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# Spectrophotometric Determination of Ambroxol by Chromotropic acid disodium salt dihydrate

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## Abstract

An easy and prompt spectrophotometric method was proposed for the limitation of the drug Ambroxol substance containing the amine group by the organic reagent (4,5-Dihydroxynaphthalene-2,7-disulfonic acid disodium salt dihydrate).

When the reaction product gave a wavelength(469nm), and in optimal conditions for the reaction, a calibration curve was generated. The result was found that Beer's law was obeyed within concentration ranges of 2-18µg.mL$^{-1}$ of Ambroxol, and the other parameter was calculated. The molar absorptivity was 0.8287X10$^{4}$ L.mol$^{-1}$.cm$^{-1}$, Sandell's sensitivity was 0.0128 µg.cm$^{-2}$, R.S.D% was 0.6520 %, with a correlation coefficient of 0.9967 for Ambroxol. The interferences were studied; they didn't affect on concentration limitation of Ambroxol. The method was successfully applied for the determination of Ambroxol in pharmaceuticals.

**Keywords:** Ambroxol, Chromotropic acid disodium salt dihydrate, pharmaceutical formulation, spectrophotometry.

## 1. Introduction

Amino compounds can be classified as organic compounds where the amine group is linked to carbon groups, and the hydrogen atom in the ammonia compound is replaced by an organic group, such as an aryl or alkyl. Amines can be classified into three types: primary, secondary, and tertiary amines, as shown in the figure below (Fig. 1).

There is another class of amino compounds called quaternary ammonium ions, which carry a positive electrical charge $[NR_4]^+$. It is formed by the association of four organic alkyl groups or aryl groups. These cations are characterized by a regular charge distribution, which gives them a very high stability, as the nitrogen atom uses all of its orbitals when forming this ion.

### 1.1. Classification of Amine

Amines can be divided into two types: aliphatic, aromatic, or heterocyclic compounds, depending on the nature of the nitrogen substituents and the types of bonds, which depend on the nature of the carbon groups attached to them. Aliphatic amines contain simple hydrocarbon substituents represented by alkyl groups, while aromatic amines, known as “anilines,” contain an aromatic ring substituent (benzene). We note that each substituent contains at least one carbon atom (Fig. 2) [1].

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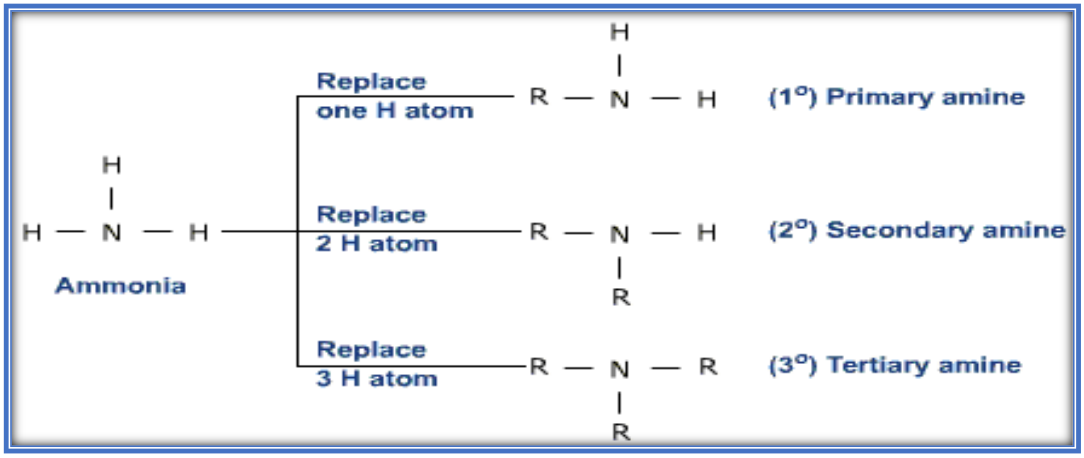



Fig. (1). Amines are classified into several types.

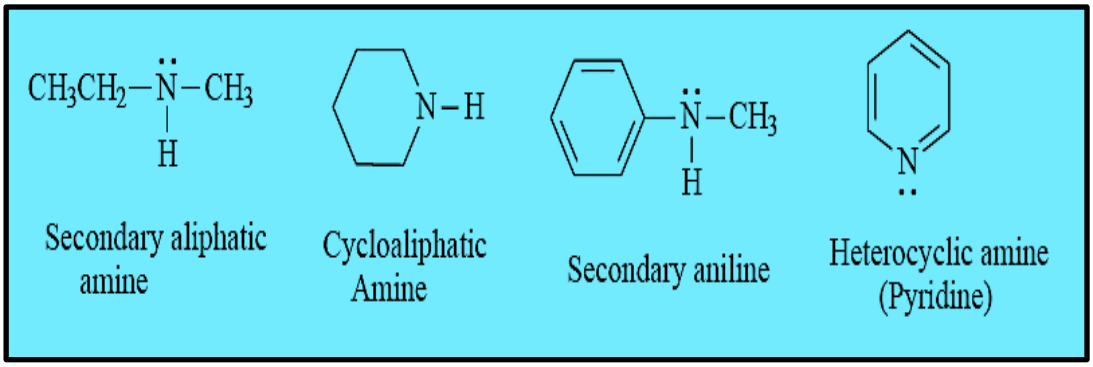



Fig. (2). Sub-Classification of Amine.

Many pharmaceutical compounds (perhaps 85% of the drugs on the market) contain covalently linked amine groups where they are attached to relatively hydrophobic groups. Most of these drugs are fixed in the ammonium ion form, which makes the drug more soluble in water (very important if the drug is to be injected into a vein) and converts the amine (in liquid oil form) into a more ionic form, ammonium ion (in solid form).

Amines play a very important role in the preparation of pharmaceutical preparations, and the principle of using amines as pharmacologically active substances goes back at least a century. Amino drugs have been manufactured to mimic or interfere with the action of natural amino neurotransmitters. Amines have a wide range of functions that include stimulants, decongestants, antihistamines, and antidepressants [2].

