Original Article

# DEVELOPMENT AND VALIDATION OF NEW SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF ANTIPSYCHOTIC DRUG ASENAPINE MALEATE IN PURE AND DOSAGE FORMS

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## ABSTRACT

**Objective:** Three sensitive, simple, precise, reproducible, and validated spectrophotometric methods have been developed for the determination of anti-psychotic drug (asenapine maleate) in pure and pharmaceutical dosage forms.

**Methods:** The methods are based on the formation of yellow-colored ion-pair complex between asenapine maleate and three acid dyes, namely, bromocresol purple (BCP), bromophenol blue (BPB) and bromothymol blue (BTB) with absorption maxima at 410, 414 and 416 nm, respectively. Several parameters such as pH, buffer type and volume, reagent volume, the sequence of addition and effect of extracting solvent were optimized.

**Results:** Under the optimum experimental conditions, beer's law is obeyed over the concentration ranges of 1.0–20, 1.0–14, and 1.0-16 µg/ml for BCP, BPB and BTB, respectively, with good correlation coefficients (0.9994-0.9998). The apparent molar absorptivity and Sandell's sensitivity values are reported for all methods. The limit of detection (LOD) and the limit of quantification (LOQ) values are found to be 0.27, 0.30, and 0.25 µg/ml and 0.90, 1.0, and 0.83 µg/ml for BCP, BPB and BTB, respectively. The stoichiometric ratio of the formed ion-pair complexes was found to be 1:1 (drug: reagent) for all methods, as deduced by Job's method of continuous variation.

**Conclusion:** The proposed methods were successfully applied for the determination of asenapine maleate in pharmaceutical formulations with good accuracy and precision. Statistical comparison of the results was performed using Student's t-test and variance ratio F-test at the 95% confidence level and there was no significant difference between the reported and proposed methods regarding accuracy and precision. Further, the validity of the proposed methods was confirmed by recovery studies via standard addition technique in accordance with ICH guidelines.

**Keywords**: Asenapine maleate, Ion-pair complex, Spectrophotometry, Method validation, Dosage forms

## INTRODUCTION

Asenapine Maleate (ASP) is chemically designated as (3aRS,12bRS)-rel-5-Chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz (2,3:6,7) oxepino4,5-c] pyrrole maleate (fig. 1) [1]. ASP is an atypical antipsychotic drug and it was approved for use in the USA in for the treatment of schizophrenia and bipolar disorder. It shows a high affinity for numerous receptors like serotonin, adrenergic, dopamine, histamine [2].
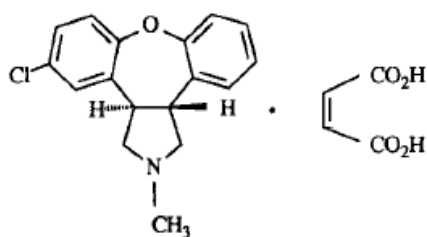


**Fig. 1: The chemical structure of asenapine maleate (ASP)**

The literature survey revealed that many methods were described for the determination of ASP in pure, tablet dosage forms and biological fluids such as spectrofluorimetry [3], high-performance liquid chromatography (HPLC) [4-15], stability indicating HPLC methods [16-21], thin layer chromatography [22], gas chromatography [23], potentiometry [24] and titrimetric method [25]. Most of these reported methods are either not appropriately sensitive or tedious and utilized expensive instruments that are not available in most quality control laboratories.

Visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure form, pharmaceutical formulations and biological samples, due to its simplicity, less expensive, less time consuming and reasonable sensitivity with significant economic advantages.

To the best of our knowledge, there are few methods that have been reported for the quantification of ASP in commercial dosage forms using a spectrophotometric technique [26-31] (table 1). However, these previously reported methods suffer from one or more disadvantage such as poor sensitivity, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid-liquid extraction step; some other methods have a relatively narrow dynamic linear range, involve a heating step, and/or use of expensive reagent or large amounts of organic solvents. For these reasons, it was worthwhile to develop a new, simple, cost-effective and selective spectrophotometric method for the determination of ASP their pharmaceutical dosage forms.

The aim of the present work is to develop simple, sensitive, accurate, precise, low-cost and validated extractive spectrophotometric methods for the determination of ASP in pure and dosage forms. The proposed methods are based on the ability of ASP to form stable ion-pair complexes with bromocresol purple (BCP), bromophenol blue (BPB) and bromothymol blue (BTB). No interference was observed in the assay of ASP from common excipients in levels found in dosage forms. These methods are validated by statistical data.

