ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Analytical Method Validation Report

(Terbutaline Sulphate, Bromhexine Hydrochloride and Guaiphenesin Syrup)

Quality Control Department

This document is an exercise on Analytical Method Validation of the various analytical methods used in determination of active ingredients in Quality Control Laboratory**”**.

PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

CONTRIBUTIONS:

This protocol is a team effort of Quality control Laboratory chemists to achieve the objective of validating the analytical methods carried out to estimate the contents of pharmaceutical products manufactured by**:** …….

Analytical Method Validation Protocol Number			
Validation Frequency	Analytical Methods should be validated during and at the end of development process and after any significant change in analytical method.		
	Designation	**Name of the Person**	**Sign /Date**
Prepared By	Officer QC		
Checked By	Manager QC		
Reviewed By	Manager QA		
Approved By	Operation Head		

What is Validation?

Validation is the evaluating of processes, products or analytical methods to ensure compliance with product or method requirements. One of the most popular definitions of Validation came from the 'US FDA' General Principle of Validation **"Establishing documented evidence which provides a high Degree of assurance that a specific process will consistently produce a product meeting its Predetermined specifications and quality attributes."**

The term Validation & Qualification are often mixed up and there is also some overlap. Equipment Qualification means checking an instrument for compliance with previously defined functional and performance specifications. For Operational Qualification generic standards and analytical conditions are used rather than real sample conditions. Validation relates more to the entire but sample specific process including sample preparation, analysis, and data evaluation.

Validation efforts in the analytical laboratory should be broken down into separate components addressing the equipment and the analytical methods run on that equipment. After these have been verified separately they should be checked together to confirm expected performance limits **(System Suitability Testing),** and finally the sample analysis data collected on such a system should be authenticated with suitable validation checkouts. All methods / equipment that are used to create, modify, maintain, archive or distribute critical data for cGMP/GLP.
Analytical method should be validated prior to routine use and after changing method parameters.
Peoples involved in Validation exercise should be qualified for their jobs. This includes education, training and/or experience.
Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Typical analytical performances characteristics that should be considered in the validation of the types of methods are as follows.

- **Accuracy**
- **Precision**
- **Specificity**
- **Detection Limit**
- **Quantitation Limit**
- **Linearity**
- **Range**

USP 30 in "(1225) Validation of compendial procedures" says Category I (Analytical methods for Quantization of major components of bulk drug substances or active ingredients including preservative in finished pharmaceutical products) should comply with **Accuracy, Precision, Specificity, Linearity, Robustness, & Range.**

However after discussions with many experts & referring some of the IDAM – APA magazines, we have decided to at least comply with **Accuracy,, Linearity, Precision, Robustness .**

Validation Report

Once the method has been validated, a validation report should be prepared that includes.

- Objective & scope of the method (applicability, type).
- Summary of the methodology.
- Type of compound & matrix.
- All chemical, reagents, reference standards, detailed instruction on their preparation.
- Method parameters.
- Detailed condition on how the experiments were conducted including sample preparation. The report must be detailed enough to ensure that it can be reproduced by a competent technician with comparable equipment.
- Statistical procedures & representative calculations.
- Representative plots
- Performance data for acceptance limit
- Criteria for revalidation
- Summary & conclusions
- Approval with name, designations, date & signatures of those responsible for the review & approval of the analytical test procedure.

Validation Report For Terbutaline Sulphate, Bromhexine Hydrochloride and Guaiphenesin Syrup

OBJECTIVE: The efficacy & safety of a medicinal product can only be assured by analytical monitoring of its quality.

SCOPE: The scope of analytical validation is to ensure that the procedure under consideration is capable of giving reproducible and reliable results.

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Product Name Terbutaline Sulphate, Bromhexine Hydrochloride and Guaiphenesin Syrup

Ingredient Terbutaline Sulphate, Bromhexine Hydrochloride and Guaiphenesin

Label Claim Each 5ml contains:
Terbutaline Sulphate IP……..1.25 mg
Bromhexine Hydrochloride IP……..4.0 mg
Guaiphenesin IP……...50 mg

(A)Test Method UV-Spectrophotometer.
Bromhexine HCl IP

Specificity (**Diluents Interference**)

Placebo Preparation: A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here, the product contains no inactive ingredients. So, here the mobile phase is used as the placebo solution. Absorbance at 525 nm, Observation Result: Nil

Conclusion for Specificity: We observed that at wavelength 525 nm there is no significant Absorbance for placebo (Diluent) for Bromhexine hydrochloride assay method. Therefore specificity of the method considered acceptable.

Bromhexine Hydrochloride:-

System Accuracy

The system precision of the above method was carried out by taking Absorbance for six times of the sample preparation.

Serial No.	Absorbance of Bromhexine hydrochloride
1.	0.485
2.	0.482
3.	0.483
4.	0.484
5.	0.485
6.	0.483
Mean	0.484
RSD	**0.269%**

Acceptance Criteria: RSD is not more than 2.0%.

