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HPTLC Determination of Amoxicillin Trihydrate and Bromhexine Hydrochloride in Oral Solid Dosage Forms

M.V. Dhoka*[1],V.T. Gawande[1], P.P.Joshi[1].

[1]Department of Quality Assurance, AISSMS college of pharmacy, kennedy road ,near R.T.O. , pune – 411001, Maharashtra, India.

Abstract:

A simple, accurate, precise and rapid high-performance thin-layer chromatographic method for determination of Amoxicillin Trihydrate and Bromhexine Hydrochloride in Bulk and combined Pharmaceutical Dosage Form was developed and validated. The method employed TLC aluminum plates precoated with silica gel $60F_{254}$ as the stationary phase. The solvent system consisted of Butyl Acetate: Glacial acetic acid: Methanol: Water (5:2.5:2.5:1) (v/v/v/v) as mobile phase. Densitometric analysis was carried out at 320nm For Amoxicillin Trihydrate and at 260nm for Bromhexine Hydrochloride-260 nm. The system was found to give compact spots for Amoxicillin Trihydrate and Bromhexine Hydrochloride at R_f of 0.51 ± 0.05 and 0.74 ± 0.05 respectively. The linear regression analysis data showed good linear relationship in the concentration range 10-30 (µg/band) and 200-1000 (ng/band) for Amoxicillin Trihydrate and Bromhexine Hydrochloride respectively. Percent Recovery for Amoxicillin Trihydrate was 98.59-101.58 and that for Bromhexine Hydrochloride was 99.14-101.45. 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. The limits of quantitation for Amoxicillin Trihydrate and Bromhexine Hydrochloride were 0.033(µg/band) and 4.51 (ng/band) respectively. The method was validated for precision, accuracy, specificity and robustness. The method has been successfully applied in the analysis of combined capsule dosage form.

Keywords: *Amoxicillin Trihydrate, Bromhexine Hydrochloride, densitometry, HPTLC, Validation.*

Introduction:

Amoxicillin [AMOX] (6*R*)-6-(a -D-4-hydroxy- phenylglycylamino) penicillanate. And Bromhexine (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 Spectrophotometry[2-4], HPLC[5-9], HPLC with Fluorimetric detection[10] , HPLC with photo diode array detection[11] , voltametry[12] methods have been developed.

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 thin layer 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 [31].

Materials and Methods:

Amoxicillin Trihydrate was kindly provided by Maxim Pharmaceuticals, Pune, India and Bromhexine Hydrochloride was obtained from NuLife Pharmaceuticals, Mumbai, India. Butyl Acetate, Glacial acetic acid, Methanol, (all AR grade) were purchased from Sisco Research Laboratories Ltd, Mumbai.

Instrumentation

Chromatographic separation was performed on a Merck TLC plates precoated with silica gel 60 F_{254} (10 cm

×10 cm with 250 µm thickness, E. Merck, Darmstadt, Germany, purchased by Anchrom Technologies, Mumbai, India). The samples were applied onto the plates using Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe as a band with 6 mm width using a Camag Linomat 5 applicator (Camag, Muttenz, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm). Densitometric scanning was performed on Camag TLC scanner 3 at 235 nm for all measurements and operated by WINCATS software (V 1.4.2, Camag).

Preparation of standard stock solutions

10mg of each drug BROM and AMOX were weighed separately and dissolved in 10 ml of AR grade methanol and then volume was made up to 10ml so as to get the concentration 1000 µg/ml.

Selection of analytical wavelength

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), selection of proper wavelength was a challenge. It was observed that at 320nm AMOX shows comparatively low but still considerable absorbance than other wavelengths hence it was selected as detection wavelength for AMOX. But as Concentration of BROM is low in capsule, the wavelength at which it shows maximum absorbance (**λ max)** was of concern and it was observed from spectra that it is at 260nm, this wavelength was selected for its detection.

Preparation of calibration curves:

From stock solution of AMOX 10- 30 µL was spotted on the TLC plate to obtain final concentration of 10-30 µg/band. Stock solution of BROM was diluted 10 times to get concentration of 100 µg/ml. 2-10 µL of this solution was spotted on the TLC plate to get concentrations of 200-1000 ng/band. The plate was developed in ascending vertical manner using solvent system Butyl Acetate: Glacial acetic acid: Methanol: Water (5:2.5:2.5:1) (v/v/v/v) after 15 min of chamber saturation. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm). The length of chromatogram run was 90 mm. The developed plates were dried and densitometric scanning was performed in the absorbance mode at 260 and 320 nm. The slit dimension was kept at 5 x 0.45 mm. After completion of chromatographic analysis, peak areas of both drugs were noted and plotted against corresponding concentrations and least square regression analysis was performed to generate the calibration equation.

