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A Validated Isocratic Method for The Simultaneous Estimation of Terbutaline Sulphate and Guaifenesin in Cough Syrup by RP-HPLC

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Keywords:
Terbutaline sulfate, Guaifenesin, ICH, Cough syrup.

ABSTRACT

A simple, specific, accurate, and precise high-performance liquid chromatography method for the simultaneous estimation of Terbutaline sulfate and Guaifenesin in cough syrup was studied in this experiment. The separation was achieved by using Phenomenex Luna C18 (4.6x250 mm 5µm) and methanol: buffer (Ammonium Formate buffer at pH 4.2) (45:55 v/v) as eluent, at a flow rate of 1.5 ml/min. Detection was carried out at 280nm. The retention time of Terbutaline sulfate and Guaifenesin was found to be 7.520 and 15.315 min respectively. The respective calibration plots were linear over the ranges 0.9µg/ml to 2.1 µg/ml for Terbutaline sulfate and 39.6µg/ml to 93.1 µg/ml for Guaifenesin, The percentage purity of Terbutaline sulfate and Guaifenesin was found to be 99.6390 and 99.7746respectively and the proposed method is validated as per the ICH

1. INTRODUCTION

Terbutaline acts as a stimulant of beta-adrenergic receptors of intracellular adenyl cyclase, the enzyme that catalyses the conversion of adenosine triphosphate (ATP) to cyclic AMP. Increased cyclic AMP levels are associated with relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells.

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Guaifenesin may act as an irritant to gastric vagal receptors, and recruit efferent parasympathetic reflexes that cause glandular exocytosis of a less viscous mucus mixture. A cough may be provoked. This combination may flush tenacious, congealed mucopurulent material from obstructed small airways and lead to a temporary improvement in dysponea or the work of breathing.

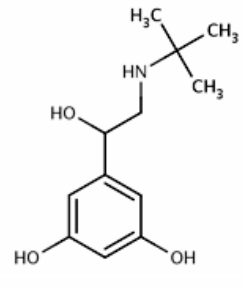

Figure 1. structure of Terbutaline Sulphate

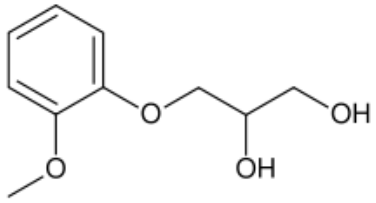

Figure 2.Structure of Guaifenesin

2. Materials and Methods

Terbutaline sulphate and Guaifenesin API produced from Chandra labs, Hyderabad. All the chemicals used were of analytical grade and HPLC grade. The chemicals used for this study are Methanol, Water, Acetonitrile of HPLCgrade and Ammonia, Formic acid of Analytical grade. HPLC (Shimadzu) with UV detector was used for this analysis.

Method Development and Optimization of Chromatographic Conditions:

The detection wavelength was based on the scanned absorption spectrum for terbutaline sulfate and guaifenesin. The spectrum was scanned over the range of 200-400 nm and was obtained by measuring the absorption of 0.1mg/ml solution of terbutaline sulfate and guaifenesin in methanol prepared from a stock

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solution. The spectrum was obtained by using a 1cm silica cell and the reference cell contained methanol as a result a wavelength of 280nm was chosen.

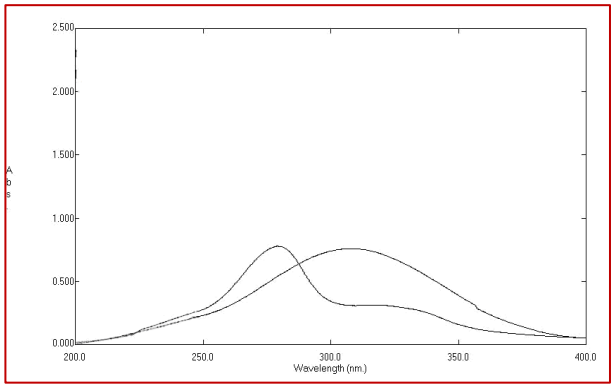

Figure 3.UV spectrum of Terbutaline sulfate and Guaifenesin.

Method Development Trials:

The method was developed by trial and error method .The first trial was performed by using ACN:Methanol (50:50 % v/v) with flow rate 2 ml/min but Only one drug was eluted and poor peak shape was found by this method. The trial 2 was performed by using mobile phase ACN: Methanol (40:60% v/v) with flow rate 2 ml/min. In this trial also terbutaline sulfate peak was not observed and some impurity peaks also found. The trial 3 was performed by using mobile phase ACN: Methanol: ammonium Formate buffer pH 5.0 (20:60:20% v/v) and flow rate 2.0 ml/min. In this trial the retention time of terbutaline sulfate and guaifenesin was found to be 6.570 and 8.221 respectively. The peaks were not sharp and tailing occurs and some additional peak also found. The trial 4 was performed by using mobile phase Methanol: buffer pH4.8 (60:40% v/v) and flow rate 1.7ml/min but the peaks obtained by this method were not sharp. The trial 5 was performed by using Methanol: buffer pH4.5 (55:4 % v/v) and flow rate 1.5ml/min. The peak shape was poor in nature.