### 1.2. Ambroxol

The chemical composition of the pharmaceutical compound Ambroxol is in the formula (trans-4-(2-amino-3,5-dibromobenzylamine) cyclohexanol hydrochloride (Fig. 3). It is a compound with strong activity in treating respiratory diseases and dissolving phlegm and is a therapeutic expectorant and bronchodilator [3-5].

Ambroxol's effectiveness stems from its ability to move viscous secretions in the respiratory organs, preventing their stagnation within the respiratory organs, i.e., making them more fluid. The treatment is administered in varying doses ranging from 30 – 120 mg, repeated daily. It is commercially available

in the form of syrup, granules, tablets, or solutions for use by injection or inhalation [6,7].

There are many varied methods for the determination of Ambroxol in pharmaceutical formulations, including LC-MS, [8] spectrophotometry, [9] HPLC, [10-12] flow injection analysis, [13,14] capillary electrophoresis, and isophoresis [15,16].

### 1.3. Organic Reagent

#### 1.3.1. Chromotropic acid disodium salt dihydrate

Chromotropic acid dihydrate salt can be utilized as a chromogenic reagent for the limitation of several amines in different pharmaceutical preparations. 4,5-dihydroxynaphthalene-2,7-disulfonic acid dihydrate (Fig.4), applied to progress the conception of trichothecenes on developed TLC plates. It serves as an intermediate in the offspring of dyes, dyes, chelating reagents, sulfonic and sulfonic acids.

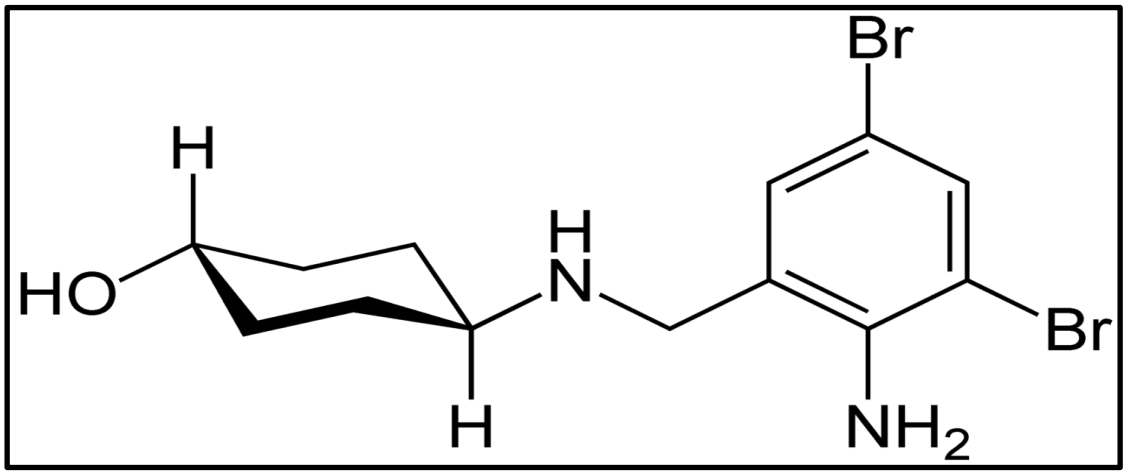



Fig. (3). Structure of Ambroxol.

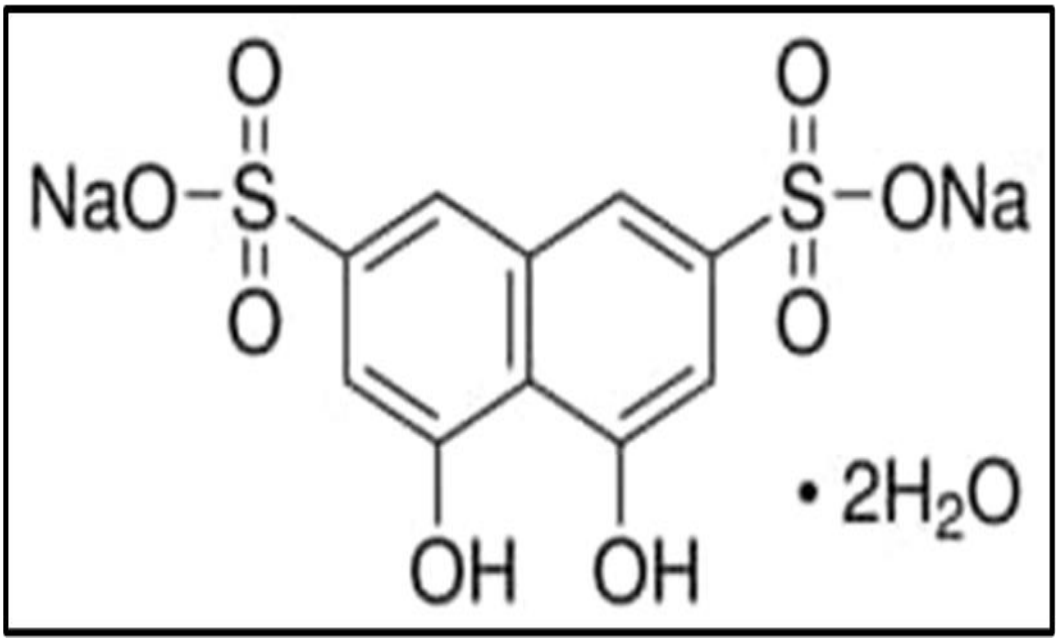



Fig. (4). 4,5-Dihydroxynaphthalene-2,7-disulfonic acid disodium salt dihydrate.

