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VALIDATED RP-HPLC METHOD FOR DETERMINATION OF BROMHEXINE HYDROCHLORIDE, TERBUTALINE SULFATE AND GUAIPHENESINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and accurate reversed phase HPLC method for simultaneous estimation of Syrian cough syrup containing Bromhexine Hydrochloride, Terbutaline Sulfate, and Guaiphenesin was developed. Separations were carried out on Column: C18(100^5) (25X4.6mm, with precolumn). The mobile phase was methanol: buffer (600:400 v/v). The elution of the analytes was achieved in less than 10 min with a flow rate of 1.5 ml/min. Detection was by using UV absorbance at a wavelength of 276 nm. Different analytical performance parameters such as linearity, precision, accuracy, and robustness were determined and found in the acceptance range of American pharmacopoeia (USP34).

KEYWORDS

Bromhexine Hydrochloride, Guaiphenesin, Reversed phase HPLC, Economical, Terbutaline Sulfate and Validation.

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INTRODUCTION

Cough syrups are combination of bronchodilator, mucolytic and expectorant for the control of productive cough in a palatable syrup dosage form[1]. Many pharmaceutical cough syrup formulations are a combination of Bromhexine hydrochloride, Terbutaline Sulfate and Guaiphenesin[2]. Bromhexine HCl is a mucolytic used in the treatment of respiratory disorders associated with productive cough[3], it has antioxidant properties[4], and it is an expectorant[5]. Terbutaline Sulfate is a direct-acting sympathomimetic with mainly beta-adrenergic

activity and a selective action on beta2 receptors (a beta2 agonist)[6]. It 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[4]. Guaiphenesin is reported to increase the volume and reduce the viscosity of tenacious sputum[5]. It is used as an expectorant for productive cough[7]. It may help controlling symptoms but does not treat the cause of symptoms or speed recovery[4]. The aim of this work is to develop and validate a simple, precise and accurate method for simultaneous estimation of cough syrup containing Bromhexine HCl Terbutaline Sulfate, and Guaiphenesin.

MATERIAL AND METHOD

Chemicals and Reagents

Potassium di hydrogen phosphate, disodium hydrogen phosphate and Methanol HPLC grade were procured from Sham Lab, Laboratory Chemicals. Bromhexine HCl Terbutaline Sulfate and Guaiphenesin were submitted from Ministry Of Health in Syria. Water is prepared by doubled distilled water system.

Instrumentation

HPLC Condition
Column: C18 (100-5) (25X4.6mm, with pre column)
Wavelength: 276nm
Injection volume: 20 µL
Flow rate: 1.5 mL/min
Temperature: 25°C
Run time: 10min
Mobile phase: Methanol: Buffer
(600:400 v/v; pH 3.5)

Step1

Buffer preparation

Dissolve 0.6g of disodium hydrogen phosphate and 0.4 g of Potassium dihydrogen phosphate in sufficient water to produce 1000mL. Adjust the pH 3.5 with glacial acetic acid.

Step 2

Preparation of standard stock solutions

Standard stock solution were prepared separately by accurately weighed (100.0, 31.25, 1250.0) mg of Bromhexine HCl, Terbutaline Sulfate and Guaiphenesin (working standards), respectively. Transferred to 250.0 ml volumetric flask, and dissolving with Mobile phase. Sonicated in bath sonicator for 10 minutes to ensure complete solubilization. After sonication, the volume was made up to the mark 250.0 ml. with same solvent, to result in a final concentration of (400, 125, 5000) µg/ml of Bromhexine HCl Terbutaline Sulfate and Guaiphenesin, respectively.

Step 3

Preparation mixed standard solutions

A combined standard solution containing Bromhexine HCl, Terbutaline sulfate and Guaiphenesin was prepared by accurately taking 5.0 ml of each standard solution and transferring to a 25.0ml volumetric flask and completing the volume with mobile phase. The solution was filtered with 0.45µ filter.

Step 4

Preparation of sample solutions

1.0ml of cough syrup is accurately taken and transferred to a 10.0 ml volumetric flask and added a mobile phase makes up the volume. Sonicated for 10 min and cool to room temperature. The solution was filtered with 0.45 µ filter.

Step 5

Validation Criteria

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components[8].

The specificity of the method 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 American pharmacopoeia (USP34).

System suitability was performed daily during entire validation of this method.

Linearity

The linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range[8].

Linearity of the method was evaluated by preparing from a stock solution, standard solutions (80, 90, 100, 110, and 120) %.

The 100% standard solution contains 80 µg/ml of Bromhexine HCl, 25 µg/ml of Terbutaline sulfate, and 1000 µg/ml of Guaifenesin.

These were injected in triplicate and the peak areas used to plot calibration curves.

Accuracy

The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value[8].

Accuracy of the method was studied by recovery investigation, by application of the analytical procedure to synthetic mixtures of the drug product components to which known amounts of analyte have been added within the range of the procedure.

Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample[8].

Precision was investigated by injecting six separate freshly prepared standard solutions and obtaining the peak areas 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.

Ruggedness

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

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and provides an indication of its suitability during normal usage[8].

