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**Research Article**

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# Method Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Terbutaline Sulphate and Guaiphenesin in Bulk and Tablet Dosage Form

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**ABSTRACT**

*A simple, precise, accurate and rapid Reverse phase liquid chromatographic method has been developed and validated for the analysis of Terbutaline and Guaiphenesin in Bulk as well as in Tablet dosage form. The analysis was carried out using Hexon C18 (250mm x 4.6ID, Particle size: 5 micron) column with UV-3000-M Detector by using the Gradient system. The mobile phase used was Methanol and Water in the ratio of 60:40 containing phosphate buffer adjusted with pH 3 was pumped at a flow rate of 0.8 ml/min with UV-detection at 275 nm. The retention time for Terbutaline and Guaiphenesin was found to be 2.278 and 4.414 minutes respectively. The method has been validated in accordance with the ICH guidelines such as Linearity, Accuracy, Precision, LOD, LOQ, and Robustness. The method was found to be linear over a concentration range of 1-5µg/ml and 40-200µg/ml with $R^2$ value 0.994 and 0.998 for Terbutaline and Guaiphenesin Respectively. The present work shows that this RP-HPLC method is useful for the routine analysis of Terbutaline and Guaiphenesin in bulk as well as its Tablet dosage form.*

**Keywords**: Terbutaline; Guaiphenesin; RP-HPLC; Validation

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## INTRODUCTION

Terbutaline is Bronchodilator drug acting by stimulation through beta-adrenergic receptors of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic- 3',5'-adenosine monophosphate (c-AMP).Chemically it is 5-[2-(tert-butylamino)-1-hydroxyethyl] benzene-1, 3-diol (Figure 1) [1-3].

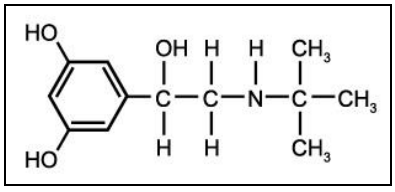


**Figure 1: Chemical structure of Terbutaline**

Guaiphenesin (GUAI) is chemically 3-(2-methoxyphenoxy)-1,2-propanediol (Figure 2)[1]. Guaifenesin acts as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi. It has been said to aid in the flow of respiratory tract secretions, allowing ciliary movement to carry the loosened secretions

upward toward the pharynx. Thus, it may increase the efficiency of the cough reflex and facilitate removal of the secretions [2,3].

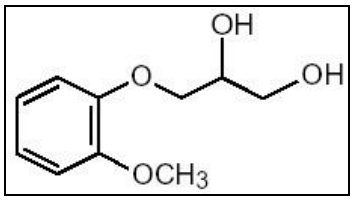


**Figure 2: Chemical strucure of Guaiphenesin**

There are number of UV Spectrophotometric [4-8], HPLC [8-11], HPTLC [17] methods have been developed for the drugs Terbutaline and Guaiphenesin in individual and in combination with other drugs in Bulk and Tablet dosage form. Also there are many methods developed on syrup dosage form also [17-19]. According to the Literature survey, there is no RP-HPLC method has yet been developed for the simultaneous estimation of Terbutaline and Guaiphenesin in Bulk as well as in Tablet Dosage form. Hence the present paper includes the work which we have carried out to develop simple, precise, Accurate, economic, sensitive and rapid RP-HPLC method for this combination. Also the method has been validated in accordance with ICH Guidelines.

## MATERIALS AND METHODS

### Reagents and chemicals

All chemicals used were of HPLC grade chemicals. Methanol and Water were of HPLC grade and were purchased from Fischer Ltd, Mumbai. Terbutaline and Guaiphenesin were obtained from Astazeneca Ltd, Bangalore, India respectively.

### Equipment and chromatographic conditions

A high-performance liquid chromatographic system, Analytical Technologies Ltd. Equipped with UV-3000-M detector was used for separation. All measurements were performed on Wenser High Precision Balance. The column used for separation was Hexon C18 (250mm x 4.6ID, Particle size: 5 micron) column with P-3000-M Reciprocating pump (40MPa).

