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# Method Development And Validation For Terbutaline Sulfate, Guaifenesin And Bromhexine Hydrochloride In A Syrup Formulation By Ion Pair RP-HPLC

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## ABSTRACT

A simple, rapid and precise RP-HPLC method was developed and validated for terbutaline sulfate, guaifenesin and bromhexine hydrochloride in a syrup formulation. A Phenomenex Luna $C_{18}$ column, 250×4.6mm, 5µm in gradient mode, with mobile phase ammonium formate buffer pH 3.0 and acetonitrile were used. The flow rate was 1 ml/min and individual components were measured at 270nm. The retention time of terbutaline sulfate, guaifenesin and bromhexine hydrochloride was found to be around 10.79, 19.06 and 15.55 min respectively. Linearity for terbutaline sulfate, guaifenesin and bromhexine hydrochloride were in the range of 0.015 to 0.045 mg/ml, 0.005 to 0.015 mg/ml and 0.02 to 0.06 mg/ml respectively. Percentage recoveries were found to be within 99.62 to 100.97%w/w for terbutaline sulfate, 100.52 to 100.81% w/w for guaifenesin and 99.98 to 101.34% w/w for bromhexine hydrochloride respectively. The proposed method is precise, selective and rapid for estimation of terbutaline sulfate, guaifenesin and bromhexine hydrochloride.



## KEYWORDS

Terbutaline sulfate;
Guaifenesin;
Bromhexine hydrochloride;
HPLC;
Syrup.

## INTRODUCTION

Terbutaline sulfate[1-8] is chemically known as bis[(1RS)-1-(3,5-dihydroxyphenyl)-2-[(1,1dimethylethyl)amino]ethanol] sulphate salt. It prevents and reversal of bronchospasm in patients with asthma and reversible bronchospasm associated with bronchitis and emphysema. Guaifenesin[9-13] is chemically known as (2RS)-3-(2-methoxyphenoxy) propane-1,2-diol salt. It is an expectorant, the action of which promotes or facilitates the removal of secretions from the respiratory tract. Bromhexine hydrochloride[14-20]

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is chemically known as N-(2-amino-3,5-dibromo-benzyl)-N-methylcyclohexana-mine hydrochloride salt. It supports the body's own natural mechanisms for clearing mucus from the respiratory tract. This medication is a secretolytic, which means that it increases the production of serous mucus in the respiratory tract and makes the phlegm thinner and less sticky. This combination is mainly used in the treatment of respiratory disease. Fixed dose combination containing terbutaline sulfate, guaifenesin and bromhexine hydrochloride is available in tablet and syrup form in the market. For this combination no analytical methods have been reported. The aim of this work was to develop a simple, rapid, precise, and accurate reverse phase HPLC method for the determination of terbutaline sulfate, guaifenesin and bromhexine hydrochloride in syrup dosage form.

## MATERIALS AND METHODS

### Instrument

HPLC Shimadzu prominence with phenomenex Luna $C_{18}$ column, (250×4.6mm), 5µm particle size. The detector used was photo diode array detector.

### Chemicals and reagents

In syrup formulation, each 5ml contains 1.50mg of terbutaline sulfate, 50 mg of guaifenesin and 2mg of bromhexine hydrochloride were procured from fourrts(India) laboratories private ltd.

Hexane-1-sulfonic acid sodium salt HPLC grade, formic acid HPLC grade, ammonium formate HPLC grade, acetonitrile HPLC grade were used.

### Chromatographic conditions

The mobile was delivered gradiently at a flow rate of 1ml/min. All solutions were filtered through a 0.45µ membrane filter before use. Phenomenex Luna $C_{18}$ column, 250×4.6mm, 5µm, particles is used as stationary phase. The column was maintained at ambient temperature. The injection volume was 50µl and the total run time was not more than 35min. The detection was carried out at 270nm.

### Mobile phase

The solvent system composed of buffer of pH 3.0 and acetonitrile is used as mobile phase. The mobile phase composition is programmed for gradient elution. The gradient programming is tabulated in (TABLE 1).

**TABLE 1: Gradient program**

| Time (min) | Buffer (ml) | Acetonitrile (ml) |
|---|---|---|
| 0 | 85 | 15 |
| 8 | 85 | 15 |
| 15 | 15 | 85 |
| 25 | 15 | 85 |
| 27 | 85 | 15 |
| 35 | 85 | 15 |

### Preparation of buffer

Weighed accurately 3.15gm of ammonium formate and transferred in to 1000ml volumetric flask, dissolved and diluted to volume with water and pH of the resulting solution is adjusted to 3.0 with formic acid. Then 5.49gm of sodium-1-hexane sulfonate is added and diluted to required volume with water. Mixed and filtered through 0.45µ membrane filter and degassed.

### Diluent

Buffer and acetonitrile are mixed in the ratio of 85:15, respectively and used as diluent.

### Preparation of standard

### Preparation of standard stock solution-1

Accurately 40mg of bromhexine hydrochloride and 30mg of terbutaline sulfate are weighed and transferred to 100ml volumetric flask. 30ml of diluent is added, dissolved and the volume is made up to mark with diluent.

### Preparation of standard stock solution-2

About 50mg of guaifenesin is accurately weighed in to a 100ml volumetric flask. 30ml of diluent is added, dissolved and made up to mark with diluent.

### Preparation of standard solution

From standard stock solution-1, accurately 10ml is pipetted out in to 100ml volumetric flask. Then 2ml of standard stock solution-2 is added to the same, mixed well and made up to the volume with diluent.

### Preparation of sample

### Preparation of sample solution-1(TERB and BROM)

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10 g of sample is accurately weighed and transferred to a 100ml volumetric flask. Then 30ml of diluent is added and sonicated for 10 minutes. The volume is made up to the mark with diluent and filtered.

### Preparation of sample solution-2(GUAI)

1 ml of the filtered sample solution-1 was diluted to 100ml with diluent.

### Estimation method

The standard solution is injected 5 times; similarly sample solutions 1 and 2 are also injected in duplicate. The chromatograms are recorded, and the responses for the peaks are measured. The amount of drug present in each 5ml is calculated. Then the assay values are calculated in terms of percentage label claim and the values are statistically validated.

