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## Analytical method development and validation for the simultaneous estimation of Terbutaline sulphate and Guaiphenesin in tablet dosage form by RP-HPLC

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### ABSTRACT

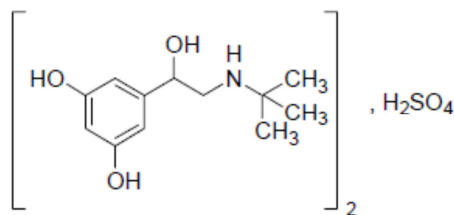
A simple, rapid, accurate and precise reverse phase-high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous determination of Terbutaline sulphate and Guaiphenesin in combined dosage form. Separation was performed using Phenomex C18 column (250\*4.5 mm, 0.5  $\mu$ ) on LC-20 AD Prominence Liquid chromatograph (Shimadzu, Japan) attached with Spd-20A/20AV Prominence SPD-20A prominence UV/Vis detector. By using mobile phase 0.02 M Potassium dihydrogen orthophosphate (pH 3.0) and Acetonitrile (40:60 V/V) isocratic elution using a flow rate of 1 mL/min. Good sensitivity was observed with UV detection at 215 nm. Retention times of Terbutaline sulphate and Guaiphenesin were found to be about 2.1 and 2.8 min, respectively. The method was validated over the range from 1.25-15  $\mu$ g/mL for Terbutaline sulphate and 12.5-150  $\mu$ g/mL for Guaiphenesin with correlation coefficients of 0.998 and 0.998, respectively. This method was shown to be accurate, specific, robust, linear, and repeatable and can be successfully employed in routine quality control for the simultaneous analysis of Terbutaline sulphate and Guaiphenesin in tablets.

**Keywords:** Terbutaline sulphate, Guaiphenesin, RP-HPLC, Tablet dosage form.

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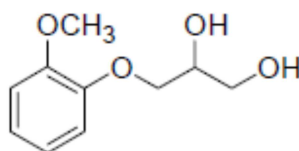
### INTRODUCTION

Terbutaline sulphate is a selective beta-2 adrenergic agonist (1). It is chemically known as (RS)-2-(tert-butylamino)-1-(3,5-dihydroxyphenyl)ethanol sulphate (Fig 1) (2). It helps in the relaxation of the smooth muscle found principally in bronchial, vascular and uterine tissue; wheezing and shortness of breath, troubled breathing caused by asthma, chronic bronchitis, emphysema and other lung diseases. Terbutaline has little effect on  $\beta_1$  receptors; thus direct cardiovascular stimulation occurs. Terbutaline use during pregnancy is associated with development of autism in humans (3-6).



**Figure 1: Structure of Terbutaline sulphate**

Guaiphenesin is an expectorant that also has some muscle relaxing action (7). It is chemically known as (RS)-3-(2-methoxyphenoxy) propane-1,2-diol (Fig 2) (8). It is used to reduce chest congestion caused by common cold, infections, or allergies, including medical, veterinary and personal, treatment of primary dysmenorrhea. Guaiphenesin may cause side effects like headache, nausea and vomiting (9-11).



**Figure 2: Structure of Guaiphenesin**

## MATERIAL AND METHODS

### Chemicals and reagents

Terbutaline sulphate was gift sample from Shimoga Life Sciences Pvt. Ltd. and Guaiphenesin was purchased from Yarrow Chem Products.

Potassium dihydrogen orthophosphate (HPLC grade) from LobaChemie Laboratory Reagents and Fine Chemicals; orthophosphoric acid (Analytical grade) from S D Fine Chem Limited; and Acetonitrile (HPLC grade) from Thomas Baker were purchased.

X-PAR tablet (manufactured by Bombay Tablet Mfg. Co. Pvt. Ltd., Mumbai) is a tablet containing Terbutaline Sulphate 2.5 mg and Guaiphenesin 100 mg was commercially purchased from local pharmacy.

### Chromatographic conditions:

The HPLC was performed using Phenomenex C18 column (250\*4.5 mm, 0.5  $\mu$ ) on LC-20 AD Prominence Liquid chromatograph (Shimadzu, Japan) attached with Spd-20A/20AV Prominence SPD-20A prominence UV/Vis detector.

The optimized chromatographic conditions were as follows: volume injected 20  $\mu$ l, flow rate 1.0 ml/min; detector was set at a wavelength of 215 nm. The mobile phase comprises of .02 M Potassium dihydrogen orthophosphate (pH 3.0): acetonitrile (40:60). The retention time of Terbutaline sulphate obtained was about 2.1 min and for Guaiphenesin it was about 2.8 min.