The mobile phase was prepared by dissolving 60ml of Methanol in 40ml of Water and sonicated to remove dissolved gases. The pH of the mobile phase was adjusted to 3±0.05 with Phosphate buffer solution. Finally the mobile phase was filtered through a 0.45µm membrane filter and degassed for 10 minutes. The injection volumes for samples and standards were 20µl and eluted at a flow rate of 0.8mL/min at 30°C. The eluents were monitored at 275 nm.

### Preparation of standard stock solutions

A working standard solution containing Terbutaline and Guaiphenesin was prepared by weighing 10mg of both the drugs dissolved in 10mL methanol and solution was sonicated for 10 min the volume was made up to the mark with methanol to obtain a stock solution of 1000 µg/mL of TER and GUAI respectively.

### Preparation of working standard solutions

From the above stock solution of TER and GUAI 0.01ml and 0.4ml was pipette out in to a 10ml volumetric flask which contain the mobile phase and then made up to the final volume with mobile phase to get mixed standard solution. UV spectrum showing isobestic point is shown in Figure 3.

### Method Validation

The developed analytical method was further subjected to validation in accordance to the ICH guidelines. The parameters evaluated were linearity, system suitability, precision, accuracy, robustness LOD, LOQ. SD and % RSD <2% were considered acceptable.

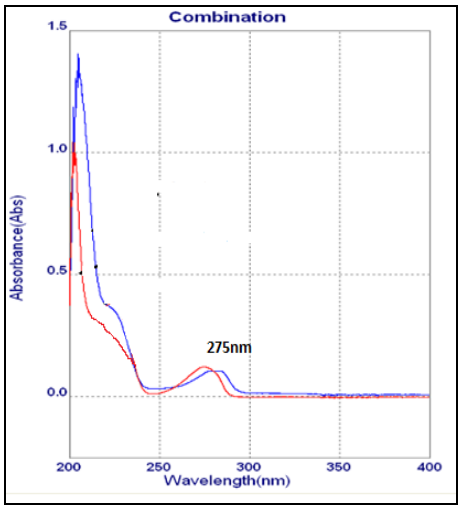


**Figure 3: Isobesitic point of Terbutaline and Guaiphenesin**

## Linearity

A set of five solutions of TER and GUAI at concentration ranging from 1-5µg/ml and 40-200µg/ml were prepared. Calibration curve was constructed by plotting the peak area against concentration. The correlation coefficient was found to be 0.994 and 0.998 for Terbutaline and Guaiphenesin respectively.

**Table 1: Linearity of Terbutaline**

| Standard concentration | Peak area of Terbutaline |
|---|---|
| 1 | 82802 |
| 2 | 102090 |
| 3 | 134737 |
| 4 | 164080 |
| 5 | 190394 |
| Regression equation | Y= 27714 x + 51683 |
| Regression coefficient | $R^2$ = 0.994 |

**Table 2: Linearity of Guaiphenesin**

| Standard concentration | Peak area of Guaiphenesin |
|---|---|
| 40 | 1886032 |
| 80 | 3722511 |
| 120 | 5466224 |
| 160 | 7644189 |
| 200 | 9568147 |
| Regression equation | Y= 48215 x -12835 |
| Regression coefficient | $R^2$ = 0.998 |

The calibration curves for both drugs given in Figure 4 and Figure 5.

## LOD and LOQ

The calculated LOD and LOQ concentration confirmed that the method were sufficiently sensitive. The value of LOD (µg/ml) 0.610 and 0.849 and value of LOQ (µg/ml) were 1.87 and 2.40 respectively for Terbutaline and Guaiphenesin.

