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Simultaneous determination of amoxicillin trihydrate and bromhexine hydrochloride in pharmaceutical dosage by reverse phase high performance liquid chromatography

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ABSTRACT

Rapid and accurate high performance liquid chromatography method is described for simultaneous determination of amoxicillin trihydrate and bromhexine hydrochloride from the combine formulation i.e. capsules. The separation of two drugs was achieved on a ZORBAX Eclips XDB C18 (150 x 4.6 mm i.d.) 5 µ particle size. The mobile phase consisted of methanol, water and triethylamine in the ratio of 50: 50: 0.1(v/v). The pH was adjusted to 3.0 with orthophosphoric acid. The detection was carried out at wavelength 248 nm. The ZORBAX Eclips XDB column showed the most favorable chromatographic parameters for analysis. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of solution. The linear range for amoxicillin trihydrate and bromhexine hydrochloride was 625 - 1875 µg / ml and 20 - 60 µg / ml respectively. The method has been successfully used to analyse commercial solid dosage i.e. capsules containing 250 mg of amoxicillin trihydrate and 8 mg. of bromhexine hydrochloride with good recoveries.

Keywords : Amoxicillin trihydrate, Bromhexine hydrochloride, RP – HPLC

INTRODUCTION

In this communication a new HPLC method is developed for assay of amoxicillin trihydrate and bromhexine hydrochloride in their combined dosage form. Amoxicillin trihydrate described chemically as 6 – (D – 4 hydroxy phenyl glycyl amino) penicillinic acid trihydrate. It is semi- synthetic penicillin that belongs to the class of β – lactam antibiotics. It is generally used as antibacterial. Bromhexine hydrochloride is 2,4 dibromo -6-{(cyclohexyl (methyl amino) methyl)} aniline hydrochloride. It is broncho-secretolytic and mucolytic. Bromhexine hydrochloride is used with antibiotics to enhance their efficiency in the treatment of respiratory infections.

Amoxicillin trihydrate is official in USP [1], IP [2] and BP [3]. Bromhexine hydrochloride is also official in IP [2] and BP [3]. Literature survey reveals HPLC [4], HPTLC [5], capillary electrophoresis [6] and spectrophotometric [7] methods for assay of combined dosage form. In this communication a new simple, rapid HPLC method is reported for simultaneous determination of amoxicillin trihydrate and bromhexine hydrochloride in combination dosage form. This simple method can also be used for the routine analysis of this combination formulation. In the proposed work development, optimization and validation of the method are presented.

MATERIALS AND METHODS

Reagents and chemicals

Reference standards of amoxicillin trihydrate and bromhexine hydrochloride were obtained from reputed firm with certificate of analysis. HPLC grade methanol from Qualigens fine chemicals was used for chromatographic procedure. Triethylamine and orthophosphoric acid were used of analytical reagent grade from S. D. fine chemicals, HPLC grade water was obtained using Millipore system. Standard and sample solutions were prepared in mobile phase i.e. methanol, water and triethylamine in the ratio of 50: 50: 0.1(v/v).

Chromatography apparatus and conditions

The HPLC system used was Water's Alliance HPLC system equipped with separation module and dual absorbance UV-detector (2487). The chromatogram was recorded and peaks quantified by means of PC based Empower 2 software.

Chromatographic separation was performed at ambient temperature on a reverse phase ZORBAX Eclips XDB C18 (150 x 4.6 mm id) 5 micron particle size. Mobile phase was consisted of methanol, water and triethylamine (50: 50: 0.1 v/v), the pH has been adjusted to 3.0 with orthophosphoric acid. The mobile phase was filtered and degassed before use. The flow rate of the mobile phase was adjusted to 1.0 ml /min. The detector wavelength was set at 248 nm. The injection volume of the standard and sample solutions was 20 µl.

Preparation of solutions

Preparation of Standard Solution

Standard Solution was prepared by transferring 125 mg of amoxicillin trihydrate and 4 mg of bromhexine hydrochloride in 100 ml volumetric flask and making volume with mobile phase to get concentration of 1250 µg / ml amoxicillin trihydrate and 40 µg / ml bromhexine hydrochloride.

