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# Utilization of ion-pair complexation reaction for the spectrophotometric determination of milnacipran HCl as anti-depression drug in pharmaceutical formulations

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***ABSTRACT :*** **The present part aims to develop and validate a simple, rapid, cost-effective, sensitive and extractive spectrophotometric methods for the determination of anti-depression drug; milnacipran HCl in pure form and pharmaceutical formulations. The developed methods are based on the formation of ion-pair complexes between milnacipran HCl with three dyes, namely, bromocresol purple (BCP), bromophenol blue (BPB), or methyl orange (MO) in acidic buffer solutions. Different factors affecting the reaction between milnacipran HCl and the dyes were studied and optimized. The formed complexes were extracted with methylene chloride and measured at 408, 413, and 423 nm using BCP, BPB and MO, respectively. The beer's law was obeyed in the ranges 1.0-14, 2.0-20 and 1.0-18 µg mL$^{-1}$ using BCP, BPB and MO, respectively under the optimum conditions. The composition of the ion pairs was found 1:1. The molar absorptivity's, Sandell's sensitivity, the limits of detection and quantification were calculated. Other method validation parameters, such as accuracy, intra-day and inter-day (1.60 and 1.80%) as precision, robustness, ruggedness and selectivity, have been evaluated. The proposed methods have been applied successfully for the analysis of milnacipran HCl in pure form and pharmaceutical formulations. The reliability of the methods was further ascertained by performing recovery studies using the standard addition method. Statistical comparison of the results with the reported method was performed by applying student's t- and F-tests and no significant statistical differences were obtained.**

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## I. INTRODUCTION

Depression is a mood disturbance that is significantly distinguishable from the usual mood fluctuations of everyday life. It may be accompanied by other mental or somatic symptoms representing several depressive syndromes (1). Up to half of all patients with depression may attempt suicide during their lifetime (2). Risk factors for developing depression include; female gender and a positive family history (3). Milnacipran hydrochloride (MCN): chemically named as 1-phenyl-1-(diethylaminocarbonyl)-2-(aminomethyl) cyclopropane (Figure 1). MCN is a norepinephrine and serotonin reuptake inhibitor, for cure of depression and fibromyalgia (2).

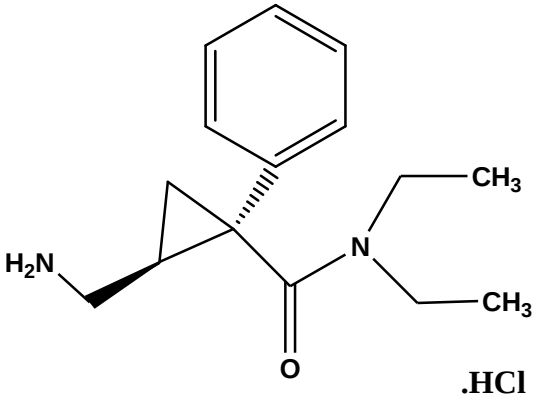



Figure (1). The chemical structure of milnacipran hydrochloride (MCN).

Several analytical assays were described for the detection of MCN in dosage forms and in biological fluids such as spectrophotomety (4-14) (Table 1), spectrofluorimetry (15, 16), high-performance liquid chromatography (HPLC) (17-24), high-performance Thin layer chromatography (HPTLC) (25-27). Most of the reported procedures for the determination of MCN suffer from the use of complex instruments, the need high expertise in their use, in

addition the unavailability of these instruments in several quality control laboratories. Accordingly, there is a need for simpler, less costly as well as easier to be applied methods for the routine analysis of MCN as a drug of widespread use.

Table (1): Comparison between the reported methods for spectrophotometric determination of MCN.

| Reagent | Wavelength (nm) | Beer's law (µg $mL^{-1}$) | Limit of detection (µg $mL^{-1}$) | Molar absorpitivity ($Lmol^{-1}cm^{-1}$) | References |
|---|---|---|---|---|---|
| Folin-ciocaltaeu | 780 | 15-25 | - | $0.334 \times 10^4$ | [4] |
| P-Methylamino phenolsulphate | 540 | 10-50 | - | $0.487 \times 10^4$ | |
| 3-methyl2-benzothiazolinone hydrazone (MBTH) | 640 | - | - | - | [5] |
| 2, 2'- bipyridine | 530 | - | - | - | |
| 4-chloro-7 nitrobenzofurazan (NBD-Cl) | 465 | 1.5-12 | 0.36 | - | [6] |
| Ninhydrin in DMF | 575 | 2.5-37.5 | 0.244 | $7.692\times 10^4$ | [7] |
| Bromothymol blue | 410 | 2-12 | 0.087 | $7.965 \times 10^5$ | |
| Bromocresol Green | 412 | 50-500 | | $1.759 \times 10^3$ | [8] |
| Ninhydrin | 570 | 4-40 | 0.55 | $3.0547 \times 10^3$ | [9] |
| MBTH with sodium meta periodate | 650 | 5-25 | - | $2.45 \times 10^3$ | [10] |
| Ammonium vanadate | 740 | 2.5-12.5 | - | $7.88 \times 10^3$ | |
| UV | 220 | 2-45 | 0.27 | $2.049 \times 10^4$ | [11] |
| UV | 223 | 2-40 | 0.41 | | [12] |
| UV | 220 | 5-30 | 1.6046 | $1.148\times 10^4$ | [13] |
| BCP | 408 | 1.0-14 | 0.22 | $1.1644\times 10^4$ | Proposed work |
| BPB | 413 | 2.0-20 | 0.45 | $0.9241\times 10^4$ | |
| MO | 42 | 1.0-18 | 0.30 | $0.7475\times 10^4$ | |

