**ORIGINAL ARTICLE**

# Spectrophotometric quantification of amitriptyline hydrochloride in pharmaceutical tablets: method development and validation

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## Abstract

The development and validation of two uncomplicated, selective, and non-extraction-based visible spectrophotometric methods was carried out for the quantification of a tricyclic antidepressant amitriptyline hydrochloride as pharmaceutical tablets as well as in its pure form. The methods rely on the formation of ion pair complexes between amitriptyline base and two acidic dyes, bromothymol blue and bromocresol purple, which were subsequently measured at 410 and 400 nm, respectively. The developed methods included numerous reaction variables that were examined and improved. Beer's law is applicable under optimal conditions for the bromothymol blue and bromocresol purple methods, respectively, over concentrations from 0.5 to 12.5 µg mL$^{-1}$ and from 0.5 to 10.0 µg mL$^{-1}$ amitriptyline hydrochloride. The computed values of molar absorptivity were $1.79 \times 10^4$ and $2.32 \times 10^4$ L.mol$^{-1}$.cm$^{-1}$ for bromothymol blue and bromocresol purple methods, respectively, with Sandell's sensitivity values of 0.0155 and 0.0119 µg cm$^{-2}$. Both detection limits and quantification limits were computed and determined to be 0.55 and 1.67 µg mL$^{-1}$ for bromothymol blue method, and 0.49 and 1.49 µg mL$^{-1}$ for bromocresol purple method, respectively. The developed methods were successfully applied to estimate the concentration of amitriptyline hydrochloride in bulk form and commercial pills. Therefore, these methods demonstrate promising potential for testing this drug in labs for quality control.

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**Graphical abstract**

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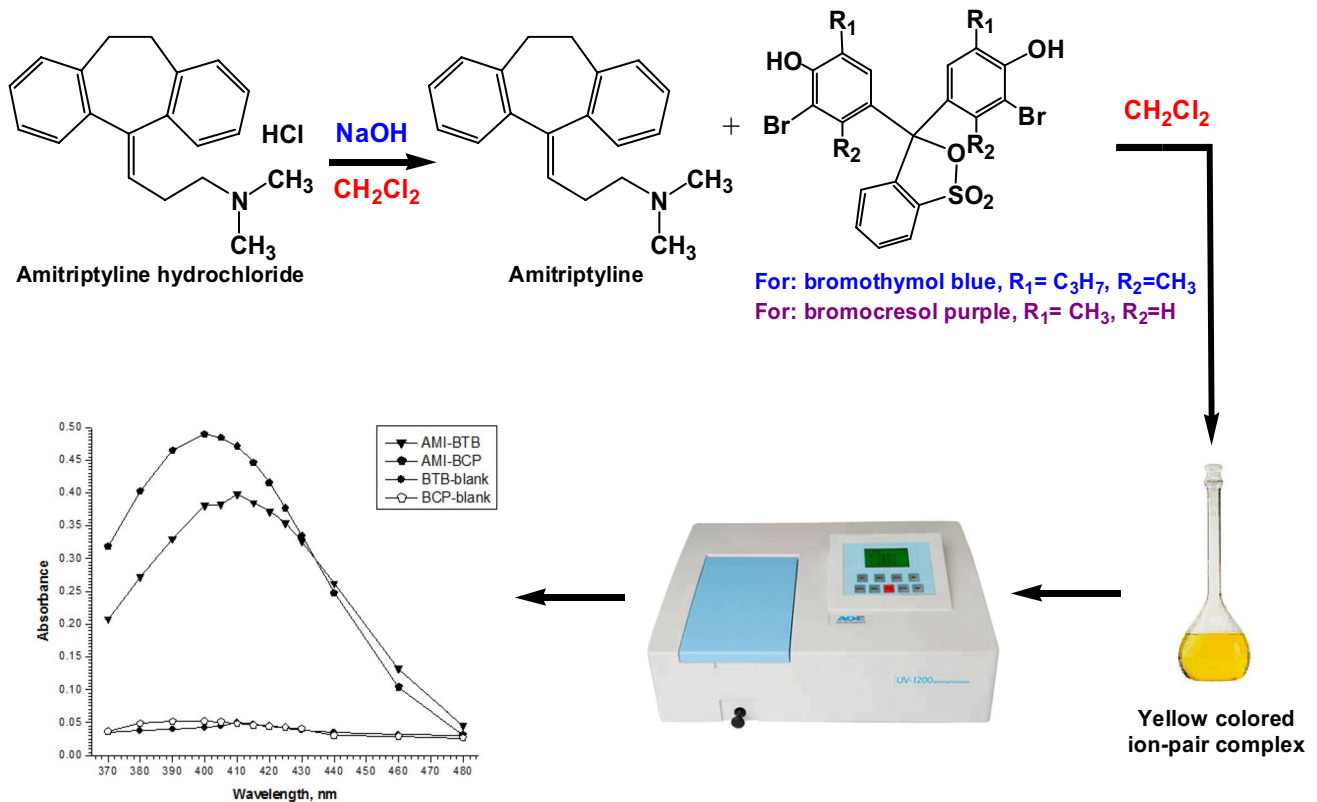


**Keyword** Amitriptyline hydrochloride · Estimation · Ion pair complexes · Spectrophotometry · Pharmaceutical tablets

# 1 Introduction

Amitriptyline hydrochloride (AMIH) is known as 3-(10,11-dihydro-5H-dibenzo[a,d][7]annulen-5-ylidene)-N,N-dimethylpropan-1-aminehydrochloride (Fig. 1). AMIH, also known as Elavil, is a tricyclic antidepressant (TCA) that appears as almost white in powder form or as colorless crystals. It is soluble in 96% ethanol, water, and dichloromethane, as reported in European Pharmacopoeia (EP) [1]. This drug works by inhibiting the uptake of serotonin and other transmitters at presynaptic nerve terminals [2].