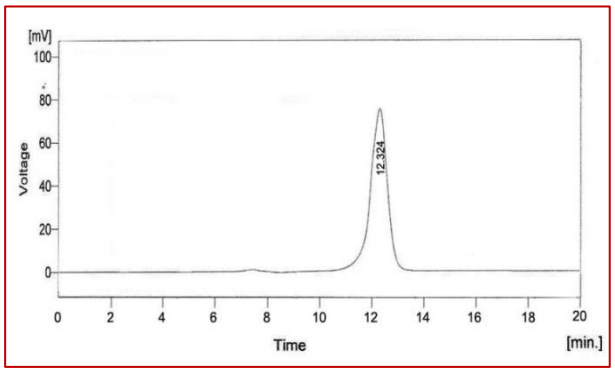

Figure 4: Chromatograph for trial 1

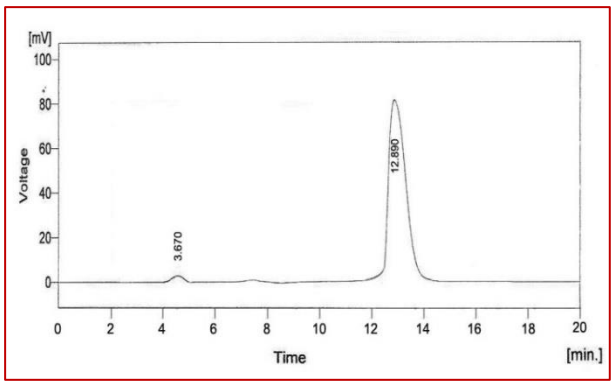

Figure 5: Chromatograph for trial 2

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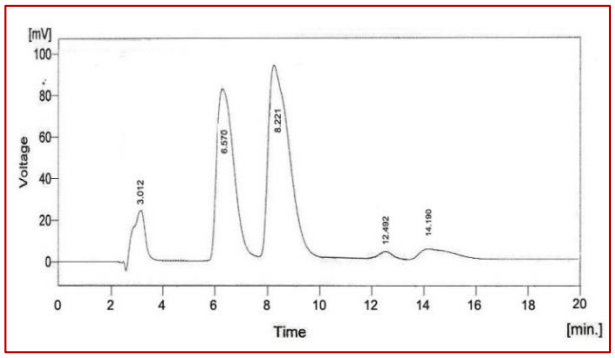

Figure 7: Chromatograph for trial 4

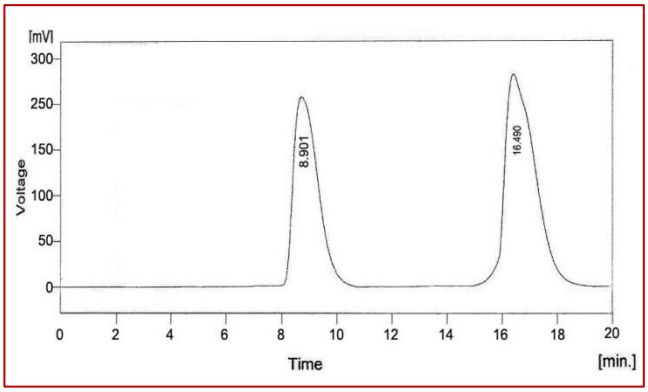

Figure 8: Chromatograph for trial 5

Optimized Chromatographic Conditions:

Stationary Phase: Phenomenex Luna C18 (4.6x250 mm 5µm)
Injector : Rheodyne
Flow rate : 1.5ml
Operating temperature: Ambient temperature
Selected wave length: 280 nm
Mobile phase ratio : Methanol: Buffer (ammonium formate buffer)(45:55 %V/V)
Diluent : Mobile Phase
Injection Volume : 20µl
Run Time : 20 min

Preparation of Buffer Solution:

3.4ml of concentrated ammonia was diluted with 3ml of water. To this 3ml of formic acid added slowly and make up the volume with water up to 50ml and adjust the pH 4.2 with ammonia and sonicated for 15 min and cool to room temperature. The solution was filtered with 0.45 µ filter.

Preparation of mobile phase:

Prepare the mobile phase with Methanol: Buffer (ammonium Formate buffer) (45:55 % V/V).