The equations of the regression line for AMOX was

$$Y= 195.7+793.3 \quad r^2=0.996$$

And that for BROM

$$Y=2.459x-45.47 \quad r^2=0.999$$

Analysis of Capsule formulation

Sample Details: Bromolin -250

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

Mfg. By: Okasa Pvt. Ltd

Twenty Capsules, each containing 8 mg BROM and 250 mg AMOX were emptied and contents were finely powdered. A quantity of powder equivalent to 10 mg of AMOX was weighed and transferred to 10 ml volumetric flask. Methanol was added to the same flask and sonicated for 10 minutes. The volume was made up to 10 ml with methanol. The solution was filtered using whatmann filter paper. The stock solution was spotted with the help of applicator to get final concentration of 400ng/band for BROM and 12.5 µg/band for AMOX. The solutions were spotted keeping 10mm distance between bands. The amount of each drug present per capsule was estimated from the respective calibration curves.

Method Validation:

As per ICH guidelines, method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and specificity.

Linearity

Linearity of the method was studied by spotting five concentrations of each drug prepared in the methanol, in the range of 10-30 (µg/band) and 200-1000 (ng/band) for Amoxicillin Trihydrate and Bromhexine Hydrochloride respectively. and noting the peak areas.

Accuracy

For accuracy of method, recovery study was carried out by applying the method to drug sample to which known amount of both drugs were added separately at level of 80, 100 and 120% of label claim (standard addition method). At each level of the amount, three determinations were performed and the results obtained were compared with expected results.

Precision

The precision of the method was demonstrated by system precision and repeatability.

In System precision 6 repeated measurements of standard solutions of both drugs were made and percentage RSD was calculated. Repeatability was demonstrated by intra-day and inter-day variation studies. In the intra day studies, 3 repeated measurements of standard and sample solutions were made in a day and percentage RSD were calculated. In the inter day variation studies, 3 repeated measurements of standard and sample solutions were made on 3 consecutive days and percentage RSD were calculated.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

$$LOD = \frac{3.3 \times \text{S. D of the response}}{\text{Slope of calibration curve}}$$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$$LOQ = \frac{10 \times \text{S. D of the response}}{\text{Slope of calibration curve.}}$$

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which time of spotting to development, time of development to scanning were altered and the changes in the area values were noted by calculating % RSD values.

Specificity

The specificity of the method was ascertained by comparing R_f values and spectra of Standard and sample.

Result and Discussion:

Optimization of Solvent System and Chromatographic Conditions

Chromatographic separation studies were carried out on the stock solution of BROM and AMOX. Initially both the drugs were spotted in concentration to get 100 ng/band and were developed by linear ascending development using solvents like hexane, toluene, methanol, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, and buffers such as acetate, phosphate buffer etc. with chamber saturation. Based on the results of these initial chromatograms binary and ternary mixtures of solvents were tried to achieve optimum resolution between BROM and AMOX. After several trials, mixture of Butyl Acetate: Glacial acetic acid:Methanol:Water (5:2.5:2.5:1) (v/v/v/v) was chosen as the mobile phase since optimum resolution and good peaks for both the drugs was obtained as shown in Fig .The samples were applied in form of bands of width 6 mm on precoated aluminum sheets of silica gel 60 $F_{254.}$

Table 1: Summary of linearity, LOD and LOQ

Parameters	AMOX	BROM
Wavelength (nm)	320	260
Beer's Law Limit	10-30(µg/band)	200-1000(ng/band)
Correlation coefficient (r^2)	0.996	0.999
Linear regression Equation (y = mx + c)	Y= 195.7+793.3	Y=2.459x-45.47
Slope (m)	195.7	2.459
Intercept (c)	793.3	45.47
Limit of detection	0.011(µg/band)	1.48 (ng/band)
Limit of quantitation	0.033(µg/band)	4.51 (ng/band)
Precision indicated by %RSD	< 1.5%	< 1.5%

Table 2: Analysis of capsule formulation

Sr. No	**Amount present (µg/ml)**		**Peak Area**		**Amount Found (µg/ml)**		**% Assay**	
	AMOX	BROM	AMOX	BROM	AMOX	BROM	AMOX	BROM
1	12.5	400	3267.9	926	12.64	395.06	101.15	98.76
2	12.5	400	3255.6	940.9	12.58	401.12	100.65	100.28
3	12.5	400	3280.3	928.6	12.70	396.12	101.66	99.03
4	12.5	400	3277.3	922.3	12.69	393.56	101.54	98.39
5	12.5	400	3260	932.2	12.60	397.58	100.83	99.39

The application position (X) and (Y) were kept at 10 mm and 10 mm respectively to avoid edge effect. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm), using 15 mins of chamber saturation. The length of chromatogram run was 90 mm. The plate was dried and scanned at 260 and 320nm over 90 mm distance.