After conducting a comprehensive survey of the literature on the quantitative estimation of AMIH in various pharmaceutical preparations, it was found that numerous quantitative assays have been conducted to evaluate AMIH. In this investigation, our primary emphasis will be on the evaluation of established methodologies that solely determine AMIH in pharmaceutical formulations. The previous studies utilized several techniques, such as high performance liquid chromatography (HPLC) [3, 4], reverse phase ultra performance liquid chromatography (RP-UPLC) [5], potentiometry [6, 7], voltammetry [8], flow injection analysis (FIA) [9], chemiluminescence [10], spectrofluorometry [11], ultraviolet (UV) spectrophotometry [12–16], and visible (Vis) spectrophotometry [11, 17–27].

Visible spectrophotometry is a commonly used technique for quantitative determination of active materials in bulk form in addition to pharmaceutical pills, because of its simplicity, selectivity, and sensitivity. To the best of our knowledge, twelve studies reported the employment of visible spectrophotometry for AMIH estimation alone in pharmaceutical preparations. However, many of these reports suffered from certain disadvantages, such as the need for an extraction step [17, 19, 22, 25], or a heating step [11, 18, 27], lower sensitivity [17, 18, 20, 22, 23], use of toxic and hazardous substance [21], and a narrow linear range [26] (Table 1).

As far as we know, only two published reports [9, 25] described the use of BTB dye and/or BCP dye for estimating AMIH in pharmaceutical formulations. However, the
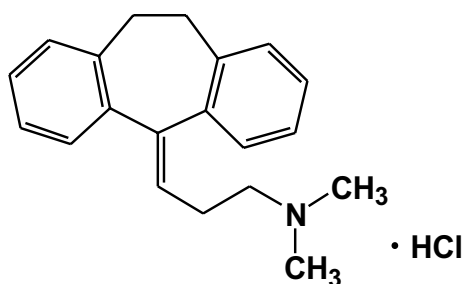
**Fig. 1** Structure of amitriptyline hydrochloride

method developed by Calatayud and Pastor [9] involves the production of an ion pair compound between AMIH and BCP (a precipitating reagent) and requires pH adjustment. This method employs FIA with turbidimetric detection, which has several limitations, including limited sensitivity, interference from the sample matrix, lack of specificity, and the need for specialized instrumentation. The methods reported by Susmitha et al. [25] relied on the production of ion pair complex between AMIH and bromophenol blue (BPB), BTB, and BCP dyes in a buffer, and the resulted complexes were then quantitatively extracted into chloroform. However, these methods require adjustment of different experimental parameters, including pH adjustment, the selection of a suitable buffer solution, efficient extraction using various solvents, significant duration of contact time, predetermined volumes of aqueous and organic phases, and specific extraction period. These requirements make the analytical method somewhat cumbersome. Moreover, incomplete extraction and emulsion formation can lead to difficulties and inaccuracies in ion pair extraction methods.

The primary goal of the present study is the development and validation of two uncomplicated, selective, and extraction-free visible spectrophotometric methods for the quantification of AMIH in tablets. Both of the developed methods rely on the production of ion pair complexes between the amitriptyline (AMI) base and two acidic dyes, bromothymol blue (BTB) and bromocresol purple (BCP).

# 2 Experimental

## 2.1 Instrument

The absorbance measurements were performed using ultraviolet–visible (UV–Vis) spectrophotometer, model UV-1200 (manufactured by AOE Instruments, Shanghai, China), equipped with quartz cuvettes with a path length of 1 cm.

## 2.2 Chemicals and reagents

Only laboratory reagent grade chemicals were utilized in the present study.

Fresh solutions of 0.1% (w/v) BTB and 0.1% (w/v) BCP (Loba Chemie Pvt. Ltd., Mumbai, India) were made using dichloromethane (DCM) (Scharlab S. L., Spain). A 0.1 N solution of sodium hydroxide (NaOH) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was produced using distilled water. In addition, a solid anhydrous sodium sulfate ($Na_2SO_4$) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was utilized as a drying agent.

Pharmaceutical grade AMIH was gifted by the Shiba Pharmaceuticals & Chemicals Mfg. Co. Ltd., Sana'a, Yemen, and was used as received. The commercial sample of the AMIH drug used for estimating AMIH in tablets was Amtil 25 mg tablets, which are marketed by the Shiba Pharmaceuticals & Chemicals Mfg. Co. Ltd., Sana'a, Yemen,

A standard amitriptyline base solution was prepared by accurately weighing (0.0283 g) of pure AMIH and dissolving it in 20 mL of distilled water, followed by thorough mixing. Subsequently, the solution was made alkaline by adding 0.1 N NaOH and then 30 mL of distilled water. A quantitative transferring of the resulting solution to a 125 mL separating funnel was done, and the free amitriptyline base (AMI) was taken away by shaking the solution with four consecutive 20 mL volumes of DCM. The combined DCM extracts and DCM washings were dried by passing them through $Na_2SO_4$ and transferring them to a 100 mL volumetric flask. The resulting solution was subsequently made up to the mark with DCM. To obtain the desired concentration of 25 μg mL$^{-1}$ AMI base for use in the developed methods, the resultant solution (250 μg mL$^{-1}$ AMI base) was appropriately diluted with DCM. The applied extraction procedure is similar to the method described by Taha et al. [28] for preparing free bases of some tricyclic antidepressant drugs.

## 2.3 Procedures

### 2.3.1 Spectrophotometric determination of AMI using BTB

Using a micro burette, various volumes ranging from 0.2 to 5.0 mL of a standard 25 μg mL$^{-1}$ AMI base solution were transferred to a set of 10 mL volumetric flasks. To adjust the total volume to 5.0 mL, DCM was used, followed by the addition of 1.0 mL of a 0.1% (w/v) BTB solution to each flask. Following a 5 min interval, the mixture was brought to the mark using DCM and then thoroughly blended. Each solution's absorbance was measured at 410 nm against a corresponding blank.