## 2. EXPERIMENTAL SECTION

The chemicals used in this research have high purity and are from various international companies, such as Orlistat/chine, chromotropic acid salt dihydrate (India), sodium hydroxide (BDH), and ethanol( UK**).**

### 2.1. Instruments

A Shimadzu UV-visible 1800 spectrophotometer with an identical 1 cm quartz cell was used for the absorbance measurements. An ABS 120 - 4 KERN electronic scale was also used to weigh the samples.

### 2.2. Preparation of standard solutions

Ambroxol solution (1000 µg/ml) was initially prepared by dissolving 0.1 g of the drug in a 100 ml volumetric flask with distilled water.

### 2.3. Preparation Solution of 4,5-Dihydroxynaphthalene-2,7-disulfonic acid disodium salt dihydrate (reagent).

The reagent solution (Chromotropic acid disodium salt dihydrate) is prepared by dissolving 0.05 g in 10 ml of deionized water using a 10 ml volumetric flask and keeping the solution away from light.

### 2.4. Preparation of solutions of interferences

A 1% (w/v) reagent solution (disodium salt dihydrate of chromotropic acid) was prepared by dissolving 0.2500 g in 25 mL of deionized water, transferring to a 25 mL volumetric flask, and diluting to the mark with deionized water and protected from light.

### 2.5. Sample solution

Weigh ten tablets of the drug Ambroxol after grinding them well, dissolve 100 mg of the powder in distilled water, transfer it to a 100 ml volumetric flask, and dilute it to 100 ml with the same solvent. Filter the solution resulting from the dissolution process using Whatman high-quality filter paper and transfer the resulting solution to a 100 ml volumetric flask. Then wash the remainder and dilute it to volume with the same solvent to obtain 1000 micrograms/ml of each drug. taking 1 mL from the pharmacological material and 2 mL (Chromotropic acid disodium salt dihydrate) with 2 mL of buffer solution (pH=10), and adding into volumetric flasks, completed to the mark with Deionized water. Ambroxol Mucosal tablets were also taken and prepared at the same concentration.

### 2.6. Preparation of solutions of interferences

Take 0.1 g of each interfering substance is dissolve it in 100 ml of solvents such as cellulose, glucose, hydrochloric acid, magnesium stearate, povidone, silica, talc (talc) etc. These interfering substances are prepared in high concentrations to determine their effect on absorbance using the same reagent.

## 3. Results and Discussion

### 3.1. Absorption Spectra

The absorption spectrum of Ambroxol against water was determined. It was found that Ambroxol gave a maximum absorption peak (λ max) at 212 nm. The reaction between Ambroxol and Chromotropic acid sodium salt dihydrate was carried out, and the absorption spectrum of the product was observed against the detector vacuum (Fig. 5). Where the reaction product was colored and wavelength (λmax) at 469 nm, and (λmax) from. Chromotropic acid dihydrate has a wavelength of 365 nm. The displacement (λmax) of the Ambroxol derivative and the Chromotropic acid dihydrate salt was shifted, avoiding any possible interference. Therefore, the measurements were made at a wavelength of 469 nm.

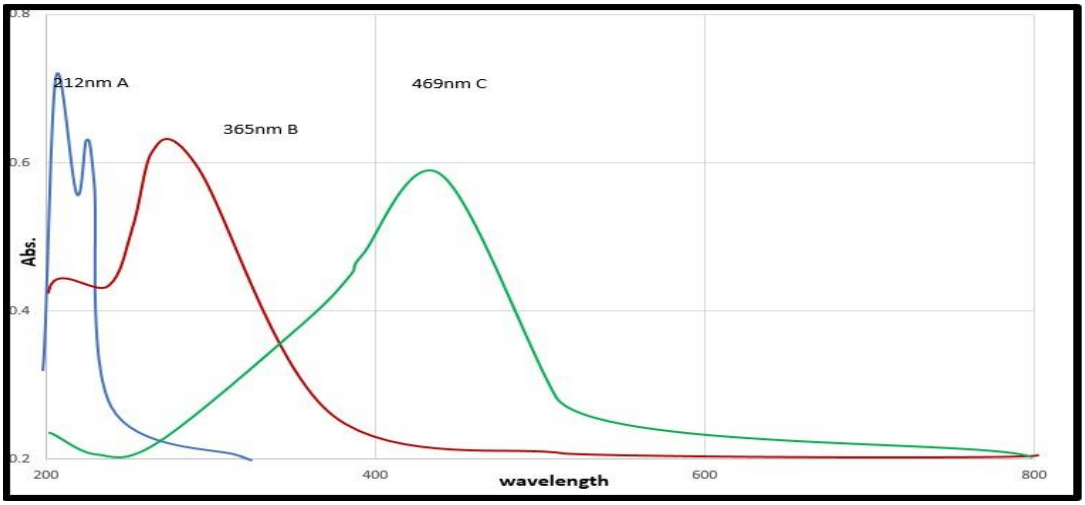



Fig. (5). Absorption spectrum of the Ambroxol, blank, and colored product.

### 3.2 Optimization of the Reaction Conditions

#### 3.2.1 Effect of (Chromotropic acid disodium salt dihydrate) Concentration.

Different concentrations of disodium reagent (chromotropic acid) were prepared as shown in Fig. 6. The difference in the reagent leads to a clear change in the reaction process and an increase in absorbance with increasing concentration of disodium salt. Experiments were conducted at a concentration of 0.6% (w/v). The highest value was recorded for absorbance. It was also noted that the absorbance values were not affected by concentrations higher than 1%.

#### 3.2.2 Effect of the volume of reagent

Different quantities of the reagent were taken, and at the concentration that was determined in the experiment above, a concentration of 0.6 percent (w/v) Chromotropic acid disodium salt dihydrate with the Ambroxol was used to find out From observing the absorption intensity, we conclude that the size of the reagent has a clear effect on the course of the reaction at a volume of 1.0 ml. (Fig.7) shows the highest absorption intensity.