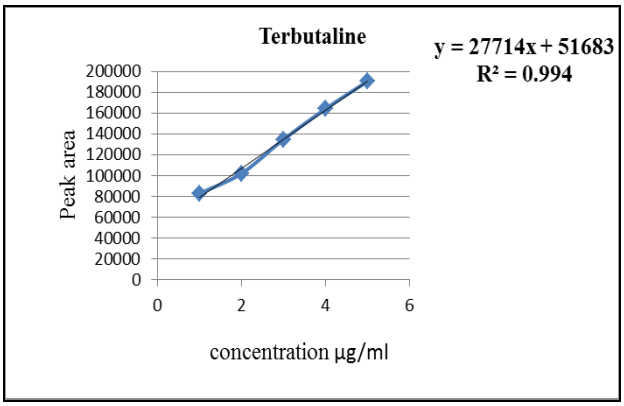


**Figure 4: Calibration curve of Terbutaline**

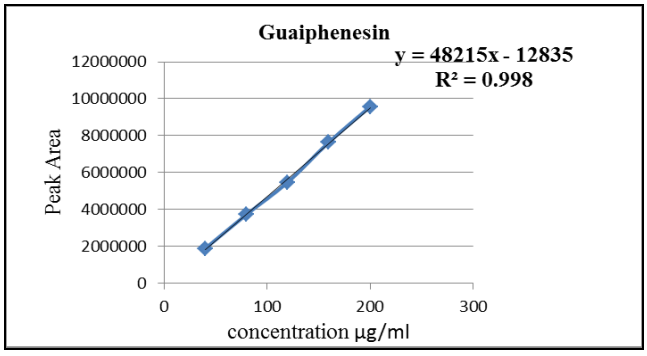


**Figure 5: Calibration curve of Guaiphenesin**

## Accuracy

The Accuracy was determined by Standard Addition method. Three different levels (50%, 100%, 150%) of standard were spiked to commercial Tablets in Triplicates. The mean of % Recoveries and the % RSD was calculated.

**Table 3**: **Accuracy study of Terbutaline**

| Sr.no | Conc. | Area | Standard Deviation | | Accuracy | Precision |
|---|---|---|---|---|---|---|
| | | | Mean | SD | %SD | %RSD |
| 1 | 1 | 82802 | 83783 | 929.66499 | 1.1096105 | 1.1096105 |
| | 1 | 83896 | | | | |
| | 1 | 84651 | | | | |
| 2 | 3 | 134737 | 135776.67 | 900.49449 | 0.6632174 | 0.6632174 |
| | 3 | 136282 | | | | |
| | 3 | 136311 | | | | |
| 3 | 5 | 200394 | 210330 | 13547.633 | 6.4411322 | 6.4411322 |
| | 5 | 204834 | | | | |
| | 5 | 225762 | | | | |

**Table 4: % Recovery of study of Terbutaline**

| Sr. no. | % Composition | Area of Standard | Area of Sample | % Recovery |
|---|---|---|---|---|
| 1 | 50% Recovery | 134737 | 134506 | 99.82 |
| 2 | 100% Recovery | 164064 | 164458 | 100.24 |
| 3 | 150% Recovery | 200394 | 200151 | 99.87 |

Limit: IP=98-102, USP=98-105

**Table 5: Accuracy study for Guaiphenesin**

| Sr no | Conc. | Area | Standard Deviation | | %SD | %RSD |
|---|---|---|---|---|---|---|
| | | | Mean | SD | | |
| 1 | 40 | 1886032 | 1861771 | 21014.16 | 1.1287189 | 1.1287189 |
| | 40 | 1849256 | | | | |
| | 40 | 1850025 | | | | |
| 2 | 120 | 5466224 | 5466433.3 | 5051.2542 | 0.0924049 | 0.0924049 |
| | 120 | 5461490 | | | | |
| | 120 | 5471586 | | | | |
| 3 | 200 | 9568147 | 9558988.3 | 30382.579 | 0.317843 | 0.317843 |
| | 200 | 9525080 | | | | |
| | 200 | 9583738 | | | | |

**Table 6: % Recovery study of Guaiphenesin**

| Sr. no. | % Composition | Area of Standard | Area of Sample | % Recovery |
|---|---|---|---|---|
| 1 | **50% Recovery** | 5466224 | 5463476 | 99.94 |
| 2 | **100% Recovery** | 7644198 | 7627347 | 99.77 |
| 3 | **150% Recovery** | 9568147 | 9564752 | 99.96 |

Limit: IP=98-102, USP=98-105

## Precision

Precision was measured by the analysis of sample solutions in triplicates, to check the intraday and inter day variations of the method. The results are furnished in Tables 7 and 8.