**Table 1: Comparison between the reported spectrophotometric methods and the present method for the determination of ASP**

| Method | Wavelength (nm) | Beer's law (μg/ml) | Molar absorptivity (L/mol. cm) | LOD (μg/ml) | Reference |
|---|---|---|---|---|---|
| UV-spectroscopy | 215 | 5.0-20 | NA | 0.929 | [26] |
| | 230 | 5.0-20 | NA | 0.471 | |
| | Absorption ration at 215 and 230 | 5.0-20 | NA | 1.75 | |
| UV-spectroscopy | | | | | |
| a-   Fixed wavelength | 269 | 10-60 | $6.39 \times 10^3$ | 0.0122 | [27] |
| b-   Area under curve | 263-274 | 10-60 | $6.39 \times 10^3$ | 0.0122 | |
| UV-spectrophotometry | 270 | 10-60 | NA | 1.4 | [28] |
| UV-spectrophotometry | 220 | 2.0-10 | $1.1 \times 10^4$ | 0.20381 | [29] |
| UV-spectrophotometry | 270 | 1.0-90 | NA | NA | [13] |
| Bromocresol green | 415 | 5.0-30 | NA | 0.39 | [30] |
| Folin ciocalteu (FC) | 769 | 4.0-20 | $7.5 \times 10^4$ | 0.271 | [31] |
| Bromocresol purple (BCP) | 410 | 1.0-16 | $2.136 \times 10^4$ | 0.27 | Proposed |
| Bromophenol blue (BPB) | 414 | 1.0-14 | $2.607 \times 10^4$ | 0.30 | work |
| Bromothymol blue (BTB) | 416 | 1.0-20 | $1.539 \times 10^4$ | 0.25 | |

NA: not available.

## MATERIALS AND METHODS

### Instruments

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10 mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm. The pH values of different buffer solutions were checked using a Hanna pH-meter instrument (pH 211) (Romania) equipped with a combined glass-calomel electrode.

### Materials and reagents

All reagents, chemicals and solvents used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Pure sample of ASP was kindly supplied by Hikma Pharma SAE, 6th October, Egypt, with a purity of 99.60±1.20% by applying the reported method [30]. Asenadia tablets, labeled to contain 10 mg ASP per sublingual tablet, a product of Western Pharmaceutical Industries Company, Cairo, Egypt. Asenapine-Hikma Pharma tablets, labeled to contain 5.0 mg ASP per sublingual tablet, a product of Hikma Pharma SAE, 6th October, Egypt were purchased from local pharmacies.

### Preparation of stock standard solution

Stock standard solutions (100 μg/ml) and ($1.0 \times 10^{-3}$ mol/l) of ASP were prepared by dissolving 10 and 40.18 mg of pure ASP in the least amount of methanol and further diluted with bidistilled water to the mark in a 100 ml volumetric flask. The standard solutions were stable for at least 7.0 d when kept in the refrigerator. Serial dilution with the same solvent was performed to obtain the appropriate concentration range.

### Reagents

BCP, BPB and BTB (BDH Chemicals LTD, Poole, England) and used without further purification. Stock solutions (0.1%, w/v) or ($1.0 \times 10^{-3}$ mol/l) of reagents were prepared by dissolving the appropriate weight of each reagent in10 ml of 96% ethanol and diluted to 100 ml with bidistilled water. These solutions were kept in the refrigerator.

Series of buffer solutions of NaOAc–HCl (pH=1.99-4.92), NaOAc–AcOH (pH=3.4-5.6) and potassium hydrogen phthalate–HCl (pH=2.0-7.0) were prepared by following the standard methods [32]. The pH of each solution was adjusted to an appropriate value by the addition of 0.2 mol/l hydrochloric acid or sodium hydroxide with the help of the pH meter. Freshly prepared solutions were always employed. Chloroform, methylene chloride, and carbon tetrachloride

were obtained from (BDH Chemicals Ltd., Poole, England) and anhydrous sodium sulfate was obtained from (Prolabo).

