro and animal findings to humans is unknown. Glycopyrronium (as the bromide salt 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. In October 2015, glycopyrrolate was approved by the FDA for use as a standalone treatment for Chronic obstructive pulmonary disease (COPD), as SeebriNeohaler. 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. These peripheral cholinergic receptors are present in the autonomic effector cells of smooth muscle, cardiac muscle, the sinoatrial 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 secretions and controls excessive pharyngeal, tracheal, and bronchial secretions (1-5).

The above review indicates that there are fewer methods for the simultaneous estimation of Formoterol fumarate and Glycopyrrolate but that **methods were** found to be cost effective and time consuming. So, my aim was to develop a new method with minimum run time and less solvent consumption for the estimation of Formoterol fumarate and Glycopyrrolate_ in combination of drugs. Hence the present study aims to develop simple, precise and accurate methods for the determination of Formoterol fumarate and Glycopyrrolate_ by RP-UPLC in tablet dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λ_{max})

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of Phosphate bufferpH4.5 (6-8)

1.36 gm of Potassium hydrogen Phosphate Monobasicwas weighed and dissolved in 1000 mL of water. then Adjust the pH to2.5±0.02 using diluted Orthophosphoric solution acid. Buffer was filtered through 0.45µm filters to remove all fine particles and gases.

Preparation of Standard solution

About 10 mg of glycopyrolate and 10mg of formoterol fumarate were weighed into a 50 mL volumetric flask, to this 50 mL of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Preparation of Sample solution

Crush 20tablets then weigh a quantity of powder equivalent to 50mg of glycopyrolate and 100mg of formoterol Fumarate in 100 mL volumetric flask and add70mL of mobile phase then sonicated it for 30minintermittent shaking after 30min make up volume with mobile phase. Pipetted 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.

System Suitability& System precision

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 100µg/mLof Glycopyrolateand100µg/mLof formoterol fumarate were injected six times and the chromatograms were recorded for the same.

RESULTS AND DISCUSSION

The glycopyrolate peak was observed at 1.208 min and formoterol fumarate peak was observed at 5.934 min with good efficiency, peak shape and good resolution. So, this trail was considered and validated according to ICH guidelines (Fig-1).

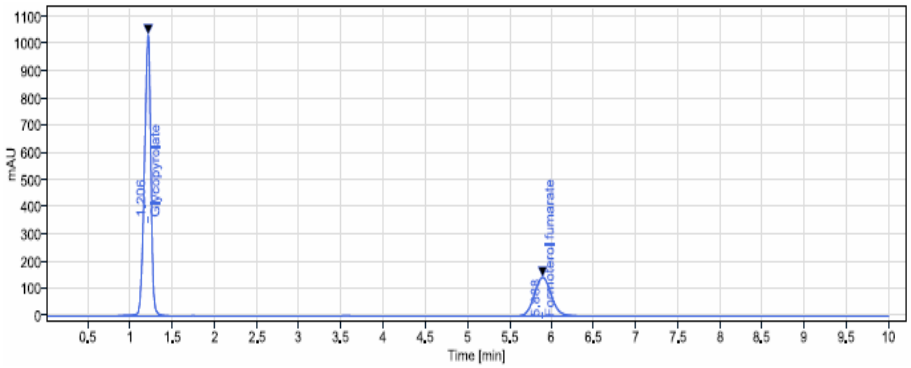

Fig-1 Chromatogram of Assay

Both drugs % assay found to be within the limits. The percentage purity of both Glycopyrolateand formoterol fumarate were found to be within the limits that is 98-102% (Table-1).

Table-1 Results for glycopyrolate and formoterol fumarate

Glycopyrolate			Formoterol Fumarate	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	924225	926498	577339	567626
Injection-2	916710	928201	572957	564175
Injection-3	922097	922456	570728	572334
Injection-4	921097	920306	571728	573527
Injection-5	919838	923706	569261	574574
Average Area	919810	914952	570393	553709
Assay(%purity)	99.81		99.60	

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of glycopyrolate and formoterol fumarate is 0.999 and 0.999 respectively (Fig-2 and 3).