Linearity

When peak area was plotted Vs Concentration, good correlation coefficients were obtained in concentration range of 10-30 (µg/band) and 200-1000 (ng/band) for AMOX and BROM respectively. Linearity was evaluated by determining five standard working solutions.

Precision

The proposed method was found to be precise as indicated by percent RSD (Relative Standard Deviation) for system precision and repeatability not more than 1.5.

Limit of Detection and Limit of Quantification

LOD was found to be 0.011(µg/band) and 1.48 (ng/band) for AMOX and BROM respectively. LOQ was found to be 0.033(µg/band) and 15.63ng/band for AMOX and BROM respectively.

Table.1 summarizes results of linearity, precision LOD, and LOQ for the method.

Table 3: Recovery Studies of AMOX and BROM

Drug		Level of Recovery		
		80	100	120
Amoxicillin Trihydrate	Mean % Recovery	101.35	99.94	99.68
	% RSD (n=3)	0.75	1.25	0.99
Bromhexine Hcl	Mean % Recovery	100.60	100.35	100.14
	% RSD (n=3)	0.63	0.72	1.18

Table 4: Robustness Study for AMOX and BROM

Sr no	Parameters Varied	% RSD of Peak Area	
		CEF	ERDO
1	Time from Spotting to development	1.3	0.86
2	Time from development to scanning	1.34	1.04

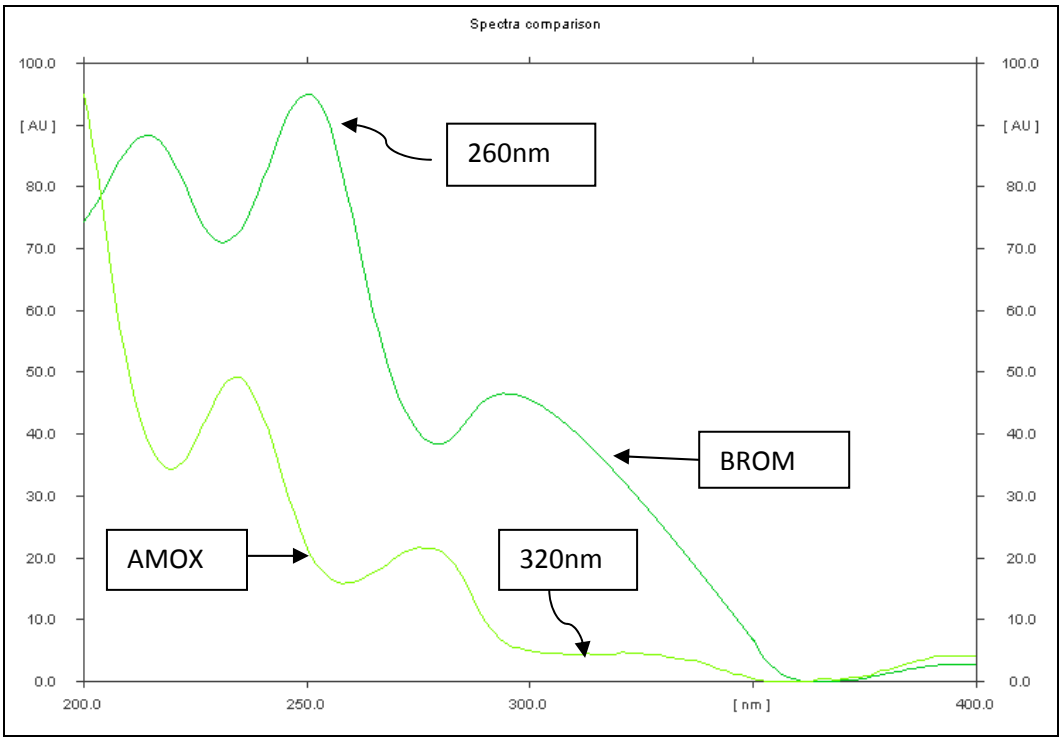

Fig. 1: Ovelain Spectra of and AMOX and BROM (10 µg/ml each)