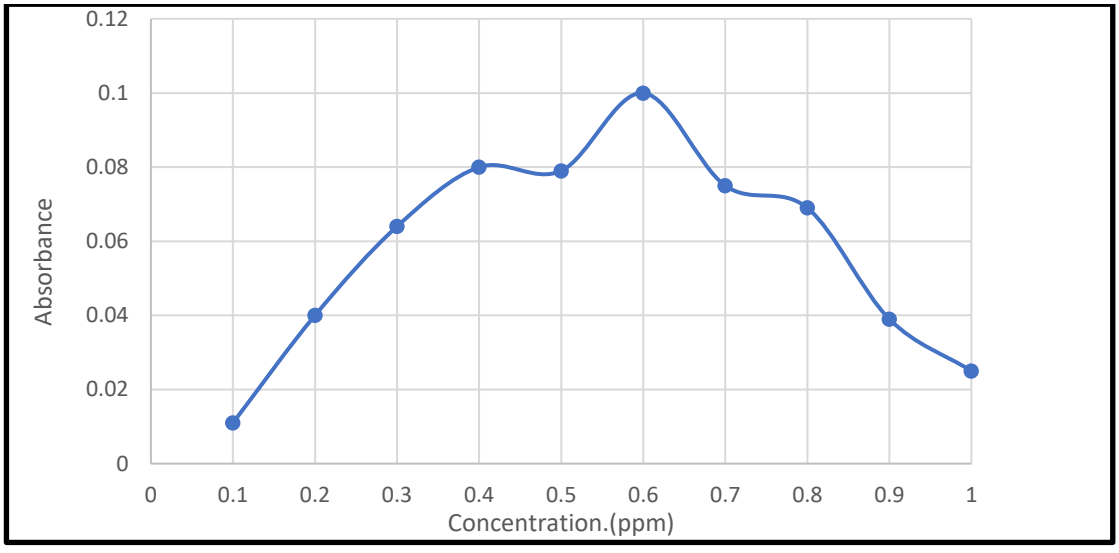



Fig. (6). The effect of Chromotropic acid disodium salt dihydrate concentration on the Ambroxol reaction.

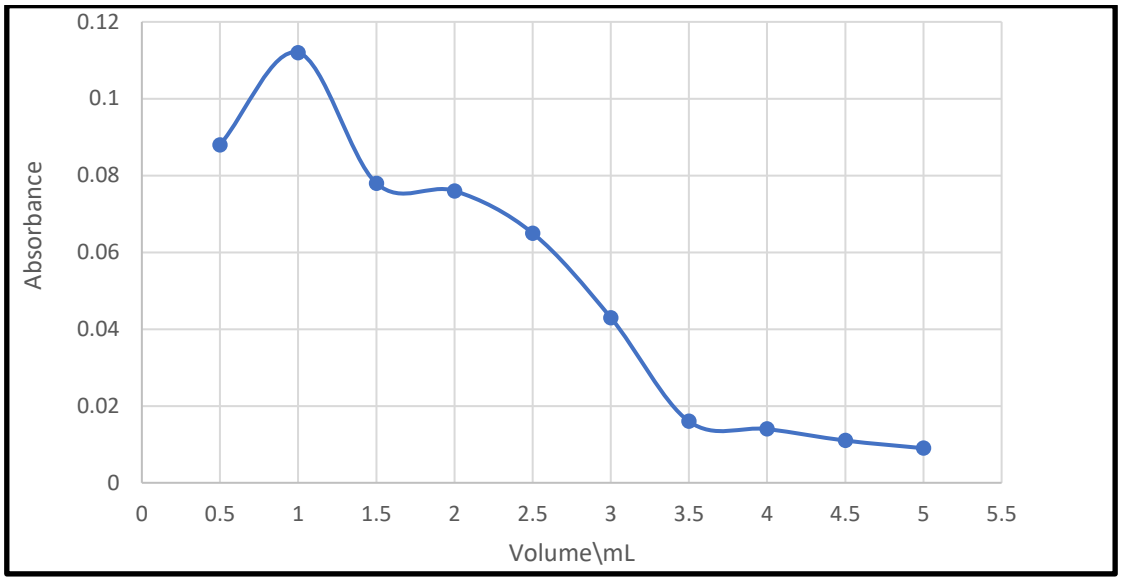



Fig. (7). The reaction is affected by the volume of Chromotropic acid disodium salt dihydrate used.

**3.2.3 Effect of pH of the buffer solution**

The pH was measured to obtain a high intensity of absorption as several solutions were taken, ranging from 7 to 13, to see how it affects the interaction between Ambroxol and Chromotropic acid disodium salt dihydrate. The product absorbance was low at pH 10, as shown in (Fig.8), showing that the antiviral had trouble interacting with the Chromotropic acid disodium salt dihydrate at this pH. The amino group of antivirals is present in the form of a salt, which makes it unable to replace the nucleosome. At pH 10, Chromotropic acid disodium salt dihydrate was found to react with compounds containing primary amines [17].

**3.2.4 Effect of the Amount of the Buffer**

Different quantities of buffer solutions were used when measuring pH (10), as this quantity gave absorption according to the chart below (Fig. 9).