**Table 1** Comparison of the developed methods and the previously reported visible spectrophotometric methods for the quantification of AMIH

| Sl No | Reagent (s) used | Methodology | $\lambda_{max}$ (nm) | Linear range, ($\mu$g mL$^{-1}$) and $\varepsilon$ (l mol$^{-1}$ cm$^{-1}$) | DL, ($\mu$g mL$^{-1}$) | Reaction time | Remarks | Ref |
|---|---|---|---|---|---|---|---|---|
| 1 | Malonic acid anhydride | A condensation yellow-colored product measured | 485 | 0.5 – 10 ($\varepsilon = 3.2 \times 10^5$) | 0.168 | 15 min | It requires heating for 15 min at 90 °C in a water bath | 11 |
| 2 | TiCl$_4$ and potassium thiocyanate | A yellow-colored ion-association compound measured | 360 | 3 – 60 ($\varepsilon = 8.96 \times 10^3$) | 0.27 | 7 min | Extraction is required using a mixture of chloroform and butanol (4:1), and less sensitive | 17 |
| 3 | Ammonium molybdate and concentrated sulphuric acid | A green-colored complex measured | 660 | 1 – 140 ($\varepsilon = 2.408 \times 10^3$) | 1.0 | 20 min | It needs to be heated for 20 min at 100 °C in a water bath and it is less sensitive | 18 |
| 4 | a) Eriochrome cyanine R b) Pyrocatechol violet | A colored ion-association complex measured / A colored ion-association complex measured | 518 / 445 | 8 – 80 ($\varepsilon = 4.6 \times 10^3$) / 1.5 – 15 ($\varepsilon = 2.4 \times 10^4$) | 4.43 / 0.78 | NR / NR | Extraction is required using either butanol or a mixture of chloroform and butanol (2:1) | 19 |
| 5 | Chloranilic acid | A purple-colored charge transfer complex measured | 520 | NA ($\varepsilon = 4.666 \times 10^3$) | NA | NA | Less sensitive | 20 |
| 6 | Ferric ammonium sulphate and mercuric thiocyanate | A pink-colored complex measured | 460 | 0 – 60 ($\varepsilon = $ NR) | NR | 10 min | Use of toxic and hazardous substance | 21 |
| 7 | 1,2-naphthoquinon-4-sulphonate | A yellow-colored product measured | 450 | 2 – 18 ($\varepsilon = 3.238 \times 10^3$) | NR | 2 min | Extraction is required using chloroform and less sensitive | 22 |
| 8 | Iron(III) and ammonium thiocyanate | A red–orange color of Fe(III)-SCN$^-$ complex measured | 460 | 1 – 10 ($\varepsilon = 2.82 – 3.36 \times 10^3$) | 0.3 | NR | Less sensitive | 23 |
| 9 | Bromate-bromide mixture and eriochrome blue black R | A violet-colored of unbleached dye measured | 530 | 0.3 – 15 ($\varepsilon = 1.345 \times 10^4$) | 0.0802 | 8 min | Many reagents are used | 24 |
| 10 | a) Bromothymol blue b) Bromophenol blue c) Bromocresol purple d) Iodine e) Alkaline KMnO$_4$ | Measurement of a yellow ion-pair complex in methods (a, b & c) / A triiodide ion (I$_3^-$) measured / A green colored manganate measured | 415 410 415 366 610 | 1.25 – 25 ($\varepsilon = $ NR) / 1.5 – 25 ($\varepsilon = $ NR) / 2 – 25 ($\varepsilon = $ NR) / 2.5 – 25 ($\varepsilon = $ NR) / 6.25 – 50 ($\varepsilon = $ NR) | 0.8 1.3 0.97 0.9 5.4 | 10 min 10 min 10 min NR 25 min | Methods (a, b & c) required a step of extraction using chloroform and buffer solution was used. Measurements at shorter wavelength in method (d). Rate measurement requires control of several variables in method (e) | 25 |
| 11 | Iron(III) and ammonium thiocyanate | A blood-red color of Fe(III)-SCN$^-$ drug complex measured | 490 | 0.5 – 5 ($\varepsilon = $ NR) | 0.0038 | NR | Narrow linear range | 26 |
| 12 | 1,2-diamino benzene and sodium periodate | A colored oxidative coupling product measured | 464 | 2 – 32 ($\varepsilon = 1.36 \times 10^4$) | 0.626 | 20 min | It requires heating in a water bath at 30 °C for 20 min | 27 |

**Table 1** (continued)

| SI No | Reagent (s) used | Methodology | $\lambda_{max}$ (nm) | Linear range, (μg mL$^{-1}$) and $\varepsilon$ (l mol$^{-1}$ cm$^{-1}$) | DL, (μg mL$^{-1}$) | Reaction time | Remarks | Ref |
|---|---|---|---|---|---|---|---|---|
| **13** | **a) Bromothymol blue (BTB)** | **Measurement of the formed ion-pair complex** | **410** | **0.5 – 12.5** ($\varepsilon = 1.79 \times 10^4$) | **0.25** | **5 min** | **Simple, sensitive, selective, only one reagent is required, no pH-adjustment, and no stage for extraction or heating in the assay procedure** | **This work** |
| | **b) Bromocresol purple (BCP)** | **Measurement of the formed ion-pair complex** | **400** | **0.5 – 10.0** ($\varepsilon = 2.32 \times 10^4$) | **0.29** | **5 min** | | |

$\lambda_{max}$: maximum wavelength, $\varepsilon$: molar absorptivity, DL: detection limit, NR: Not reported, NA: Not available

## 2.3.2 Spectrophotometric determination of AMI using BCP

Six volumetric flasks (10 mL) were used to transfer various volumes ranging from 0.2 to 4.0 mL of a 25 μg mL$^{-1}$ AMI base standard solution. DCM was then added to bring the volume of the overall solution to 4.0 mL, and afterwards, 1.0 mL of a 0.1% (w/v) BCP solution was introduced into each flask. Then, the resulting mixture was left aside for 5 min and then it was thoroughly mixed and diluted with DCM to the mark. The measurement of the absorbance of all solutions was read at 400 nm against a corresponding blank.

