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Research Article

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF AMOXICILLIN TRIHYDRATE AND BROMHEXINE HYDROCHLORIDE IN ORAL DOSAGE FORMS

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ABSTRACT

A simple high-performance liquid chromatographic method is reported for the simultaneous determination of Amoxicillin Trihydrate and Bromhexine Hydrochloride in oral dosage forms. Investigated drugs were resolved on HiQ Sil C18 (4.6 × 250mm, 5µm) reverse-phase column, utilizing a mobile phase of Methanol: 0.02M Ammonium acetate, pH5 (adjusted with orthophosphoric acid 10% aqueous) 90:10v/v. Mobile phase was delivered at the flow rate of 1.0 ml/minute. Ultra violet Detection was carried out at 254nm. Separation was completed within 10 minutes. Calibration curves were linear with correlation coefficient 0.993 and 0.995 over a concentration range of 100-300 µg/ml for Amoxicillin Trihydrate and 2-10 µg/ml for Bromhexine Hydrochloride respectively. Recovery was between 99.5-101.32 percent and 98.93-101.18 percent for Amoxicillin Trihydrate and Bromhexine Hydrochloride respectively. Method was found to be reproducible with relative standard deviation (R.S.D) for intra and interday precision to be <1.5% over the said concentration range.

Keywords: Amoxicillin Trihydrate, Bromhexine Hydrochloride, high performance liquid chromatography

INTRODUCTION

Amoxicillin [AMOX] (6*R*)-6-(a-D-4-hydroxy-phenylglycylamino) penicillanate. And Bromhexine [BROM](2-Amino-3, 5-dibromo-N-cyclohexyl-N-thylbenzylamine hydrochloride; N-(2-Amino-3, 5-dibromobenzyl)-N-ethylcyclohexylamine hydrochloride) are used clinically for the treatment of acute exacerbations of chronic bronchitis. Amoxicillin trihydrate is a broad spectrum antibiotic and is official in U.S.P[1]. Literature survey reveals that for Amoxicillin Trihydrate has been determined by Spectrophotometry[2-4], HPLC[5-9], HPLC with Fluorimetric detection[10], HPLC with photo diode array detection[11], voltametry[12].

Bromhexine hydrochloride [BROM] is a mucolytic used in the treatment of respiratory disorders associated with productive cough. It is official in B.P[13]. It has been determined by different techniques including spectrophotometry[14-16], HPLC[17-19], colorimetry [20.21], TLC[22], Flow-injection-spectrophotometry[23], GC[24], Ion-Selective Electrode (ISE) [25], Hybrid Linear Analysis [26], capillary isotachophoresis[27], Absorption Spectrophotometry and Electrophoresis[28,29].

It was found that though individually these drugs have been analyzed by many methods, only one method of microbore HPLC was reported for this combination which makes use of Spherisorb, CN Microbore (150mm×2mm) column and Mobile Phase of 20% Acetonitrile[30].

In this paper we report simple, accurate, precise and sensitive Reverse phase high performance liquid chromatography method for simultaneous determination of Amoxicillin Trihydrate and Bromhexine Hydrochloride in combined solid oral dosage form. The proposed method is optimized and validated according to ICH guidelines.

MATERIALS AND METHODS

Amoxicillin trihydrate and Bromhexine Hydrochloride were kindly supplied as gift samples from Maxim Pharmaceuticals, pune and NuLife Pharmaceuticals, pune. Methanol (HPLC grade) and Ammonium acetate (AR grade), Orthophosphoric acid (AR grade) were obtained from S.d.Fine Chem Ltd. Mumbai

Equipments

HPLC was performed using a Jasco HPLC system 2000 consisting of a pump PU2080 Plus, Rheodyne sample injection port with 20 microlitre loop, UV detector 2075 plus, Borwin Software version 1.5 and Column used was C-18 (4.6 × 250mm, 5µ). Weighing was done on Shimadzu Model AY-120 balance. Delux 101 pH meter was used for checking and adjusting pH. All Calibrated glasswares were used for the study.

Preparation of standard stock solution

10 mg of each AMOX and BROM was taken in 10ml volumetric flask separately and dissolved in mobile phase and volume was made with mobile phase to get final concentration of 1mg/ml.

Preparation of mobile phase

Methanol: 0.02M Ammonium acetate pH adjusted to 5 with orthophosphoric acid (10% aqueous) (90:10 v/v) was prepared, filtered through 0.45 μm membrane filter and sonicated on ultra sonic bath.

Preparation of solutions for calibration curve

Standard Stock solution of AMOX was further diluted to get solutions of concentrations 100, 150, 200, 250, 300 μg/ml .Standard solution of BROM was diluted as 1ml to 10ml with mobile phase. This solution was further diluted to get solutions of concentrations 2, 4, 6, 8, 10 μg/ml.