**TABLE 2: Estimation of active ingredients in syrup**

| Sample | Label claim (mg/5ml) | Amount present (mg/5ml)* | % Label claim* | %R.S.D |
|---|---|---|---|---|
| TERB | 1.5 | 1.501 | 100.08 | 0.18 |
| GUAI | 50 | 50.169 | 100.34 | 1.00 |
| BROM | 2 | 2.020 | 100.98 | 0.84 |

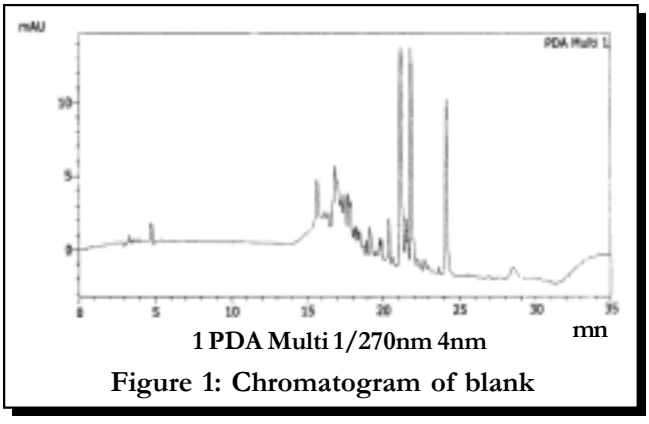



**Figure 1: Chromatogram of blank**

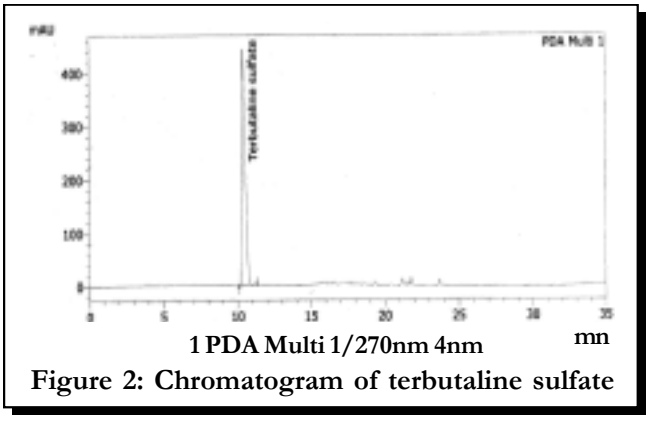



**Figure 2: Chromatogram of terbutaline sulfate**

The results are tabulated in (TABLE 2) and chromatograms in (Figures 1& 2).

### Calculation

Terbutaline and bromhexine hydrochloride:

$$\text{Amount of drug present} = \frac{\text{Sample area} \times \text{std. wt} \times 10 \times 100 \times \text{Purity of std.} \times \text{wt / ml} \times 5}{\text{Stan ard area} \times 100 \times 100 \times \text{Sample wt} \times 100}$$

Guaifenesin:

$$\text{Amount of drug present} = \frac{\text{Sample area} \times \text{Std. wt} \times 2 \times 100 \times 100 \times \text{Purity of std.} \times \text{wt / ml} \times 5}{\text{Stan dard area} \times 100 \times 100 \times \text{sample wt} \times 1 \times 100}$$

### Validation[21-23]

Analytical method validation for the estimation of terbutaline sulfate(TERB), guaifenesin(GUAI) and bromhexine hydrochloride(BROM) in syrup by HPLC has been conducted as per the ICH guidelines, to ensure that the performance characteristics of the method meet the requirements for its intended application.

### Specificity

Placebo solution was prepared separately at a concentration of 100mg/ml of excipients blend. A solution of placebo was spiked with the TERB, GUAI and BROM at the working concentration. And the solution was analyzed as per the assay method described. (TABLE 3) summarizes the retention time, relative retention time and the resolution values.

### System suitability

**TABLE 3: Specificity data**

| Peak name | Retention time (min) | Relative retention time (min) | Resolution |
|---|---|---|---|
| Placebo | 16.98 | - | - |
| Terbutaline sulfate | 10.79 | 0.63 | 26.98 |
| Guaifenesin | 15.55 | 0.91 | 9.10 |
| Bromhexine hydrochloride | 19.06 | 1.12 | 14.56 |

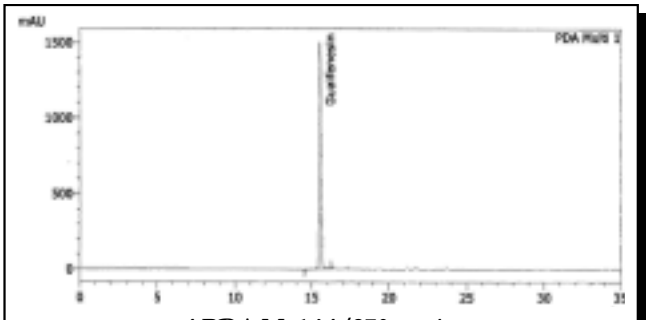
For system suitability, parameters like tailing factor and the number of theoretical plates were noted down and are tabulated in (TABLE 4).

### System precision

The standard solution prepared at the working

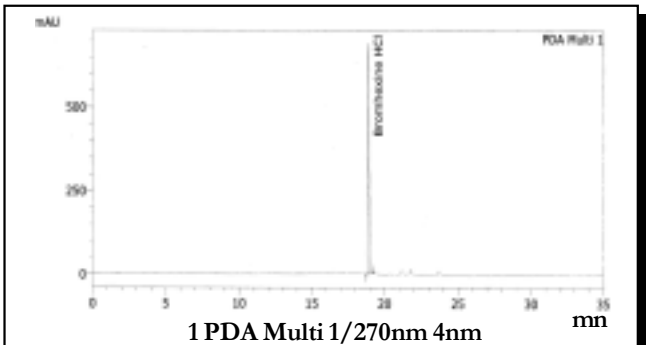
**TABLE 4: System suitability**

| Sample | Tailing factor | No. of Theoretical plates |
|---|---|---|
| TERB | 1.24 | 12922 |
| GUAI | 1.40 | 103660 |
| BROM | 1.57 | 222172 |



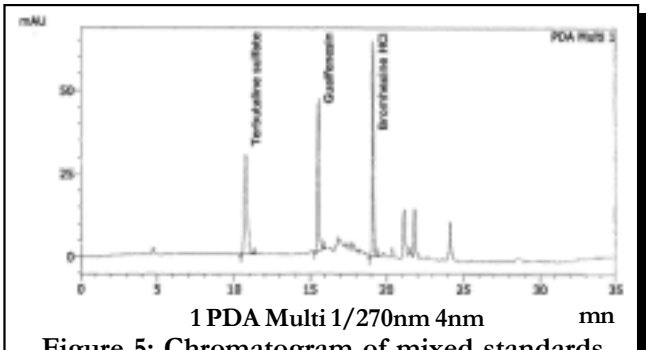

1 PDA Multi 1/270nm 4nm

**Figure 3: Chromatogram of guaifenesin**




1 PDA Multi 1/270nm 4nm

**Figure 4: Chromatogram of bromhexine hydrochloride**




1 PDA Multi 1/270nm 4nm

**Figure 5: Chromatogram of mixed standards**

concentration is taken and analyzed in replicate. The values of mean and percentage relative standard deviation are calculated and are tabulated in (TABLE 5-7), respectively.