### Preparation of 0.02 M Potassium dihydrogen orthophosphate:

2.7218 g of Potassium dihydrogen orthophosphate dissolved in 1000 ml of water.

### Preparation of Mobile Phase:

Prepared 0.02 M Potassium dihydrogen orthophosphate, adjusted pH to 3 with Orthophosphoric acid (filter) and mixed with Acetonitrile in the ratio of 40:60. Then it is sonicated.

**Standard Preparation:**

**Terbutaline sulphate:** 100 mg of Terbutaline sulphate was weighed accurately in 100 ml volumetric flask. 80 ml of water added into it, sonicated to dissolve and diluted to volume. 1 ml of this solution further diluted to 10 ml with mobile phase. Then 0.25 ml of this solution diluted to 10 ml with mobile phase.

**Guaiphenesin:** 100 mg of Guaiphenesin was weighed accurately in 100 ml volumetric flask. 80 ml of water added into it, sonicated to dissolve and diluted to volume. 1 ml of this solution further diluted to 10 ml with mobile phase.

**METHOD VALIDATION**

The optimized chromatographic method was validated according to the procedure described in ICH guidelines Q2 (R1) for the validation of analytical methods. (12)

**Accuracy:** The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

**Precision:** The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

System precision, method precision and intermediate precision has done.

**Specificity:** Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

**Linearity:** The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

**Range:** The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

**Robustness:** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

**RESULTS AND DISCUSSION****Accuracy:**

Standard drug solution was added to the pre-analysed tablet sample solution at the three different concentration level (80%, 100%, 120%) within the range of linearity for both the drugs.

**Table 1: Accuracy data for Terbutaline sulphate and Guaiphenesin**

Terbutaline sulphate			Guaiphenesin		
% Level	Mean	% RSD	% Level	Mean	% RSD
80	99.87 %	0.31%	80	100.43 %	1.02%
100	101.02 %	0.06%	100	100.52 %	0.16%
120	101.18 %	0.15%	120	101.50 %	0.72%

**Acceptance criteria:** Accuracy should be between 98-102% and % RSD should not be more than 2.0.

**Precision:**

**System precision:** % RSD for Terbutaline sulphate and Guaiphenesin was found to be 0.72 % and 0.33 % respectively.

**Method precision:** % RSD for Terbutaline sulphate and Guaiphenesin was found to be 0.65 % and 0.31 % respectively.

**Intermediate precision or ruggedness:** % RSD for Terbutaline sulphate and Guaiphenesin was found to be 0.34 % and 0.41 % respectively.

**Acceptance criteria:** % RSD should not be more than 2.0.

**Specificity:**

This was evaluated by injecting the blank (mobile phase), standard and sample solution prepared as per the proposed method and injected into the HPLC system to check interference if any at the retention time of Terbutaline sulphate and Guaiphenesin.