Procedure for Sample Preparation/capsule analysis

Sample Details: Bromolin -250

Label Claim: Each capsule contains Amoxicillin Trihydrate IP equivalent to Amoxicillin 250mg Bromhexine Hydrochloride IP 8 mg

Mfg. By: Okasa Pvt. Ltd

Twenty Capsules, each containing 250mg AMOX and 8mg BROM were emptied and contents were finely powdered. A quantity of powder equivalent to 25 mg AMOX was weighed and transferred to 25 ml volumetric flask. 20 ml mobile phase was added to the same flask and sonicated for 10 minutes. The volume was made up to 25 ml with mobile phase. The solution was first filtered using whatmann filter paper No. 41 and then through 0.45μ filter paper in order to remove the excipients. From the filtrate, appropriate dilution was done in mobile phase to get a solution of 125 μg/ml of AMOX and 4 μg/ml of BROM. Such five replicates were made and injected in to the system

Dilutions for precision studies

Precision of the method was checked by system precision and repeatability (Intra day and Inter day studies). In system precision 6 replicates of mixed standard (containing AMOX 125μg/ml and BROM 4μg/ml) were used. Repeatability was done by using 3 replicate readings at 3 concentration levels. For Intra day variability trials are taken in a day and for Inter day variability studies were done on 3 consecutive days. Concentration levels used for AMOX were 100, 150, 200 μg/ml and that for BROM were 2, 4, 6 μg/ml.

Dilutions for Recovery studies

To study accuracy of the method, recovery studies were carried out by addition of standard drug solution to sample at 3 different levels, 80%, 100% and 120% of the test concentration (test concentration is 125μg/ml for AMOX and 4 μg/ml for BROM)

Robustness studies

Robustness of the method was determined by small, deliberate changes in flow rate, mobile phase ratio, Wavelength of detection and pH of mobile phase. Flow rate was changed to 1 $\pm$ 0.05 ml/min. The mobile phase ratio was changed to $\pm$ 1% for methanol, pH of mobile phase was changed to 5 $\pm$ 0.1

LOD and LOQ Determination

Limit of detection can be calculated by using following formula

$$LOD = 3.3\ \sigma/S$$

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

$$LOQ = 10\ \sigma/S$$

Where σ = Standard deviation of the response

S = Slope of the calibration curve

System Suitability Testing

System suitability testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. Parameters such as theoretical plates, tailing factor, resolution are determined and compared against the specifications.

RESULTS AND DISCUSSION

The solutions of Amoxicillin Trihydrate (AMOX) and Bromhexine Hydrochloride (BROM) working standards were injected into the HPLC system and run in different solvent systems as mobile phases. Different mobile phases containing methanol, Buffers (phosphate, ammonium acetate) in different proportions were tried and finally Methanol: 0.02MAmmonium acetate. Adjusted to pH-5 with ortho phosphoric acid (90:10 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for both AMOX and BROM. Representative chromatogram of mixed standard of AMOX and BROM is shown in Fig 1.

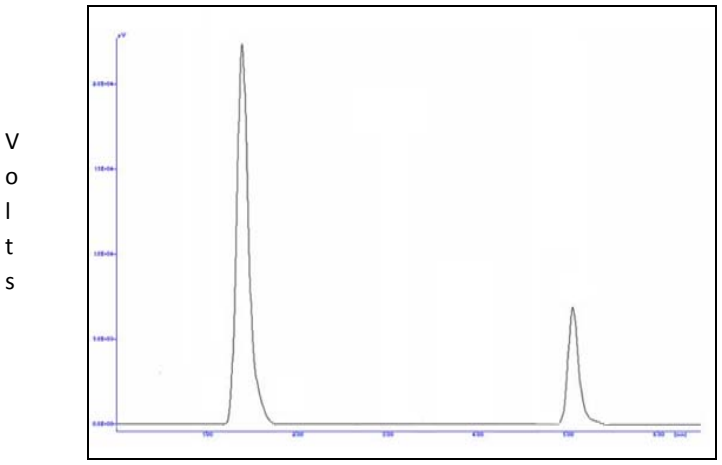

Fig.1: Chromatogram of working standard mixture of AMOX, BROM

From the standard stock solution further dilutions (AMOX 125 µg/ml and BROM 4 µg/ml) were done using mobile phase and scanned over the range of 200-400 nm and the spectra were overlain. As in marketed formulations content of AMOX is far greater (125mg) than BROM (4mg), a wavelength at which AMOX shows comparatively low absorbance than BROM was of concern. It was observed that at 254nm Both AMOX and BROM showed considerable absorbance and AMOX shows comparatively low absorbance than BROM and therefore it was selected as detection wavelength. Overlain spectra of both drugs are shown in Fig.2

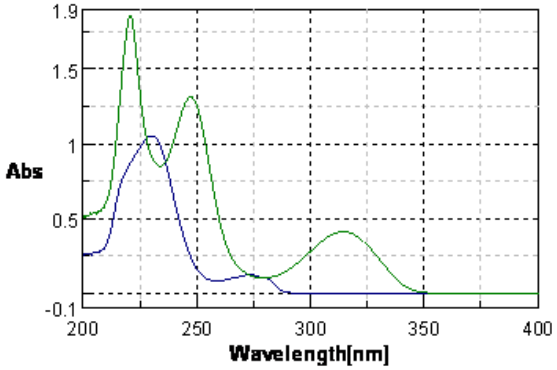

Fig. 2: Overlain spectra of AMOX and BROM (10 µg/ml each)