## Method precision

The method precision was performed by analyzing sample solution at working concentration five times(five replicate sample preparation). (TABLE 8) shows the values for method precision.

**TABLE 5: System precision for terbutaline sulfate**

| S.No | Retention time (min) | Peak area |
|---|---|---|
| 1 | 10.76 | 425092 |
| 2 | 10.76 | 424877 |
| 3 | 10.76 | 425511 |
| 4 | 10.76 | 425822 |
| 5 | 10.76 | 424922 |
| | **Average** | 425245 |
| | **%RSD** | 0.096 |

**TABLE 6: System precision for guaifenesin**

| S.No | Retention time (min) | Peak area |
|---|---|---|
| 1 | 15.53 | 313508 |
| 2 | 15.53 | 313483 |
| 3 | 15.54 | 313994 |
| 4 | 15.59 | 313356 |
| 5 | 15.53 | 313579 |
| | **Average** | 313584 |
| | **%RSD** | 0.077 |

**TABLE 7: System precision for bromhexine hydrochloride**

| S.No | Retention time (min) | Peak area |
|---|---|---|
| 1 | 19.11 | 369807 |
| 2 | 19.10 | 369619 |
| 3 | 19.11 | 370082 |
| 4 | 19.10 | 369829 |
| 5 | 19.09 | 369940 |
| | **Average** | 369855 |
| | **%RSD** | 0.046 |

## Linearity and range

### (a) Linearity of terbutaline sulfate

The linearity of the HPLC method was demonstrated for terbutaline sulfate solutions ranging from 0.015mg/ml to 0.045mg/ml, which is equivalent to 50% to 150% of the working strength. The standard solutions at five different concentrations within the mentioned range are prepared and analyzed.

Thus for terbutaline sulfate, linearity exists in the concentration range of 0.0150 to 0.04mg/ml with a regression coefficient of 0.9999.

### (b) Linearity of guaifenesin

The linearity of the HPLC method was demon-

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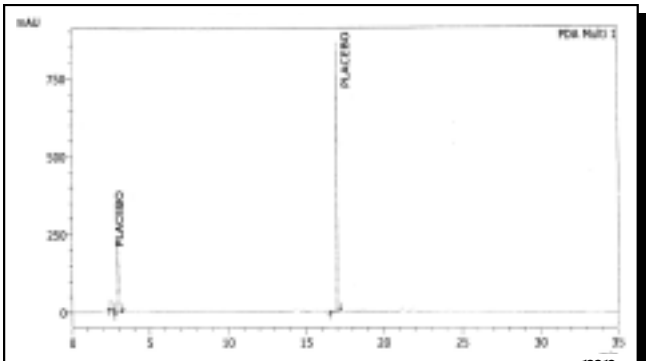


Figure 6: Chromatogram of placebo

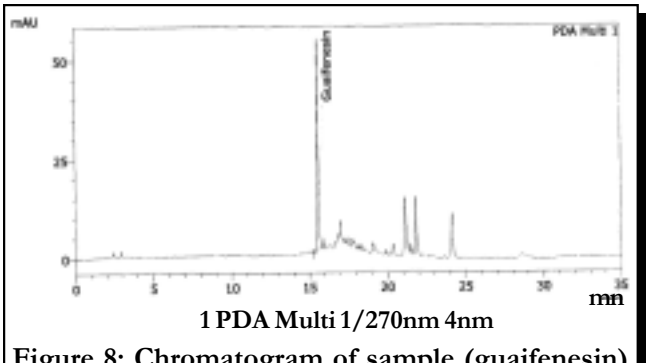


Figure 8: Chromatogram of sample (guaifenesin)

TABLE 8: Method precision

| S. No. | Terbutaline sulfate | Guaifenesin | Bromhexine hydrochloride |
|---|---|---|---|
| 1 | 101.94 | 100.49 | 102.46 |
| 2 | 99.66 | 100.85 | 102.58 |
| 3 | 99.37 | 100.90 | 102.01 |
| 4 | 101.41 | 101.29 | 101.64 |
| 5 | 102.26 | 100.87 | 99.34 |
| Mean(%) | 100.92 | 100.88 | 101.60 |
| %RSD | 1.31 | 0.28 | 1.29 |

strated for guaifenesin solutions ranging from 0.005mg/ml to 0.015mg/ml, which is equivalent to 50% to 150% of the guaifenesin working strength. Five standard solutions at various concentrations within the mentioned range are prepared and analyzed. Thus for guaifenesin linearity exists in the range of 0.005 to 0.015mg/ml with a regression coefficient of 1.0.

### (c) Linearity of bromhexine hydrochloride

The linearity of the HPLC method was demonstrated for bromhexine hydrochloride solutions ranging from 0.02mg/ml to 0.06mg/ml, which is equivalent to 50% to 150% of the bromhexine hydrochloride working strength. Five standard solutions at various concentrations are prepared within the above mentioned range and analyzed as per the method.

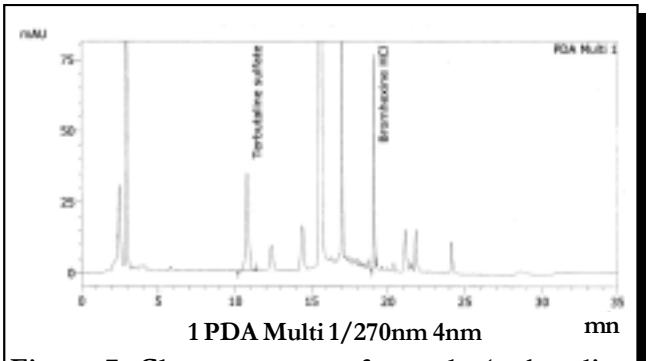


Figure 7: Chromatogram of sample (terbutaline sulfate and bromhexine hydrochloride)

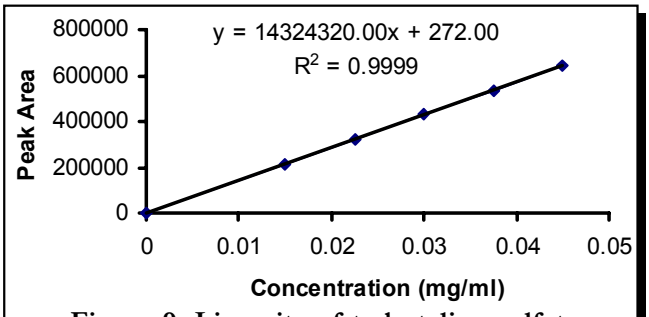


Figure 9: Linearity of terbutaline sulfate

TABLE 9: Linearity

| Sample | Linearity range (mg/ml) |
|---|---|
| Terbutaline sulfate | 0.015 to 0.045 |
| Guaifenesin | 0.005 to 0.015 |
| Bromhexine hydrochloride | 0.02 to 0.06 |

Thus for bromhexine hydrochloride linearity exists in the range of 0.02 to 0.06mg/ml with a regression coefficient of 0.9994. The linearity results for the three drugs are tabulated in TABLE 9.

