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Research Article

REVERSE PHASE HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TERBUTANILE SULPHATE, BROMHEXINE HCL AND GUAIFENESIN IN COUGH SYRUP

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ABSTRACT

The present paper describes a simple, accurate and precise reversed phase HPLC method for rapid and simultaneous quantification of Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin in a cough syrup formulation. Separations were carried out on a phenomenex Luna C18 column (250 X 4.6 mm ID), 5 µm particle size. A isocratic elution system was developed using acetonitrile-methoanol-buffer (350:450:250 v/v). The elution of the analytes was achieved in less than 15 min with a flow rate of 1.2 ml/min. Detection was by UV absorbance at a wavelength of 220 nm. Quantification of the components in actual syrup formulations was calculated against the responses of freshly prepared external standard solutions. Different analytical performance parameters such as linearity, precision, accuracy, limit of detection, limit of quantification, and robustness were determined according to international conference on harmonization ICH Q2B guidelines. All the parameters of validation were found in the acceptance range of ICH guideline.

Keywords: RP-HPLC, Terbutaline sulphate, Bromhexine Hcl, Guaifenesin.

INTRODUCTION

Terbutaline Sulphate [1-3] is used to prevent and treat wheezing, shortness of breath troubled breathing caused by asthma, chronic bronchitis, emphysema and other lung diseases. It relaxes and opens air passage in the lungs, making it easier to breathe.Bromhexine Hcl is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. In addition, bromhexine Hcl has antioxidant properties.

Guaifenesin [3-5] is used to relive chest congestion. Guaifenesin may help control symptoms but does not treat the cause of symptoms or speed recovery. Guaifenesin is in a class of medications called expectorants. It works by thinning the mucus and clear the airways.The combination of 1.5mg of Terbutaline Sulphate, 4mg of Bromhexine Hcl and 50mg of Guaifenesin is available commercially as syrup formulations.Few Spectrophtometric methods have reported for the qualitative and quantitative determination of Terbutaline Sulphate, Bromhexine Hcl, and Guaifenesin in tablet dosage form [6-8].

Various HPLC[9-11], Voltammetric assay [12], Capillary gas chromatography[13], Isotachophoresis [14] and Capillary zone electrophoresis [14] methods are also reported in the literature for the estimation of Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin individually and in combination with other drugs. According to literature survey there is no method reported for the simultaneous estimation of Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin in combined liquid dosage forms.

The objective of the present work was the development and validation of a method for the estimation of cough syrup containing is Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin.

EXPERIMENTAL

Chemicals and reagents

Potasssium di hydrogen phosphate was procured from S.D fine chemicals. Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin was procured from Tablets India Pvt. Ltd. Methonal and Acetonitrile HPLC grade was procured from Spectrochem Pvt. Ltd. Water is prepared by mili Q system.

Instrumentation

HPLC condition

Column : ODS C18 (250X4.6mm, 5µm)

Wavelength : 220nm

Injection volume : 20 µL

Flow rate : 1.2 mL/min

Temperature : Ambient temperature

Run time : 15min

Mobile phase : Acetonitrile: Methanol: Buffer (300:250:450 v/v; pH 4.2)

Buffer preparation

Dissolve 5.04 gm of disodium hydrogen phosphate and 3.01 gm of potassium dihydrogen phosphate in sufficient water to produce 1000mL. Adjust the pH 4.2 with glacial acetic acid.

Preparation mixed standard solutions

A combined standard solution containing Terbutaline sulphate, Bromhexine Hcl and Guaifenesin was prepared by accurately weighing 15, 40 and 500 mg of each powder and transferring to a 100-ml volumetric flask, mixing until dissolved and mobile phase added to make up the volume. Take 5ml of above mixed standard solution is transferred to 50ml volumetric flask and mobile phase added to make up the volume. Sonicated for 10 min and cool to room temperature. The solution was filtered with 0.45 µ filter.

Preparation of sample solutions

30.54 mg of cough syrup is accurately weighed and transferred to a 50 ml volumetric flask and added a mobile phase make up the volume. Take 5ml of above solution and transferred to a 50ml volumetric flask and added a mobile phase make up the volume. Sonicated for 10 min and cool to room temperature. The solution was filtered with 0.45 µ filter.