Linearity/ Accuracy:

Definition:

The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Range:

Definition:

The Range of an analytical method is the interval between the upper & lower level of analyte that have been demonstrated with precision, accuracy & linearity using the method as written. The Range

is normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay:

Limit: Bromhexine hydrochloride IP

Label Claim 4.0mg / 5ml

(Limit: 90.0 % to 110.0 % of the labeled amount).

Reagents:

1) Methanol
2) 5 M HCl
3) 2 %w/v solution of Sodium Nitrite.
4) 5 %w/v solution of Ammonium Sulphamate.
5) 0.1%w/v solution of NED Dye.

Standard Solution:

Weigh accurately to 80.1 mg of Bromhexine HCl working standard into 100 ml V.F. add 50 ml of Methanol, sonicate for dissolving and make up to mark with same solvent. Filter, and dilute 5 ml into 50 ml with 5 M HCl.

Sample Preparation:

Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter

Procedure:

Take 5 ml of filtrate of standard And sample in 50 ml volumetric flask, add 1 ml of 2 %w/v solution of sodium nitrite, add 1 ml of 5 M HCl solution, add 1 ml of 5 %w/v solution of ammonium sulphamate after 3 minutes add 2 ml of NED Dye solution.

Take the absorbance at 525 nm of both solution using Regent blank and calculate the result by comparison.

$$\frac{\text{Sample Abs}}{\text{Standard Abs}} \times \frac{\text{Std. Wt.}}{100} \times \frac{5.0}{50} \times \frac{50}{5.0} \times \frac{\text{Potency}}{100} \times \frac{5.0}{\text{claim}} \times 100 = \%$$

Sr No	Standards	Absorbance
	Standard-1	0.486
	Standard-2	0.486
	Standard-3	0.486
	Standard-4	0.486
	Standard-5	0.485
	Standard-6	0.486
	Mean	0.486
	RSD	**0.092%**

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Samples	Sample absorbance	Mean
Sample-A-01 56 mcg	0.341	
Sample-A-02 56 mcg	0.341	0.341
Sample-A-03 56 mcg	0.341	
Sample-B-01 64 mcg	0.388	
Sample-B-02 64 mcg	0.388	0.388
Sample-B-03 64 mcg	0.388	
Sample-C-03 72 mcg	0.437	
Sample-C-02 72 mcg	0.437	0.437
Sample-C-03 72 mcg	0.437	
Sample-D-01 80 mcg	0.484	
Sample-D-02 80 mcg	0.484	0.484
Sample-D-03 80 mcg	0.485	

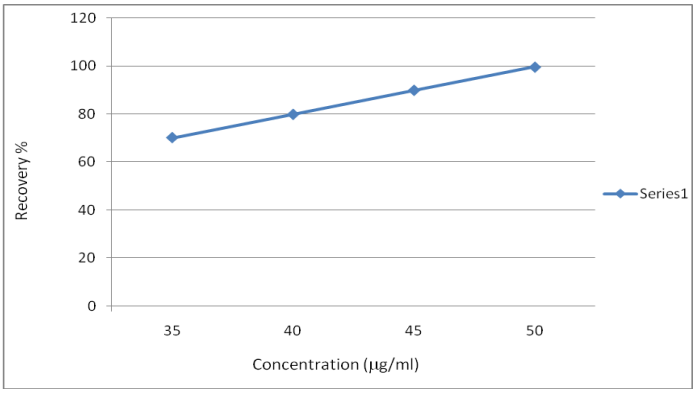
Data Collection:

Concentration (µg/ml)	Concentration in %	Sample Mean Abs	Recovery%
35	70%	0.341	70.10%
40	80%	0.388	79.83%
45	90%	0.437	89.91%
50	100%	0.484	99.60%

From the above results, draw a curve.

Linearity plot for Bromhexine HCl

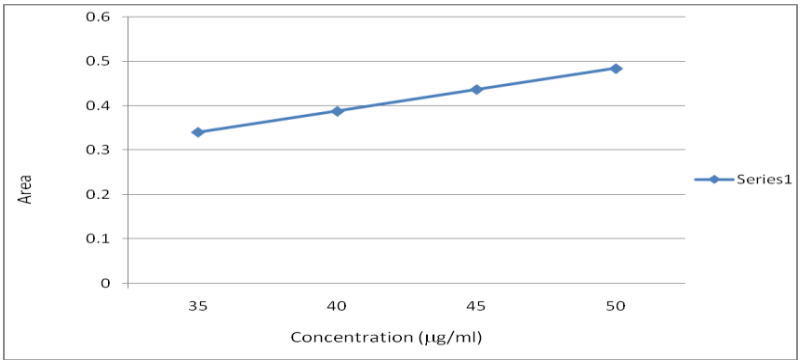
Concentration (µg/ml)	Recovery %
35	70.10
40	79.83
45	89.91
50	99.60



ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Linearity plot for Bromhexine HCl

Concentration (µg/ml)	Absorbance
35	0.341
40	0.388
45	0.437
50	0.484



R-squared value (R^2)

The R-squared value, also known as the coefficient of determination, is an indicator that ranges in value from 0 to 1 and reveals how closely the estimated values for the trend line correspond to your actual data. A trend line is most reliable when its R-squared value is at or near 1.