### Accuracy

Accuracy of the method is determined by analyzing three solutions containing terbutaline sulfate, guaifenesin and bromhexine hydrochloride at approximately 50%, 100% and 150% of the working strengths spiked with placebo. Each level was analyzed. The percentage recovery results obtained are listed in(TABLE 10-12) for terbutaline sulfate, guaifenesin and bromhexine hydrochloride, respectively. For chromatograms at 50, 100 and 150% levels, (Figure 12-14).

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TABLE 10: Accuracy of terbutaline sulfate

| % Level of TERB working strength | Theoretical Conc. (mg/ml) | Measured Conc. (mg/ml) | % Recovery |
|---|---|---|---|
| 50 | 0.0157 | 0.0158 | 100.97 |
| 100 | 0.0370 | 0.0368 | 99.62 |
| 150 | 0.0462 | 0.0466 | 100.92 |

TABLE 11: Accuracy of guaifenesin

| % Level of GUAI working strength | Theoretical Conc. (mg/ml) | Measured Conc. (mg/ml) | % Recovery |
|---|---|---|---|
| 50 | 0.00620 | 0.00625 | 100.81 |
| 100 | 0.0127 | 0.0128 | 100.78 |
| 150 | 0.0189 | 0.0190 | 100.52 |

TABLE 12: Accuracy of bromhexine hydrochloride

| % Level of BROM working strength | Theoretical Conc. (mg/ml) | Measured Conc. (mg/ml) | % Recovery |
|---|---|---|---|
| 50 | 0.0209 | 0.0211 | 101.34 |
| 100 | 0.0402 | 0.0401 | 99.98 |
| 150 | 0.0613 | 0.0613 | 100.03 |

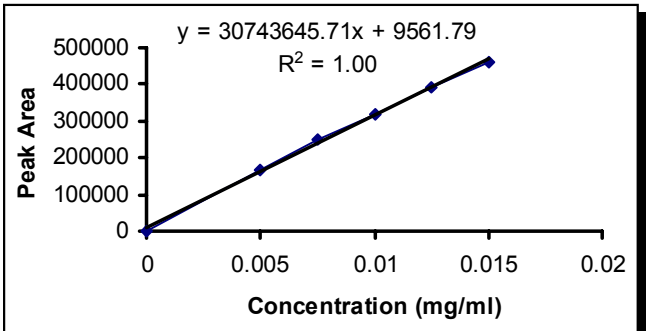


Figure 10: Linearity of guaifenesin

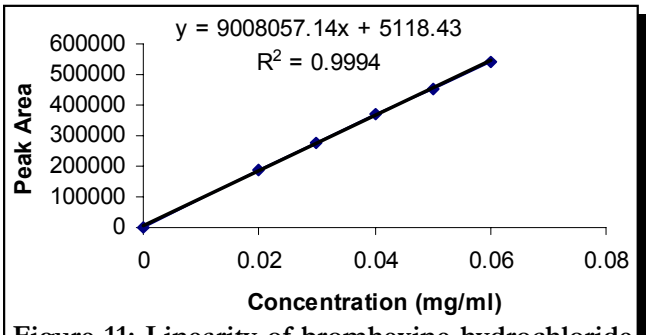


Figure 11: Linearity of bromhexine hydrochloride

## Ruggedness (Intermediate precision)

The method ruggedness is assessed by evaluating the variability of the results obtained with the analysis of two samples of syrup by different analyst on different days with different instruments. Results are summarized in(TABLE 13) & (Figure 15-18).

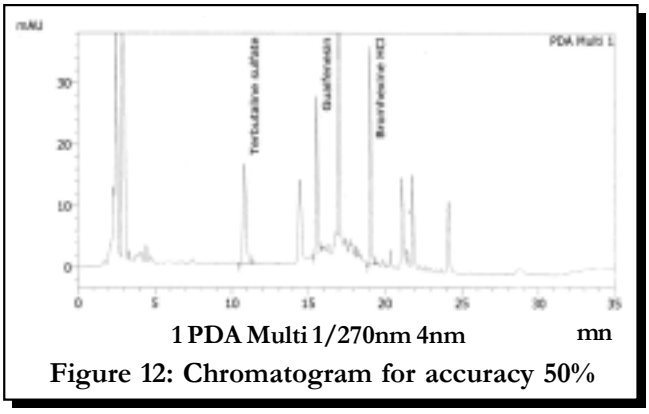


Figure 12: Chromatogram for accuracy 50%

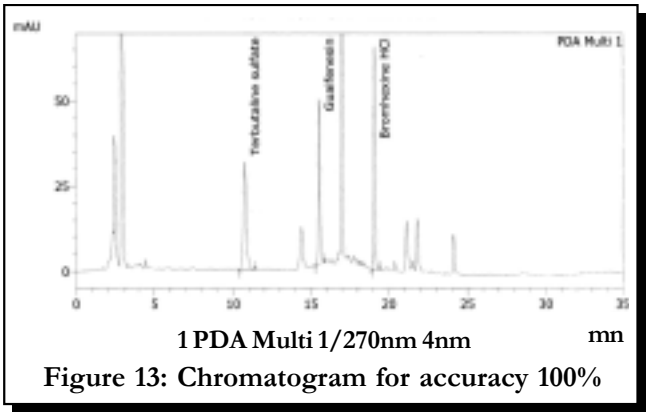


Figure 13: Chromatogram for accuracy 100%

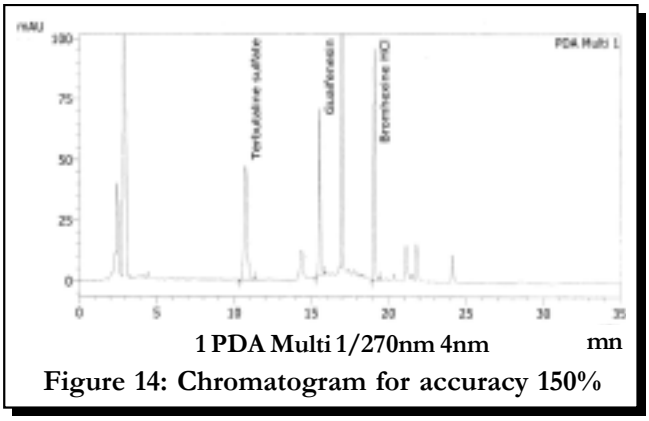


Figure 14: Chromatogram for accuracy 150%

## Robustness

To test the robustness the parameters of the method are altered. System suitability is performed along with the sample analysis to assess if these changes had a significant effect on the chromatography and assay values. The parameters altered for robustness testing are listed in (TABLE 14). The results obtained for testing robustness are in (TABLE 15-19) and (Figure 19-24).