In contrast, 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, pharmaceutical formulations and biological samples due to its simplicity, reproducibility, speed, less analysis time and reasonable sensitivity with significant economic advantages.

The present investigation aims to develop simple, sensitive and cost-effective methods for the determination of MCN in pure form and pharmaceutical formulations using spectrophotometric technique. The methods based on the formation of ion–associate complexes between MCN and the dyes bromocresol purple (BCP), bromophenol blue (BPB), and methyl orange (MO).

## . II.Materials and Methods

### 2.1. Apparatus:

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10 mm quartz cell 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.

### 2.2. Materials and reagents:

All chemicals, solvents and reagents used in this work were of analytical reagent or pharmaceutical grade and all solutions were prepared fresh daily. Double distilled water was used throughout the investigation.

### 2.2.1. Materials:

Pharmaceutical grade MCN was kindly supplied by D Averroes pharma for Pharmaceutical Industries, 6th industrial zone, Sadat City, Menoufia, Egypt. Averomilan® tablets; labeled to containing 50 mg of MCN

(B.N. # 171100) provided by Averroes pharma for Pharmaceutical Industries, 6th industrial zone, Sadat City, Menoufia, Egypt.

Bromocresol purple (BCP), bromophenol blue (BPB), and methyl orange (MO) (BDH Chemicals LTD, Poole, England) were used without further purification.

**2.2.2. Stock standard solutions:**

A stock standard solution (100 µg $mL^{-1}$) of MCN was prepared by dissolving 10 mg of pure MCN in 100 mL double distilled water. The standard solutions were found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use.

Stock solutions (0.1 w/v) and ($1.0\times10^{-3}$ mol $L^{-1}$) of each reagent (BCP, BPB, and MO) were prepared by dissolving the appropriate weight of each reagent in10 mL of 96% ethanol and diluted to 100 mL with double distilled water. These solutions were stable for at least one week if kept in the refrigerator.

Series of buffer solutions of KCl–HCl (pH=1.5-4.2), NaOAc–HCl (pH=1.99-4.92), NaOAc–AcOH (pH=3.0-5.6) and potassium hydrogen phthalate–HCl (pH=2.0-7.0) were prepared [28].

**2.3. General procedures:**

Accurately measured aliquots (0.1–2.0 mL) of standard MCN solution (100 µg $mL^{-1}$) was transferred into 10 ml measuring flasks. 3.0 mL NaOAc–AcOH buffer at the optimum pH 3.0 and 3.5 using (BCP or BPB) and MO, respectively were added. Then, 2.0 mL of each reagent (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 408, 413 and 423 nm using BCP, BPB and MO, respectively against 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 MCN to calculate the amount of drug in unknown analyte samples.

**2.4. Application to pharmaceutical formulations:**

Twenty tablets of MCN formulation were weighed accurately and ground into a fine powder in a mortar and mixed well. An accurate weight of the powdered tablets equivalent to 10 mg MCN as added into a 100-mL volumetric flask, and dissolved in methanol and the flask was sonicated for 20 min and completed to the mark with double distilled water with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with double distilled water in a 100 mL measuring flask to give 100 µg $mL^{-1}$ stock solution of MCN for analysis by the recommended spectrophotometric methods. The contents of tablets were calculated using the corresponding regression equations of the appropriate calibration graphs.

**2.5. Stoichiometric relationship:**

The stoichiometric ratios of the ion-associates formed between the drugs under investigation and the reagents were determined by applying the continuous variation (29) and the molar ratio (30) methods at the wavelengths of maximum absorbance. In continuous variation method, equimolar solutions were employed: a 1.0 x $10^{-3}$ mol $L^{-1}$ standard solution of MCN and 1.0 x $10^{-3}$ mol $L^{-1}$ solution of dye was used. A series of solutions was prepared in which the total volume of the studied drugs and the dye was kept at 2.0 mL. The drug and reagent were mixed in various complementary proportions (0:2, 0.2:1.8, 0.4:1.6,……..2:0, inclusive) 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 MCN is kept constant to 1.0 mL of (1.0 x $10^{-3}$ mol $L^{-1}$) while that of dye (1.0 x $10^{-3}$ mol $L^{-1}$) is regularly varied (0.2–2.4 mL). The absorbance of the prepared solutions was measured at the optimum wavelength for each complex.

