

Development and Validation of RP-HPLC Method for The Simultaneous Estimation of Amoxicillin Trihydrate and Bromhexine Hydrochloride from Oily Suspension

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Abstract

A new simple, rapid and precise reverse phase high pressure liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of amoxicillin trihydrate and bromhexine hydrochloride from oily suspension. An ODS C₁₈ (250 X 4.5mm ID), 5 μ particle size with mobile phase methanol and glacial acetic acid (50:50 v/v) were used. The flow rate was 1.0ml/min and responses were measured at 254 nm. The retention time for amoxicillin trihydrate and bromhexine hydrochloride were observed at 3.04 and 8.18 min. respectively. Linearity for amoxicillin trihydrate and bromhexine hydrochloride were in the range of 8-50 mcg/ml and 5-25 mcg/mL respectively. Percent recovery was 99.54% and 98.65% for amoxicillin trihydrate and bromhexine hydrochloride respectively. The proposed method can be applied for the routine analysis of amoxicillin trihydrate and bromhexine hydrochloride in combination.

Keywords: RP-HPLC; Amoxicillin trihydrate; Bromhexine hydrochloride; Oily suspension

Introduction

Amoxicillin trihydrate is official in the IP [1], BP [2] and the USP [3], which is 6-(D-4hydroxyphenylglycylamino) penicillanic acid trihydrate chemically. It is generally used as antibacterial whereas bromhexine hydrochloride is official in the IP [1], BP [2] & USP [3] and used as bronchodilator. The pharmacopoeias describe non-aqueous potentiometric titration methods for the determination of the drugs from bulk drug. No RP-HPLC method is reported for the simultaneous estimation of amoxicillin trihydrate and bromhexine hydrochloride from their combined formulation; though there are various methods for the determination of amoxicillin trihydrate by spectrophotometric [4,5] and bromhexine hydrochloride by spectrophotometric [6,7], simultaneous estimation by HPLC [8,9]. The aim of this research work is the development of a simple, rapid and precise RP-HPLC method for the simultaneous estimation of amoxicillin trihydrate and bromhexine hydrochloride from oily suspension.

Material and Methods

Chemicals

Oily suspension containing Amoxicillin trihydrate I. P., 125 mg and Bromhexine hydrochloride I.P., 4 mg per 5 ml was procured from the market. Methanol, HPLC grade (Rankem), HPLC grade water, Glacial acetic acid and phosphoric acid AR grade were obtained from S.D. Fine chem. Mumbai.

Instrument and conditions

Chromatographic separation was achieved using High Performance Liquid Chromatograph, JASCO equipped with pump PU 980, universal injector (Rheodyne) with injection volume of 20 mL, U.V. / Visible detector U.V.975, Borwin software. Intersil C₁₈ Column (250 x 4.6 mm) 5 μ particle size was used as the stationary phase. SHANUPRO software was used for calculation. The analysis was carried out at room temperature and the flow rate maintained at 1.0ml/min. The column effluent was monitored at 254nm.

Mobile phase

Mobile Phase consisted of Methanol and glacial acetic acid (1%w/w) in the proportion of 50:50 was prepared and pH adjusted to 3.0 with phosphoric acid. Mobile phase was filtered through 0.45 μm membrane filter and degassed.

- Preparation of Standard Solution: 100mg of amoxicillin trihydrate was accurately weighed, transferred in 100mL volumetric flask and 25mL of mobile phase was added, sonicated for 15min. and diluted up to mark with mobile phase.
- 100mg of bromhexine hydrochloride was accurately weighed, transferred in 100mL volumetric flask and 25mL of mobile phase was added, sonicated for 15min. and diluted up to mark with mobile phase.

Preparation of working standard

25 mL of solution (a) and 0.8 mL of solution (b) were taken in 50 mL volumetric flask and mixed well. The volume was made up to the mark with mobile phase to get the concentration of 25 mg/mL and 0.016 mg/mL of Amoxicillin trihydrate I. P and Bromhexine hydrochloride I.P. respectively. The resultant solution was filtered through 0.2 μm membrane filter paper.

Preparation of sample solution

Accurately measured 5 mL of oily suspension (uniformly dispersed) was taken in 100 mL separating funnel. The content of oily suspension was extracted with 2 x 25 mL of mobile phase. The aqueous part was transferred to 250 mL volumetric flask and volume was made up to mark with mobile phase. The resultant solution was filtered through 0.2 μm membrane filter paper.

Assay

The working standard solution and sample solution were injected (20 μL) through Rheodyne injector of liquid chromatograph and

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peaks were recorded. The amount of drug samples were calculated from the peak area (response factor) obtained.

Recovery

The analytical recovery of amoxicillin trihydrate and bromhexine hydrochloride was determined at concentrations of 100,150 and 200 mcg/mL. The test solution was spiked with known amounts of the drug to achieve the concentration previously specified. These samples were processed by the analytical method described above and peak heights were compared with the peak heights obtained by direct injection of the drugs in the mobile phase. The linearity study was carried out in the range of 50 to 250 mcg/mL and 25 to 200 mcg/mL.

Validation of method

As per ICH [10] guideline the method is validated and following parameters were evaluated.