### General recommended procedure

Accurately measured aliquots (0.1–2.0 ml) of standard ASP solution (100 μg/ml) was transferred into 10 ml measuring flasks. 3.0 ml NaOAc–AcOH buffer at the optimum pH 4.0, 3.5 and 4.0 using BCP, BPB and BTB, respectively, were added. Then, 2.0 ml of the BCP, BPB and BTB (0.1%, w/v) was added and the volume was completed to 10 ml with bidistilled water. The formed ion associate complexes were extracted with 10 ml methylene chloride. The solution was shaking for 2.0 min, then allowed to stand for clear separation of the two phases and the methylene chloride layer was passed through anhydrous sodium sulfate. The absorbance of the yellow-colored ion-pair complexes was measured at 410, 414 and 416 nm using BCP, BPB and BTB, respectively, against the corresponding reagent blank similarly prepared. All measurements were made at room temperature (25±2 °C). In the three proposed methods, a standard curve was prepared by plotting the absorbance values versus concentrations of ASP to calculate the amount of drug in unknown analyte samples.

### Applications for dosage forms (tablets)

Twenty tablets containing ASP were finely pulverized and weighed. A weighed quantity of the powdered tablets equivalent to 10 mg of ASP was transferred into a 100 ml volumetric flask, about 20 ml of methanol was added and the flask was sonicated for 30 min. The volume was completed to the mark with bidistilled water, mixed well and filtered through a Whatman No.1 filter paper into 100 ml volumetric flask, discarding the first 10 ml; then, the conical flask was washed with bidistilled water. The wash was added to the same volumetric flask, and then the flask was made up to volume with bidistilled water. Aliquots containing ASP in the final concentration ranges were analyzed as described under "General recommended procedure". The concentration of ASP was determined either from the calibration curve or using the corresponding regression equation. The method of standard addition was used for the accurate determination of ASP content.

### Stoichiometric relationship

The stoichiometric ratios of the ion-associates formed between ASP and the reagents were determined by applying the continuous variation [33] and the molar ratio [34] methods at the optimum wavelengths. In the continuous variation method, equimolar solutions were employed: a $1.0 \times 10^{-4}$ mol/l standard solution of ASP and $1.0 \times 10^{-4}$ mol/l solution of dye were used. A series of solutions was prepared in which the total volume of ASP and the dye was kept at 2.0 ml. The drug and reagent were mixed in

various complementary proportions (0.2:1.8, 0.4:1.6, 0.6:1.4, 0.8:1.2, 1.0:1.0, 1.2:0.8, 1.4:0.6, 1.6:0.4, 1.8:0.2) and completed to volume in a 10 ml calibrated flask with the appropriate solvent for extraction following the above mentioned procedure. In the molar ratio method, the concentration of ASP is kept constant to 1.0 ml of (1.0 x10$^{-4}$ mol/l) while that of dye (1.0 x10$^{-4}$ mol/l) is regularly varied (0.2–2.4 ml). The absorbance of the prepared solutions was measured at optimum condition and at the optimum wavelength for each complex.

## RESULTS AND DISCUSSION

### Absorption spectra

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes present mainly in anionic form at pH ≥ 2.5. So, when treated with an acid dye at acidic pH using buffer solutions, a yellow ion-pair complex which is extracted with methylene chloride is formed. The absorption spectra of the ion-pair complexes, which were formed between ASP and reagents, were measured in the range 350–550 nm against the blank solution and the maximum absorbances were measured at wavelengths 410, 414, and 416 nm using BCP, BPB, and BTB, respectively.

### Optimum reaction conditions for complex formation

The optimization of the methods was carefully studied to achieve the complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH of the buffer, the type of organic solvent, volumes of the dye, reaction time and temperature for the extraction of ion-pair complexes.