Linearity Equation

Equations for calculating trend line

Calculates the least squares fit for a line represented by the following equation:

y = m x + b

Where m is the slope and b is the intercept.

x = concentration

y = Absorbance Value

Sample

Therefore, from Linearity Equation, **y = mx + b, m** ⟶ 0.999x

b ⟶ 0.163

We can arrive sample concentration from the above equation is 100 mcg

$$\frac{\text{Sample Abs}}{\text{Standard Abs}} \times \frac{\text{Std. Wt.}}{100} \times \frac{5.0}{50} \times \frac{\text{Potency}}{5.0} \times \frac{5.0}{100} \times \frac{100}{\text{claim}}$$

= %

Conclusion for Linearity:

The graphical representation & data collected during this exercise proves Bromhexine HCl for demonstrate linearity in the range of 70% to 100% when determined by UV- method.

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

Precision – Method precision

Limit: Bromhexine hydrochloride

Label Claim 4.0 mg / 5.0ml
(Limit: 90.0 % to 110.0 % of the labeled amount).

Reagents:

1) Methanol
2) 5 M HCl
3) 2 %w/v solution of Sodium Nitrite.
4) 5 %w/v solution of Ammonium Sulphamate.
5) 0.1%w/v solution of NED Dye.

Standard Solution:

Weigh accurately to 80.1 mg of Bromhexine HCl working standard into 100 ml V.F. add 50 ml of Methanol, sonicate for dissolving and make up to mark with same solvent. Filter, and dilute 5 ml into 50 ml with 5 M HCl.

Sample Preparation:

Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

Procedure:

Take 5 ml of filtrate of standard And sample in 50 ml volumetric flask, add 1 ml of 2 %w/v solution of sodium nitrite, add 1 ml of 5 M HCl solution, add 1 ml of 5 %w/v solution of ammonium sulphamate after 3 minutes add 2 ml of NED Dye solution.
Take the absorbance at 525 nm of both solution using Regent blank and calculate the result by comparison.

$$\frac{\text{Sample Abs}}{\text{Standard Abs}} \times \frac{\text{Std. Wt.}}{100} \times \frac{5.0}{50} \times \frac{50}{5.0} \times \frac{\text{Potency}}{100} \times \frac{5.0}{\text{claim}} \times 100 = \%$$

Sample Dilutions:

By ……..

(A) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

(B) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

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(C) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.
(D) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.
(E) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.
(F) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

Procedure:
Take 5 ml of filtrate of standard And sample in 50 ml volumetric flask, add 1 ml of 2 %w/v solution of sodium nitrite, add 1 ml of 5 M HCl solution, add 1 ml of 5 %w/v solution of ammonium sulphamate after 3 minutes add 2 ml of NED Dye solution.
Take the absorbance at 525 nm of both solution using Regent blank and calculate the result by comparison.

Test Data Collection:

Standards	Absorbance
Standard 1	0.486
Standard 2	0.486
Standard 3	0.486
Standard 4	0.486
Standard 5	0.485
Standard 6	0.486
Mean	0.486
RSD	**0.092%**

Samples		Sample Absorbance	Mean
Sample A	T1	0.485	0.485
	T2	0.485	
Sample B	T1	0.484	0.484
	T2	0.484	
Sample C	T1	0.485	0.485
	T2	0.485	
Sample D	T1	0.486	0.486
	T2	0.486	
Sample E	T1	0485	0.485
	T2	0.485	
Sample F	T1	0.485	0.485
	T2	0.485	

Estimated Amount of Bromhexine Hydrochloride:-

- Assay on % of Theory for sample A---- 99.79%
- Assay on % of Theory for sample B---- 99.60%
- Assay on % of Theory for sample C-----99.56%
- Assay on % of Theory for sample D-----99.85%
- Assay on % of Theory for sample E-----99.56%

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- Assay on % of Theory for sample F-----99.56%

Table for Six Replicate Assays

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.79%	99.67%	0.138%
Sample B	99.60%		
Sample C	99.56%		
Sample D	99.85%		
Sample E	99.56%		
Sample F	99.56%		

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Conclusion for precision: The overall % Relative standard deviation for Bromhexine hydrochloride there is no significant difference. Therefore Repeatability of the method considered acceptable as it well within 2 % Relative Standard Deviation.

Intermediate Precision – (Within laboratory variations such as different days, analyst & equipments):

Analyst: "……..."

Standard Solution:

Weigh accurately to 80.4 mg of Bromhexine HCl working standard into 100 ml V.F. add 50 ml of Methanol, sonicate for dissolving and make up to mark with same solvent. Filter, and dilute 5 ml into 50 ml with 5 M HCl.