Preparation of sample solution

Twenty capsules of the product under study were accurately weighed and removed powder blend from all capsules. A portion equivalent to 250 mg amoxicillin trihydrate and 8 mg bromhexine hydrochloride was weighed accurately. It was transferred into a 200 ml volumetric flask. It was dissolved in small quantity of mobile phase and further diluted to volume using mobile phase to get 1250 µg / ml of amoxicillin trihydrate and 40 µg / ml bromhexine hydrochloride solution. It was mixed and filtered through whatman filter paper no. 41. First few ml of the filtrate was discarded. The resulting solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Method Development

Different columns containing octyl and octadecyl silane stationary phase were tried for the separation and resolution. It was found that ZORBAX Eclips XDB C18 column offered more advantages over the ODS column. Individual drug solutions were injected into the column and elution pattern of both the drugs and resolution parameters were studied as a function of pH. In addition to this, UV spectra of individual drugs were recorded at the wavelength range from 200 to 300 nm and the response for optimization was compared. The choice of wavelength 248 nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity. The overlain spectra of two drugs are given in figure 1.

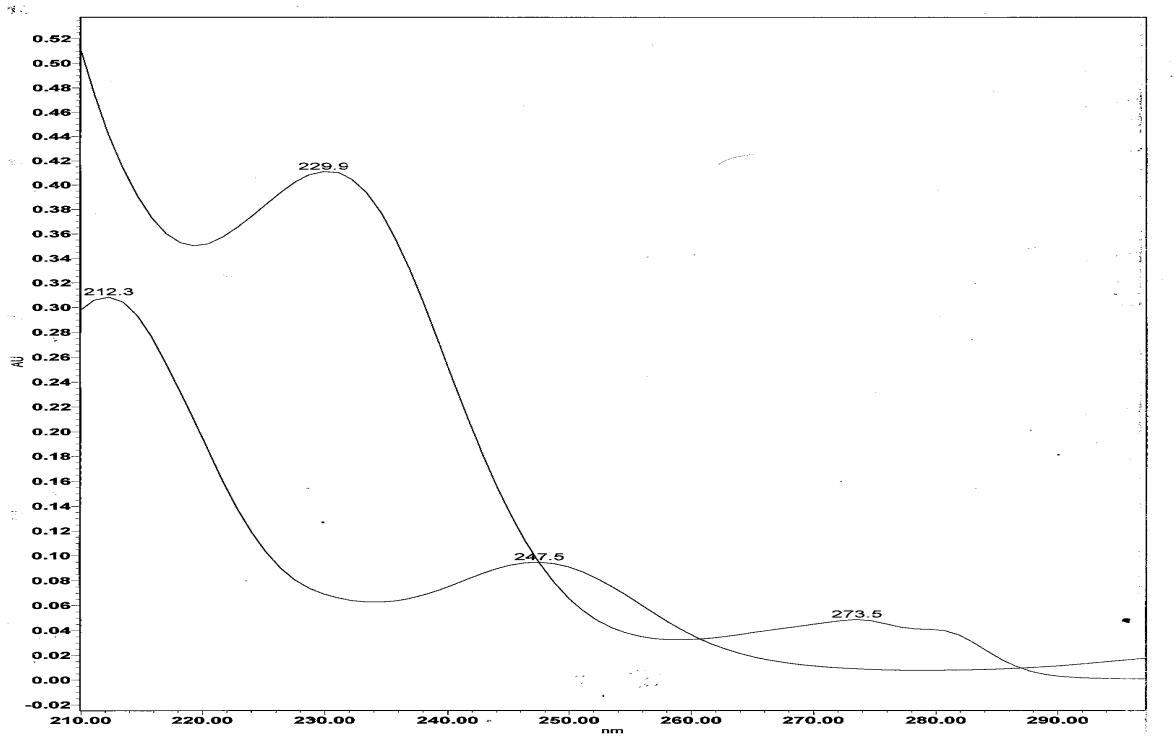
The pH effect showed that optimized conditions were reached at pH 3.0. It produces well-shaped peaks for both the drugs assayed. A typical chromatogram of the two drugs is given in figure 2. The relative chromatographic figures of merit are reported in table 1. The good chromatographic separation indicated that any of these drugs could be used as internal standard for assay of other drugs.