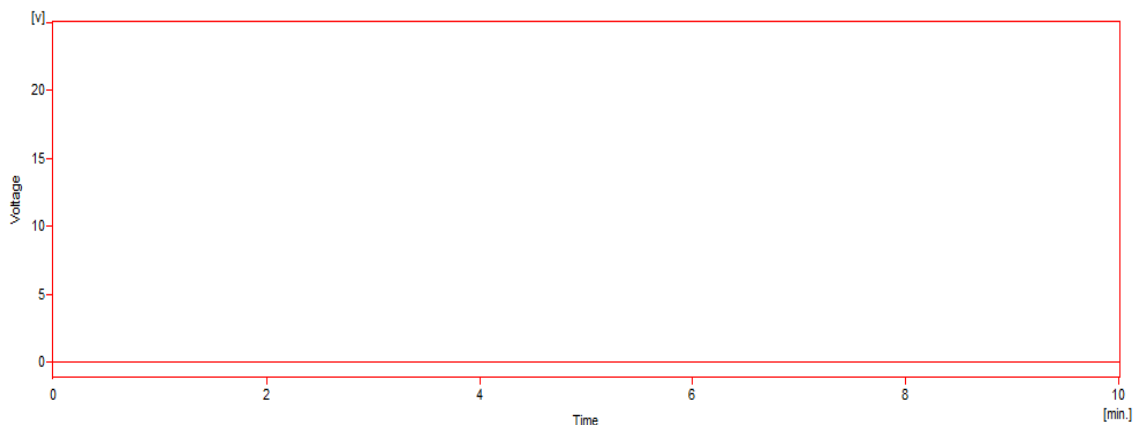


Figure 3: Chromatogram of blank

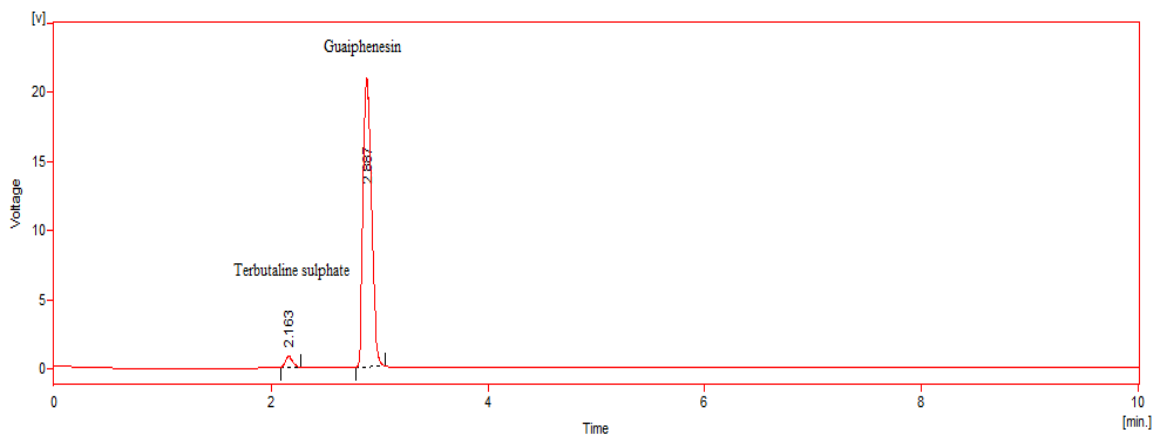


Figure 4: Chromatogram of standard solution

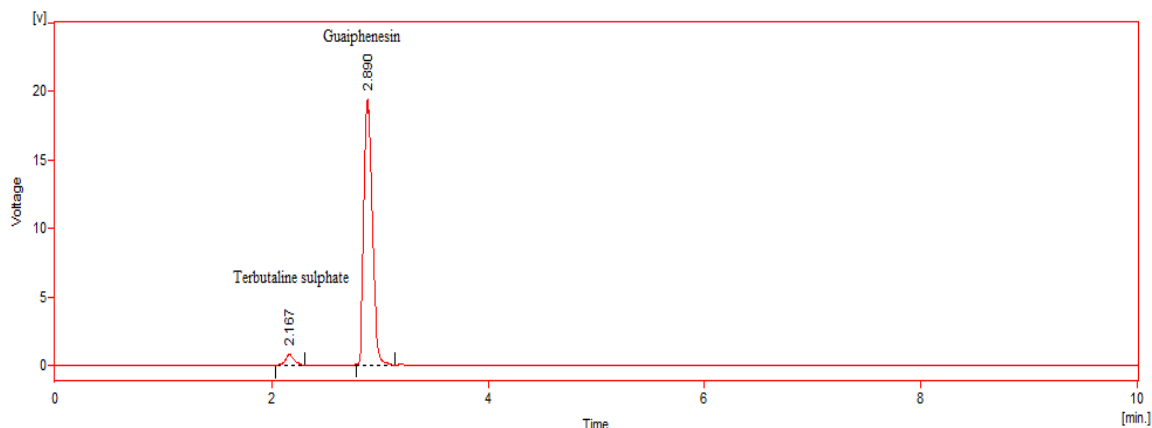


Figure 5: Chromatogram of sample solution

**Acceptance criteria:** No peaks shall be eluted at the retention time of Terbutaline sulphate and Guaiphenesin in blank.