Sample Dilutions:

Analyst: "……."

(A) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

(B) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

(C) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

(D) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

(E) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

(F) Take 5 ml of sample into 50 ml volumetric flask, add 5 ml of methanol , sonicate for dissolving and make up to mark with 5 M HCl and Filter.

Procedure:

Take 5 ml of filtrate of standard And sample in 50 ml volumetric flask, add 1 ml of 2 %w/v solution of sodium nitrite, add 1 ml of 5 M HCl solution, add 1 ml of 5 %w/v solution of ammonium sulphamate after 3 minutes add 2 ml of NED Dye solution.

Take the absorbance at 525 nm of both solution using Regent blank and calculate the result by comparison.

Test Data Collection:

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Standards	Absorbance
Standard 1	0.487
Standard 2	0.487
Standard 3	0.487
Standard 4	0.486
Standard 5	0.487
Standard 6	0.487
Mean	0.487
RSD	0.091%

Samples		Sample Absorbance	Mean
Sample A	T1	0.486	0.486
	T2	0.486	
Sample B	T1	0.485	0.485
	T2	0.485	
Sample C	T1	0.485	0.485
	T2	0.485	
Sample D	T1	0.485	0.485
	T2	0.485	
Sample E	T1	0.485	0.485
	T2	0.485	
Sample F	T1	0.485	0.485
	T2	0.485	

Calculation:
Bromhexine hydrochloride Content

$$\frac{\text{Sample Abs} \times \text{Std. Wt.} \times 5.0 \times 50 \times \text{Potency} \times 5.0 \times 100}{\text{Standard Abs} \times 100 \times 50 \times 5.0 \times 100 \times \text{claim}} = \ \%$$

Estimated Amount analyst by "……."

- Assay on % of Theory for sample A ----99.80%
- Assay on % of Theory for sample B ----99.58%
- Assay on % of Theory for sample C ----99.58%
- Assay on % of Theory for sample D ----99.58%
- Assay on % of Theory for sample E ----99.58%
- Assay on % of Theory for sample F -----99.58%

Relative standard deviation of two different analysts and days:
Test Data analyst by "……."

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Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.80%	99.62%	0.098%
Sample B	99.58%		
Sample C	99.58%		
Sample D	99.58%		
Sample E	99.58%		
Sample F	99.58%		

Test Data analyst by "……."

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.79%	99.67%	0.138%
Sample B	99.60%		
Sample C	99.56%		
Sample D	99.85%		
Sample E	99.56%		
Sample F	99.56%		

Acceptance Criteria: NMT 2 % (% of Relative Standard Deviation).

Conclusion for Intermediate Precision:

The overall % Relative standard deviation of two different analysts are 0.098% & 0.138% Bromhexine hydrochloride there is no significant difference between two analysts Within laboratory variations such as different days, analyst & equipments.
Therefore reproducibility of the method considered to be acceptable.

CONCLUSION:

All the analytical parameter are checked as per the approved validation process and found well within specified acceptance criteria.Hence,It is concluded that ,this method is suitable for accurate & precise results for routine analysis.

(B) Test Method By Liquid Chromatography.
Terbutaline Sulphate and Guaiphenesin

Specificity (**Diluents Interference**)

Placebo Preparation: A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here, the product contains no inactive ingredients. So, here the mobile phase is used as the placebo solution. Area at 276nm, Observation Result: Nil

Conclusion for Specificity: We observed that at wavelength 220nm there is no significant area for placebo (Diluent) for Terbutaline Sulphate and Guaiphenesin syrup assay method. Therefore specificity of the method considered acceptable.

System Accuracy

The system precision of the above method was carried out by taking area for six times of the sample preparation of exact weight.

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Serial No.	Area of Terbutaline Sulphate	Area of Guaiphenesin
1.	41264.1	26131.5
2.	41198.6	26198.6
3.	41231.8	26228.1
4.	41512.7	26201.7
5.	41438.3	26307.8
6.	41309.5	26179.9
Mean	41325.83	26207.93
% RSD	0.2997	0.223363

Acceptance Criteria: RSD is not more than 2.0%

Linearity/ Accuracy:

Definition:

The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Range:

Definition:

The Range of an analytical method is the interval between the upper & lower level of analyte that have been demonstrated with precision, accuracy & linearity using the method as written. The Range is normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay:

Limit: Terbutaline Sulphate and Guaiphenesin Syrup

(Limit: 90.0 % to 110.0 % of the labeled amount of both).

Chromatographic Condition:-

Wavelength	**:** 276 nm
Column	**:** 4.6 mm x 25 cm 5µm C8
Flow Rate	**:** 1.0 ml/minute
Injection Volume	**:** 20µl

Mobile Phase:

Take 1.4 ml of Orthophosphoric acid in to 1000 ml volumetric flask and makeup to mark with water and mix Well. Take 750 ml of this solution and add 250 ml of Acetonitrile mix well. Then filter and after degassing Use for analysis.