Method validation

System suitability

System performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates, tailing factor and relative standard deviation were determined. The results are shown in table 1 indicating good performance of the system.

Figure 1. Overlain spectra of amoxicillin trihydrate and bromhexine hydrochloride.



Linearity

Under the experimental conditions described above, linear calibration curves for both the drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the two drugs i.e. (y) v/s concentration (x). The regression analysis data obtained is tabulated in table 2. The linear ranges were 625 – 1875 µg / ml of amoxicillin trihydrate and 20 - 60 µg / ml of bromhexine hydrochloride.

Accuracy

Accuracy of the proposed method was determined by applying the described method to synthetic mixture containing known amount of each drug corresponding to 50 %, 75 %, 100 %, 125 % and 150 % of the nominal concentration. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table 3.

Precision

The method precision was established by carrying out the analysis of capsule powder blend containing two drugs. The assay was carried out of the two drugs using proposed analytical method in six replicates. The value of relative standard deviation lie well within the limit (0.49 % for amoxicillin trihydrate and 0.73 % bromhexine hydrochloride), indicating the sample repeatability of the method. The results obtained are tabulated in table 4.

Robustness

The robustness of the method is determined as a measure of the analytical methods capability to be unaffected by small variation in method parameters.

The different variations are as given below:

Variation in flow rate by ± 0.2 ml /min.
Variation in pH by ± 0.2 units
Variation in wavelength by ± 2.0 nm

Figure 2. A Typical chromatogram of mixture of amoxicillin trihydrate and bromhexine hydrochloride.

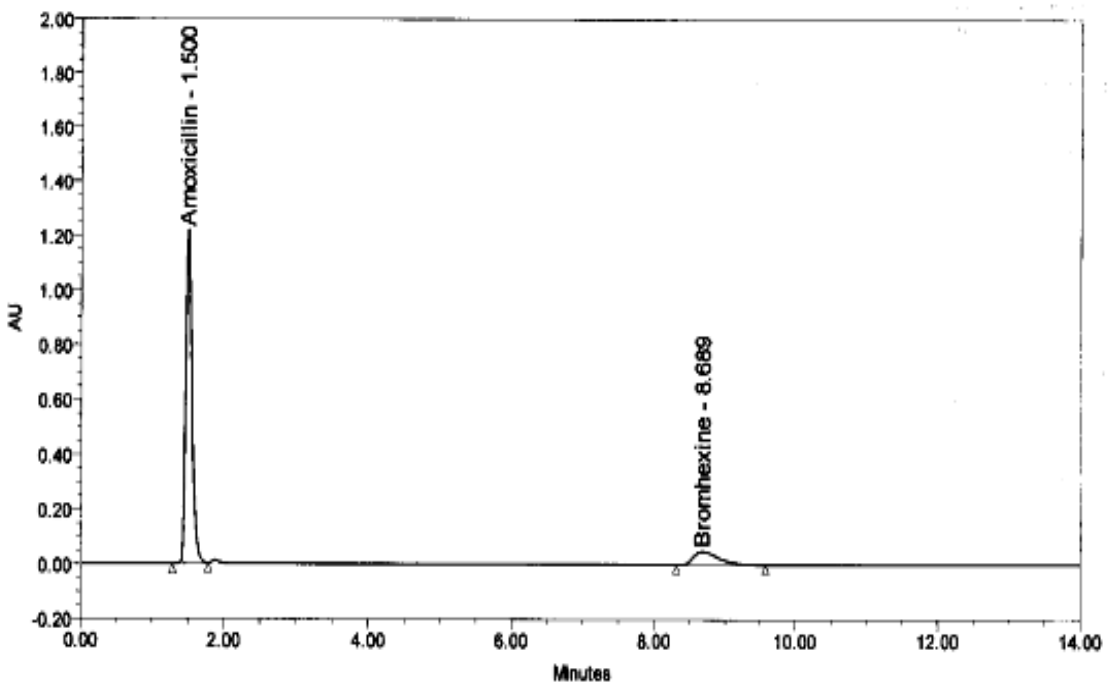


Table – 1. System performance parameters for amoxicillin trihydrate and bromhexine hydrochloride (n = 6).