## III.RESLUTS AND DISCUSSIONS

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes present mainly in anionic form at a pH ≥ 2.5. So, when treated with an acid dye at pH range (2.8-6.0) of acidic buffers solutions, a yellow ion-pair complex is formed which is then extracted with methylene chloride. The absorption spectra of the ion-pair complexes, formed between MCN and reagents, were measured in the range 350–650 nm

against the blank solution. The maximum absorbances of ion-pair complexes were recorded at 408, 413, and 423 nm using BCP, BPB, and MO, respectively.

**3.1. 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 formation were realized by carrying out the following preliminary experiments: effect of pH of buffer, the type of organic solvent, volumes of the dye, reaction time and temperature for the extraction of ion-pair complexes.

**3.1.1. Effects of pH on ion-pair formation**

The effect of pH on the drug–reagent complex formation was studied by extracting the colored complexes in the presence of various buffers. It was noticed that the maximum color intensity and highest absorbance value were observed in NaOAc- AcOH buffer of pH 3.0, and 3.5 for (BCP or BPB) and MO, respectively (Figure 2). Different acetate buffer volumes (0.5-4.0 mL) were checked for choosing the optimum buffer volume. The buffer volume of 3.0 mL was chosen as the optimum volume that it gives the maximum absorbance and reproducible results.

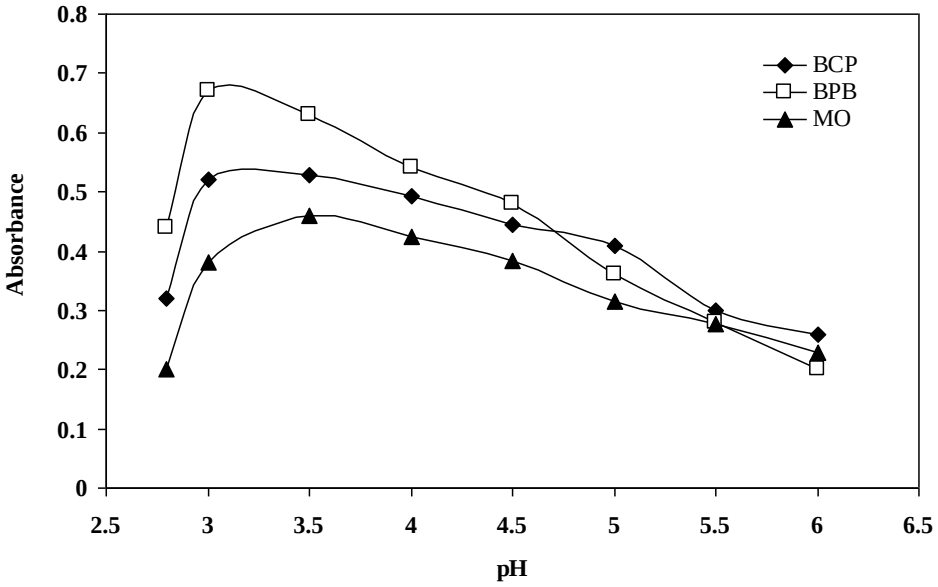



**Figure (2)** Effect of pH of acetate buffer solution on ion pair complex formation between MCN and (0.1 %, w/v) reagents.

**3.1.2. Effect of reagents concentration**

The effect of reagent concentration was studied by measuring the absorbance of solutions containing a fixed concentration of MCN and various volumes (0.5-4.0 mL) of the respective reagents. 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 (Figure 3).