Standard preparation:

Weigh to 31.5 mg. of Terbutaline Sulphate add 10 ml of mobile phase, sonicate to dissolve, dilute to 25 ml with Mobile phase. Take 1 ml in 25 ml V.F. add 50.2 mg of Guaiphenesin dissolve and makeup with mobile phase.

Sample Preparation

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

Chromatographic system: Inject standard solution. The test is not valid unless the tailing factor is Not more than 2.0 and column efficiency is not less than 2000 theoretical plates and the relative Standard deviation for replicate injection is not more than 2.0 %. Inject the test solution and reference solution

Procedure:
Inject in HPLC and collect the DATA from received chromatogram from HPLC and calculate quantity of Terbutaline Sulphate and Guaiphenesin by the formula given below:-

Terbutaline Sulphate:-

$$\frac{\text{Sample area X Std.Wt. X 1.0 X 25 X Potency X 5.0 X 100}}{\text{Standard area X 25 X 25 X 5.0. X 100 X claim}}$$

= %

Guaiphenesin: -

$$\frac{\text{Sample area X Std.Wt. X 25 X Potency X 5.0 X 100}}{\text{Standard area X 25 X 5.0. X 100 X claim}}$$

= %

S.No.	Standards	Area of Terbutaline Sulphate	Area of Guaiphenesin
1.	Standard-1	41169.7	26101.3
2.	Standard-2	41151.9	26119.9
3.	Standard-3	41119.6	26198.7
4.	Standard-4	41296.5	26121.8
5.	Standard-5	41334.2	26156.5
6.	Standard-6	41139.5	26183.5
	Mean	41201.9	26146.95
	%RSD	0.219	0.149

Acceptance Criteria: RSD is not more than 2.0%

Samples	Sample Area of Terbutaline Sulphate	Mean	Sample Area of Guaiphenesin	Mean
Sample-A-01 70%	28819.3		18345.1	
Sample-A-02 70%	28801.3	28819.97	18398.7	18340.97
Sample-A-03 70%	28839.3		18279.1	
Sample-B-01 80%	32961.5		20921.8	
Sample-B-02 80%	32915.2	32938.8	20861.7	20898.33
Sample-B-03 80%	32939.7		20911.5	
Sample-C-01 90%	37119.8		23531.8	

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Sample-C-02 90%	37079.8	37122.8	23501.1	23510.53
Sample-C-03 90%	37168.8		23498.7	
Sample-D-01 100%	41189.6		26121.8	
Sample-D-02 100 %	41296.5	41273.43	26116.5	26124.0
Sample-D-03 100 %	41334.2		26133.7	

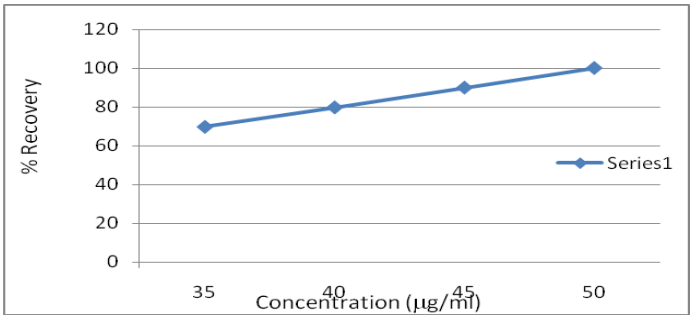
Data Collection:-

Concentration (µg/ml) of Terbutaline Sulphate	Concentration (µg/ml) of Guaiphenesin	Concentration in %	Sample mean area of Terbutaline Sulphate	Sample mean area of Guaiphenesin	Recovery% of Terbutaline Sulphate	Recovery% of Guaiphenesin
35.0	1400	70%	28819.97	18340.97	69.95	70.15
40.0	1600	80%	32938.8	20898.33	79.94	79.93
45.0	1800	90%	37122.8	23510.53	90.10	89.92
50.0	2000	100%	41273.43	26124.0	100.17	99.91

From the above results, draw a curve.

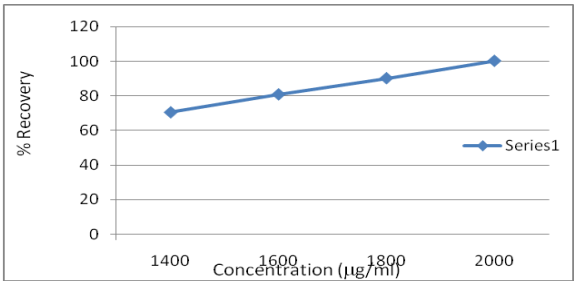
Linearity plot for Terbutaline Sulphate -