## 2.3.3 Assay of AMIH in the tablets

The weight of ten Amtil 25 mg AMIH tablets was determined and these tablets were crushed into a powder. An accurate amount of the powder, corresponding to 25 mg of AMI base, was weighed and then transferred into a 50 mL volumetric flask. A 30 mL of distilled water was added to the same flask, which was shaken for about 15 min. Subsequently, the flask was filled to the mark using distilled water, and the resulting mixture was thoroughly blended. After that, the filtration was done utilizing a Whatman No. 42 filter paper. After obtaining the filtrate, it was rendered alkaline by adding 0.1 N sodium hydroxide. The resulting solution was then moved to a 125 mL separatory funnel, and the AMI base was extracted using four portions of DCM (20 mL). The final steps were the same as those mentioned in the preparation of the "standard amitriptyline base solution" to get a concentration of 25 μg mL$^{-1}$ AMI base. Once the working concentration was obtained, appropriate portions of this solution were analyzed using the BTB and BCP methods as outlined in the assay procedure.

## 2.3.4 Procedures for method validation

Validation was performed based on the current International Conference on Harmonization (ICH) guidelines [29]. It covered linear ranges, detection limits (DL), quantification limits (QL), precision, accuracy, robustness, and selectivity.

To evaluate linearity, a set of seven standard solutions of AMI base was analyzed for the BTB method, and a set of six standard solutions of AMI base were analyzed for the BCP method. Calibration curves were created as reported above in the "assay procedure of AMI using BTB and BCP."

Three solutions of AMI were prepared at varying concentrations to assess the developed methods' accuracy and precision. These concentrations were within the working limits of the methods, which were 5, 7.5, and 10 μg mL$^{-1}$ AMI for the BTB method and 2.5, 5, and 7.5 μg mL$^{-1}$ AMI for the BCP method. To assess precision and accuracy, these solutions were analyzed in seven duplicates on the same day (intra-day) and on five consecutive days (inter-day). The

percentage relative standard deviation (%RSD) and percentage relative error (%RE) were computed to evaluate precision and accuracy, respectively.

The robustness of the methods was assessed by making minor adjustments to some variables. Specifically, the volume of the dye was varied at 0.9, 1.0, and 1.1 mL for both dyes, and time of the reaction was varied at 4, 5, and 6 min in both methods. Assays were performed under the optimum experimental circumstances, and the %RSD values were calculated to evaluate the methods' robustness.

The sensitivity of the developed methods was determined in terms of molar abosorptivity ($\varepsilon$) and Sandell's sensitivity. Molar absorptivity is a physical constant of the species under study and is the absorbance at the selected wavelength of solution for a compound of unit molar concentration measured in 1 cm path length. Molar absorptivity can be calculated from the following equation [30]:

*Molar absorptivity = Slope × Molecular weight × 1000*

Sandell's sensitivity is the concentration of the analyte in ($\mu$g mL$^{-1}$), which gives an absorbance of 0.001 when measured in a cuvette of cross-sectional area 1 cm$^2$ and 1 cm path length. Sandell's sensitivity can be calculated from the following equation [30]:

$$Sandell's\ sensitivity = \frac{Molecular\ weight}{Molar\ absorptivity}$$

To determine the methods' selectivity, predetermined amounts of frequently used additives including starch, acacia, talc, glucose, gelatin, and trisodium citrate were mixed to prepare a placebo blank. This mixture was then poured into a 50 mL calibrated flask holding 30 mL of distilled water, and the produced solution was prepared as stated above in the "Assay procedure for tablets". The resulting placebo blank solution was analyzed using aliquots of 2.0 and 3.0 mL of the solution by the BTB and BCP methods, respectively. To further test the selectivity, a synthetic mixture solution was produced by the addition of 28.3 mg of pure AMIH to a placebo mixture of the same composition and it was thoroughly mixed. A 25 $\mu$g mL$^{-1}$ AMI base solution of the synthetic mixture was then made following the same procedure described before in the "Assay procedure for tablets". This solution was analyzed five replicates using 2.0 and 3.0 mL of the synthetic mixture solution by the BTB and BCP methods, respectively, to evaluate their selectivity.

### 2.3.5 Procedures for stoichiometric ratio

The ion pair complexes' stoichiometry in both methods was ascertained using Job's continuous variations method [31]. To prepare the required solutions, specified quantities of drug and dyes were dissolved in DCM to obtain solutions equivalent to $9.01 \times 10^{-5}$ M AMI base and $9.01 \times 10^{-5}$ M

BTB or BCP dye. Subsequently, different solutions were made by mixing AMI base and dye in complementary proportions of 0:10, 2:8, 4:6, 5:5, 6:4, 8:2, and 10:0, while keeping the final volume at 10 mL of AMI base and dye. Next, the absorbance of the produced ion pair complexes in each solution was measured after 5 min at the respective wavelengths versus DCM to determine the complexes' stoichiometry using both methods.

### 2.3.6 Procedures for improving the reaction conditions

Various reaction parameters were fine-tuned to accurately measure the ion pair complexes absorbance, for instance, the selection of an organic solvent, the dye's concentration, and the time required to complete the reaction.

Initially, the AMIH drug was treated with sodium hydroxide and converted to AMI base, which was later extracted into DCM. To identify the optimal solvent for preparing BTB and BCP solutions and also for dilution, different solvents such as chloroform and DCM were tested using a constant amount of 7.5 $\mu$g mL$^{-1}$ AMI in DCM for the BTB and BCP methods. The AMI base was then reacted separately with both dyes using these solvents, and each solution's absorbance was measured at respective wavelengths after 5 min against the reagent blank.

The concentration effect of BTB and BCP dyes on the produced color intensity at the chosen wavelengths ($\lambda_{max}$) was also investigated by the measurement of absorbance of solutions containing 7.5 $\mu$g mL$^{-1}$ AMI and varying volumes of 0.1% (w/v) BTB and BCP solutions, ranging from 0.25 to 2.00 mL, separately.

