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

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## 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. The Retention time of Glycopyrrolate and Formoterol fumarate were 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 and 50-120 µg/mL for Formoterol 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

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## 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. HPLC technology simply doesn't have the capability to take full advantages of sub-2µm particles. UPLC can be regarded as new 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 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 linearvelocities Therefore 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 separations Using 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. 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 (1-3).

Every day a number of diseases are being diagnosed. So, various pharmaceutical organizations are working to develop new drug molecules and new combinations of Beta2-agonistand Antispasmodicfor better treatment. This is the reason for a greater competition in the pharmaceutical sector, and the future scenario is likely to be the same. The scope of developing and validating a method is to ensure a suitable strategy for evaluation of a particular analyte which is more specific, accurate and precise. The main focus is drawn to achieve improvement in the manufacturing

and analytical conditions and making proper amendments in the standard operating procedures being followed. The above review indicates that there are fewer methods for the simultaneous estimation of Formoterol fumarate and Glycopyrrolatebut 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 ($\lambda_{max}$)
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 samples for Assay (4-6)
### Preparation of Standard solution
About 50mg of Glycopyrolate and 100mg of Formoterol fumarate were weighed into a 100 mL volumetric flask, to this 70mL of mobile phase was added, sonicated and the volume was made up with the mobile phase.Pipetted 5 mL of the clear solution in to 50 mL volumetric flask and make up volume with mobile phase.

### Preparation of Sample solution
Sample name: Formoterol fumarate and GlycopyrrolateTablets(50 mg of Glycopyrolate and100mg of Formoterol fumarate). Crush 20tablets then weigha quantity of powder equivalent to 50mg of Glycopyrolate and 100mg of Formoterol Fumaratein 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.

## RESULTS AND DISCUSSION

The wavelength of maximum absorption ($\lambda_{max}$) of the solution of the drugs in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 241 nm for Glycopyrolate, 238 nm for Formoterol fumarate and at 279 nm same absorbance for both the drugs i.e., isobestic point. Thus 279 nm was selected as detector wavelength for the UPLC chromatographic method.
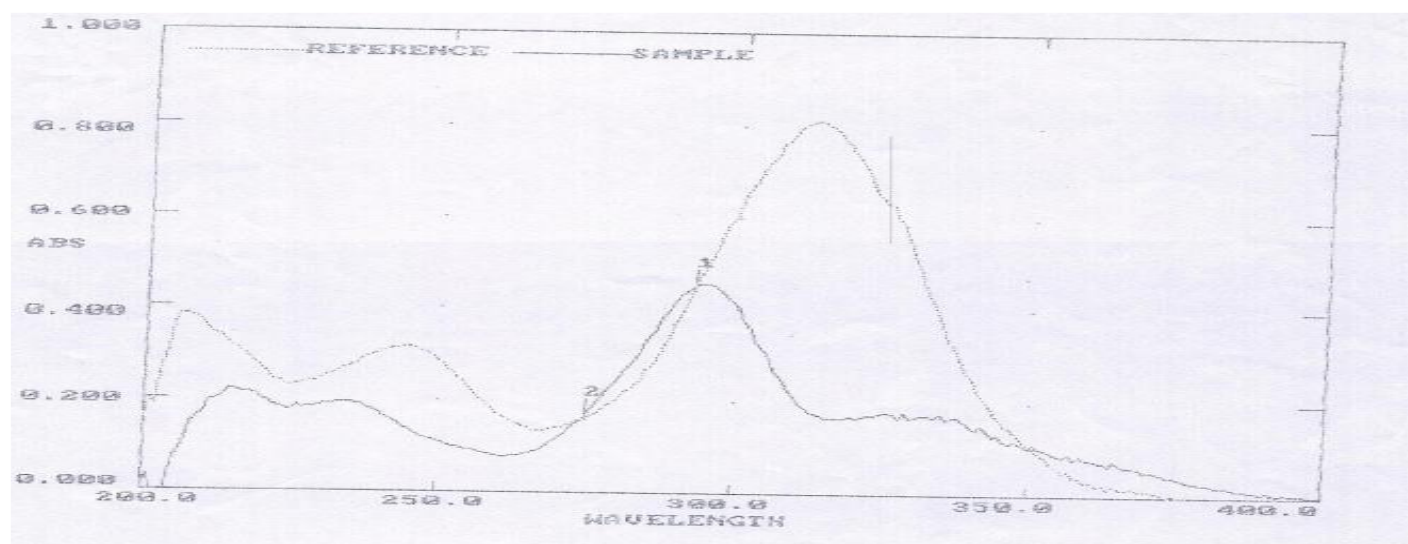


**Fig-1 UV-VIS overlay Spectrum of glycopyrolate and formoterol fumarate (279 nm)**

The percentage purity of both Glycopyrolate and 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 | |