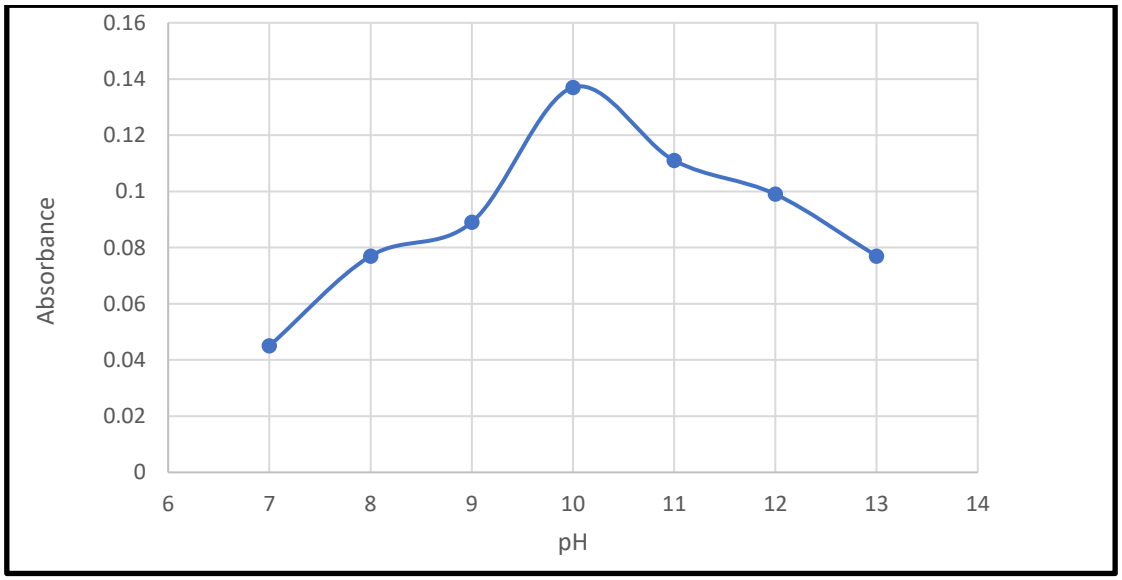



Fig. 8 shows the effect of pH of the buffer solution.

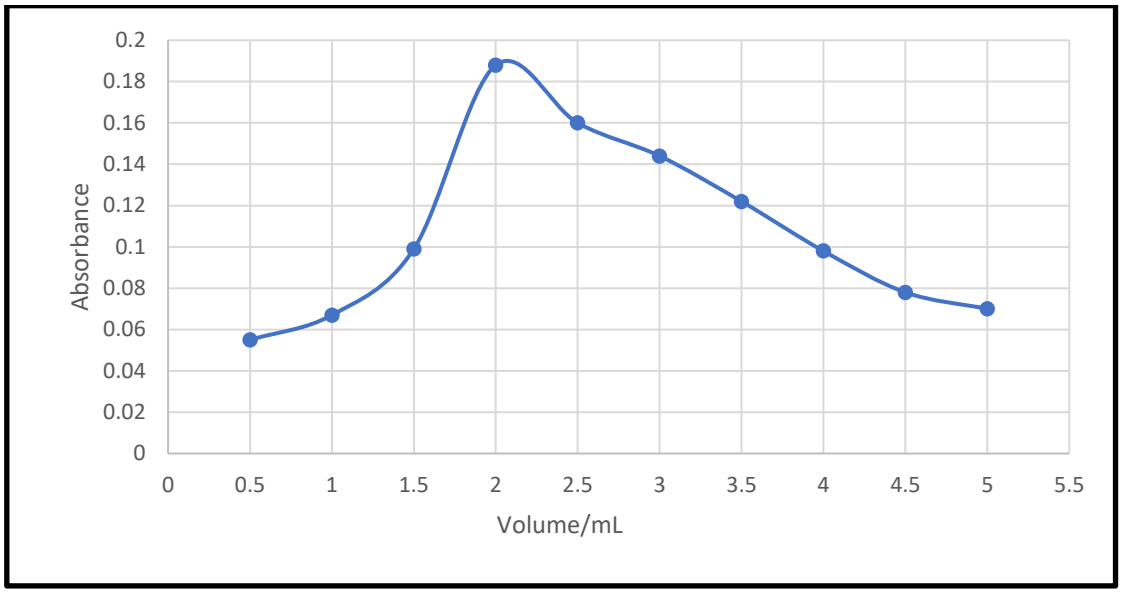



Fig. 9 shows the effect of the volume of the Buffer solution.

### 3.2.5 Effect of reaction time

Time has an important role in determining the course of the interaction between Ambroxol and Chromotropic acid disodium salt dihydrate and the reagent (Fig.10), where the absorption was measured at different time intervals to determine the best absorbency that the product reaches.

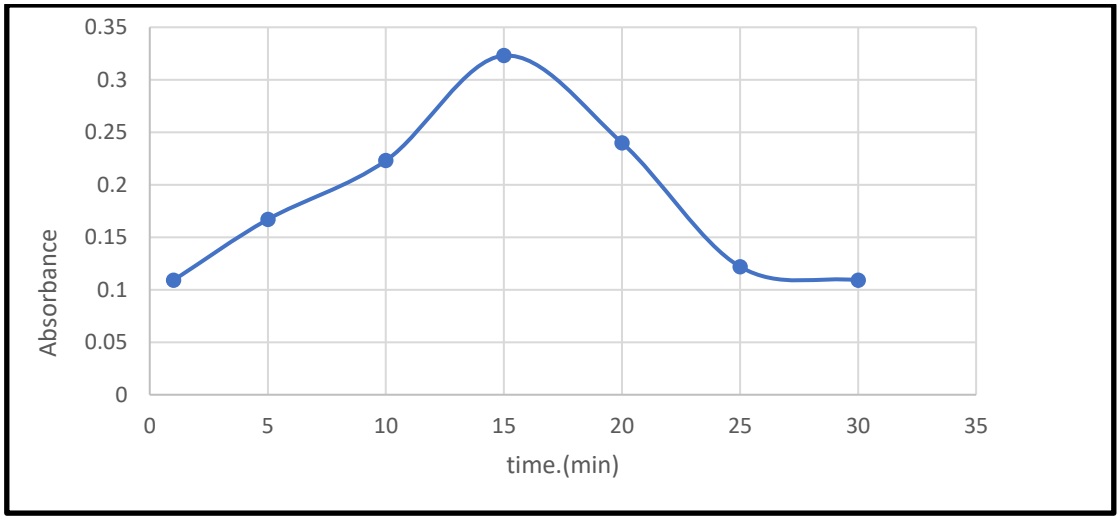



Fig. (10). Ambroxol reaction with the Reagent and the effect of standing time.

### 3.2.6 Effect of temperature

The effect of temperature on the course of the reaction was studied, where the results of the absorbance at a temperature 45ºC were the highest according to Figure 11.