To determine the reaction time between AMI and BTB or BCP, 1 mL of 0.1% (w/v) BTB or BCP solution was added to a constant amount of 7.5 $\mu$g mL$^{-1}$ AMI solution. The mixture was then allowed to react for various time durations ranging from 0 to 20 min, followed by measuring the absorbance of the produced ion pair complexes at room temperature.

## 3 Results and discussion

### 3.1 Proposed reaction pathway

To facilitate the formation of ion pair complexes in nonpolar solvents, AMIH was initially neutralized with sodium hydroxide, and the produced AMI free base was extracted into DCM, after which the assay procedures were carried out. The AMI base can form ion pair complexes with acidic dyes, specifically BTB and BCP, due to the presence of a nitrogen atom in AMI linked to two methyl groups, which are electron-donating moities that can be easily protonated. The lactoid ring in the chemical structure of BTB and BCP

dyes is opened, and a quinoid group is then formed, giving these dyes their color [32]. There is an equilibrium between the two tautomers, however the predomination of the quinoid form is attributed to the strong acidity of the sulfonic acid group. Upon protonation of the drug, the resulting protonated AMI can combine with dye anions to form complexes, resulting in the formation of AMI-BTB and AMI-BCP ion pair complexes. The possible reaction pathway has been proposed and presented in Scheme 1.

## 3.2 Absorption spectra

Either BTB or BCP reacts with AMI to produce strongly yellowish ion pair complexes. In comparison to the reagent blank solutions, the absorption spectra of these ion pair complexes (Fig. 2) were measured at 370–480 nm. The maximum wavelengths of these ion pair complexes were 410 nm and 400 nm for the BTB and BCP methods, respectively, and these two wavelengths were used to meausre the absorbance.

## 3.3 Improvement of reaction variables

### 3.3.1 Effect of organic solvents

The findings of this investigation indicated that chloroform and DCM could be used both for preparing BTB and BCP solutions and as diluting solvents. However, DCM was prefered since it was more readily available. As a result,
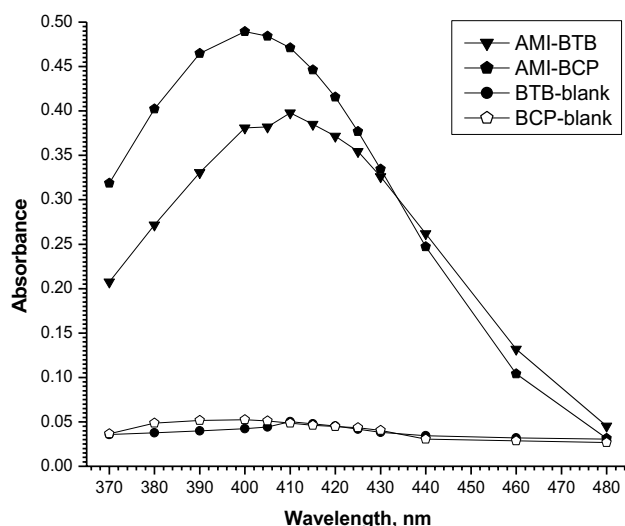


**Fig. 2** Absorption spectra of AMI-BTB and AMI-BCP ion pair complexes (6 μg mL$^{-1}$ AMI) versus the reagent blank

DCM was employed for preparing dyes solutions, drug solution (AMI base), and as a solvent for dilutions.

### 3.3.2 Dye concentration's effect

The outcomes indicated that the ion pair complexes achieved the highest color intensity with one milliliter of both BTB and BCP solutions. The use of higher concentrations of both
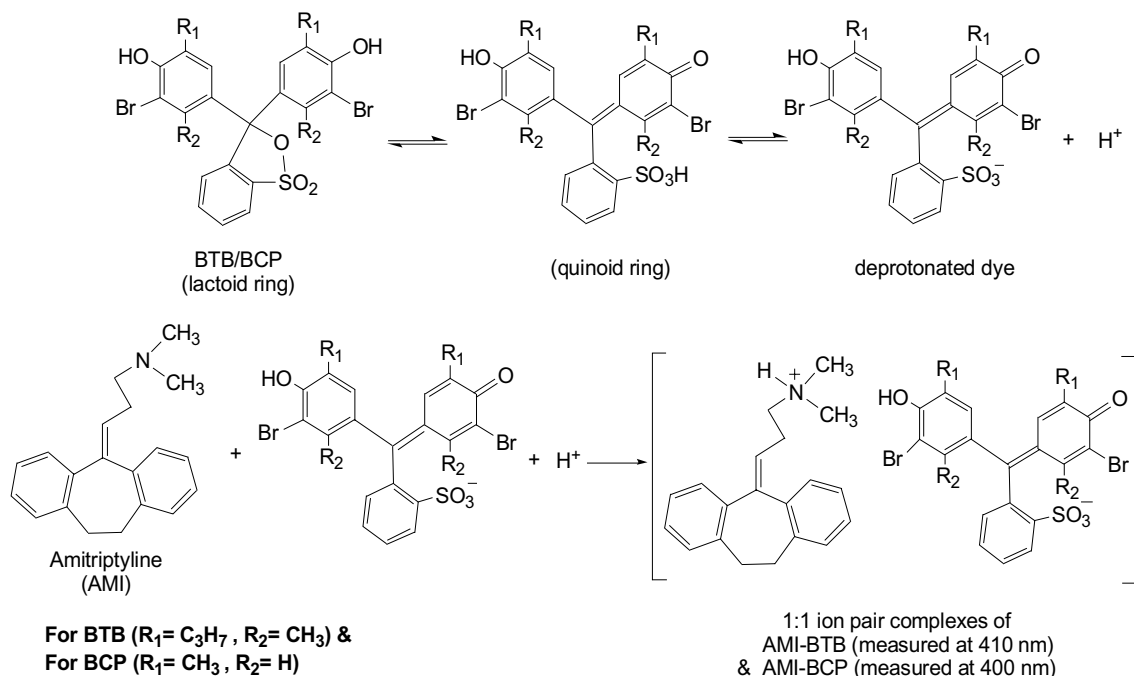