Preparation of Standard Solution:

Accurately weighed quantity of 15mg Terbutaline sulfate and 665mg Guaifenesin was transferred to a 100ml volumetric flask, dissolved in 25ml of mobile phase, sonicated for 15 min. Cool to room temperature and the solution was made up the volume with the mobile phase. From the above stock solution take 5ml was transferred to 50ml volumetric flask and make up the volume with the mobile phase. From the second dilution 5ml was transferred to 50ml volumetric flask and made up the volume with the mobile phase. The solution was filtered with 0.45µ filter.

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Preparation of Sample Solution:

For estimating the liquid dosage form 5.878gm of syrup (containing 1.5mg terbutaline sulphate and 66.5mg of guaifenesin) were taken in a 100 ml volumetric flask. To this 25ml of the mobile phase was added. The mixture was subjected to sonication for 15 min. Cool to room temperature and the solution was made up to the mark with mobile phase. From the above dilution, take 5ml was transferred to 50ml of volumetric flask, the volume was made up with the mobile phase. Then the solution was filtered with 0.45µ filter.

Diluent: Mobile phase is used as diluent.

3. Results and Discussion

Terbutaline sulphate and guaifenesin were separated and quantitated either in pure form or simultaneously in the presence of other interfering substances present in cough syrup. Several trials were carried out to obtain good separation among terbutaline sulphate and guaifenesin in mixture. These trials involved the use of different ratios of the mobile phase components, different pH of buffer and different flow rates. A series of mobile phases were tried, among the various mobile phase methanol: Ammonia Formate buffer (pH 4.2) with a ratio of 45:55 % v/v was found to be an ideal mobile phase since it gave a good resolution and peak shapes with perfect optimization. The flow rate was found to be optimized at 1.5 ml/min on Phemenex Luna C_{18} column with an Internal diameter 250×4.6mm, and 5 micron particle size with UV detection at 280 nm gave a satisfactory Chromatogram with terbutaline sulphate and guaiphenesin of retention time of 7.520 min and 15.320 min (Mixed standard) respectively.

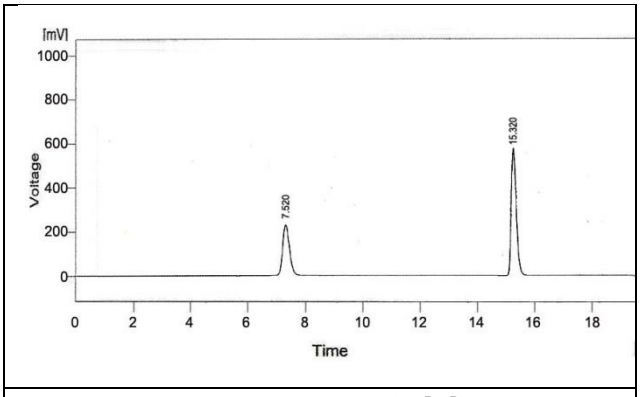

Figure 9. System suitability

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Table 1. Results for System suitability parameters.

Parameters	Terbutaline sulfate	Guaifenesin
Resolution	-	24.5
Tailing factor	1.12	2.0
Number oftheoretical plate	6554.6	7088.6
Retention time	7.54	15.32
%RSD	0.51	0.34

Accuracy:

The recovery was carried out at 80%, 100% and 120% level and the contents were determined from the respective chromatogram. From the results obtained we can conclude that the method was accurate.

Table 2. Recovery Studies for Terbutaline sulfate and Guaifenesin.

S. No	Sample	Spike level	Amount added (gm)	Amount Recovered (gm)	% Recovered
1	**Terbutaline sulfate**	80 %	0.0121	0.0120	99.9035
2		100 %	0.0151	0.0150	99.6390
3		120 %	0.0179	0.0177	99.0460
4.	**Guaifenesin**	80 %	0.5340	0.5329	99.8007
5		100 %	0.6680	0.6664	99.7746
6		120 %	0.7985	0.7976	99.8878

Precision:

The precision of test method was done by performing assay on six replicate determination of sample preparation at the test concentration level (as per the method of analysis) and calculated relative standard deviation of assay results.

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Stability of The Sample Solution:

Stability of the solution used in Hplc method is required for a reasonable time to generate reproducible and reliable results. The stability of the drug solution was subjected to short term stability at room temperature and it was found that the solution was stable for approximately eight hours.