**Table 7: Precision of Terbutaline by the proposed methods**

| Intraday Morning Conc (µg/ml) | Area | Intraday Evening Conc (µg/ml) | Area |
|---|---|---|---|
| 2 | 102090 | 2 | 102075 |
| 2 | 102098 | 2 | 102060 |
| 2 | 102033 | 2 | 102061 |
| Mean= 102236.2 | | | |
| %RSD= 0.38% | | | |

Limit: %RSD value should be less than 2%

| Interday Day 1 Conc (µg/ml) | Area | Interday Day 2 Conc (µg/ml) | Area |
|---|---|---|---|
| 2 | 103045 | 2 | 101996 |
| 2 | 102096 | 2 | 10196 |
| 2 | 102081 | 2 | 101998 |
| Mean= 101998 | | | |
| %RSD= 0.41% | | | |

Limit: %RSD value should be less than 2%

**Table 8: Precision of Guaiphenesin by the proposed methods**

| Intraday Morning Conc (µg/ml) | Area | Intraday Evening Conc (µg/ml) | Area |
|---|---|---|---|
| 80 | 1886035 | 80 | 1886029 |
| 80 | 1886039 | 80 | 1886047 |
| 80 | 1886152 | 80 | 1886028 |
| Mean=1886055 | | | |
| %RSD= 1.2% | | | |

Limit: %RSD value should be less than 2%

| Interday Day1 Conc (µg/ml) | Area | Interday Day2 Conc (µg/ml) | Area |
|---|---|---|---|
| 80 | 1886039 | 80 | 1886031 |
| 80 | 1188609 | 80 | 1886065 |
| 80 | 1886057 | 80 | 1886029 |
| Mean= 1886029 | | | |
| %RSD= 0.98% | | | |

Limit: %RSD value should be less than 2%

**Robustness**

The Robustness of the method was evaluated by deliberately varying the Chromatographic conditions of the method such as flow rate, and wavelength and effect was seen on parameters like Tailing Factor and Retention Times. They showed adherence to the limits represent method was Robust. The result of Robustness study discussed in (Table 9).

**Table 9: Robustness study of Terbutaline and Guaiphenesin**

| Chromatographic changes | | | | Chromatographic changes | | | |
|---|---|---|---|---|---|---|---|
| **Factor** | **Level** | **Assay %** | **%Deviation** | **Factor** | **Level** | **Assay** | **% Deviation** |
| **Flow Rate (ml/min)** | | | | **Flow Rate (ml/min)** | | | |
| 0.6 | -2 | 99.23 | 0.06 | 0.6 | -0.2 | 100.21 | 0.08 |
| 0.8 | 0 | 99.15 (USP limit=98-102%) | 0 | 0.8 | 0 | 99.2 (USP limit is= 92.5-107.5%) | 0 |
| 1 | 2 | 100.5 | 0.5 | 1 | 0.2 | 99.89 | 0.7 |
| % Deviation at flow rate 0.6ml is 0.06 and at 1ml is 0.5% respectively. | | | | % Deviation at flow rate 0.6ml is 0.08 and at 1ml is 0.7% respectively. | | | |
| **Wavelength (nm)** | | | | **Wavelength (nm)** | | | |
| 271.4 | -5 | 99.66 | 0.23 | 269 | -5 | 98.55 | 0.72 |
| 276.4 | 0 | 99.89 | 0 | 274 | 0 | 99.26 | 0 |
| 281.4 | 5 | 98.95 | 0.94 | 279 | 5 | 99.35 | 0.09 |

**Assay**

The average tablet mass was calculated from the mass of 20 Tablets of Bricarex (2.5mg Terbutaline and 100mg of Guaiphenesin) Tablet. They were then finely ground, homogenized. Accurately weighed 48.78 mg powder which contains Terbutaline and Guaiphenesin. The powder was transferred into a 10ml of Methanol. The mixture was sonicated for atleast 20min to aid dissolution and then filtered through a whattman no.42 paper. This Tablet solution further diluted to obtain 3µg/ml of Terbutaline and 120µg/ml of Guaiaphenesin. The assay results were compiled, found satisfactory and show that there is a no interference of tablet matrix with the drug and the results are summarized in Table 8, and the standard and test chromatograms are given in Fig. 6 and 7. Low % RSD shows that this method can be easily applied for the estimation of TER and GUAI in bulk drug and in a Tablet.