**Scheme 1** Proposed reaction pathways for deprotonation of the dyes and formation of AMI-BTB and AMI-BCP ion pair complexes
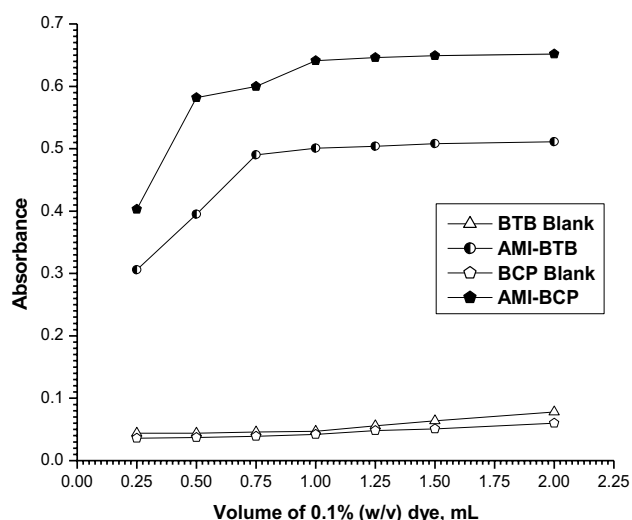
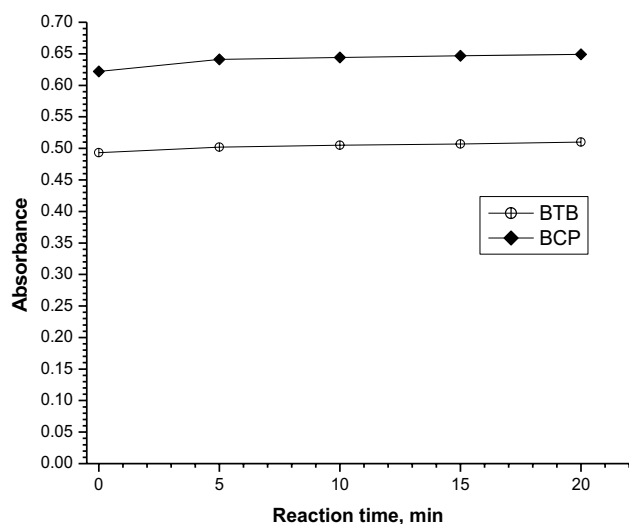**Fig. 3** Dyes concentration's effect on the ion pair complexes' absorbance (7.5 µg mL.$^{-1}$ AMI)



**Fig. 4** Reaction time's effect between AMI (7.5 µg mL$^{-1}$) and BTB & BCP dyes

dyes did not show any effect on the absorbance of the produced complexes, as demonstrated in Fig. 3.

### 3.3.3 Reaction time's effect

The ion pair complexes that were formed between AMI and BTB or BCP were monitored over time. The color began to develop immediately after the addition of the dye's solution to the AMI solution, and the reaction between AMI and BTB or BCP was observed to be fully completed within 5 min (Fig. 4). The resulting ion pair complexes remained stable for a minimum duration of half an hour in both methods.

## 3.4 Stoichiometry of the formed ion pair complexes

According to the Job's continuous variations graph, the interaction between equal molar concentrations of AMI and BTB or BCP occurred through the production of ion pair complexes. The absorbance of each solution was measured and plotted versus $V_{AMI}/(V_{AMI} + V_{dye})$, as illustrated in Fig. 5. The highest value at a mole fraction of 0.5 was obtained, indicating the creation of 1:1 (AMI:BTB and AMI:BCP) ion pair complexes. The computed conditional stability constants for the resulted ion pair complexes were $2.39 \times 10^8$ and $7.25 \times 10^7$ for AMI:BTB and AMI:BCP complexes, respectively [33].

## 3.5 Method validation

### 3.5.1 Analytical parameters

After optimizing the experimental variables for the estimation of AMIH, the calibration curves for AMI with BTB and BCP were established by drawing the absorbance against the concentration of AMI (Fig. 6). Many parameters such as the standard deviation of the slope ($S_b$) and the intercept ($S_a$) were computed from the calibration graph data and presented in (Table 2). Beer's law was applicable in the concentration ranges of 0.5–12.5 and 0.5–10.0 µg mL$^{-1}$ AMIH for BTB and BCP methods, respectively. The values of the molar absorptivity of the produced ion pair complexes, along with Sandell sensitivities, DL, and QL were calculated and listed in (Table 2).

### 3.5.2 Precision and accuracy

Table 3 summarizes the findings of the intra-day and inter-day assays, revealing that the %RSD values were less than or equal to 2.62% (intra-day) and 2.74% (inter-day). These results suggest that the developed methods are highly precise. Additionally, %RE values were less than or equal to 2.80%, confirming the excellent accuracy of both methods (Table 3).

### 3.5.3 Robustness

The impact of altering the reaction time and dye volume on the absorbance of each produce complex in the two methods was examined. The outcomes were shown in Table 4 and demonstrate the robustness of the developed methods (RSD% values were less than 1.72%).