Concentration (µg/ml)	Recovery %
35.0	69.95
40.0	79.94
45.0	90.10
50.0	100.17



Linearity plot for Guaiphenesin -

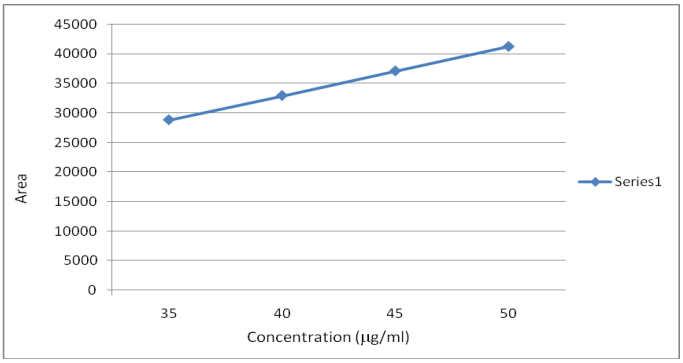
Concentration (µg/ml)	Recovery %
1400	70.15
1600	79.93
1800	89.92
2000	99.91



ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

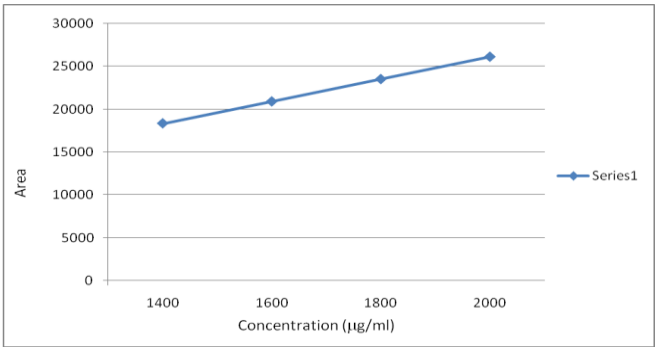
Linearity plot for Terbutaline Sulphate -

Concentration (µg/ml)	Area
35.0	28819.97
40.0	32938.8
45.0	37122.8
50.0	41273.43



Linearity plot for Guaiphenesin -

Concentration (µg/ml)	Area
1400	18340.97
1600	20898.33
1800	23510.53
2000	26124.0



R-squared value ($R^{2)}$

The R-squared value, also known as the coefficient of determination, is an indicator that ranges in value from 0 to 1 and reveals how closely the estimated values for the trend line correspond to your actual data. A trend line is most reliable when its R-squared value is at or near 1.

Linearity Equation

Equations for calculating trend line

Calculates the least squares fit for a line represented by the following equation:

$y = m x + b$

Where m is the slope and b is the intercept.

x = concentration
y = Area Value
Sample

Therefore, from Linearity Equation, **y = mx + b, m** ⟶ 0.999x
b ⟶ 0.163

Terbutaline Sulphate:-

$$\frac{\text{Sample area X Std.Wt. X 1.0 X 25 X Potency X 5.0 X 100}}{\text{Standard area X 25 X 25 X 5.0. X 100 X claim}}$$

= %

Guaiphenesin: -

$$\frac{\text{Sample area X Std.Wt. X 25 X Potency X 5.0 X 100}}{\text{Standard area X 25 X 5.0. X 100 X claim}}$$

= %

Conclusion for Linearity: The graphical representation & data collected during this exercise proves Terbutaline Sulphate and Guaiphenesin Syrup for demonstrate linearity in the range of 70% to 100% when determined by Liquid Chromatographic method.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

Precision – Method precision:

Terbutaline Sulphate and Guaiphenesin Syrup

Label Claim per tablet (Limit: 90.0 % to 110.0 % of the labeled amount of both).

Chromatographic Condition:-

Wavelength	**:** 276 nm
Column	**:** 4.6 mm x 25 cm 5µm C8
Flow Rate	**:** 1.0 ml/minute
Injection Volume	**:** 20µl

Mobile Phase:

Take 1.4 ml of Orthophosphoric acid in to 1000 ml volumetric flask and makeup to mark with water and mix Well. Take 750 ml of this solution and add 250 ml of Acetonitrile mix well. Then filter and after degassing

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Use for analysis.

Standard preparation:
Weigh to 31.5mg. of Terbutaline Sulphate add 10 ml of mobile phase, sonicate to dissolve, dilute to 25 ml with Mobile phase. Take 1 ml in 25 ml V.F. add 50.2 mg of Guaiphenesin dissolve and makeup with mobile phase.