Drug Substances	Retention time	Symmetry Factor	No. of theoretical plates	Relative standard deviation
Amoxicillin Trihydrate	1.5	1.5	1478	0.15
Bromhexine Hydrochloride	8.6	1.7	2981	0.27

** Calculated at 5% peak height, + Calculated as* $N = 16 (t_R/w)^2$

Table 2. Linearity – regression analysis data

Parameters	Amoxicillin Trihydrate	Bromhexine Hydrochloride
Correlation Coefficient (r)	0.99	0.99
Intercept (y)*	205864	76294
Slope (m)*	5788	31027

**For equation y = mx + c*

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Stability of solution

Solution stability was checked for 24 hrs at room temperature. The drug solutions were found to be stable for the specified period. Solution of sample and standard contain 1250 µg / ml amoxicillin trihydrate and 40 µg / ml bromhexine hydrochloride.

Method application

The validated high performance liquid chromatographic method was applied to simultaneous determination of amoxicillin trihydrate and bromhexine hydrochloride. Twenty capsules mixture containing amoxicillin trihydrate (250 mg) and bromhexine hydrochloride (8 mg) per capsule were used. A portion equivalent to 125 mg amoxicillin trihydrate and 4 mg bromhexine hydrochloride was weighed accurately and was dissolved in 80 ml of mobile phase.

It was mixed well and further diluted to 100 ml to get a solution of concentration of 1250 µg / ml amoxicillin trihydrate and 40 µg / ml bromhexine hydrochloride. 20 µl of this solution was injected into the chromatograph under the specified conditions. The analyte peaks were identified by comparison with observed retention times with those of respective standards. The peaks areas obtained were used to calculate the drugs present. The assay results, expressed as mg / capsule, are shown in table 4. It indicates that the amount of each drug in the product meets the requirements.

Table 3. Accuracy - % recovery of each analyte.

Drug	Amount of drug taken (mg)	Amount of drug added (mg)	Total amount of drug found (mg)	Percentage Error (%)	% Recovery	RSD (%) N = 6
Amoxicillin trihydrate	246.68	0	-	-	-	-
	246.68	125.0	125.52	0.42	100.42	1.6
	246.68	191.5	190.75	0.39	99.61	1.5
	246.68	248.83	245.2	1.46	98.54	1.1
	246.68	298.0	292.67	1.79	98.21	0.5
	246.68	341.1	335.71	1.58	98.42	0.3
Bromhexine hydrochloride	7.49	0	-	-	-	-
	7.49	4.08	4.11	0.73	100.73	0.7
	7.49	5.95	5.93	0.34	99.67	1.4
	7.49	8.0	8.01	0.12	100.12	1.1
	7.49	10.0	10.02	0.20	100.2	0.6
	7.49	12.0	12.05	0.42	100.43	1.2

Table 4. Precision – method Precision.

Experiment No.	Sample weight taken (in mg)	Content in mg/cap obtained of Amoxicillin trihydrate	Content in mg/cap obtained of bromhexine hydrochloride
1	30.8.6	255.60	8.08
2	301.2	253.80	8.10
3	307.2	255.36	8.00
4	307.8	255.82	7.96
5	300.8	253.00	7.98
6	301.4	253.36	7.97
	% RSD	0.49	0.73

CONCLUSION

The proposed work provides a fast, accurate and rugged HPLC assay method with stability indicating potential for these two drugs in mixture capsule or in solution alone. For the proposed method both the drugs gave well define peaks. They were well separated. The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation in comparison to previous methods. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drugs to the pre analyzed formulation and reanalyzing the mixture by proposed method. The percent recovery obtained indicates non- interference from the excipients used in the formulations.

Thus the proposed RP-HPLC method for the simultaneous estimation of amoxicillin trihydrate and bromhexine hydrochloride in combined dosage forms is precise, accurate, linear, robust, simple and rapid. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, formulations and dissolution studies.

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