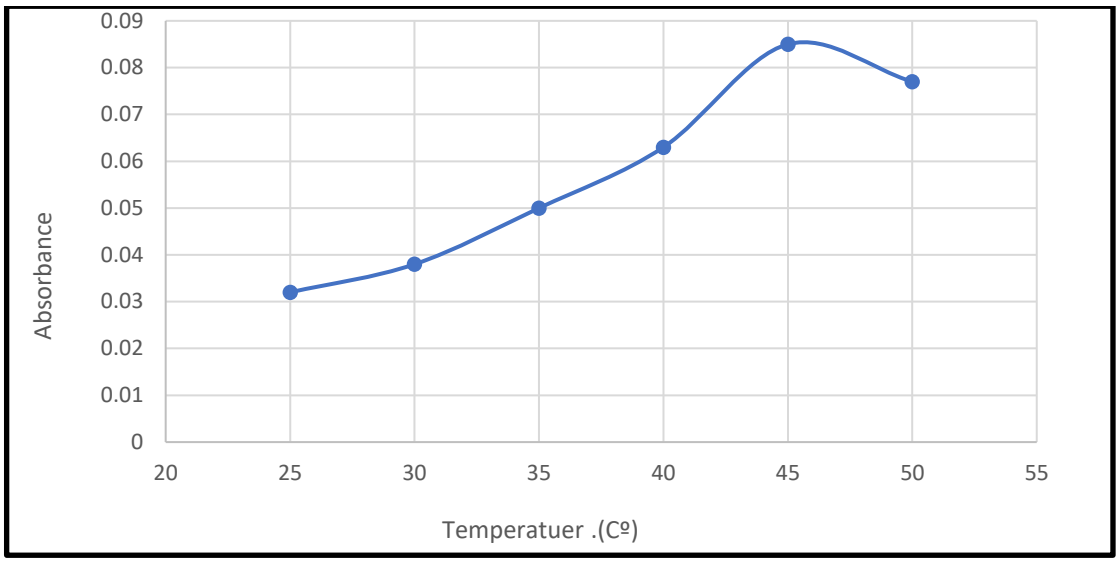



Fig. (11). The effect of temperature on Ambroxol reactivity with Reagent.

### 3.2.7 Effect of addition order

The standard addition is instrumental in obtaining a high absorbance (Table 1). Demonstrates natural substances with increased absorption as a result of the generation of a chemical reaction.

### 3.3. Calibration curve of Antiviral Acyclovir.

The calibration curve for the drug Ambroxol was drawn after determining all the optimal conditions for interaction with the reagent, where the absorption ranged from (2-18) $\mu g.ml^{-1}$ at the specified wavelength (469) with a R = 0.9967 correlation coefficient. 0.8287x104 L/mol/cm was the value of molar absorptivity (ε) (Fig.12).

Specific absorbance was determined by the straight-line equation and was equal to 0.0777 g/mL. This analytical method favors the determination of Ambroxol at low concentrations due to its high molar absorbance value and Sandell's sensitivity.

(Table.1) Addition order of Ambroxol.

| No. | Addition | Abs |
|---|---|---|
| **1** | **R + Buffer +Ambroxol** | **0.093** |
| **2** | **Ambroxol + Buffer + R** | **0.088** |
| **3** | **R+ Ambroxol + Buffer** | **0.112** |

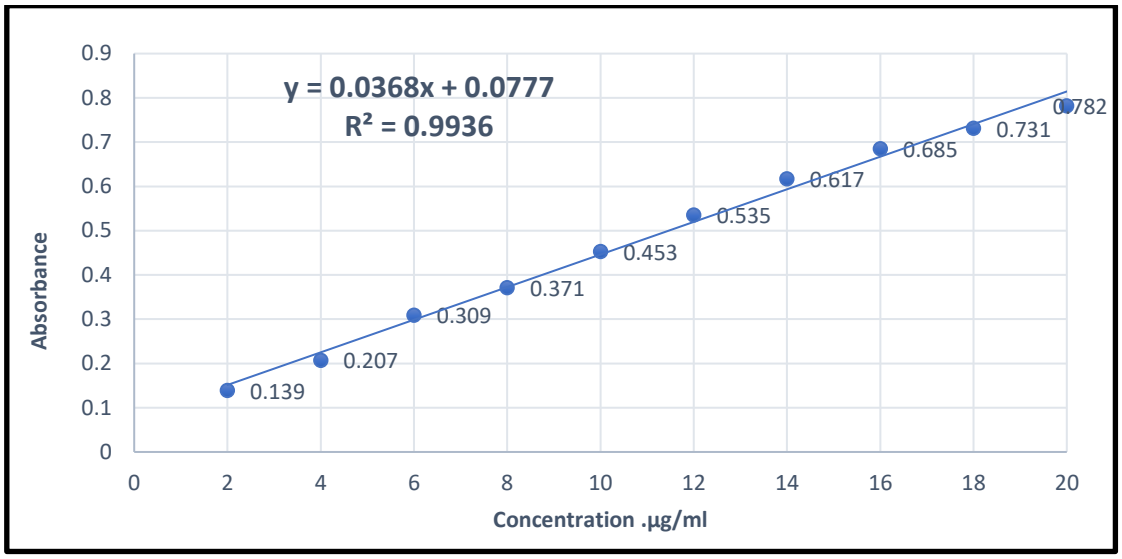



Fig. (12). Calibration curve of the drug Ambroxol.

**3.4. Evaluation of limit of detection, quantification, and linearity (LOD and LOQ).**

The linear regression analysis was applied to detect linearity, which involves providing nine concentrations of Acyclovir [18] in the range of 2-16 g/mL, through the defined equation of calibration and correlation coefficient by methods of least squares regression. By measuring concentration vs absorbance and by linear regression analysis, the calibration curves were created. A = 0.0368X + 0.0777 ($R^2$ = 0.9936) was the regression equation for the results (Table 2). The result shows the amount limit of detection and quantification. According to the ICH guidelines [19-23], to use the formulas LOD = 3.3

S. Da/b and LOQ = 10 S. Da/b,

* S. Da was the standard deviation of the blank.
* b was the slope.