### Effects of pH

It was observed that the effective extraction of the complex depends on the type of the buffer used and its pH value. The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as NaOAc–HCl (pH=2.0-4.5), NaOAc–AcOH (pH=2.8-5.5) and potassium hydrogen phthalate–HCl (pH=3.0-6.0). It is evident that the maximum color intensity and maximum absorbance were found in NaOAc-AcOH buffer. It is evident that the maximum absorbances of the ion pair complexes were obtained at pH 4.0, 3.5, and 4.0 for BCP, BPB and BTB methods, respectively (fig. 2). Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-5.0 ml). The higher absorbance value and reproducible results were obtained by using 3.0 ml of acetate buffer solutions.
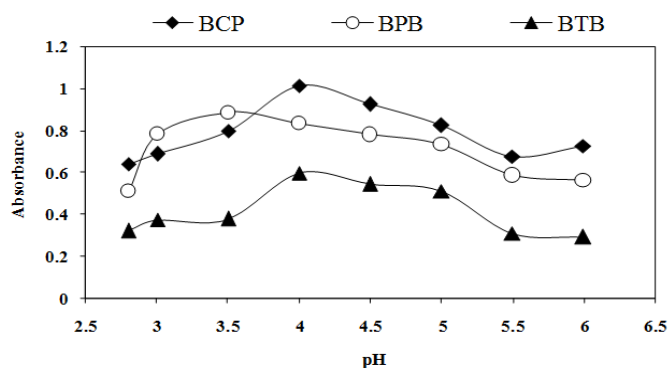


**Fig. 2: Effect of pH of buffer solution on the ion pair complex formation between (20, 14 and 16 µg/ml) ASP and (0.1%, w/v) BCP, BPB and BTB reagents, respectively, (N=3.0)**

### Effect of reagent concentration

The effect of the reagent was studied by measuring the absorbances of solutions containing a fixed concentration of ASP and various volumes of the BCP, BPB or BTB (0.1%, w/v) reagents in the range of (0.5–4.0 ml). The results showed that the

absorbance of the extracted ion-pair increased by increasing the BCP, BPB or BTB volume till 2.0 ml. So, the maximum color intensity of the complex was achieved with 2.0 ml of (0.1 %, w/v) of each reagent solution. Although a larger volume of the reagent had no pronounced effect on the absorbance of the formed ion-pair complexes (fig. 3).
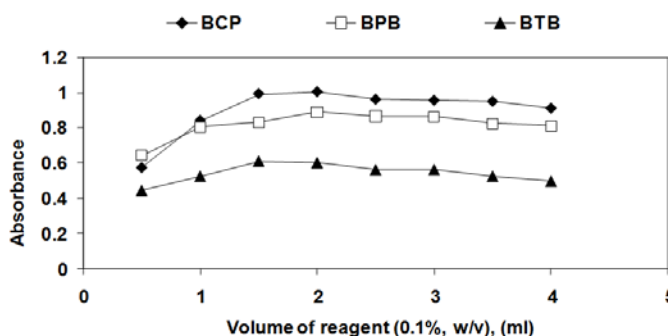


**Fig. 3: Effect of volume of (0.1%, w/v) BCP, BPB and BTB reagent on the ion pair complex formation with (20, 14 and 16 µg/ml) ASP, respectively, (N=3.0)**

### Choice of extracting solvent

The effect of several organic solvents *viz.*, chloroform, carbon tetrachloride, dichloromethane and diethyl ether were studied for

effective extraction of the colored species from the aqueous phase (fig. 4). Dichloromethane was found to be the most suitable solvent for the extraction of colored ion-pair complexes for all reagents

quantitatively. Experimental results indicated that double extraction with total volume 10 ml dichloromethane, yielding maximum absorbance intensity, stable absorbance and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.
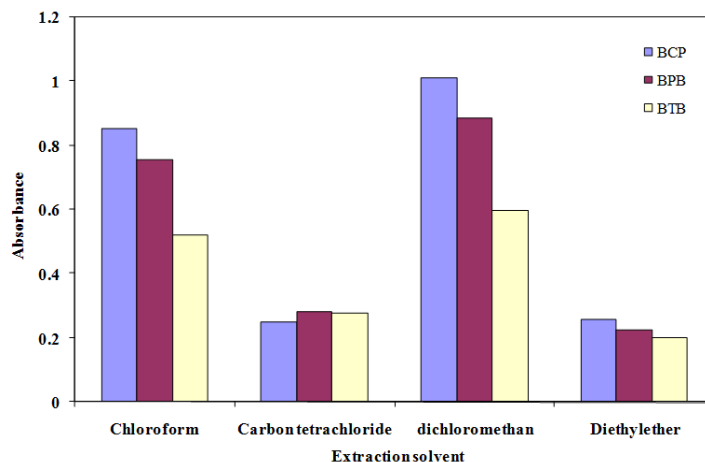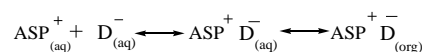