Sample Preparation
Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

Chromatographic system: Inject standard solution. The test is not valid unless the tailing factor is Not more than 2.0 and column efficiency is not less than 2000 theoretical plates and the relative Standard deviation for replicate injection is not more than 2.0 %. Inject the test solution and reference solution

Procedure:
Inject in HPLC and collect the DATA from received chromatogram from HPLC and calculate quantity of Terbutaline Sulphate and Guaiphenesin by the formula given below:-

Terbutaline Sulphate:-

$$\frac{\text{Sample area X Std.Wt. X 1.0 X 25 X Potency X 5.0 X 100}}{\text{Standard area X 25 X 25 X 5.0. X 100 X claim}}$$

= %

Guaiphenesin: -

$$\frac{\text{Sample area X Std.Wt. X 25 X Potency X 5.0 X 100}}{\text{Standard area X 25 X 5.0. X 100 X claim}}$$

= %

Sample Dilutions:

By ……..-

(A) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(B) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(C) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(D) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(E) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(F) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Test Data Collection:-

Standards	Area of Terbutaline Sulphate	Area of Guaiphenesin
Standard 1	41169.7	26101.3
Standard 2	41151.9	26119.9
Standard 3	41119.6	26198.7
Standard 4	41296.5	26121.8
Standard 5	41334.2	26156.5
Standard 6	41139.5	26183.5
Mean	41201.9	26146.95
RSD	0.219	0.149

Samples		Sample Area of Terbutaline Sulphate	Mean	Sample area of Guaiphenesin	Mean
Sample A	T1	41169.7	41160.8	26156.3	26138.1
	T2	41151.9		26119.9	
Sample B	T1	41119.6	41208.05	26198.7	26160.25
	T2	41296.5		26121.8	
Sample C	T1	41334.2	41236.85	26156.5	26170.0
	T2	41139.5		26183.5	
Sample D	T1	41189.9	41164.8	26146.4	26133.1
	T2	41139.7		26119.8	
Sample E	T1	41211.9	41184.25	26152.9	26181.15
	T2	41156.6		26209.4	
Sample F	T1	41281.5	41245.35	26184.3	26180.9
	T2	41209.2		26177.5	

Estimated Amount of Terbutaline Sulphate:-

- Assay on % of Theory for sample A---- 99.70%
- Assay on % of Theory for sample B---- 99.20%
- Assay on % of Theory for sample C-----98.94%
- Assay on % of Theory for sample D----- 99.50%
- Assay on % of Theory for sample E----- 99.15%
- Assay on % of Theory for sample F----- 99.70%

Estimated Amount of Guaiphenesin:-

- Assay on % of Theory for sample A---- 99.30%
- Assay on % of Theory for sample B---- 99.05%
- Assay on % of Theory for sample C-----98.78%
- Assay on % of Theory for sample D----- 99.05%
- Assay on % of Theory for sample E----- 99.10%
- Assay on % of Theory for sample F----- 98.80%

Table for Six Replicate Assays

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Sample Number	Estimated Amount of Terbutaline Sulphate	Mean	% RSD	Estimated Amount of Guaiphenesin	Mean	%RSD
Sample A	99.20%	99.30 %	0.303	99.30%	99.06%	0.19
Sample B	98.94%			99.05%		
Sample C	99.50%			98.78%		
Sample D	99.15%			99.05%		
Sample E	99.70%			99.10%		
Sample F	99.70%			98.80%		

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Conclusion for precision: The overall % Relative standard deviation for Terbutaline Sulphate and Guaiphenesin in Terbutaline Sulphate and Guaiphenesin Syrup, there is no significant difference. Therefore Repeatability of the method considered acceptable as it well within 2 % Relative Standard Deviation.

Intermediate Precision – (Within laboratory variations such as different days, analyst & equipments):

Analyst: …….-

Standard preparation:

Weigh to 31.3 mg. of Terbutaline Sulphate add 10 ml of mobile phase, sonicate to dissolve, dilute to 25 ml with Mobile phase. Take 1 ml in 25 ml V.F. add 50.1 mg of Guaiphenesin dissolve and makeup with mobile phase.

Sample dilution:

(A) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(B) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(C) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(D) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(E) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

(F) Take 5 ml of the sample add 10 ml of mobile phase, dilute to 25 ml with mobile phase and Filter.

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Test Data Collection:-

Standards	Area of Terbutaline Sulphate	Area of Guaiphenesin
Standard 1	41169.3	26111.7
Standard 2	41159.7	26129.3
Standard 3	41119.9	26158.9
Standard 4	41299.7	26131.7
Standard 5	41338.5	26156.2
Standard 6	41139.2	26173.3
Mean	41251.9	26145.81
RSD	0.238	0.156

Samples		Sample Area of Terbutaline Sulphate	Mean	Sample area of Guaiphenesin	Mean
Sample A	T1	41178.7	41180.87	26156.3	26158.71
	T2	41161.9		26119.9	
Sample B	T1	41149.6	41218.81	26198.7	26162.27
	T2	41216.5		26121.8	
Sample C	T1	41354.2	41216.79	26156.5	26178.09
	T2	41129.5		26183.5	
Sample D	T1	41139.9	41194.81	26146.4	26183.17
	T2	41139.7		26119.8	
Sample E	T1	41241.9	41204.39	26152.9	26161.69
	T2	41176.6		26209.4	
Sample F	T1	41291.5	41235.46	26184.3	26160.79
	T2	41219.2		26177.5	