**Table 2:** regression equation

| Parameter | Value |
|---|---|
| Measurement wavelength (nm) | 469 |
| Molar absorptivity ($L.mol^{-1}.cm^{-1}$) [20] | 0.8287X104 |
| Linear range (µg/mL) | (2-18) |
| Detection limit (µg/mL) [21] | 0.1956 |
| LOQ (µg/mL) [22] | 0.6521 |
| Sandell's sensitivity(µg/cm) [23] | 0.0128 |
| Correlation coefficient (R) | 0.9967 |
| Determination coefficient (R2) | 0.9936 |
| Slope (b) | 0.0368 |
| Intercept (a) | 0.0777 |

### 3.5. Accuracy

The accuracy of the experiment was studied by taking several different concentrations at a specific concentration (8µg.ml$^{-1}$) of the drug mixture Ambroxol with the reagent to verify the accuracy of the proposed method. The standard deviation was calculated to determine the drug [24,25] shown in Table 3.

### 3.6. Stability and Nature of the Product's Constant:

Stoichiometric ratios were calculated using the mole ratio [26-28] Jobs' method [29], as illustrated in (Fig.,13,14). The findings revealed an Ambroxol and Chromotropic acid disodium salt dihydrate. ratio of 1:1. Product production occurs in the same way. This result indicates the formation of stable compounds. We note the formation of the product by the proposed method, as in Scheme 1. The reaction product was highly stable between Ambroxol acid and the disodium salt chromotropic reagent.

Table 3- The parameters relative error and recovery are used to express the accuracy of Ambroxol determination methods.

| Parameters | Values |
|---|---|
| wavelength (nm) | 469 |
| Concentration (µg/mL) | 8 |
| R | 0.9967 |
| $\bar{X}$ | 0.3680 |
| R.S.D (%) | 0.6520 |
| Error (%) | -1.5 |
| Recovery (%) [25] | 98.5 |

R: Coefficient of correlation
RSD: relative standard deviation
$\bar{X}$: Absorbance of blank
DL: Detection limit

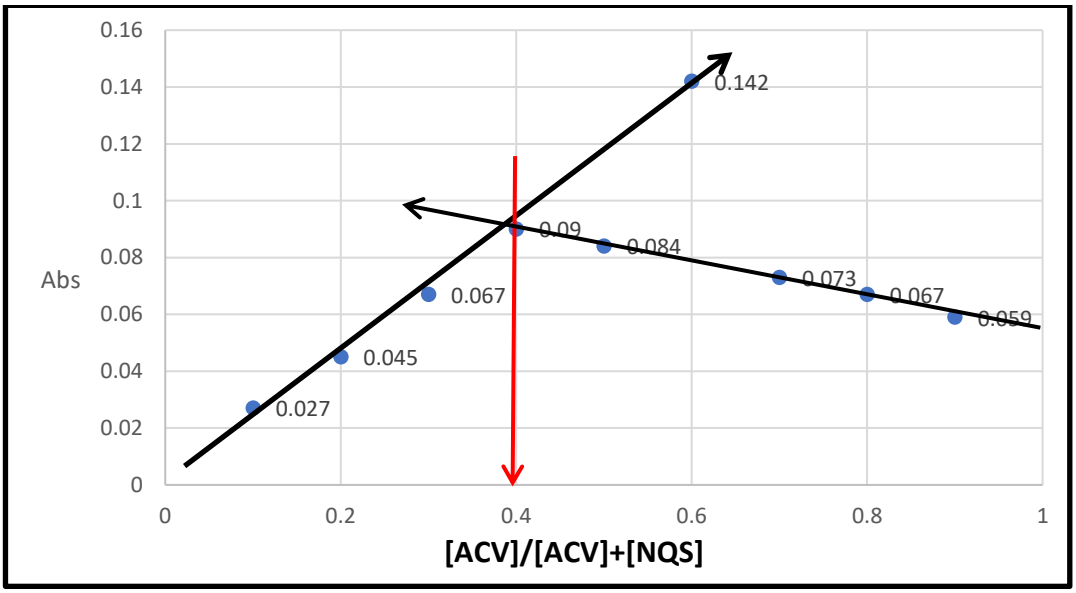



Fig. (13). The stoichiometry of the reaction between Ambroxol and Chromotropic acid disodium salt dihydrate is exhibited as a continuous variation (Job's method) plot.

