

## ANALYSIS OF AMBROXOL HYDROCHLORIDE IN FLAVAMED® TABLETS BY MEANS OF SPECTROSCOPIC ABSORPTION METHODS

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**Abstract:** Ambroxol hydrochloride (AMB), (1s,4s)-4-((2-amino-3,5-dibromocyclohexyl)methylamino) cyclohexanol hydrochloride, is semi - synthetic derivative of vasicine obtained from Indian shrub *Adhatoda vasica*. It is a metabolic product of bromhexin and it is used as broncho secretolytic and an expectorant drug. Analysis of Flavamed® tablets, in which ambroxol hydrochloride (AMB) is an active component, was performed. *UV/VIS* spectrophotometry and atomic absorption spectroscopy (*AAS*) were used. Direct and indirect *UV/VIS* spectrophotometric methods were used for quantitative analysis of AMB and the following recovery value results were obtained: 100,16% and 95,23%, successively. Content of heavy metals in Flavamed® tablets was determined by atomic absorption spectroscopy.

**Keywords:** Ambroxol hydrochloride, Flavamed tablets, Spectroscopic analysis

### Introduction

Ambroxol hydrochloride (AMB) is semi-synthetic derivative of vasicine obtained from Indian shrub *Adhatoda vasica*. It is a metabolic product of bromhexine. AMB is chemically (1s,4s)-4-((2-amino-3,5-dibromocyclohexyl)methylamino)cyclohexanol hydrochloride. Its molecular weight is 414.6 g mol<sup>-1</sup> with molecular formula C<sub>13</sub>H<sub>18</sub>Br<sub>2</sub>N<sub>2</sub>O·HCl. Dissociation constant (*pK<sub>a</sub>*) of AMB is 8.2 (Eu. Ph. 8.0; Rele and Gurav, 2012).

AMB is used as broncho secretolytic and an expectorant drug. It simulates the transportation of the viscous secretions in the respiratory organs and reduces the stand stillness of the secretions. AMB is a clinically proven systemically active mucolytic agent. AMB is completely absorbed. AMB is changed into various inactive metabolites which are mainly eliminated as water-soluble conjugates. After oral administration, 85 % of the active substance is eliminated in the urine. Less than 10 % is eliminated in the form of unchanged AMB (Hajera and Zaheer 2012; The Merck Index 777&382; British Ph. 2004).

In order to insure stability, safety and efficiency of a drug, numerous analyses of active substance and its excipients in formulation are required. Concerning literature review on ambroxol hydrochloride several optical methods, including *UV/VIS*, *IR* and atomic absorption spectroscopy (*AAS*) have been used. *UV/VIS* spectrophotometry was the first choice for the qualitative and quantitative analysis of AMB in pharmaceutical formulations (Pai, Rou and Lalitha, 2007; Raju and Kiran Babu, 2006). Also, different chromatographic methods have been reported in the literature (Rele and Gurav, 2012; Jain, 2010; Aranzazu, Sayalero and Lopez, 2001).

The aim of this paper is analysis of Flavamed® tablets, which contain AMB as an active ingredient, by *UV/VIS* spectrophotometry. Two very simple and accurate methods for analysis of ambroxol hydrochloride were used. *AAS* method was performed for heavy metal content assessment.

## Materials and methods

Flavamed® (Berlin-Chemie AG, Menarini, Germany) packaging of tablets were purchased in local pharmacy store. Each tablet contains 30 mg of AMB as an active substance with following excipients: corn starch (disintegrant and binder), the powdered cellulose (disintegrant), the croscarmellose sodium (superdisintegrant), the povidone K30 (binder) and the magnesium stearate (lubricant). The tablets are white, round with flat surfaces and faceted edges, with embedded dividing line on one side. The pure, pharmaceutical standard of AMB was obtained by Drug Agency of Bosnia and Herzegovina.

All reagents (glacial acetic acid, sodium nitrate, sodium hydroxide and  $\beta$ -naphthol) and solvents were of analytical grade, Merck.

A Shimadzu-UV 1800 double beam spectrophotometer with pair of 10 mm quartz cells was used. Absorbance was measured in 200-600 nm wavelength interval. Atomic absorption spectroscopy was performed by Perkin Elmer AAS instruments.

Flavamed® tablets were analysed by two different methods, previously validated and reported in the literature (Rele and Gurav, 2012, Bhatia *et al*, 2008). The methods are designated as Method I (direct) and Method II (indirect) spectrophotometry.

### METHOD I

Standard stock solution of 95  $\mu\text{g/ml}$  of AMB was prepared. Appropriate mass of AMB standard was dissolved in 20 ml of methanol in 100 ml volumetric flask and the volume was made up to mark with distilled water. Solution containing 25  $\mu\text{g/ml}$  of AMB was scanned in the *UV* region, in order to obtain a spectrum of AMB. Six standard solutions were prepared by diluting the stock solution with distilled water to give the final concentration range of 9.5-44.1  $\mu\text{g/ml}$ .