**Fig. 4: Effect of extraction solvent on the ion pair complex formation of ASP with dyes at the optimum conditions**
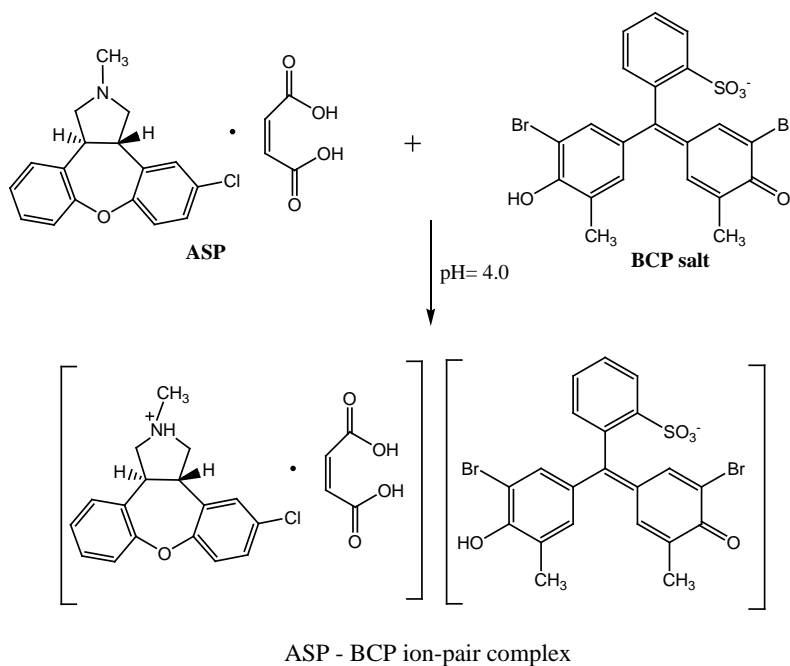
### Effect of shaking time and temperature

The optimum shaking time was investigated by shaking from 0.5-5.0 min at ambient temperature (25±2 °C). Maximum and constant absorbance values were obtained when extracted after 2.0 min of shaking for all complexes. Therefore, a shaking time of 2.0 min was maintained throughout the experiment. The effect of temperature on colored complexes was studied by measuring the absorbance values over the temperature range 20-35 °C. It was found that the absorbance of the colored ion-pair complex was constantly up to 30 °C. At higher temperatures, the drug concentration was found to increase due to the volatile nature of dichloromethane. Therefore, the temperature chosen was room temperature (25±2 °C) as the best temperature for the determination of ASP in bulk and pharmaceutical formulations. The absorbance of the complexes remains stable for at least 12 h at room temperature.

### Composition of the ion-pair complexes

The molar ratio of the ion pair complexes (ASP: dye) was determined by the continuous variations and molar ratio methods (figs. 5 and 6). The results indicate that the molar ratio of (ASP: dye) is (1:1) ion-pair complex are formed through the electrostatic attraction between the positively charged ASP$^+$and negatively charged dye, (BCP$^-$, BPB$^-$, and BTB$^-$). The extraction equilibrium can be represented as follows:

$$ASP^+_{(aq)} + D^-_{(aq)} \longleftrightarrow ASP^+ D^-_{(aq)} \longleftrightarrow ASP^+ D^-_{(org)}$$

Where ASP$^+$and D$^-$ represent the protonated drug and the anion of the dye (BCP$^-$, BPB$^-$ or BTB$^-$), respectively, and the subscript (aq) and (org) refer to the aqueous and organic phases, respectively (scheme 1).