VALIDATION CRITERIA

Specificity

The specificity defined as the ability of method to measure the analyte accurately and specifically in the presence of components present in the sample matrix, was determined by analysis of chromatograms of drug-free and drug-added placebo formulation.

System suitability

The system suitability parameters, theoretical plates (N) and asymmetry factor (As), were calculated, as reported by European

pharmacopoeia [15]. System suitability was performed daily during entire validation of this method.

Linearity and calibration curve

Linearity of the method was evaluated by preparing a standard solution containing 15 µg/ml of Terbutaline sulphate, 40 µg/ml of Bromhexine Hcl and 500 µg/ml of Guaifenesin (100% of targeted level of the assay concentration). Sequential dilutions of these solutions at 60, 80, 120 and 140 % of target assay concentration. These were injected in triplicate and the peak areas used to plot calibration curves.

Accuracy

Accuracy of the method was studied by recovery investigation. This also provided the working range for the method. Placebo syrup solution containing all components apart from Terbutaline Sulphate, bromhexine HCl and guaifenesin was used. Known amounts of each of these three were then 'spiked' in to separate 25 ml aliquots of placebo to give pseudo sample solutions of approximately 80, 100 and 120 % of stated label strength values. These samples were then analyzed according to procedure and percentage recoveries calculated.

Precision

Precision was investigated using one batch of freshly manufactured cough cold syrup formulation. From this six separate sample solutions was injected and the peak areas obtained used to calculate mean and percentage R.S.D. values. Injecting a freshly prepared standard solution six times and calculating mean and percentage R.S.D. values evaluated system repeatability.

Limits of detection and quantitation

The detection limit and quantification limit for each analyte were determined based on a signal- to- noise concept, as the lowest concentrations at which signal -to- noise ratio is between 3or 2:1 and 10:1 respectively, with defined precision and accuracy under the given experimental conditions.

Stability

Drug stability should be considered in analysis of in vitro extended release data, since released drug may degrade in due time.The stabilitiy of Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin was determined by replicate standards at concentrations of 15, 40 and 500 µg/ml, respectively, were injected at evenly spaced intervals during a single RP-HPLC assay run of 24h and the stability of these three analytes assessed at the same concentrations in the release medium of mobile phase (pH 4.2; $37^0C \pm 2^0C$) at 4h intervals until 24h.

Ruggedness

Ruggedness of the method was studied by using different sources of analysts, instruments and columns with same experimental conditions.

Robustness

Robustness of the method was studied by slightly changes in experimental conditions like pH, flow rate, temperature. Performed by same analyst with same instrument.

RESULT AND DISCUSSION

Analytical Separation performances

Figs 1 and 2 show examples of the standard and sample solution chromatograms obtained using the optimized chromatographic conditions. The retention times of Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin were found to be 2.531, 12.190 and 5.710 min, respectively. These retention times did not vary to any considerable degree during and in between analyses (% R.S.D. less than 2% for the retention time of each peak). Resolution of the Guaifenesin from the Terbutaline Sulphate was 2.21 and Bromhexine Hcl from the Guaifenesin was 15.34. Both these values meet the acceptance criteria for resolution of greater than or equal to two. The number of theoretical chromatographic plates for Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin were 3568, 3785 and 4814, respectively.