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**TABLE 13: Ruggedness**

| Parameter | Sample | | Assay value |
|---|---|---|---|
| Analyst(1 )-Day(1)-Instrument(1 ) | Sample-1 | Terbutaline sulfate | 100.30 |
| | | guaifenesin | 99.74 |
| | | bromhexine HCl | 101.73 |
| | Sample-2 | Terbutaline sulfate | 100.16 |
| | | guaifenesin | 102.74 |
| | | bromhexine HCl | 101.70 |
| Analyst(2) | Sample-1 | Terbutaline sulfate | 99.93 |
| | | guaifenesin | 99.23 |
| | | bromhexine HCl | 100.21 |
| | Sample-2 | Terbutaline sulfate | 99.94 |
| | | guaifenesin | 104.84 |
| | | bromhexine HCl | 100.28 |
| Day(2) | Sample-1 | Terbutaline sulfate | 99.19 |
| | | guaifenesin | 101.12 |
| | | bromhexine HCl | 100.03 |
| | Sample-2 | Terbutaline sulfate | 99.13 |
| | | guaifenesin | 102.19 |
| | | bromhexine HCl | 99.66 |
| Instrument(2) | Sample-1 | Terbutaline sulfate | 101.60 |
| | | guaifenesin | 100.28 |
| | | bromhexine HCl | 102.30 |
| | Sample-2 | Terbutaline sulfate | 99.97 |
| | | guaifenesin | 100.54 |
| | | bromhexine HCl | 100.87 |
| %RSD | | Terbutaline sulfate | 0.76 |
| | | guaifenesin | 1.81 |
| | | bromhexine HCl | 0.94 |

**Instrument 1: Shimadzu Prominence**
**Instrument 2: HPLC Agilent 1100 Series**

## Solution stability

A solution of sample at 100% of working concentration was kept at room temperature. The solutions are analyzed at different intervals like initial, after 24hrs and after 48hrs. The percentage relative standard deviation for the assay values at each time interval are calculated and the results obtained are tabulated in(TABLE 20).

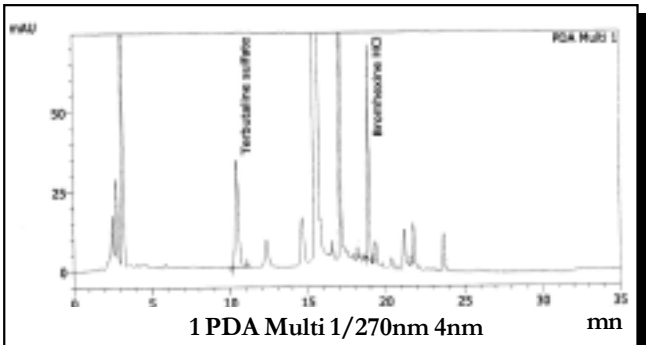


**Figure 15: Chromatogram for ruggedness-instrument 1 (terbutaline sulfate & bromhexine hydrochloride)**

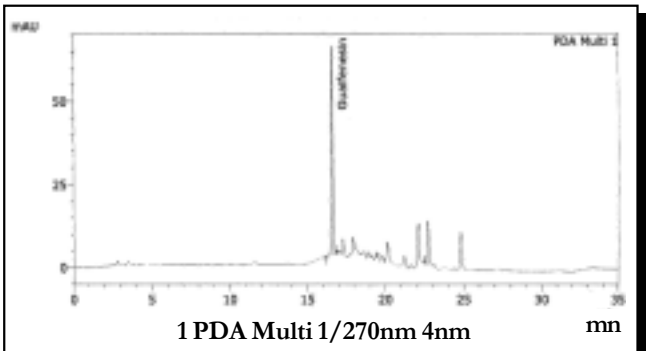


**Figure 16: Chromatogram for ruggedness-instrument 1 (guaifenesin)**

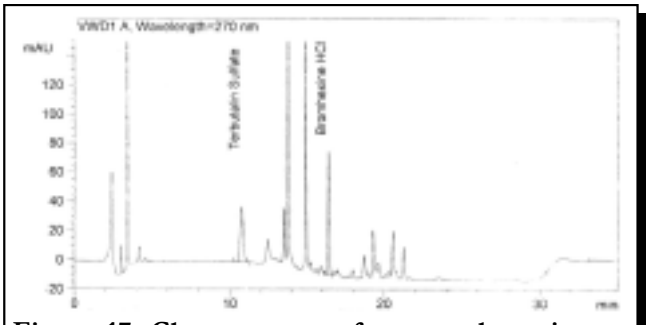


**Figure 17: Chromatogram for ruggedness-instrument 2 (terbutaline sulfate & bromhexine hydrochloride)**

**TABLE 14: Parameters altered to test method robustness**

| Parameter | Actual | Low level | High level |
|---|---|---|---|
| Flow rate | 1.00ml/min | 0.90ml/min | 1.10ml/min |
| Buffer composition | 85% | 83% | 87% |
| pH of buffer | 3.0 | 2.9 | 3.1 |

## Limits of measurement

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### Limit of detection (LOD)

The limit of detection is determined by calculating the signal to noise ratio and by comparing test results from samples with known concentrations of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. The result obtained for each active ingredient is listed in (TABLE 21) (Figure 25).