Accuracy

Accuracy of the method was checked by assaying the samples using analyte recovery method. These samples were processed by the analytical method described above and peak areas were compared with the peak areas obtained by direct injection of the drugs in the mobile phase. The analytical recovery of amoxicillin trihydrate and bromhexine hydrochloride was determined at concentrations of 100,150 and 200 mcg/mL.

Precision

Precision of the method was studied by analysis of multiple samplings of homogeneous sample and expressed as CV. It was demonstrated by intra-day and inter-day variation studies.

Ruggedness and robustness

Ruggedness of the method was determined by carrying out the experiment of different instruments by different analyst and on different days, showed that the method was rugged. Robustness of the method was determined by making slight changes in chromatographic conditions.

Result and Discussion

Optimization of chromatographic method

The mobile phase composing methanol and glacial acetic acid (1%w/w) in the proportion of 50:50 was prepared and pH adjusted

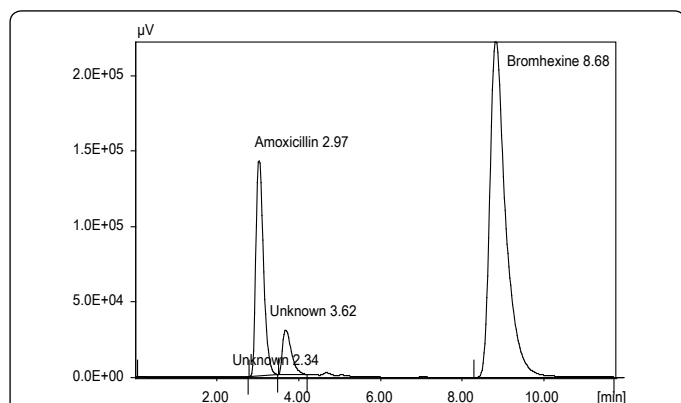


Figure 1: Typical Chromatogram of Test Sample Peak Amoxicillin – Amoxicillin trihydrate ($t_r = 2.97$ mins) Peak Bromhexine – Bromhexine hydrochloride ($t_r = 8.68$ mins).

| Formulation | Label Claim(mg) | % of Amoxicillin trihydrate | CV | % of Bromhexine hydrochloride | | CV |
|-----------------|------------------------|-----------------------------|--------|-------------------------------|-------|------|
| | Amoxicillin trihydrate | Bromhexine hydrochloride | | | | |
| Oily suspension | 125 | 4 | 100.24 | 0.64 | 99.41 | 0.85 |

CV- Coefficient of variation

Table 1: Results of HPLC assay.

| Sr. No. | Conc. of added drug in Pre analyzed formulation mcg/mL | | Recovery of added drug mcg/mL | | % Recovery | |
|---------|--|----------------|-------------------------------|----------------|------------------|----------------|
| | Amox. trihydrate | Bromhexine Hcl | Amox. trihydrate | Bromhexine Hcl | Amox. trihydrate | Bromhexine Hcl |
| 1. | 5 | 5 | 5.03 | 4.99 | 100.52 | 99.80 |
| 2. | 10 | 10 | 9.87 | 9.75 | 98.69 | 97.50 |
| 3. | 15 | 15 | 14.72 | 14.82 | 98.14 | 98.80 |

Table 2: Results of recovery study.

| Sr. No. | Parameter | Amox. trihydrate | Bromhexine Hcl |
|---------|---------------------------|------------------|----------------|
| 1 | Capacity Factor (K') | 674.25 | 984.5 |
| 2 | Resolution (Rs) | 8.39 | 11.14 |
| 3 | Tailing Factor (T) | 1.58 | 1.79 |
| 4 | Theoretical Plate(N) | 3646.35 | 4281.51 |
| 5 | Calibration Range(mcg/ml) | 50-250 | 50-200 |
| 6. | % RSD | 1.7349 | 1.6542 |

Table 3: System suitability parameters.

to 3.0 with phosphoric acid showed good resolution peaks within a short run time. The typical chromatograph is shown in Figure 1.

Linearity

The amoxicillin trihydrate shows the linearity in the range of 50 to 250mc/mL and bromhexine hydrochloride 25 - 200mc/mL. An excellent correlation was observed in the peak area and the concentration of amoxicillin trihydrate and bromhexine hydrochloride.

Assay and recovery

The Table 1 shows assay results which indicate that method is precise and accurate. The results of recovery study given in Table 2 confirm the accuracy of the method. The proposed RP-HPLC method is accurate, simple, rapid and selective for the simultaneous estimation of amoxicillin trihydrate and bromhexine hydrochloride in oily suspension form. Hence it can be conveniently adopted for the routine quality control analysis of the oily suspension.

Validation of Method

As per the current regulatory requirements, the validation of the developed method was carried out by studying different system suitability parameters, respectively for amoxicillin trihydrate and bromhexine hydrochloride were: resolution, tailing factor, theoretical plates, % RSD and capacity factor. Resolution between the two components should be more than 3.0, tailing factor should be less than 2 and theoretical plates should be more than 2000. It is evident from table 3 that method developed for these two drugs in combination is passing the standards of regulatory requirements.

Conclusion

The newly developed method for the simultaneous estimation of amoxicillin trihydrate and bromhexine hydrochloride was found to be precise and accurate with low values of coefficient of variation. Hence it can be conveniently adopted for the routine quality control analysis of the oily suspension.

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