**Linearity:**

Table 2: Linearity data for Terbutaline sulphate and Guaiphenesin

Concentration of Terbutaline sulphate in µg/ml	Peak areas	Concentration of Guaiphenesin in µg/ml	Peak areas
1.25	41.121	12.5	225.811
2.5	78.409	25	492.241
5.0	148.675	50	991.312
7.5	240.324	75	1452.193
10	305.296	100	1855.974
12.5	397.113	125	2437.12
15.0	484.251	150	2915.79
<b>Slope</b>	<b>32.091</b>	<b>Slope</b>	<b>19.349</b>
<b>Intercept</b>	<b>4.245</b>	<b>Intercept</b>	<b>4.203</b>
<b>R<sup>2</sup></b>	<b>0.9982</b>	<b>R<sup>2</sup></b>	<b>0.9987</b>

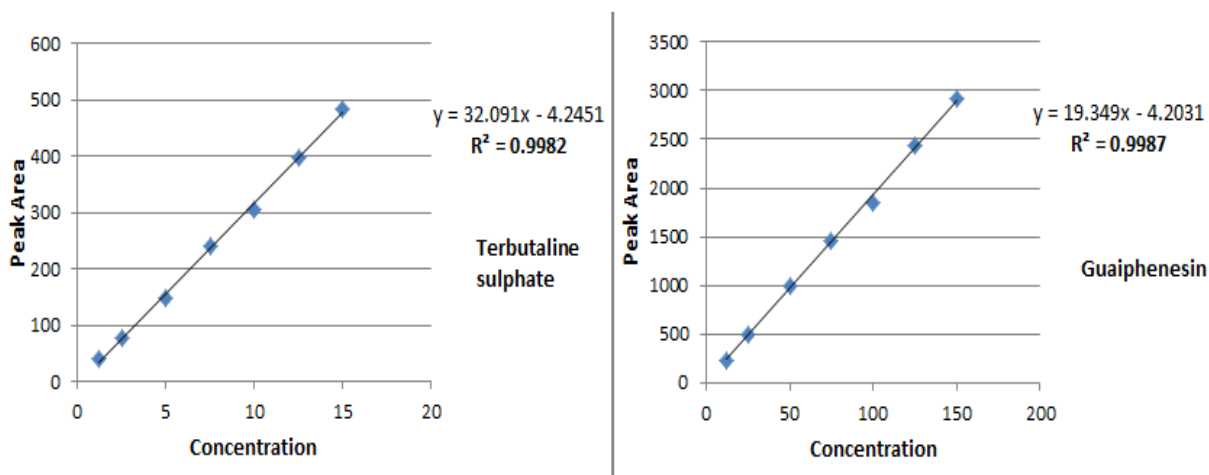


Figure 6: Calibration curve of Terbutaline sulphate and Guaiphenesin

**Acceptance criteria:** Correlation coefficient ( $R^2$ ) should be not less than 0.99.

**Range:**

Range inferred from the data of linearity, recovery and precision experiments.

**Acceptance criteria:** The range of the method based on the results from the linearity, accuracy and precision studies.

**Robustness:**

Robustness of the method was evaluated by changing the flow rate by  $\pm 10\%$  and by changing the organic content by  $\pm 2\%$  absolute.

**Table 3: Robustness data for Terbutaline Sulphate**

Sr. No.	Parameters	% Assay			Over all mean	Over all SD	Over all %RSD
		1	2	3			
I	Plus flow (1.1 ml/min)	99.95	98.35	98.55	98.95	0.872	0.88
II	Minus flow (0.9 ml/min)	98.59	98.19	98.25	98.34	0.216	0.219
III	Plus Organic (+2%)	99.32	98.92	98.37	98.87	0.477	0.482
IV	Minus Organic (-2%)	99.30	99.44	99.29	99.34	0.084	0.084

**Table 4: Robustness data for Guaiphenesin**

Sr. No.	Parameters	% Assay			Over all mean	Over all SD	Over all %RSD
		1	2	3			
I	Plus flow (1.1 ml/min)	101.12	100.89	101.06	101.02	0.119	0.118
II	Minus flow (0.9 ml/min)	100.30	100.67	100.72	100.56	0.229	0.228
III	Plus Organic (+2%)	100.96	101.17	100.93	101.02	0.131	0.129
IV	Minus Organic (-2%)	100.53	100.22	101.01	100.59	0.398	0.396

**Acceptance criteria:** % RSD should be not more than 2.0.

### CONCLUSION

A rapid, simple, reproducible and specific RP-HPLC method was developed for simultaneous estimation of Terbutaline sulphate and Guaiphenesin in tablet dosage form.

The developed method was validated as per ICH guidelines for linearity, precision, specificity, accuracy, ruggedness and robustness, and was found to be linear, accurate, precise and repeatable.

The developed method could be successfully used to analyze the drugs in marketed formulation and also the routine analysis of Terbutaline sulphate and Guaiphenesin.

### Acknowledgement

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