### 3.5.4 Selectivity

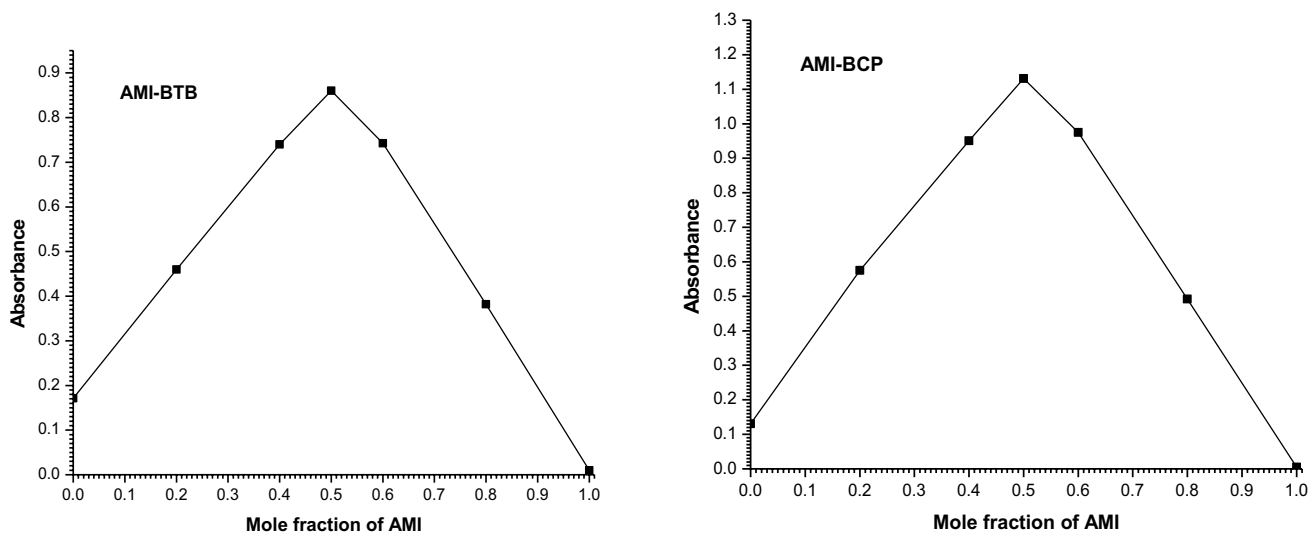Analyses of samples from synthetic mixture and placebo blank were conducted to assess the proposed methods'

**Fig. 5** Plots for the produced ion pair complexes (AMI-BTB & AMI-BCP) using Job's continuous variation approach
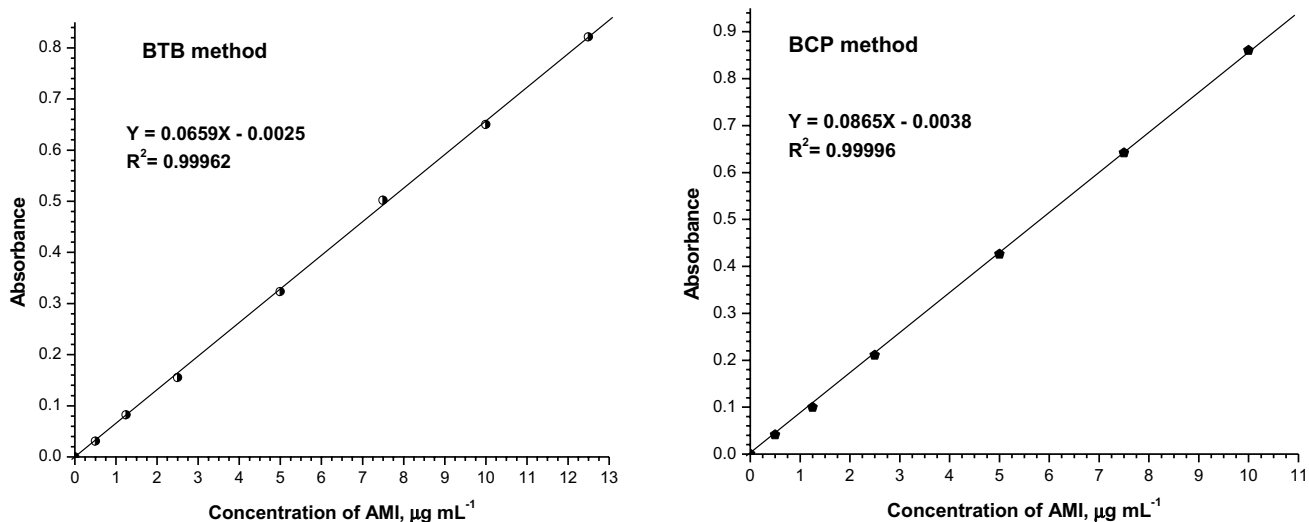


**Fig. 6** Calibration curves of AMI with BTB and BCP

selectivity. When the placebo blank extract was tested, the measured absorbance matched that of the corresponding blank, confirming that the additives used did not affect the conducted assay. The resulting synthetic mixture extract was also analyzed, and the recovery percentages of AMI were computed and determined to be $97.61 \pm 2.43\%$ and $99.01 \pm 1.96\%$ (five replicates) for the BTB and BCP methods, respectively. These findings confirmed the selectivity of the developed methods.

### 3.5.5 Application of the developed methods to the analysis of AMIH in the tablets

The developed methods were effectively used to estimate AMIH in representative tablets, namely, Amtil 25 mg/tablet. Additionally, the same tablets underwent testing using an official method [1] and the proposed methods. The obtained outcomes were compared statistically by the Student's t-test and F-test. The official procedure involves dissolving of

**Table 2** Analytical variables and regression

| Parameter | BTB method | BCP Method |
|---|---|---|
| $\lambda_{max}$ (nm) | 410 | 400 |
| Beer's law ranges ($\mu$g mL$^{-1}$) | 0.50–12.5 | 0.50–10.0 |
| Molar absorptivity (L mol$^{-1}$ cm$^{-1}$) | $1.79 \times 10^4$ | $2.32 \times 10^4$ |
| Sandell sensitivity ($\mu$g cm$^{-2}$) | 0.0155 | 0.0119 |
| Detection limit ($\mu$g mL$^{-1}$) | 0.25 | 0.29 |
| Quantification limit ($\mu$g mL$^{-1}$) | 1.67 | 1.49 |
| Regression equation: | | |
| Intercept (a) | -0.0025 | -0.0038 |
| Slope (b) | 0.0659 | 0.0865 |
| Correlation coefficient (r) | 0.99981 | 0.99998 |
| Coefficient of determination ($R^2$) | 0.99962 | 0.99996 |
| Standard deviation of intercept ($S_a$) | 0.00408 | 0.00156 |
| Standard deviation of slope ($S_b$) | 0.00058 | 0.00028 |
| Variance ($S_a^2$) | $1.66 \times 10^{-5}$ | $2.43 \times 10^{-6}$ |

an accurately weighed amount of AMIH in 96% ethanol, conducting an acid–base titration by adding 0.1 M NaOH, followed by the determination of the endpoint using a potentiometer. The results, presented in Table 5, confirmed that the practically calculated *t*- and *F*-values were lower than the theoretical values. This suggests that there were no significant differences between the proposed methods' accuracy and precision and those of the reference method.