**Table 10: Assay of Tablet solution**

| Tablet Mixture | Drug | Label claim mg/tablet | Amount added | Conc. estimated (mg) | Mean conc. Estimated (mg) | % Assay (w/w) | % RSD |
|---|---|---|---|---|---|---|---|
| TER+GUAI | TER | 2.5 | 2.5 | 2.53 | 2.48 | 99.15 | 0.1 |
| | | | | 2.54 | | | |
| | | | | 2.59 | | | |
| | | | | 2.45 | | | |
| | | | | 2.32 | | | |
| | GUAI | 100 | 100 | 100.12 | 99.91 | 99.2 | 0.27 |
| | | | | 100.25 | | | |
| | | | | 99.58 | | | |
| | | | | 99.87 | | | |
| | | | | 99.73 | | | |

% Assay of TER or GUAI = TER or GUAI Conc estimated (mg)/TER or GUAI input (mg) X 100; % RSD = SD/mean X 100

## RESULTS AND DISCUSSION

A simple, Accurate and most versatile RP-HPLC method for simultaneous estimation of Terbutaline and Guaiphenesin was successfully developed in bulk as well as in Tablet dosage form. The mobile phase used was Methanol: Water (60:40) with Hexon C18 (250mm x 4.6ID, Particle size: 5 micron) column in Gradient mode. The flow rate was 0.8 mL/min at 30°C with UV-detection at 275 nm. Linearity was assessed by plotting concentration versus area, which is shown in Table 1 and 2, and it is linear in the range of 1- 5µg / mL for Terbutaline and 40-200µg / mL for Guaiphenesin, with correlation coefficients of 0.994 and 0.998 respectively, with a good linearity response. The % recovery was found to be within limits of the acceptance criteria with a recovery range of 99.82 - 100.24% for Terbutaline and 99.77 – 99.94% for Guaiphenesin. The detection limit of the proposed method was 0.610µg/mL and 0.849µg/mL and the quantification limit was 1.87µg/mL and 2.4µg/mL for Terbutaline and Guaiphenesin respectively. A typical chromatogram of the Terbutaline and Guaiphenesin standard solution of at the

test level is shown in Fig 8. The assay procedures were repeated five times and the results were found to give 99.15% of TER and 99.20% of GUAI as shown in Table 11.

