

## **SIMULTANEOUS ESTIMATION OF AMOXICILLIN TRIHYDRATE AND BROMHEXINE HYDROCHLORIDE IN ORAL SOLID DOSAGE FORMS BY SPECTROPHOTOMETRIC METHOD**

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### **ABSTRACT**

Two accurate, precise, rapid and economical methods were developed and validated for the estimation of Amoxicillin Trihydrate and Bromhexine Hydrochloride in Bulk and combined Pharmaceutical Dosage Form. First method is First order Derivative method, wherein wavelengths selected for quantitation were 283 nm, for Amoxicillin Trihydrate and 218.6 nm, for Bromhexine Hydrochloride. Second method is area under curve method wherein two wavelength ranges chosen were 271-274nm and 244-248nm for Amoxicillin trihydrate and Bromhexine hydrochloride respectively. In both of the methods linearity for detector response was observed in the concentration range of 100-300µg/ml for Amoxicillin Trihydrate and 2-10µg/ml for Bromhexine Hydrochloride. The proposed methods were successfully applied for the simultaneous determination of both drugs in capsule dosage form. The results of the analysis have been validated statistically and by recovery studies.

**KEYWORDS:** Amoxicillin Trihydrate, Bromhexine Hydrochloride, First order Derivative method, area under curve method

### **INTRODUCTION**

Amoxicillin [AMOX] (6R)-6-(a -D-4-hydroxy-phenylglycylamino) Pencillanate and Bromhexine (2-Amino-3, 5-dibromo-N-cyclohexyl-N-thylbenzylamine hydrochloride; N-(2-Amino-3, 5-dibromobenzyl)-N-ethylcyclohexylamine hydrochloride) are used clinically in combination for the treatment of acute exacerbations of chronic bronchitis. Amoxicillin trihydrate is a broad spectrum antibiotic and is official in U.S.P<sup>1</sup>. Literature survey reveals that for Amoxicillin Trihydrate, Spectrophotometry<sup>2,4</sup>, HPLC<sup>5-9</sup>, HPLC with Fluorimetric detection<sup>10</sup>, HPLC with photo diode array detection<sup>11</sup>, voltametry<sup>12</sup> 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<sup>13</sup>. It has been determined quantitatively by different techniques including spectrophotometry<sup>14-16</sup>, HPLC<sup>17-19</sup>, colorimetry<sup>20,21</sup>, TLC<sup>22</sup>, Flow-injection-spectrophotometry<sup>23</sup>, GC<sup>24</sup>, Ion-Selective Electrode (ISE)<sup>25</sup>, Hybrid Linear Analysis<sup>26</sup>, capillary isotachopheresis<sup>27</sup>, Absorption Spectrophotometry and Electrophoresis<sup>28,29</sup>.

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<sup>30</sup>.

In this paper we report two simple, accurate, precise and sensitive spectrophotometric methods for simultaneous determination of Amoxicillin Trihydrate and Bromhexine Hydrochloride in combined solid oral dosage form. The proposed method were optimized and validated according to ICH guidelines<sup>31</sup>.

### **MATERIAL AND METHODS**

#### **Instrument**

The instrument used in the present study was JASCO double beam UV/Visible spectrophotometer (Model UV-550) with slit width fixed at 2 nm. All weighing was done on electronic balance (Model Shimadzu AY -120).

#### **Materials**

Amoxicillin Trihydrate was kindly provided by Maxim Pharmaceuticals, Pune, India and Bromhexine Hydrochloride was obtained from NuLife Pharmaceuticals, Pune, India.

Solvent: Methanol was used for preparing stock solutions and 0.1N HCl was used for further dilutions. All solvents (AR grade) were purchased from Sisco Research Laboratories Ltd, Mumbai

**Stock Solution**

25mg of each of AMOX and BROM were weighed separately and dissolved in methanol AR grade, and then volume was made up to 25 ml for both to get concentration 1mg/ml. Further dilutions were done in 0.1N HCl.