Twenty tablets were weighed accurately and ground to a fine powder. From the triturate obtained, an amount equivalent to 30 mg of AMB was weighed and transferred to 100ml volumetric flask. The content of the flask was dissolved in methanol solvent with the aid of ultrasonication for 10 min. The solution was filtered through filter paper and was made up to 100 ml with glass-distilled water to get a stock solution containing 300  $\mu\text{g/ml}$  of AMB (Bhatia *et al*, 2008).

### METHOD II

This proposed method is an indirect way of quantitative determination of AMB in which AMB is transformed by chemical reaction into a compound with different optical characteristics with  $\lambda_{\text{max}}=425\text{ nm}$ .

A stock solution of AMB was prepared by dissolving certain amount of AMB in mixture of methanol and distilled water in ratio (20:80). Appropriate amount of stock solution of AMB was taken and mixed with reagents to achieve desired chemical reaction. AMB was diazotized by sodium nitrate (0,5%) in acid medium provided by glacial acetic acid (10%) and then coupled with  $\beta$ -naphthol (0,1%) in alkaline medium (NaOH, 4%). The solution were allowed to stand for six minutes in order reaction to be completed, before absorbance measurements at 425 nm. Concentration range of AMB was between 10-50  $\mu\text{g/mL}$ .

Preparation of Flavamed® tablets was performed in a similar manner as described in method I. Upon preparation of AMB solution from Flavamed® tablets, chemical reaction proceeded.

## Results and discussions

### METHOD I

The spectrum of AMB is presented at Figure 1. with  $\lambda_{\max}$  at 245 nm and 310 nm which is in good agreement with Eur.Ph. 8.0. For determination of AMB, the wavelength 245 nm have been selected.

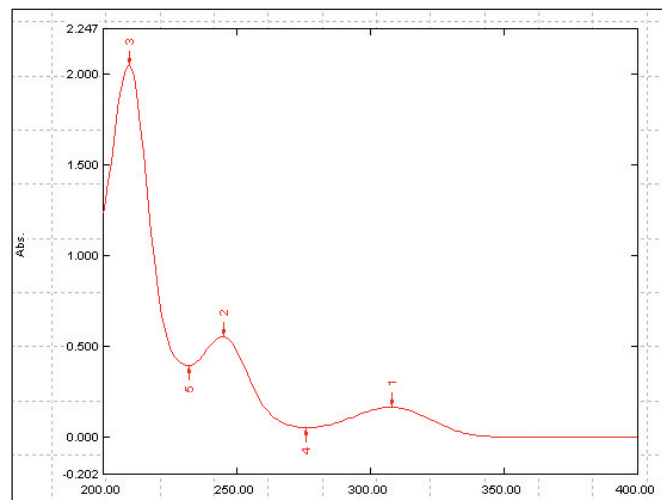


Figure 1. UV/VIS spectrum of AMB

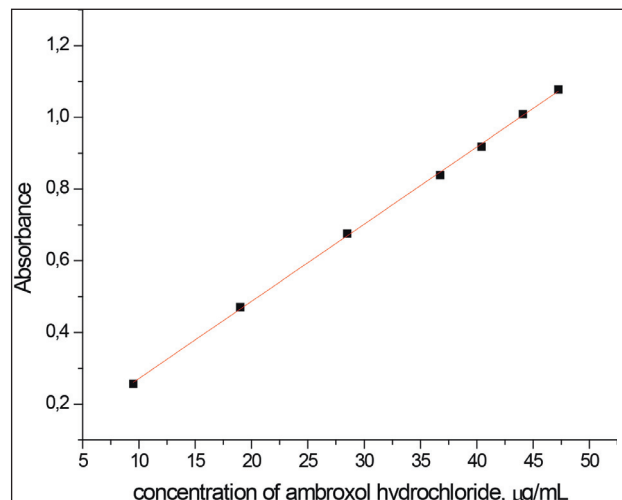


Figure 2. Calibration curve of AMB obtained by Method I at  $\lambda_{\max}$  = 245 nm