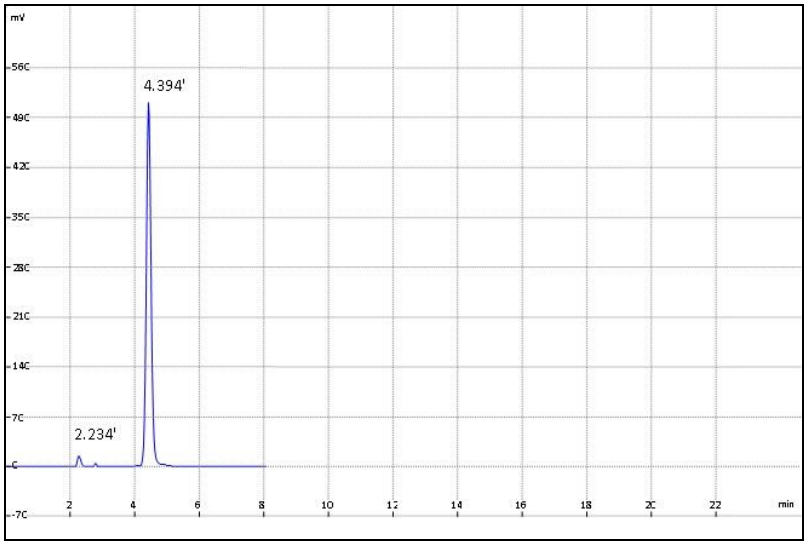


**Figure 6: Chromatogram of the standard preparation of Terbutaline and Guaiphenesin**

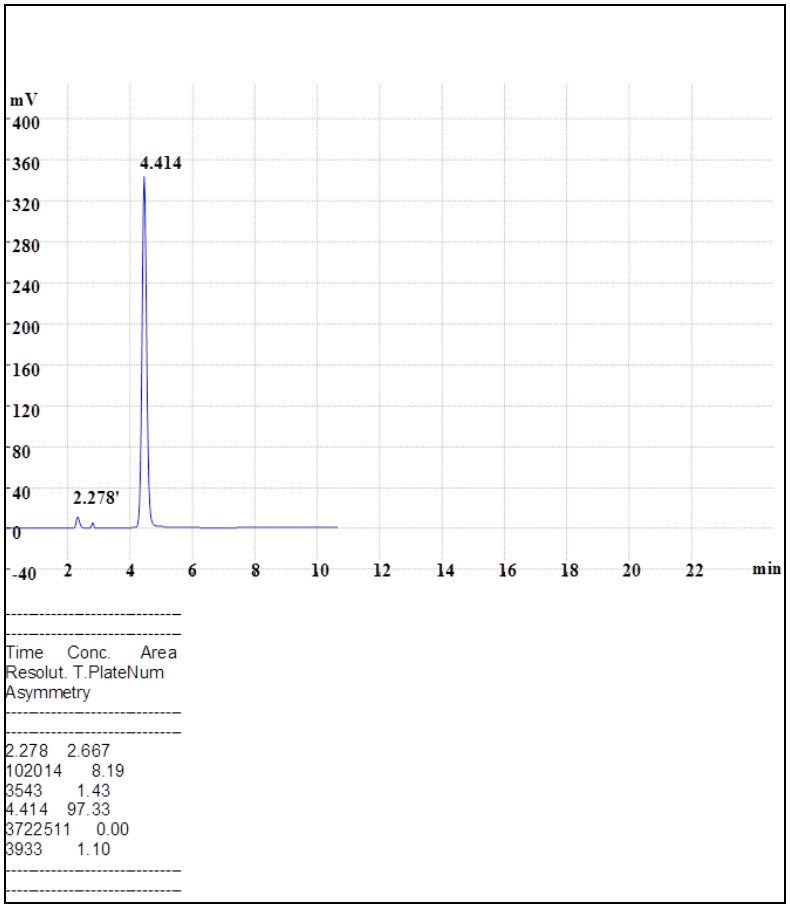


**Figure 7: Chromatogram of the test sample of Terbutaline and Guaiphenesin**

**Table 11: Validation parameters of the HPLC method of Terbutaline and Guiphenesin**

| Method Characteristics | Terbutaline | Guaiphenesin |
|---|---|---|
| Linearity | 1-5µg/ml | 40-200µg/ml |
| Regression equation | Y= 27714 x + 51683 | Y= 48215 x -12835 |
| Correlation coefficient | 0.994 | 0.998 |
| Retention Time(min) | 2.278 | 4.414 |
| Therotical plates | 3543 | 3933 |
| Tailing factor | 1.43 | 1.1 |
| LOD(µg/ml) | 0.61 | 0.849 |
| LOQ (µg/ml) | 1.87 | 2.4 |
| Accuracy(% RSD) | | |
| 50% | 99.82 | 99.94 |
| 100% | 100.24 | 99.77 |
| 150% | 99.87 | 99.96 |
| Precision(% RSD) | | |
| Intraday (n=3) | 0.38% | 1.20% |
| Interday (n=3) | 0.41% | 0.98% |
| Robustness | Robust | |

## CONCLUSION

The proposed study describes a new and simple RP-HPLC method for the estimation of Terbutaline and Guaiphenesin in bulk drug and in a Tablet. The Method is accurate, precise, rapid and sensitive. It can moreover gives the shorter duration of analysis for Terbutaline and Guaiphenesin makes these be easily and conveniently used for routine quality control analysis, particularly when large numbers of samples are encountered. The $R_t$ of TER and GUAI was 2.278 and 4.414 mins respectively. The developed method was found to be specific as there was no interference of the excipients, which is confirmed by the absence of extra peaks.

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