ASP - BCP ion-pair complex

**Scheme 1: Proposed reaction mechanism for the ion pair complex formation between ASP and BCP**
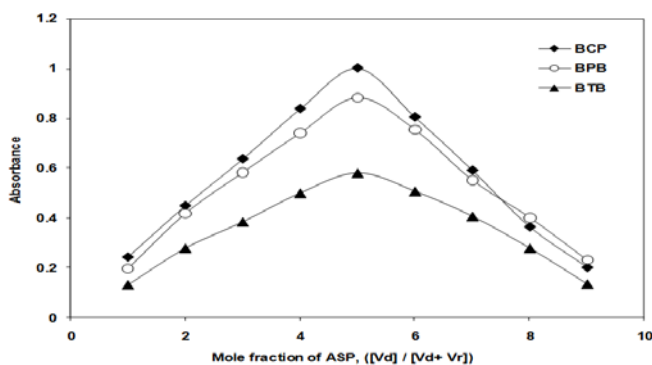
**Fig. 5: Job's method of continuous variation graph for the reaction of ASP with BCP, BPB and BTB, [drug] = [dye] = (1.0×10⁻⁴ mol/l) (N=3.0)**
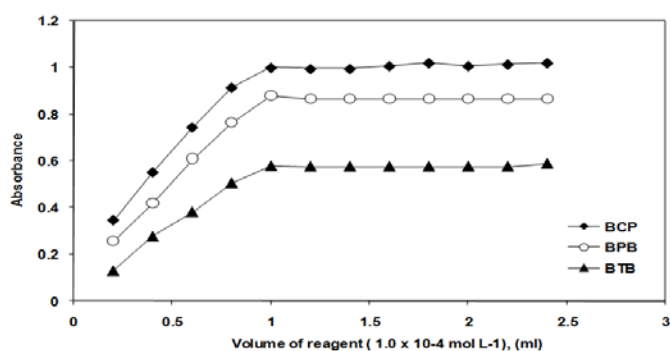


**Fig. 6: Mole ratio plots for the ion-association complexes of ASP (1.0 x 10⁻⁴ mol/l) with various volumes of reagent solution (1.0 x 10⁻⁴ mol/l) at the optimum conditions**

### Method of validation

#### Linearity

At described experimental conditions for ASP determination, standard calibration curves with reagents were constructed by plotting absorbance vs. concentration of ASP. The statistical parameters were given in the regression equations calculated from the calibration graphs A = aC+b, where A is the absorbance and C is the concentration in µg/ml. The linearity of calibration graphs was proved by the high values of the correlation coefficient ($r$) and the small values of the $y$-intercepts of the regression equations. The apparent molar absorptivity of the resulting colored ion-pair complexes and relative standard deviation of response factors for each proposed spectrophotometric method was also calculated and recorded in table 2. The molar absorptivity of BPB>BCP>BTB ion-pair complexes.

#### Sensitivity

The limits of detection (LOD) and quantitation (LOQ) for the proposed methods were calculated using the following equation [35, 36]:

LOD = $3s/k$ and

LOQ = $10 s/k$

Where s is the standard deviation of ten replicate determinations values of the reagent blank and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, LOD was found to be 0.27, 0.30, and 0.25 µg/ml for BCP, BPB and BTB methods, respectively.

According to this equation, LOQ was found to be 0.90, 1.0, and 0.83 µg/ml for BCP, BPB and BTB methods, respectively.

**Table 2: Statistical analysis of calibration graphs and analytical data in the determination of ASP using the proposed methods**

| Parameters | BCP | BPB | BTB |
|---|---|---|---|
| Wavelengths λ max (nm) | 410 | 414 | 416 |
| Beer's law limits (µg/ml) | 1.0-20 | 1.0-14 | 1.0-16 |
| Molar absorptivity ε, (L/mol. cm) x 10⁴ | 2.136 | 2.607 | 1.539 |
| Sandell·s sensitivity (ng/cm²) | 18.81 | 15.41 | 26.10 |
| Regression equation [a] | | | |
| Intercept (*a*) | 0.0085 | 0.0054 | 0.0052 |
| Standard deviation of intercept (*Sa*) | 0.012 | 0.007 | 0.009 |
| Slope (b) | 0.0499 | 0.0628 | 0.0365 |
| Standard deviation of slope (*Sb)* | 0.005 | 0.005 | 0.004 |
| Correlation coefficient (*r²*) | 0.9998 | 0.9994 | 0.9996 |
| LOD (µg/ml)[b] | 0.27 | 0.30 | 0.25 |
| LOQ (µg/ml)[b] | 0.90 | 1.0 | 0.83 |
| mean±SD | 99.79±0.80 | 100.25±0.85 | 99.85±0.93 |
| RSD% (N=6) | 0.80 | 0.85 | 0.93 |
| RE% | 0.84 | 0.89 | 0.98 |
| t-test [c] | 0.29 | 0.99 | 0.37 |
| F-test[c] | 2.25 | 1.99 | 1.66 |

[a]A = a+bC, where C is the concentration in µg ml⁻¹, A is the absorbance units, [b]LOD, limit of detection; LOQ, the limit of quantification; ε, molar absorptivity, [t]heoretical values of t and F at P= 0.05 are 2.571 and 5.05, respectively.