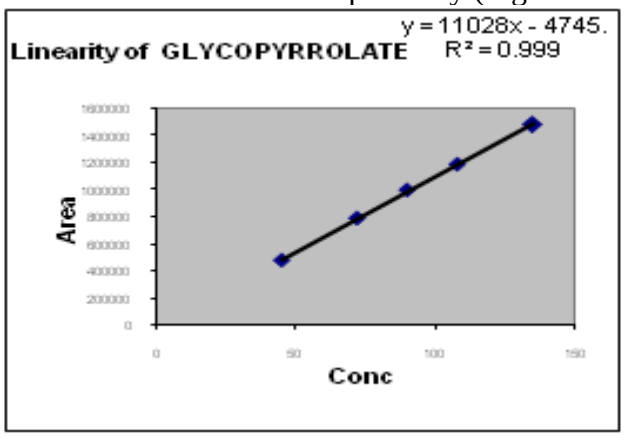

Fig-2 Graph for Linearity data of glycopyrolate

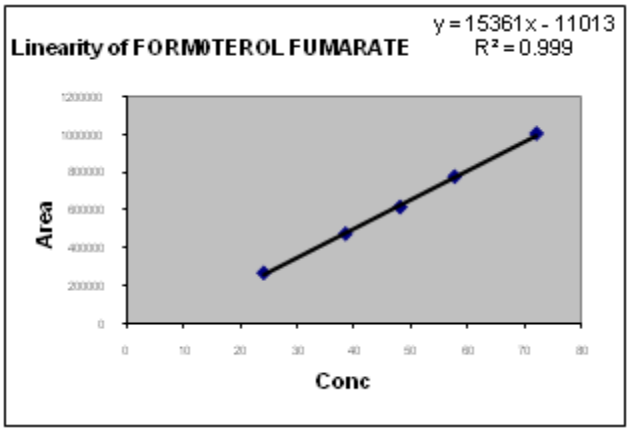

Fig-3 Graph for Linearity data of formoterol fumarate

The % mean recovery of glycopyrolate and formoterol Fumarate was founded between 98.0 to 102.0. The tailing factor was found to be within the limits on small variation of flow rate and wavelength (Table-2).

Table-2 Results for Robustness of glycopyrolate and formoterol fumarate

Chromatographic changes		**Theoretical Plates**		**Tailing factor**		**Resolution**
		GPR	**FMR**	**GPR**	**FMR**	**Between GPR & FMR**
Flow rate (mL/min)	0.8	5585	3375	1.23	1.20	4.71
	1.2	5579	3367	1.25	1.20	4.75
Wavelength (nm)	277	5592	3361	1.27	1.21	4.81
	281	5568	3372	1.25	1.25	4.75

CONCLUSION

A new precise, accurate, rapid method has been developed for the simultaneous estimation of Glycopyrrolate and Formoterol fumaratein pharmaceutical dosage form by RP-UPLC.The optimum wavelength for the determination of

Glycopyrrolate and Formoterol fumaratewas selected at 279 nm on the basis of isobestic point. Various trials were performed with different mobile phases in different ratios, but finally Water: Acetonitrile : Methanol (20:30:50) %v/v/v was selected as good peak symmetry and resolution between the peaks was observed. TheRetention time of Glycopyrrolate and Formoterol fumaratewere found to be 1.208 & 5.934 min respectively. The Retention times for both the drugs were considerably less compared to the Retention time obtained for the drugs in the other mobile phase. The different analytical performance parameters such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 50-120 µg/mLFor Glycopyrrolate and50-120 µg/mL forFormoterol fumarate. From linearity the correlation coefficient R^2 value was found to be 0.9996for Glycopyrrolateand 0.999 for Formoterol fumarate. The proposed UPLC method was also validated for system suitability, system precision and method precision. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. Hence the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of Glycopyrrolate and Formoterol fumaratein Educational institutions and Quality control laboratories.

REFERENCES

1. Chatwal**,** R**.** G**.;** Anand**,** K**.** S**.** High performance liquid chromatography**.** *Instrumental methods of chemical analysis***,** 5thed**.;** Himalaya publishers**:**Mumbai, 2010**;** 2.570-2.629**.**
2. Sharma**,** B**.** K**.** High performance liquid chromatography**.***Instrumental methods of chemical analysis***,** 24th ed**.;** Goel publishers**:**Meerut**,** 2005**;** 295 - 300**.**
3. Dong,W. M. HPLC instrumentation and trends. *Modern HPLC for practicing scientists*, USA, 2006; 5-10, 78-110.
4. Swartz,M. E.; Ira Krull, S, Analytical method development. *Analytical method development and validation*, 1st ed.; Marcel Dekker, Inc: New York, 2009; 17-80.
5. Satinder, A.; Dong, M. W. Method development and validation. *Pharmaceutical analysis by HPLC*, 15th ed.; New York, 2005; 16-70.
6. ICH, *Text on Validation of Analytical Procedures*, ICH – Q2A, International Conference on Harmonisation, IFPMA, Geneva, 1995, 2-3, A–1 to A–3.
7. ICH, *Validation of Analytical Procedures: Methodology*, ICH – Q2B, International Conference on Harmonisation, 1996, 1-3.
8. ICH Guidelines, Q2 (R1) - *Validation of Analytical Procedures*:Text and Methodology,2005, 1-6.