### 3.5.6 Recovery study

To confirm the accuracy of the developed methods, the standard addition procedure was employed. The recommended approach for assessing the recovery of an analyte in real samples is through the use of standard addition method [34]. The powder of pre-analyzed tablets was enriched with specific quantities of pure drug at three distinct concentration levels, which were 50%, 100%, and

**Table 4** Results of methods' robustness

| Method | AMI taken $\mu$g mL$^{-1}$ | % RSD Volume of the dye* | Reaction time** |
|---|---|---|---|
| BTB | 5.00 | 1.24 | 1.29 |
| | 7.50 | 1.09 | 1.14 |
| | 10.0 | 1.64 | 1.33 |
| BCP | 2.50 | 1.17 | 1.56 |
| | 5.00 | 1.42 | 1.67 |
| | 7.50 | 1.59 | 1.71 |

*The amount of dye used in both methods was 0.9, 1.0 and 1.1 mL

** In both the methods, the reaction time was taken as 4, 5 and 6 min

**Table 5** Comparison of assay results obtained by the reference and developed methods

| Commercial tablets | Nominal quantity, mg | Found % (of nominal quantity ± SD) * Reference method | Developed methods BTB method | BCP method |
|---|---|---|---|---|
| Amtil | 25 | 99.38 ± 1.18 | 97.85 ± 2.08 *t* = 1.48 *F* = 3.11 | 98.37 ± 1.93 *t* = 1.03 *F* = 2.68 |

*Average value of five measurements

Tabulated *t*-value is equal to 2.78 at the 95% confidence level

Tabulated *F*-value is equal to 6.39 at the 95% confidence level

150% of the amount found in the tablet powder. The developed methods were used to estimate the entire amount of AMI, and the percentage recovery of the extra pure AMI was estimated. The outcomes, listed in Table 6, showed that the percentage recovery values were between 97.75% and 99.43%, with corresponding standard deviation (SD) values between 1.72% and 2.41%. These findings suggest that the assay was not affected by the presence of different additives in AMIH pills.

**Table 3** Assessment of precision and accuracy

| Method | AMI taken $\mu$g mL$^{-1}$ | Precision and accuracy during the day (n=7) AMI found* $\mu$g mL$^{-1}$ | %RSD | % RE | Day-to-day precision and accuracy (n=5) AMI found* $\mu$g mL$^{-1}$ | %RSD | % RE |
|---|---|---|---|---|---|---|---|
| BTB | 5.00 | 4.92 | 1.93 | 1.60 | 4.89 | 2.09 | 2.20 |
| | 7.50 | 7.36 | 2.24 | 1.87 | 7.39 | 1.92 | 1.47 |
| | 10.0 | 9.75 | 2.62 | 2.50 | 9.72 | 2.74 | 2.80 |
| BCP | 2.50 | 2.54 | 1.69 | 1.60 | 2.55 | 2.11 | 2.00 |
| | 5.00 | 5.14 | 2.07 | 2.80 | 5.13 | 2.43 | 2.60 |
| | 7.50 | 7.66 | 2.58 | 2.13 | 7.63 | 1.97 | 1.73 |

* Average of five measurements

**Table 6** Recovery experiment results through the standard-addition method

| Method | Tablet used | AMI in tablet, $\mu g\ mL^{-1}$ | Pure AMI added, $\mu g\ mL^{-1}$ | Total found, $\mu g\ mL^{-1}$ | Pure AMI recovered*, (Percent $\pm$ SD) |
|---|---|---|---|---|---|
| BTB method | Amtil (25 mg) | 3.91 | 2.00 | 5.87 | $98.00 \pm 2.25$ |
| | | 3.91 | 4.00 | 7.82 | $97.75 \pm 2.41$ |
| | | 3.91 | 6.00 | 9.84 | $98.83 \pm 2.09$ |
| BCP method | Amtil (25 mg) | 3.44 | 1.75 | 5.18 | $99.43 \pm 1.72$ |
| | | 3.44 | 3.50 | 6.88 | $98.29 \pm 2.30$ |
| | | 3.44 | 5.25 | 8.64 | $99.05 \pm 1.95$ |

*Mean value of three determinations

## 4 Conclusion

The developed methods outperform the previously reported visible spectrophotometric methods for the estimation of AMIH in pharmaceutical tablets. They eliminate the need for extraction and offer greater simplicity, sensitivity, and selectivity. None of the developed assays involves heating or extraction steps in the assay procedure, and both of them have demonstrated a good accuracy and precision. The developed methods require only readily available and affordable dyes as reagents with no need for pH adjustments or critical reaction conditions. The developed methods exhibit greater sensitivity compared to most of the previously reported methods, as evidenced by the molar absorptivity values of $1.79 \times 10^4$ and $2.32 \times 10^4$ l. $mol^{-1}.cm^{-1}$ for BTB and BCP methods, respectively, with Sandell's sensitivity values of 0.0155 and 0.0119 $\mu g\ cm^{-2}$. The developed methods are robust, work well at 25 ºC, and are ideal for routine use in quality control laboratories of pharmaceutical industries in the developing countries. Therefore, they can be utilized as alternative methods for rapid estimation of AMIH in bulk samples and pharmaceutical tablets. Moreover, excipients that could be found in the commercial tablets did not cause any interference.

## Declarations

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