**Procedure**

**Method A- First Order Derivative Method**

For each drug, appropriate dilutions were done from standard stock solution using 0.1NHCl and were scanned separately in the UV range 200 – 400 nm. These spectra were converted to first order derivative spectra . After observing the overlain first derivative spectra of AMOX and BROM the zero crossing points of both drugs were selected for analysis of other drug. (Figure1)The first wavelength selected was 283 nm, the zero crossing point of BROM, where AMOX showed considerable absorbance. The second wavelength was 218.6 nm, the

zero crossing point of AMOX, where BROM showed considerable absorbance.

The concentration of AMOX and BROM were calculated by putting the values of absorbance in linearity equations.

A) For Amox  $Y=0.370x-0.004$

B) For Brom  $Y=0.006-0.001$

**Method B: Area Under Curve Method**

The two-wavelength range selected should be such that there is negligible change in absorbance of drug. Within these wavelength ranges peak area of both drugs were measured and used in calculations. So the two wavelength ranges chosen were 271-274nm and 244-248nm for AMOX and BROM respectively as shown in Figure 2.The area under curve of said concentrations for both the drugs were noted at selected analytical wavelength ranges. These area under curve were then divided by concentration in gm/lit to get Xamox and Xbrom values.

**Determination Of ‘X’ Values**

$$X = \frac{\text{AUC of component between selected wavelength ranges}}{\text{Concentration of that component in mg/lit}}$$

**Equation-1**

$$C_{\text{BROM}} = \frac{X^{A1} \times \text{AUC}^M_2 - X^{A2} \times \text{AUC}^M_1}{X^{B1} \times X^{A2} - X^{B2} \times X^{A1}}$$

**Equation-2**

$$C_{\text{AMOX}} = \frac{X^{B2} \times \text{AUC}^M_1 - X^{B1} \times \text{AUC}^M_2}{X^{B1} \times X^{A2} - X^{B2} \times X^{A1}}$$

$C_B$  = Concentration of BROM

$C_A$  = Concentration of AMOX

$X^{B1}$  = Area under curve of bromhexine at wavelength of 271-274nm

$X^{A2}$  = Area under curve of amoxicillin at wavelength of 244-248nm

$X^{B2}$  = Area under curve of bromhexine at wavelength of 244-248nm

$X^{A1}$  = Area under curve of amoxicillin at wavelength of 271-274nm

$\text{AUC}^M_1$  = Area under curve of mixture at wavelength of 271-274nm

$\text{AUC}^M_2$  = Area under curve of mixture at wavelength of 244-248nm

Application of proposed method for determination of AMOX and BROM in capsules

Sample Details:	Bromolin -250
Label Claim:	Each capsule contains Amoxicillin Trihydrate IP Equivalent Amoxicillin 250 mg Bromhexine Hydrochloride IP 8 mg
Mfg. By:	Okasa Pvt. Ltd

Contents of Twenty capsules were emptied and powdered. Powder equivalent to 25mg of AMOX was transferred to 25 ml volumetric flask and dissolved in

methanol AR grade. Solution was sonicated for 10 mins; volume was made up and then filtered. From this stock solution further dilution was done using 0.1 N HCl so to

get the final concentration of 4 µg/ml for BROM and 125 µg/ml for AMOX. The solution was then scanned in the range of 200-400 nm against blank. Absorbances were recorded at selected wavelengths. Calculations were done as per the equations.

#### **Method Validation**

These methods were validated according to ICH guidelines

#### **Accuracy**

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). Percent recovery for AMOX and BROM, by both the methods, was found in the range of 98.41 % to 101.94%

#### **Linearity**

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of AMOX and BROM. For both the methods, the Beer-Lambert's concentration range was found to be 100-300µg/ml for AMOX and 2-10µg/ml for BROM.