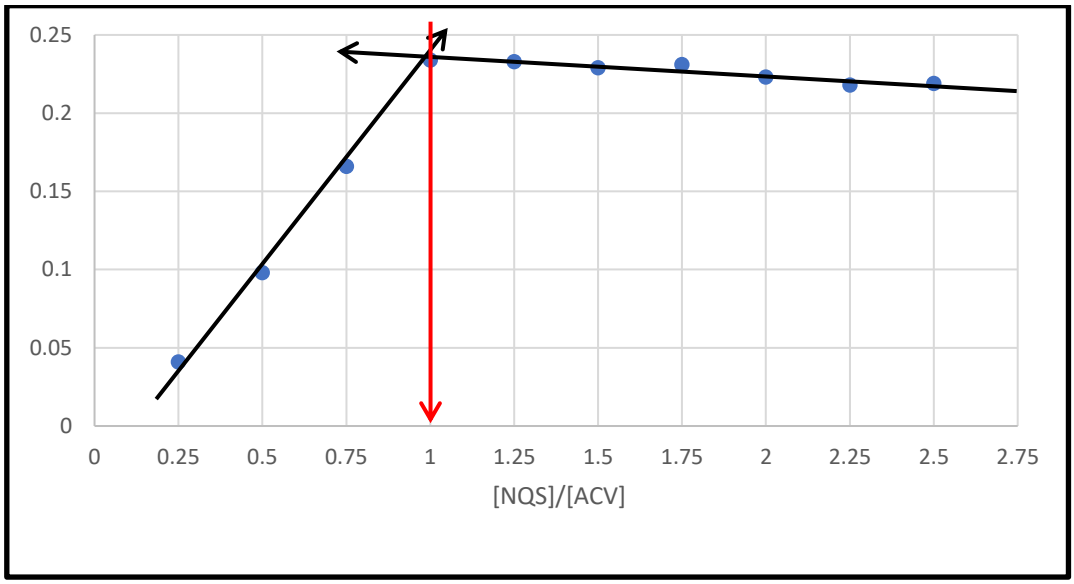
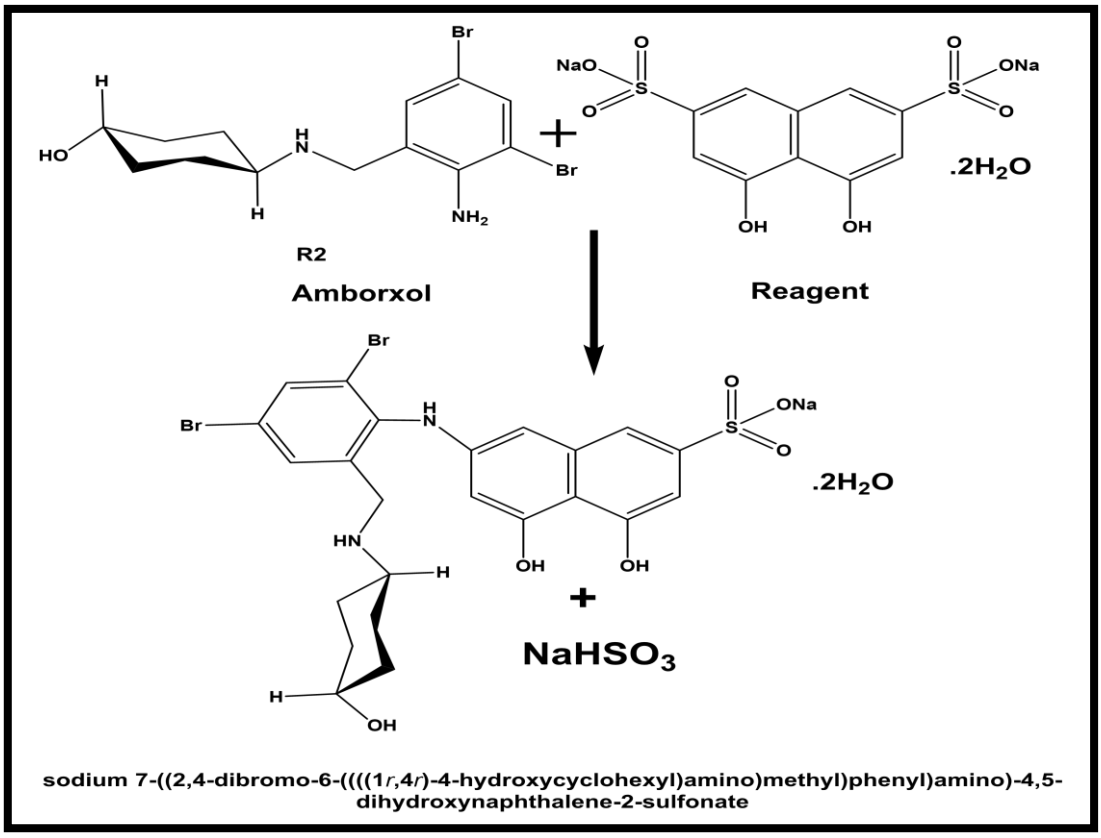



Fig. (14). The mole ratio of the reaction between Ambroxol and Chromotropic acid disodium salt dihydrate is plotted as a continuous variation plot.

The best preparation of stoichiometric ratio detection between reagent and Ambroxol was wavelength of 469 nm, and the ratio of Ambroxol to reagent at 1:1.




Scheme (1) shows the recommended Ambroxol and Chromotropic acid disodium salt dihydrate reaction formula

### 3.7. Interference Study:

The interactions present in the medicinal substance that affect the absorption were determined, as a high concentration of 1000 µg.ml$^{-1}$ was taken.

Table (4). The effect of interference on the drug.

| interferences | Abs |
|---|---|
| Cellulose | -0.02 |
| Glucose | 0 |
| Hydrochloric acid | -0.003 |
| Magnesium stearate | -0.001 |
| Povidone | -0.003 |
| Silica | 0 |
| Talcum (Talc) | - 0.005 |

### 3.8. Analyzing Ambroxol drug formulations with the proposed method

This study was used to estimate the drug Ambroxol in some medical drugs, and the results are according in the table below.

Table (5) analytical applications

| Sample | Conc. µg. mL$^{-1}$ | | Error % | Recovery % |
|---|---|---|---|---|
| | Present | Found | | |
| Ambroxol tablets | 16 | 16.02 | 0.125 | 100.125 |
| Ambroxol Mucosalvan | 10 | 10.10 | 1 | 101 |

## 4. Conclusion

In this study, the reagent (disodium chromotropic acid and dihydrate salt) was used to determine Ambroxol using spectrophotometric techniques. All optimum reaction conditions were studied by determining the drug and reagent concentrations, and determining the temperature and time based on the absorption value. Commercially available dosages were also determined. We conclude from this study that the use of spectroscopic methods in drug estimation is an important and accurate method for estimating pharmaceutical preparations at very low concentrations. These methods are characterized by being rapid, which differs from other traditional analytical methods. These methods will help in the future in detecting and monitoring drug stability during manufacturing or storage processes.

## 5. CONFLICT OF INTEREST

The authors declare that there was no conflict of interest in carrying out this work.

## 6. ACKNOWLEDGMENTS

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