Estimated Amount of Terbutaline Sulphate:-

- Assay on % of Theory for sample A---- 99.10%
- Assay on % of Theory for sample B---- 99.40%
- Assay on % of Theory for sample C-----98.64%
- Assay on % of Theory for sample D----- 99.10%
- Assay on % of Theory for sample E----- 99.75%
- Assay on % of Theory for sample F----- 99.50%

Estimated Amount of Guaiphenesin:-

- Assay on % of Theory for sample A---- 99.10%
- Assay on % of Theory for sample B---- 99.35%
- Assay on % of Theory for sample C-----98.48%
- Assay on % of Theory for sample D----- 99.25%
- Assay on % of Theory for sample E----- 99.40%
- Assay on % of Theory for sample F----- 98.97%

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Table for Six Replicate Assays

Sample Number	Estimated Amount of Terbutaline Sulphate	Mean	% RSD	Estimated Amount of Guaiphenesin	Mean	%RSD
Sample A	99.10%	99.20%	0.67	99.10%	99.12%	0.38
Sample B	99.40%			99.35%		
Sample C	99.64%			98.48%		
Sample D	98.10%			99.25%		
Sample E	99.75%			99.40%		
Sample F	99.50%			98.97%		

Table for Six Replicate Assays analyst by two different Analysts:

Test Data analyst by **…….**-

Table for Six Replicate Assays

Sample Number	Estimated Amount of Terbutaline Sulphate	Mean	% RSD	Estimated Amount of Guaiphenesin	Mean	%RSD
Sample A	99.20%	99.30 %	0.303	99.30%	99.06%	0.19
Sample B	98.94%			99.05%		
Sample C	99.50%			98.78%		
Sample D	99.15%			99.05%		
Sample E	99.70%			99.10%		
Sample F	99.70%			98.80%		

Test Data analyst by **……..**-

Table for Six Replicate Assays

Sample Number	Estimated Amount of Terbutaline Sulphate	Mean	% RSD	Estimated Amount of Guaiphenesin	Mean	%RSD
Sample A	99.10%	99.20%	0.67	99.10%	99.12%	0.38
Sample B	99.40%			99.35%		
Sample C	99.64%			98.48%		
Sample D	98.10%			99.25%		
Sample E	99.75%			99.40%		
Sample F	99.50%			98.97%		

Acceptance Criteria: NMT 2 % (% of Relative Standard Deviation).

Conclusion for Intermediate Precision:

The overall % Relative standard deviation of two different analysts are 0.303% & 0.19% of Terbutaline Sulphate and Guaiphenesin 0.67 % & 0.38% there is no significant difference between two analysts Within laboratory variations such as different days , analyst & equipments.

Therefore reproducibility of the method considered to be acceptable.

Robustness:

To demonstrate the analytical method is capable to yield reproducibility results under;

Small but deliberate variations in method parameters during normal usage such as composition & Flow rate of mobile phase.

ANALYTICAL METHOD VALIDATION REPORT FOR TERBUTALINE SULPHATE, BROMHEXINE HYDROCHLORIDE AND GUAIPHENESIN SYRUP

Procedure:

Perform the robustness study by injecting single of resolution solution & standard solution for six times for the following parameters.

- Change in ratio of the mobile phase. Record the observation in below observation table.
- Change in Flow rate of mobile phase. Record the observation in below observation table.

OBSERVATION TABLE:-

Change ratio in the mobile phase at 220nm						
Mobile phase		Flow rate ml/min	System suitability			
Buffer	**Acetonitrile**		**Retention time of Terbutaline Sulphate**	**Retention time of Guaiphenesin**	**Tailing Factor Terbutaline Sulphate**	**Tailing Factor Guaiphenesin**
750ml	250ml	1.0 ml/min.	3.827	7.123	1.22	1.29
740:ml	260ml	1.0 ml/min.	3.734	7.016	1.12	1.18
760ml	240ml	1.0 ml/min.	3.912	6.274	1.56	1.61

Change in flow rate at 220 nm					
Mobile phase		System Suitability			
Ratio of Mobile Phase (Buffer:Acetonitrile)	**Change in flow rate**	**Retention time of Terbutaline Sulphate**	**Retention time of Guaiphenesin**	**Tailing Factor Terbutaline Sulphate**	**Tailing Factor Guaiphenesin**
750 : 250	0.8 ml/min.	3.827	7.323	1.37	1.31
750 : 250	1.0 ml/min.	3.734	7.176	1.29	1.27
750 : 250	1.2ml/min.	3.912	6.979	1.24	1.16

Acceptance criteria:

Analytical method validation shall be robust (Tailing factor is not more than 2.0).

Conclusion for Robustness:

There is no significant difference for Terbutaline Sulphate and Guaiphenesin in Terbutaline Sulphate and Guaiphenesin syrup for different conditions, such as composition & Flow rate of mobile phase. Therefore Robustness of the method considered acceptable.

CONCLUSION:

All the analytical parameter are checked as per the approved validation process and found well within specified acceptance criteria. Hence, it is concluded that, this method is suitable for accurate & precise results for routine analysis.