Table 3. Results for Stability of sample solution

Stability	Area of Terbutaline sulfate (mV)	Area of Guaifenesin (mV)
0 hour	1128.342	7876.514
8thhour	1128.104	7876.321

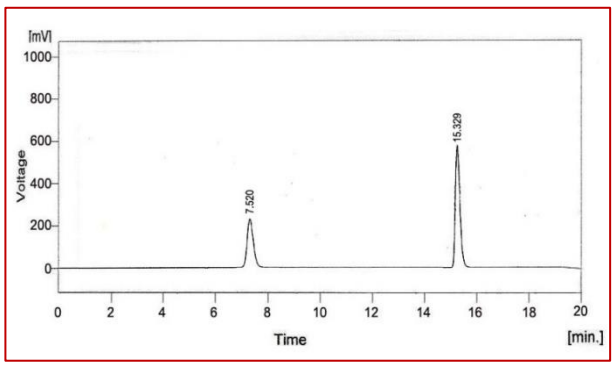

Figure 10: STABILITY-(O-HOUR

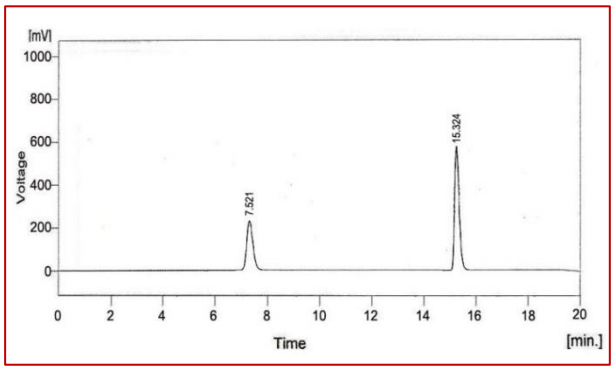

Figure 11: STABILITY-(8th-HOUR)

The lowest concentration of terbutaline sulphate that can be detected, was determined from standard curve was 0.002 µg/ml.

The lowest concentration of guaifenesin that can be detected was determined from standard curve was0. 022 µg/ml.

Limit of Quantitation (LOQ)

The lowest concentration at which peak can be quantified is called LOQ, was found to be 0.006 µg for terbutaline sulphate and for guaifenesin was found to be 0.065µg/ml.

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Table 4. Results for LOD and LOQ

Sample	LOD	LOQ
Terbutaline sulfate	0.002	0.006
Guaifenesin	0.022	0.065

Table.5.Results for Linearity Parameters

Parameters	Terbutaline sulfate	Guaifenesin
Linearity Range	0.9-2.1µg/ml	**39.6-93.1µg/ml**
CorrelationCoefficient	0.9990	0.9990
Slope (m)	756.32	117.96
Intercept	2.15	18.49

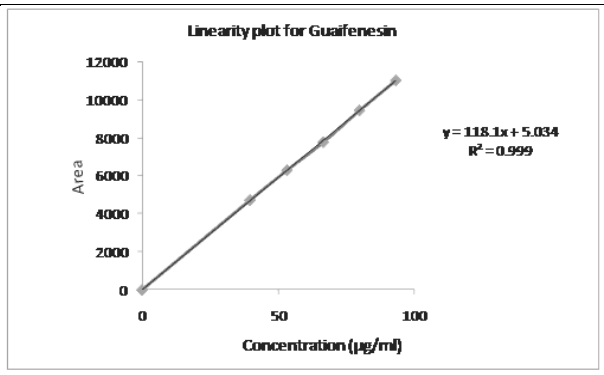

Figure.12.Linearity plot for Guaifenesin

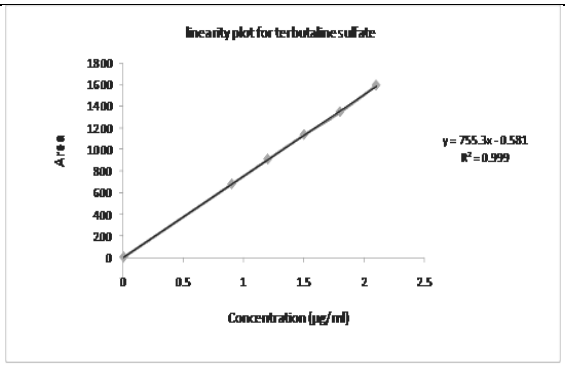

Figure.13.Linearity plot for Terbutaline sulphate

4. Conclusion

The linearity and range was found to be in the range of 0.9-2.1 µg/ml for terbutaline sulphate and 39.6-93.1 µg/ml for guaifenesin. The correlation coefficient of terbutaline sulphate and guaifenesin were found to be 0.999 and 0.999 respectively, which indicates a perfect correlation. The developed method was validated for accuracy, precision, and system stability. The percentage recovery of terbutaline sulphate and guaifenesin were found to be 99.7883 %and 99.5174 % respectively. The good percentage recovery of the sample clearly indicates, that the reproducibility and accuracy of the developed method. Similarly the % RSD value of precision was also found to be within the acceptable limit.

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Hence, the developed chromatographic method for terbutaline sulphate and guaifenesin was found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in drug research, quality control department in industries and approved testing laboratories and etc.

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