#### **Precision**

The reproducibility of the proposed method was determined by performing capsule assay at different time intervals on same day (Intra-day precision) and on three different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD. Percent RSD for Intraday precision was found to be 0.84 (for AMOX) and 1.20 (for BROM) in first order derivative method; 0.44 (for AMOX) and 0.84 (for BROM) in Area under curve method. Inter-day precision was found to be 1.66 (for AMOX) and 1.75 (for BROM) in first order derivative method; 0.97 (for AMOX) and 1.60 (for BROM) in Area under curve method

#### **RESULTS**

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of AMOX and BROM. In both the methods linearity for detector response was observed in the concentration range of 100-300 µg/ml for Amoxicillin Trihydrate and 2-10 µg/ml for Bromhexine Hydrochloride. In method A, concentration of the individual drug present in the mixture was determined against the calibration curve. In method-B X' values were calculated for both the drugs at selected wavelengths and substituted in equations for determining concentration of AMOX and BROM in capsule dosage form.

Percent assay for AMOX and BROM in capsule analysis, by both the methods, was found in the range of 98.33 % to 101.66 %. Standard deviation and coefficient of variation for three determinations of individual drugs, by both the methods, was found to be less than ± 2.0 indicating the precision of both the methods. Accuracy of

proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for AMOX and BROM, by both the methods, was found in the range of 98.41 % to 101.94 %, values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of both the methods. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can be employed for routine quality control of Amoxicillin Trihydrate and Bromhexine Hydrochloride in combined oral solid dosage forms.

#### **DISCUSSION**

The proposed spectroscopic methods allow for accurate, precise and reliable measurement of AMOX and BROM simultaneously in combined dosage form. Both the developed methods were found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in respective two-component oral dosage forms of AMOX and BROM. The methods were evaluated for validation parameters, linear relation including coefficient of correlation, accuracy, reproducibility and precision. The RSD for all parameters and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of AMOX and BROM in pharmaceutical preparations.

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Table. 1 X Value Measurement

Components	271-274nm	244-248nm
BROM (X <sup>B</sup> )	9.705 (XB1)	100.7(XB2)
AMOX (X <sup>A</sup> )	9.42024(XA1)	26.16544(XA2)

Table 2: Capsule analysis and recovery studies of amoxicillin trihydrate and bromhexine hydrochloride

Method	Label Claim (mg/cap)		% Assay		% Recovery*		(% ) RSD	
	AMOX	BROM	AMOX	BROM	AMOX	BROM	AMOX	BROM
A	250	8	99.98	99.87	100.54	100.05	0.84	1.47
B	250	8	100.56	100.76	100.76	100.45	0.62	0.96

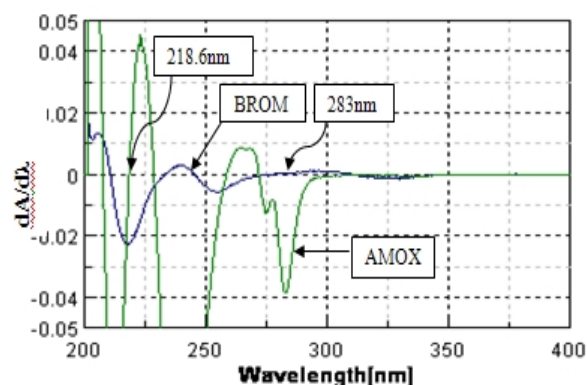
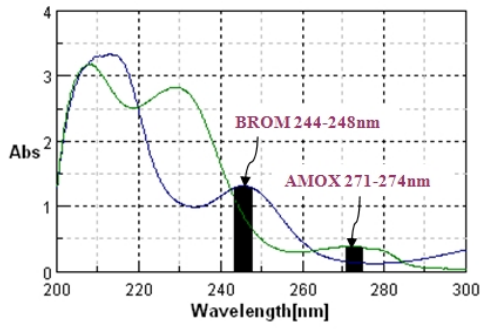


Figure 1. Overlain First order Derivative spectra of AMOX and BROM



**Figure2. Overlain spectra of AMOX and BROM (10 µg/ml each)**