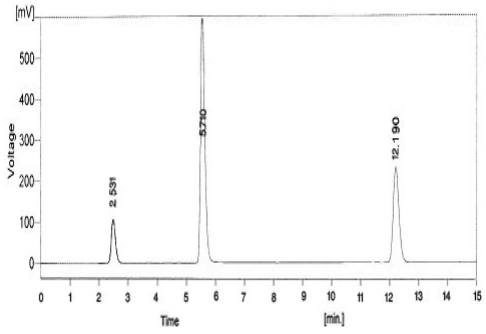

Fig. 1: Standard Chromatogram

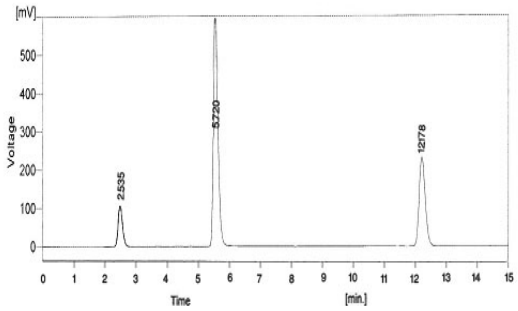

Fig. 2: Sample Chromatogram

Calibration graphs and linearity study

Linearity of the method was evaluated by preparing a standard solution containing 15 µg/ml of Terbutaline Sulphate, 40 µg/ml of Bromhexine Hcl and 500 µg/ml Guaifenesin (100% of targeted level of the assay concentration). Sequential dilutions were preformed to give solutions at 60, 80,100, 120 and 100% of the target assay concentration. These were injected in triplicate and the peak areas used to plot calibration curves against the concentration. The correlation coefficient values of these three analytes were 0.999. The results shown in Table 1.

Table 1: Linearity study results.

Analyte (n=5)	Linearity Range	Equation of calibration curve	Correlation Coefficient
Terbutaline sulfate	9-21µg/ml	y =4617x+25.1	0.999
Bromhexine hcl	245621µg/ml	y =2807x+351.7	0.999
Guaifenesin	300700µg/ml	y =2007x+2375	0.999

Limits of detection and quantitation

The limits of detection and quantification is decide about the sensitivity of the method and were calculated from the peak -to-noise ratios. In the present study, the LOD values for Terbutaline Sulphate was 0.06 µg/ml, Bromhexine Hcl was 0.08 µg/ml and Guaifenesin was 0.18 µg/ml and quantification limits were found to be 0.22, 0.26 and 0.57 µg/ml, respectively.

Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch of syrup. From this six separate sample solutions was injected and the peak areas obtained used to calculate mean and percentage R.S.D. values. The results obtained are shown in Table 2. In all instances the accepted criteria of % R.S.D. of less than 2% was met.

Table 2: Method precision results

Analyte (n=6)	Amount percent (mean)	%RSD of assay
Terbutaline sulfate	1.48mg	0.15
Bromhexine hcl	3.98mg	0.39
Guaifenesin	50.12mg	0.33

Precision of the system was evaluated by injecting a freshly prepared standard solution six times. The %R.S.D. results obtained 0.72, 0.34 and 0.53 for Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin, respectively, all well below the accepted maximum of 1%.

Accuracy

Accuracy of the method was studied by recovery investigation as described in section--. The results of this investigation are shown in table 3. For all three analytes at the different concentration levels evaluated the recovery values meet the acceptance criteria of 100 ±2%.

In addition, these results provide the working range for the method. The method can accurately determine Terbutaline Sulphate levels between 12 and 18 µg/ml, Bromhexine Hcl levels between 32 and 48 µg/ml and Guaifenesin levels between 400 and 600 µg/ml.

Table 3: Accuracy (recovery) study results

Percentage of target concentration	Terbutaline sulfate (%recovery)	Bromhexine Hcl (% recovery)	Guaifenesin (% recovery)
80	99.96	99.86	100.93
100	99.99	100.89	99.77
120	100.71	99.84	100.31

Ruggedness

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage R.S.D. was below 2%, exhibits the ruggedness of developed analytical method.

Robustness

Robustness of the method was determined by small deliberate changes in flow rate, pH, and temperature. The content of the analytes was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method is robust.

Stability

In case of an unexpected delay during analysis, it is important to have information about the stability of all solutions. In this study the stability of Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin in the working standard solution and sample preparations were studied.

These three analytes did not show evidence of significant degradation for at least 24h, when kept at release medium (mobile phase; pH 4.2; $37^0C \pm 2^0C$). During this period, results do not decrease below 98%.

CONCLUSIONS

The method described enables to the quantification of Terbutaline Sulphate, Bromhexine Hcl and Guaifenesin. The advantages lie in the simplicity of sample preparation and the cost economic reagents were used. In addition all three compounds were eluted within 15 min. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result indicates that the precision and reproducibility date are satisfactory. The developed chromatographic method can be effectively applied for routine analysis in drug research.

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