**TABLE 15: Data for retention time in robustness study**

| Parameter condition | Retention time | | |
|---|---|---|---|
| | TERB | GUAI | BROM |
| Actual | 10.4 | 15.5 | 18.9 |
| Flow rate (0.90 ml/min) | 11.5 | 16.5 | 19.6 |
| Flow rate (1.10 ml/min) | 9.5 | 14.6 | 18.2 |
| Buffer (83%) | 7.7 | 12.7 | 18.7 |
| Buffer (87%) | 15.0 | 16.3 | 18.9 |
| Buffer pH (low 2.9) | 12.3 | 16.0 | 18.8 |
| Buffer pH (high 3.1) | 10.4 | 15.5 | 18.8 |

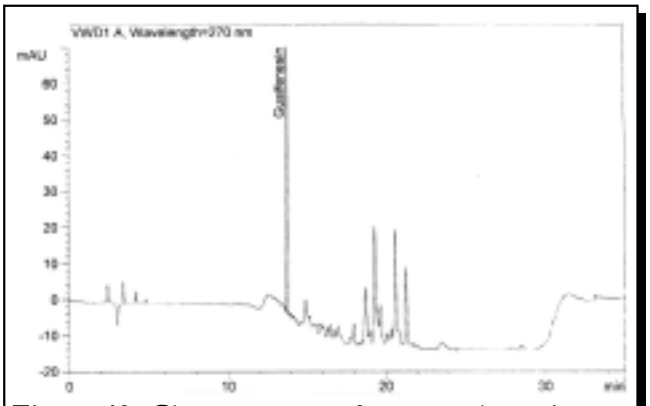


**Figure 18: Chromatogram for ruggedness-instrument 2 (guaifenesin)**

**TABLE 16: Data for robustness study (Variation of flow)**

| Parameter condition | | Retention time | | | Area | | | USP plate count | | | Tailing factor | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Flow rate | | TERB | GUAI | BROM | TERB | GUAI | BROM | TERB | GUAI | BROM | TERB | GUAI | BROM |
| 1.00 ml/min | | 10.4 | 15.5 | 18.9 | 487886 | 329467 | 390818 | 12387 | 80952 | 226212 | 1.24 | 1.14 | 1.55 |
| | | 10.4 | 15.5 | 18.9 | 485764 | 324613 | 389140 | 12401 | 81363 | 228202 | 1.24 | 1.15 | 1.56 |
| | %RSD | | | | 0.31 | 1.05 | 0.30 | | | | | | |
| 0.90 ml/min | | 11.5 | 16.5 | 19.6 | 532296 | 411830 | 445019 | 12650 | 11948 | 203752 | 1.27 | 1.22 | 1.54 |
| | | 11.5 | 16.5 | 19.6 | 532681 | 411870 | 445272 | 12672 | 11961 | 203263 | 1.27 | 1.22 | 1.56 |
| | %RSD | | | | 0.05 | 0.01 | 0.04 | | | | | | |
| 1.10 ml/min | | 9.5 | 14.6 | 18.2 | 436433 | 310993 | 366668 | 11387 | 50957 | 219536 | 1.27 | 1.15 | 1.85 |
| | | 9.5 | 14.6 | 18.2 | 436359 | 311177 | 366398 | 11365 | 51472 | 220254 | 1.27 | 1.15 | 1.86 |
| | %RSD | | | | 0.01 | 0.04 | 0.05 | | | | | | |

**TABLE 17: Data for robustness study (Variation of buffer composition)**

| Parameter condition | | Retention time | | | Area | | | USP plate count | | | Tailing factor | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Buffer composition | | TERB | GUAI | BROM | TERB | GUAI | BROM | TERB | GUAI | BROM | TERB | GUAI | BROM |
| 85% | | 10.4 | 15.5 | 18.9 | 487886 | 329467 | 390818 | 12387 | 80952 | 226212 | 1.24 | 1.14 | 1.55 |
| | | 10.4 | 15.5 | 18.9 | 485764 | 324613 | 389140 | 12401 | 81363 | 228202 | 1.24 | 1.15 | 1.56 |
| | %RSD | | | | 0.31 | 1.05 | 0.30 | | | | | | |
| 83% | | 7.7 | 12.7 | 18.7 | 464553 | 318652 | 400541 | 12923 | 16871 | 237052 | 1.25 | 1.19 | 1.73 |
| | | 7.7 | 12.7 | 18.7 | 462760 | 318309 | 399319 | 12852 | 16774 | 239385 | 1.25 | 1.19 | 1.74 |
| | %RSD | | | | 0.27 | 0.08 | 0.22 | | | | | | |
| 87% | | 15.0 | 16.3 | 18.9 | 479941 | 328410 | 378483 | 32329 | 27118 | 254577 | 1.39 | 1.34 | 1.52 |
| | | 15.0 | 16.3 | 18.9 | 488851 | 329374 | 379529 | 31614 | 38022 | 254398 | 1.34 | 1.33 | 1.52 |
| | %RSD | | | | 1.30 | 0.21 | 0.20 | | | | | | |

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**TABLE 18: Data for robustness study (Variation of buffer pH)**

| Parameter condition | | Retention time | | | Area | | | USP plate count | | | Tailing factor | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | | TERB | GUAI | BROM | TERB | GUAI | BROM | TERB | GUAI | BROM | TERB | GUAI | BROM |
| | | 10.4 | 15.5 | 18.9 | 487886 | 329467 | 390818 | 12387 | 80952 | 226212 | 1.24 | 1.14 | 1.55 |
| 3.0 | | 10.4 | 15.5 | 18.9 | 485764 | 324613 | 389140 | 12401 | 81363 | 228202 | 1.24 | 1.15 | 1.56 |
| | %RSD | | | | 0.31 | 1.05 | 0.30 | | | | | | |
| | | 12.3 | 16.0 | 18.8 | 467988 | 318704 | 415138 | 14332 | 19332 | 258957 | 1.24 | 1.23 | 2.26 |
| 2.9 | | 12.3 | 16.0 | 18.8 | 464580 | 323664 | 415884 | 14144 | 19757 | 254896 | 1.24 | 1.23 | 2.26 |
| | %RSD | | | | 0.52 | 1.09 | 0.13 | | | | | | |
| | | 10.4 | 15.5 | 18.8 | 478725 | 309113 | 390860 | 14162 | 87137 | 233649 | 1.24 | 1.13 | 1.83 |
| 3.1 | | 10.5 | 15.5 | 18.8 | 478289 | 308489 | 407452 | 14228 | 85430 | 227765 | 1.24 | 1.13 | 1.85 |
| | %RSD | | | | 0.06 | 0.14 | 1.94 | | | | | | |