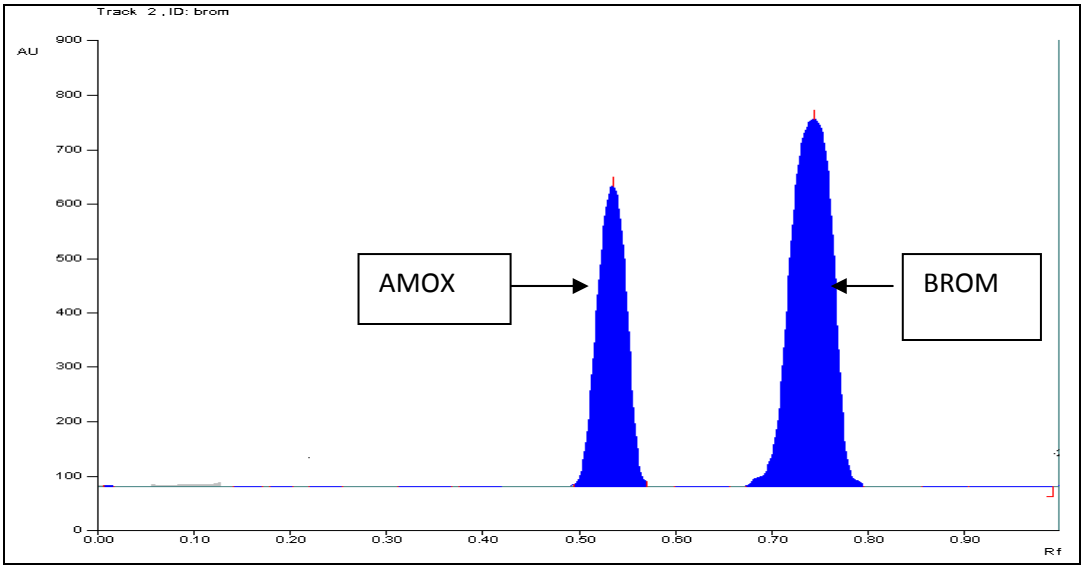

Fig. 2: Representative Densitogram of AMOX and BROM at 260nm (100ng/band each)

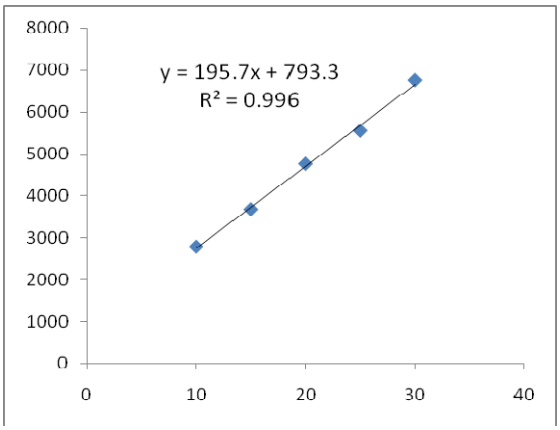

Fig. 3: Calibration curve of AMOX

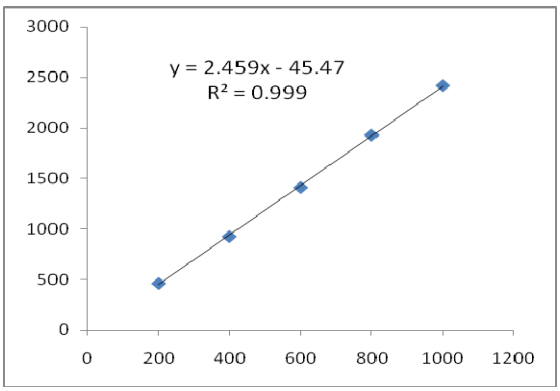

Fig. 4: Calibration curve of BROM

Analysis of capsule formulation

The proposed method was also evaluated in terms of assay of formulated and optimized Cefixime Trihydrate and Erdosteine capsules. Six replicate determinations were performed on the accurately weighed amounts of capsules. The results obtained are shown in Table. 2.

Accuracy

The proposed method when used for estimation of AMOX and BROM from capsule dosage form after spiking with working standard afforded recovery of 98–102% and result of recovery for both drugs from the developed formulation are listed in Table 3

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which time of spotting to development, time of development to scanning were altered and the changes in the area values were noted by calculating % RSD values. The result obtained is shown in Table.No.4

Specificity

The method was found to be specific since no interfering spots were seen when R_f values of standard and sample were compared. There is no difference in the spectra of sample and standard solution which indicate the specificity of the method.

Conclusion:

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and sensitive, thus can be used for

routine analysis of AMOX and BROM in combined solid oral dosage forms.

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