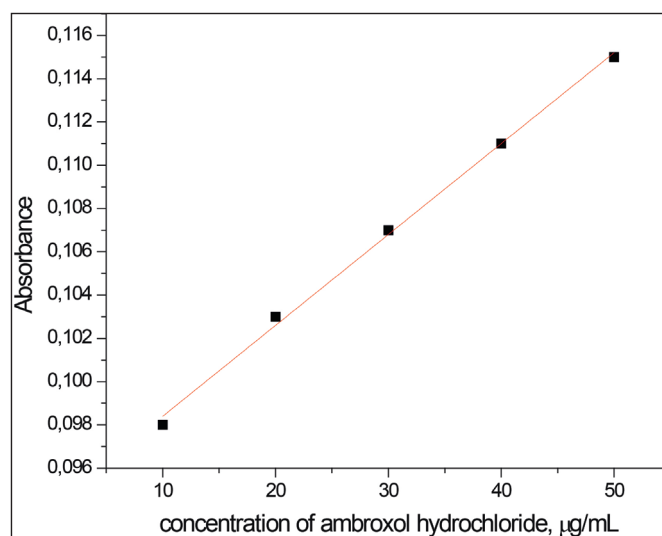


Figure 3. Calibration curve of AMB obtained by Method II at  $\lambda_{\max}$  = 425 nm

### METHOD II

This method is based upon ability of AMB solution to be convert by some proper chemical reaction into compound with some different optical characteristics, therefore, absorbance measurement was conducted at  $\lambda_{\max}$  = 425 nm. The calibration curve is presented at Figure 3. It was in 10-50  $\mu\text{g/mL}$  concentration range. The linear regression was done and all the parameters are presented in Table 1.

For quantitative analysis calibration curve in range 9.5- 44.1  $\mu\text{g/mL}$  was constructed (Figure 2). Linear regression analysis was performed and parameters of regression analysis are given in Table 1. Afterwards, the absorbance of the obtained drug solution was measured and the concentration of AMB in Flavamed® tablets was determined using the equation generated from the calibration curve (Figure 2). The experiment was carried out in triplicate. SD and RSD values are given in Table 2, along with estimated concentration of AMB in Flavamed® tablets by Method I. The recovery value was 100,16%,

**Table 1.** Regression analysis parameters of AMB

| Parameteres             | Method I                | Method II               |
|-------------------------|-------------------------|-------------------------|
| Absorption maximum, nm  | 245                     | 425                     |
| Beer's law limit, µg/mL | 9,50 – 44,10            | 10,00 – 50,00           |
| Regression equation     | $y = 0,02152x + 0,0565$ | $y = 0,00042x + 0,0942$ |
| Correlation coefficient | 0,9994                  | 0,9970                  |

The concentration of AMB drug in tablets was determined using calibration curve. A proper amount of AMB solution from Flavamed® tablets was transferred in flask, with all other reagents (sodium nitrate, glacial acetic acid, β-naphtol and NaOH) needed to perform chemical reaction and then diluted up to 25 mL. Finally, AMB concentration from Flavamed® tablets was 20 µg/mL. Using linear regression data, the results of analysis of Flavamed® tablets (average of five determination) were in very good agreement with value of AMB sample. The recovery value was 95,23%. Table 2 contains data concerning concentration of AMB in Flavamed® tablets obtained by Method II.

**Table 2.** Analysis parameters of AMB in Flavamed® tablets

| Parameteres   | Method I                      | Method II   |
|---|-------------------------------|---|
| Amount of AMB in Flavamed® tablets added, µg/mL     | 15                            | 20  |
| Amount of AMB in Flavamed® tablets estimated, µg/mL | 15,0420<br>15,0280<br>15,0140 | 18,5714<br>20,9523<br>16,1904<br>18,5714<br>20,9523 |
| *Recovery value, %                                  | 100,16                        | 95,23   |
| SD  | 0,0003                        | 0,000837  |
| RSD, %  | 0,0789                        | 0,818   |

\*estimation based upon average value of AMB concentration

Furthermore, Flavamed tablets were analysed by atomic absorption spectroscopy for determination of presence of heavy metals. Concerning the Eu. Ph. 8.0, the amount of heavy metals should not exceed 20 ppm which is in a well agreement with obtained values for heavy metal determination in Flavamed® tablets. The results are presented in Table 3.

**Table 3.** Content of heavy metals in Flavamed® tablets

|               |        |       |
|---------------|--------|-------|
| Content of Pb | < 0,01 | mg/kg |
| Content of Cd | < 0,03 | mg/kg |
| Content of As | < 0,02 | mg/kg |
| Content of Fe | 0,05   | mg/kg |
| Content of Zn | < 0,03 | mg/kg |
| Content of Cu | < 0,03 | mg/kg |
| Content of Cr | < 0,03 | mg/kg |
| Content of Ni | < 0,07 | mg/kg |
| Content of Mn | < 0,06 | mg/kg |
| Content of Co | < 0,1  | mg/kg |

## Conclusions

AMB in Flavamed® tablets was investigated with two spectroscopic absorption methods: *UV/VIS* spectrophotometry and atomic absorption spectroscopy. Using *UV/VIS* spectrophotometry, qualitative and quantitative analysis was performed. Direct and indirect approach were successfully used. The recovery values were in good agreement with values declared at Flavamed® package. Using atomic absorption spectroscopy the presence of heavy metals was proved, but in a proper range.

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