**TABLE 19: Data for retention time and assay values of active ingredients in robustness study**

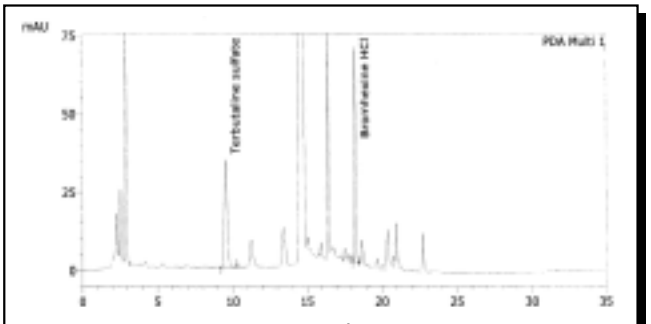
| Parameter condition | Retention time | %Assay |
|---|---|---|
| Actual | | |
| TERB | 10.4 | 100.30 |
| GUAI | 15.5 | 99.74 |
| BROM | 18.9 | 101.73 |
| Low flow (0.90 ml/min) | | |
| TERB | 11.5 | 98.84 |
| GUAI | 16.5 | 99.32 |
| BROM | 19.6 | 101.57 |
| High flow (1.10 ml/min) | | |
| TERB | 9.5 | 98.84 |
| GUAI | 14.6 | 101.33 |
| BROM | 18.2 | 98.05 |
| Low Buffer (83%) | | |
| TERB | 7.7 | 98.51 |
| GUAI | 12.7 | 98.53 |
| BROM | 18.7 | 101.75 |
| High Buffer (87%) | | |
| TERB | 15.0 | 102.0 |
| GUAI | 16.3 | 101.11 |
| BROM | 18.9 | 101.64 |
| Buffer pH (Low 2.9) | | |
| TERB | 12.3 | 98.38 |
| GUAI | 16.0 | 98.61 |
| BROM | 18.8 | 101.94 |
| Buffer pH (High 3.1) | | |
| TERB | 10.4 | 100.42 |
| GUAI | 15.5 | 100.0 |
| BROM | 18.8 | 101.60 |

**TABLE 20: Solution stability**

| Sample | % Assay | | | |
|---|---|---|---|---|
| | Initial | After 24hrs | After 48hrs | %RSD |
| TERB | 101.94 | 100.30 | 98.84 | 1.55 |
| GUAI | 100.49 | 99.74 | 101.37 | 0.81 |
| BROM | 102.46 | 101.73 | 101.49 | 0.50 |

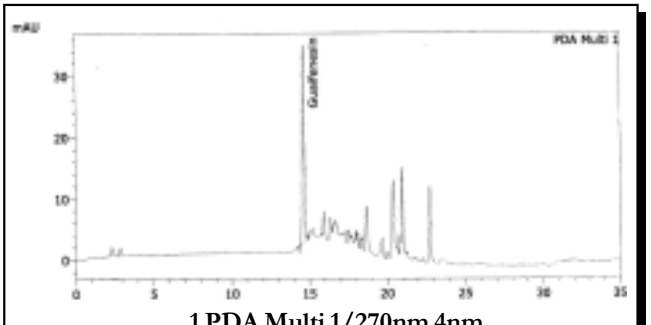
**TABLE 21: Limits of measurement**

| Active ingredients | LOD (ng/ml) | LOQ (ng/ml) |
|---|---|---|
| Terbutaline sulfate | 462 | 768 |
| Guaifenesin | 150 | 250 |
| Bromhexine HCl | 604 | 1008 |




1 PDA Multi 1/270nm 4nm

**Figure 19: Chromatogram for robustness- varied flow rate (terbutaline sulfate & bromhexine hydrochloride)**




1 PDA Multi 1/270nm 4nm

**Figure 20: Chromatogram for robustness-varied flow rate (guaifenesin)**

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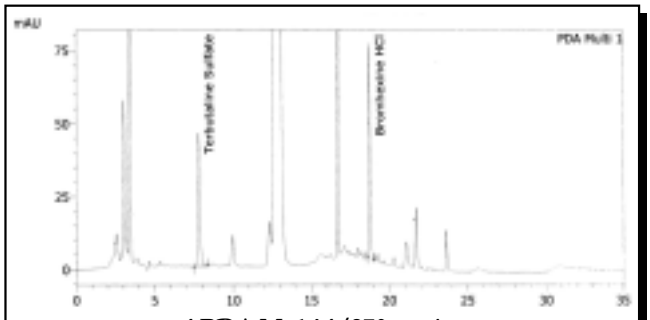
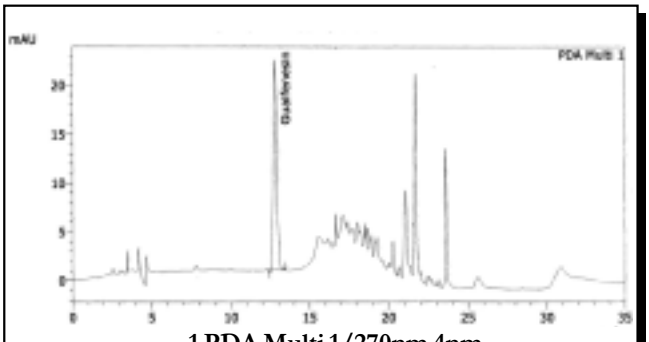