**Accuracy and precision**

In order to evaluate the accuracy and precision of the proposed methods, solutions containing three different concentrations of ASP were prepared and the assay procedure was analyzed in six replicates, and percentage relative standard deviation (RSD%) values were obtained within the same day to evaluate the repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision). The percentage relative error (RE%) was calculated using the following equation:

$$RE\% = \frac{(Found - Added)}{Added} \times 100$$

The analytical results of intra-day and inter-day precision (RSD%) and accuracy (RE%) were summarized in table 3. These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

**Table 3: Intra-day and Inter-day precision and accuracy data for ASP obtained by the proposed methods**

| Method | Added concentration (µg/ml) | Intra-day | | | | Inter-day | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Recovery % | Precision RSD % [a] | Accuracy RE % | Confidence limit [b] | Recovery % | Precision RSD % [a] | Accuracy RE % | Confidence limit [b] |
| BCG | 6.0 | 99.00 | 0.48 | -1.0 | 5.94±0.03 | 98.90 | 0.53 | -1.10 | 5.934±0.033 |
| | 12 | 99.20 | 0.70 | -0.80 | 11.904±0.087 | 99.80 | 0.81 | -0.20 | 11.976±0.102 |
| | 18 | 100.40 | 1.30 | 0.40 | 18.072±0.247 | 99.20 | 0.95 | -0.80 | 17.856±0.178 |
| BPB | 4.0 | 99.20 | 0.67 | -0.80 | 3.968±0.028 | 99.10 | 0.50 | -0.90 | 3.964±0.021 |
| | 8.0 | 99.00 | 1.10 | -1.0 | 7.92±0.091 | 99.30 | 0.47 | -0.70 | 7.944±0.039 |
| | 12 | 99.60 | 1.50 | -0.40 | 11.952±0.188 | 99.50 | 0.80 | 0.50 | 11.94±0.1 |
| BTB | 5.0 | 99.10 | 0.80 | -0.90 | 4.955±0.042 | 98.70 | 0.40 | -1.30 | 4.935±0.021 |
| | 10 | 100.50 | 0.90 | 0.50 | 10.05±0.095 | 99.30 | 0.66 | -0.70 | 9.93±0.069 |
| | 15 | 99.70 | 0.75 | -0.30 | 14.955±0.118 | 100.80 | 1.20 | 0.80 | 15.12±0.19 |

[a]Mean of six determination, RSD%, percentage relative standard deviation; RE%, percentage relative error, [b]Confidence limit at 95% confidence level and five degrees of freedom (t = 2.571).

**Robustness and ruggedness**

For the evaluation of the method robustness, some parameters were interchanged; pH, dye concentration, wavelength range, and shaking time. The capacity remains unaffected by small deliberate variations.

Method ruggedness was expressed as RSD% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical differences between different analysts and instruments, suggesting that the developed methods were robust and rugged (table 4).

**Table 4: Results of method robustness and ruggedness expressed as intermediate precision (RSD %) for ASP-dye ion-pair complex**

| Methods | Nominal concentration (µg/ml) | RSD% | | | |
|---|---|---|---|---|---|
| | | Robustness | | Ruggedness | |
| | | Variable alerted[a] | | | |
| | | pH[b] | Dye concentration[c] | Inter-analysts | Inter-instruments |
| BCP | 6.0 | 0.80 | 0.75 | 1.20 | 1.10 |
| | 12 | 1.20 | 1.0 | 1.90 | 1.70 |
| | 18 | 2.0 | 1.70 | 2.30 | 2.40 |
| BPB | 4.0 | 0.65 | 0.90 | 0.85 | 0.70 |
| | 8.0 | 0.90 | 1.50 | 1.30 | 1.60 |
| | 12 | 1.80 | 2.10 | 1.90 | 2.0 |
| BTB | 5.0 | 0.95 | 1.20 | 1.0 | 0.80 |
| | 10 | 1.70 | 1.80 | 1.10 | 1.80 |
| | 15 | 2.20 | 2.30 | 1.75 | 2.40 |

[a]Mean of three determinations, [b]pH (±0.2), [c]The volumes of dye used were 2.0±0.2 ml.