A graph was plotted for glycopyrolate and formoterol Fumarate against the concentrations of the solutions and the peak areas. The correlation coefficient $R^2$ was determined and was found to be 0.999 for Glycopyrolate and 0.999 for formoterol fumarate (Fig-2 and 3).
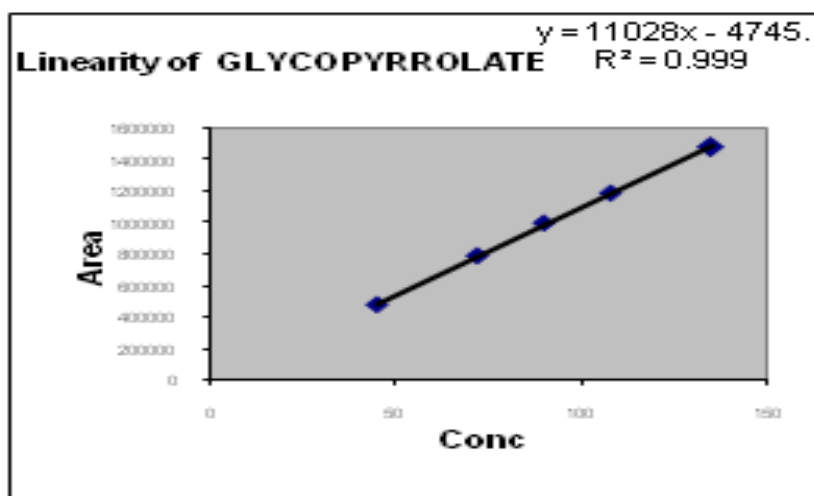
$$y = 11028x - 4745.$$
$$R^2 = 0.999$$

**Fig-2 Graph for Linearity data of Glycopyrolate**
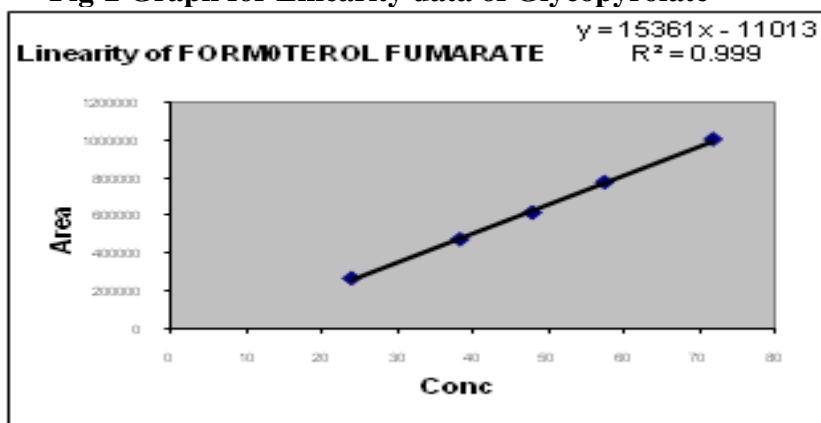


$$y = 15361x - 11013$$
$$R^2 = 0.999$$

**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 (Table-2 and 3).

**Table-2 Results for Recovery of glycopyrolate**

| %Recovery | Amount present (µg/mL) | Amount found (µg/mL)* | Percent Recovery * | % Mean Recovery |
|---|---|---|---|---|
| 50% | 45 | 45.39 | 100.9 | |
| 100% | 90 | 90.45 | 100.5 | 101.0 |
| 150% | 135 | 138.15 | 101.6 | |

**Table-3 Results for Recovery of formoterol fumarate**

| % Recovery | Amount present (µg/mL) | Amount found (µg/mL)* | Percent Recovery * | % Mean Recovery |
|---|---|---|---|---|
| 50% | 24 | 24.37 | 101.6 | |
| 100% | 48 | 48.84 | 101.7 | 101.0 |
| 150% | 72 | 71.77 | 99.7 | |

The % Degraded for Glycopyrrolate and Formeterol Fumarate from these stability methods should be not more than 1.0 % (Table-4 and 5).

### Table-4 Results of Degradation of Glycopyrrolate

| Method | std area | Degradation area | % Obtained | % Degraded |
|---|---|---|---|---|
| Peroxide | 920630 | 932189 | 101.256 | 0.644 |
| Photolytic | 920630 | 932174 | 101.255 | 0.645 |
| Acidic | 920630 | 932171 | 101.254 | 0.646 |
| Alkaline | 920630 | 932174 | 101.254 | 0.646 |
| Thermal | 920630 | 932189 | 101.255 | 0.645 |

### Table-5 Results of Degradation of Formeterol Fumarate

| Method | std area | Degradation area | % Obtained | % Degraded |
|---|---|---|---|---|
| Peroxide | 572068 | 582684 | 101.856 | 0.044 |
| Photolytic | 572068 | 582675 | 101.854 | 0.046 |
| Acidic | 572068 | 582668 | 101.853 | 0.047 |
| Alkaline | 572068 | 582674 | 101.854 | 0.046 |
| Thermal | 572068 | 582684 | 101.856 | 0.044 |

## 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 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. 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.

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