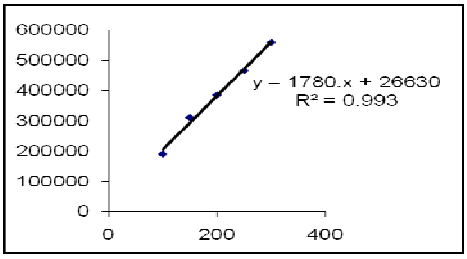

Fig 3 Calibration Curve for AMOX

Table 1: Summary of chromatographic conditions

S No	Parameters	Conditions
1	Column	HiQ Sil C-18 (4.6 × 250mm, 5µm)
2	Mobile phase	Methanol :0.02M Ammonium acetate pH5 90:10v/v
3	Flow rate	1 ml/min
4	Detection Wavelength	254 nm
5	Sample injector	20 µl loop

Method validation

The linear relationship was observed between the peak area and concentration over the range of 100-300 µg/ml for AMOX and 2- 10 µg/ml for BROM. The linearity was expressed as correlation coefficient, which was 0.993 for AMOX and 0.995 for BROM. Correlation coefficient, y- intercept, slope of regression line are shown in Figure -3 and 4. Precision was carried out as system precision and repeatability as per ICH guidelines. It was determined at 3 concentration levels with 3 replicates at each level. For all three concentration levels % RSD obtained was less than 2 % for both the drugs. The results of precision are given in table no.2 and 3. Robustness studies were carried out after deliberate alterations of flow rate, mobile phase compositions, and mobile phase pH. It was observed that the small changes in these operational parameters, did not lead to changes of retention times of peak of interest. Results of robustness studies are shown in table no.5

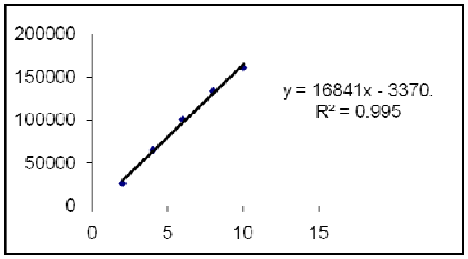

Fig 4 Calibration Curve for BROM

Table 2: System precision of AMOX and BROM

Replicate	AMOX 125µg/ml)	BROM (4 µg/ml)
1	255224.1	62953.13
2	248650	63639.35
3	250468.8	63421.6
4	251568.8	64164.7
5	255501	63648.36
6	249894.4	63021.56
Mean	251884.5	63474.78
Std Dev	2855.12	450.23
% RSD	1.13	0.70
Std Error of Mean	1170.13	184.52

Table 3: Intraday and Interday variability of AMOX and BROM

S No		Concentration (µg/ml)					
		AMOX			BROM		
		100	150	200	2	4	6
1	Intra day precision	1.06	0.55	0.80	1.02	0.94	0.93
2	Inter day precision	1.53	1.39	1.60	1.90	1.44	1.002

Table 4: Recovery Studies of AMOX and BROM

Drug		Level of Recovery		
		80	100	120
AMOX	Mean % Recovery	100.93	100.55	100.53
	% RSD (n=3)	0.32	0.62	0.93
BROM	Mean % Recovery	99.94	100.14	100.09
	% RSD (n=3)	1.14	0.85	0.83

Table 5: Robustness study of AMOX and BROM

Drug	% RSD *Found for robustness study					
	Flow rate(1 ml/min)		pH (5)		Ratio of mobile phase (90:10v/v)	
	+0.05	-0.05	+0.1	-0.1	+1%	-1%
AMOX	0.72	1.23	0.53	1.39	1.93	1.8
BROM	0.39	1.37	0.65	0.48	1.27	1.49

Table 6: Results of assay

Amount present (µg/ml)		Peak Area		Amount Found(µg/ml)		% Assay	
AMOX	BROM	AMOX	BROM	AMOX	BROM	AMOX	BROM
125	4	245224.1	62753.13	122.75	3.92	98.20	98.15
125	4	248600	63639.35	124.65	3.97	99.72	99.47
125	4	251468.8	63421.6	126.26	3.96	101.01	99.15
125	4	253568.8	64564.7	127.44	4.03	101.95	100.84
125	4	245501	63648.36	122.91	3.97	98.33	99.48

The proposed method was evaluated in the assay of capsule formulation containing AMOX and BROM. Five replicate determinations were carried out on capsules. % assay found was 98.20-101.95 for AMOX and that for BROM was 98.15-100.84 %. Results of capsule analysis was shown in table no.6

CONCLUSION

The method described enables the quantification of Amoxicillin Trihydrate and Bromhexine Hydrochloride in combined capsule dosage form. The validation data demonstrate good precision and accuracy, which prove the reliability of the proposed method. Hence, this HPLC method can be used routinely for quantitative estimation of both components in solid oral dosage form.

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