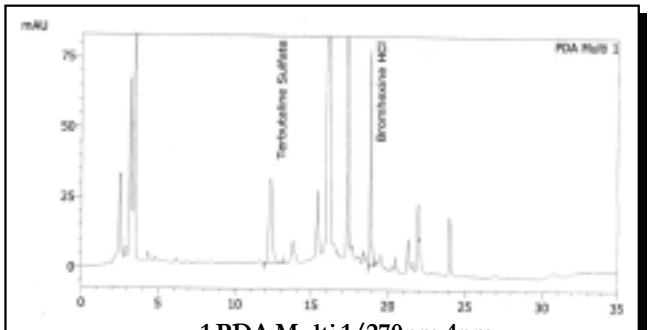

1 PDA Multi 1/270nm 4nm

**Figure 21: Chromatogram for robustness-varied buffer composition (terbutaline sulfate & bromhexine hydrochloride)**



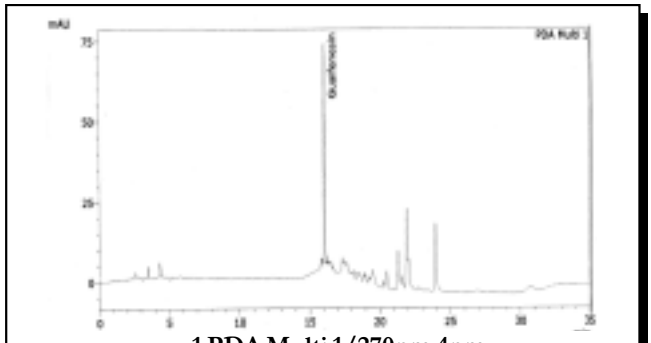

1 PDA Multi 1/270nm 4nm

**Figure 22: Chromatogram for robustness-varied buffer composition (guaifenesin)**




1 PDA Multi 1/270nm 4nm

**Figure 23: Chromatogram for robustness-varied ph (terbutaline sulfate & bromhexine hydrochloride)**




1 PDA Multi 1/270nm 4nm

**Figure 24: Chromatogram for robustness-varied ph (guaifenesin)**

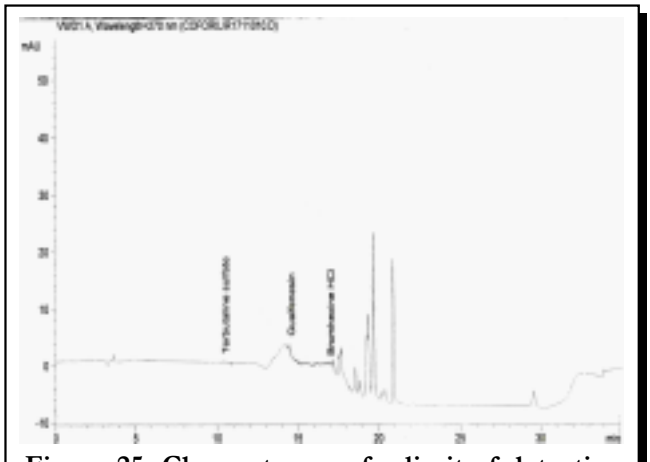



**Figure 25: Chromatogram for limit of detection**

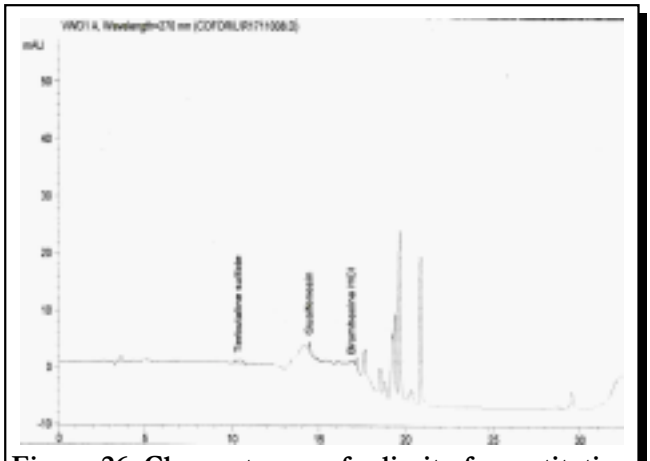



**Figure 26: Chromatogram for limit of quantitation**

## Limit of quantitation (LOQ)

Limit of quantitation value is determined by calculating the signal to noise ratio and by comparing test results from samples with known concentrations of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably quantitated. The result obtained for each active ingredient is listed in(TABLE 21). (Figure 26).

## CONCLUSIONS

The proposed HPLC method, employed for the estimation of terbutaline sulfate, guaifenesin and bromhexine hydrochloride is simple and reliable. The method was validated and the results obtained were within acceptable limits. The system suitability parameters were found to have acceptable values. The

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determination of terbutaline sulfate, guaifenesin and bromhexine hydrochloride by HPLC analysis yielded well- resolved peaks, excellent recovery(>99.0%) and good precision(%R.S.D <2.0% ). The method is also found to be rugged, robust and stable, with the values of percentage relative standard deviation not more than 2%.

This method can be successfully applied for the identification and quantitative estimation of terbutaline sulfate, guaifenesin and bromhexine hydrochloride in syrup formulations.

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## REFERENCES

[1] C.H.Sun, L.Liu, Q.Yin; Yao Xue Bao, **36(5)**, 368-72 **(2001)**.
[2] N.Daraghmeh, M.M.Al-Omari, Z.Sara, A.A.Badwan, A.M.Jaber; Journal of Pharm.Biomed.Anal., **29(5)**, 927-37 **(2002)**.
[3] K.H.Kim, H.J.Kim, J.H.Kim, S.D.Shin; Journal of Chromatogr.B.Biomed.Sci.Appl., **751(1)**, 69-77 **(2001)**.
[4] K.H.Kim, H.J.Kim, S.P.Hong, S.D.Shin; Arch.Pharm. Res., **23(5)**, 441-445 **(2000)**.
[5] V.L.Herring, J.A.Johnson; Journal of Chromatogr. B.Biomed.Sci.Appl., **741(2)**, 307-12 **(2000)**.
[6] K.H.Kim, D.S.Kim, S.P.Hong, O.S.Keon; Arch. Pharm.Res., **23(1)**, 26-30 **(2000)**.
[7] G.A.Jacobson, G.M.Peterson; Journal of Pharm. Biomed.Anal., **12(6)**, 825-32 **(1994)**.
[8] K.A.Sagar, M.T.Kelly, M.R.Smyth; Biomed.Chromatogaphy, **7(1)**, 29-33 **(1993)**.
[9] I.H.Habib, M.E.Hassouna, G.A.Zaki; Farmaco, **60(3)**, 249-54 **(2005)**.
[10] G.Bazylak, L.J.Nagels; Journal of Pharm.Biomed. Anal., **32(4-5)**, 887-903 **(2003)**.
[11] V.Tantishaiyakul, C.Poeaknapo, P.Sribun, K.Sirisuppanon; Journal of Pharm.Biomed.Anal. **17(2)**, 237-43 **(1998)**.
[12] J.P.Rauha, H.Salomies, M.Aalto; Journal of Pharm. Biomed.Anal., **15(2)**, 287-93 **(1996)**.
[13] M.Johansson, S.Lenngren; Journal of Chromatogr., **432**, 243-52 **(1988)**.
[14] V.Galli, C.Barbas; Journal of Chromatogr.A., **1048(2)**, 207-11 **(2004)**.
[15] M.H.Abdel-Hay, M.S.El-Din, M.A.Abuirjeie; Analyst, **117(2)**, 157-60 **(1992)**.
[16] T.M.Chen, J.R.Pacifico, R.E.Daly; Journal of Chromatogr.Sci., **26(12)**, 636-9 **(1988)**.
[17] L.Carnevale; Journal of Pharm.Sci., **72(2)**, 196-8 **(1983)**.
[18] W.O.McSharry, I.V.Savage; Journal of Pharm.Sci., **69(2)**, 212-4 **(1980)**.
[19] V.D.Gupta, A.G.Ghanekar; Journal of Pharm.Sci., **66(6)**, 895-7 **(1977)**.
[20] S.Gangwal, P.Trivedi; Indian Journal Pharmaceutical Sciences, **61(2)**, 128-130 **(1999)**.
[21] Validation of Analytical Procedures/Methodology, ICH hormonised triplicate guidelines, 1-8 **(1996)**.
[22] K.P.R.Chowdary, G.Himabindu; 'Validation of Analytical Methods', Eastern Pharmacist, 39-41 **(1999)**.
[23] S.K.Sharma; 'Validation of Pharmaceutical Products and process', The Eastern Pharmacist, 21 -23, **(2001)**.