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

RESULTS AND DISCUSSION

A rapid, economical and accurate RP-HPLC method was developed for the simultaneous estimation of Bromhexine Hydrochloride, Terbutaline Sulfate, and Guaiphenesin. Figure No.1 and 2 show examples of the standard and sample solution chromatograms obtained using the optimized chromatographic conditions.

The retention times of Bromhexine HCl, Terbutaline Sulfate and Guaiphenesin were found to be 8.956, 1.842 and 3.016 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 Sulfate was 6.979 and Bromhexine HCl from the Guaifenes in was 17.909. Both these values meet the acceptance criteria for resolution of greater than or equal to 2. The number of theoretical chromatographic plates for Bromhexine HCl, Terbutaline Sulfate and Guaiphenesin were 5610.047, 2407.742 and 4225.981, respectively.

Linearity of the method was evaluated. The correlation coefficient values of these three analytes were 0.999 as shown in Table No.1.

Precision of the system was investigated. The results obtained were met the acceptance criteria of R.S.D. % is less than 2%, as shown in Table No.2.

Accuracy of the method was studied by recovery investigation as described. The recovery values meet the acceptance criteria of 100±2% as shown in Table No.3.

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

exhibits the ruggedness of developed analytical method.

Robustness of the method was determined; the content of the analytes was not adversely affected by these changes as evident from the low value.

Table No.1: Linearity study results

S.No	Analyte (N=5)	Linearity Range	Equation of Calibration Curve	Correlation Coefficient
1	Bromhexine HCl	64-96 µg/ml	y = 2228x - 445.51	0.999
2	Terbutaline sulfate	20-30 µg/ml	y = 5664.3x + 238.6	0.999
3	Guaifenesin	800-1200µg/ml	y = 7474.2x	0.999

Table No.2: Precision study results

S.No	Analyte (N=6)	Amount percent (mean)	RSD% of assay
1	Bromhexine HCl	19.8 mg	0.37
2	Terbutaline sulfate	6.2 mg	0.35
3	Guaifenesin	250.8 mg	0.13

Table No.3: Accuracy (Recovery %) study results

S.No	Percentage of target concentration	Bromhexine HCl (Recovery %)	Terbutaline sulfate (Recovery %)	Guaifenesin (Recovery %)
1	80	99.33	99.64	99.50
2	100	99.42	100.05	100.09
3	120	99.95	99.39	100.09

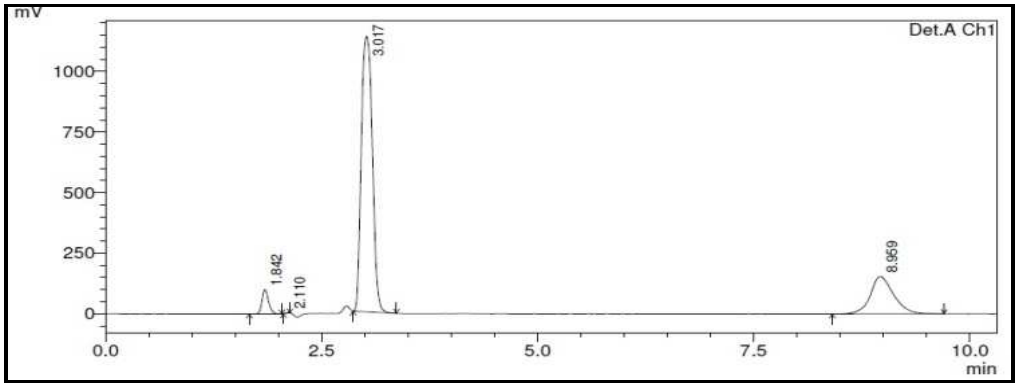

Figure No.1: Standard Chromatogram

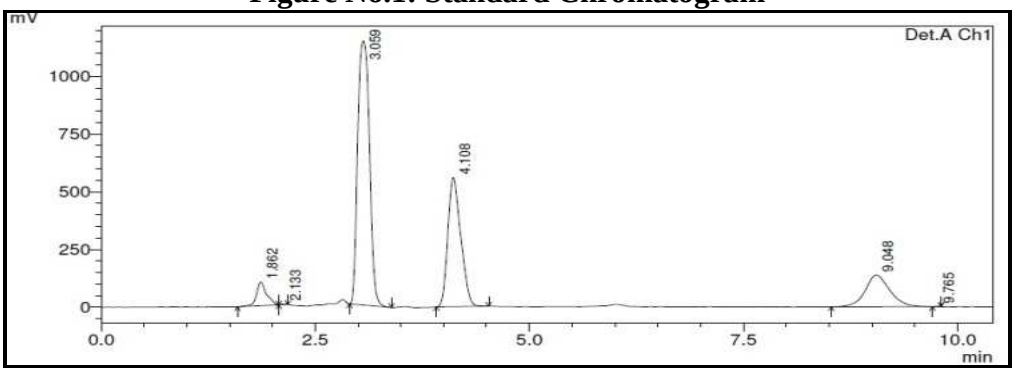

Figure No.2: Sample Chromatogram

CONCLUSION

Simultaneous analysis of Bromhexine HCl, Terbutaline Sulfate and Guaiphenesin in a single marketed formulation was performed by RP-HPLC method. The advantages of this method lie on the simplicity of sample preparation and the economic reagents were used. In addition all three compounds were eluted within less than 10 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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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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