**Table 5: Application of the standard addition method for the determination of ASP in dosage forms (tablets) using the proposed methods**

| Method | Taken drug (µg/ml) | Pure drug added (µg/ml) | Asenapine-hikma pharma tablets (5.0 mg) | | Asenadia tablets (10 mg) | |
|---|---|---|---|---|---|---|
| | | | Total found (µg/ml) | Recovery[a] (%)±SD | Total found (µg/ml) | Recovery[a] (%)±SD |
| **BCP** | 5.0 | 5.0 | 9.93 | 99.30±0.60 | 9.88 | 98.80±0.40 |
| | 5.0 | 10 | 15.075 | 100.50±0.75 | 14.93 | 99.53±0.64 |
| | 5.0 | 15 | 19.84 | 99.20±1.10 | 20.14 | 100.70±1.50 |
| **BPB** | 4.0 | 4.0 | 7.92 | 99.00±0.36 | 7.95 | 99.70±0.55 |
| | 4.0 | 6.0 | 9.98 | 99.80±0.80 | 9.95 | 99.50±0.68 |
| | 4.0 | 8.0 | 12.05 | 100.42±1.40 | 11.88 | 99.00±1.60 |
| **BTB** | 4.0 | 4.0 | 7.93 | 99.13±0.70 | 7.94 | 99.25±0.65 |
| | 4.0 | 6.0 | 9.94 | 99.40±1.30 | 10.09 | 100.90±0.80 |
| | 4.0 | 8.0 | 11.86 | 98.83±1.90 | 11.97 | 99.75±1.20 |

[a]Average of six determinations.

## Effects of interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany ASP in its dosage forms (starch, lactose, glucose, saccharose, talc, sodium chloride, titanium dioxide, and magnesium stearate) was studied. The results indicated that there is no interference from excipients and additives, indicating a high selectivity for determining ASP in its dosage forms.

## Applications to dosage forms

The proposed methods have been successfully applied to the determination of ASP in dosage forms (Asenapine-Hikma Pharma tablets and Asenadia tablets). Six replicates determinations were made. Moreover, to check the validity of the proposed methods, dosage forms were tested for possible interference with standard addition method (table 5). Therefore, it is concluded that the excipients in dosage forms of ASP did not cause any interference in the analysis of ASP. A statistical comparison of the results for the determination of ASP in tablet dosage forms using the proposed and reported methods [30] is shown in table 6. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed and reported methods at the 95 % confidence level with respect to accuracy and precision [36] (table 6).

**Table 6: Results of analysis of tablets by the proposed methods for the determination of ASP and statistical comparison with the reported method [30]**

| Samples | Recovery[a] (%)±SD | | | |
|---|---|---|---|---|
| | Proposed methods | | | Reported method [30] |
| | BCP | BPB | BTB | |
| Asenapine-Hikma Pharma tablets (5.0 mg) | 99.67±0.72 | 99.74±0.71 | 99.12±0.29 | 99.40±0.54 |
| *t-value*[b] | 0.67 | 0.85 | 1.02 | |
| *F-value*[b] | 1.78 | 1.73 | 3.47 | |
| Asenadia tablets (10 mg) | 99.68±0.96 | 99.40±0.36 | 99.97±0.85 | 99.78±0.60 |
| *t-value*[b] | 0.20 | 1.21 | 0.41 | |
| *F-value*[b] | 2.56 | 2.78 | 2.01 | |

[a]Average of six determinations, [b]The theoretical values of *t* and *F* are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

## CONCLUSION

The proposed methods describe the application of extractive ion–pair complex formation reaction with dyes for the quantification of ASP in pure and dosage forms. Compared with the existing spectrophotometric methods, the proposed methods have the advantages of relatively simple, rapid, cost-effective, and more sensitive for determining ASP in pure and dosage forms. Moreover, the proposed methods are free from tedious experimental steps such as heating unlike the previously reported spectrophotometric methods cited earlier. The most attractive feature of these methods is its relative freedom from interference by the usual diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal high precision and accuracy of the proposed methods besides being robust and rugged. Therefore, the validated method could be useful for routine quality control assay of ASP in pure and dosage forms.

## FUNDING

## AUTHORS CONTRIBUTIONS

## CONFLICTS OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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