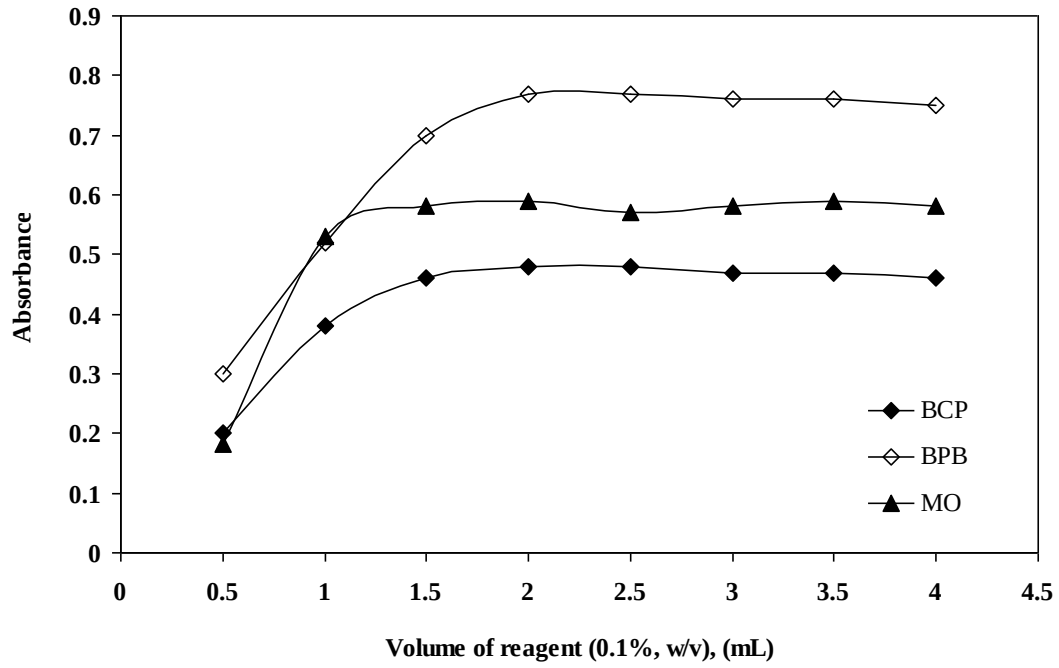



Figure (3) Effect of reagent volume (0.1%, w/v) on the ion pair complex formation with MCN.

**3.1.3. Effect of extracting solvents**

The effect of several organic solvents *viz*., chloroform, carbon tetrachloride, methylene chloride and diethylether were studied for effective extraction of the colored species from aqueous phase (Figure 4). Methylene chloride was found to be the most suitable solvent for the quantitative extraction of colored ion pair complexes, for all reagents. This may be attributed to higher polarity of methylene chloride. Experimental results indicated that double extraction with total volume 10 mL methylene chloride, 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.

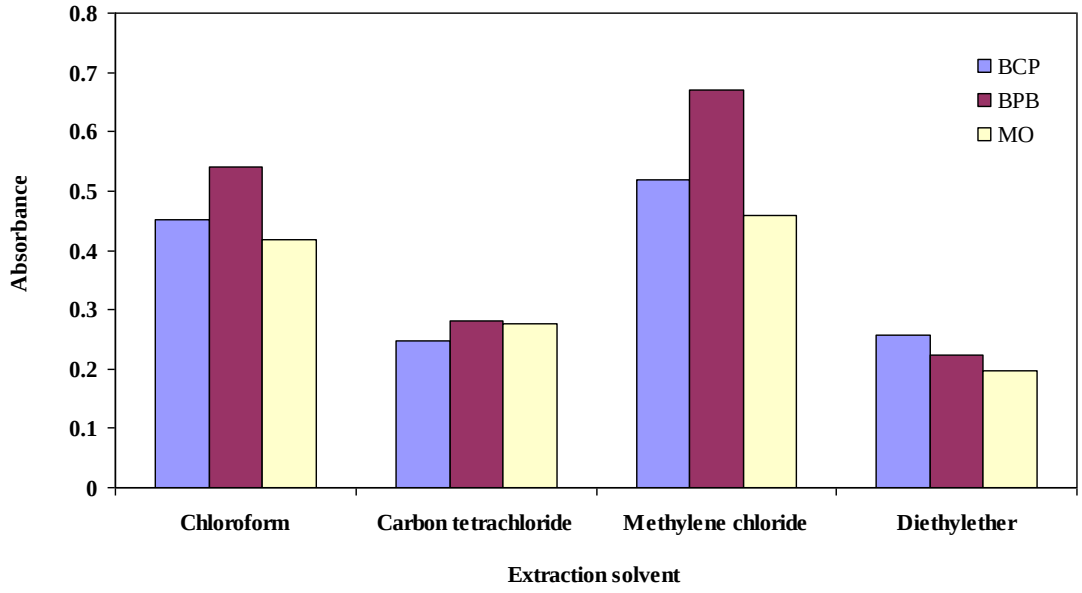


Figure (4) Effect of extraction solvent on the ion pair complex formation of MCN with dyes at the optimum conditions.

### 3.1.4. Effect of time and temperature

The optimum reaction time was investigated from 0.5 to 5.0 min by following the color development at ambient temperature (25 ± 2°C). Complete color intensity was attained after 2.0 min of mixing for all complexes. The effect of temperature on colored complexes was investigated by measuring the absorbance values at different temperatures. It was found that the colored complexes were stable up to 35°C. At higher temperatures, the MCN concentration was found to increase due to the volatile nature of methylene chloride. The absorbance remains stable for at least 12 h at room temperature for all reagents.

### 3.2. Stoichiometric relationship

Job's method of continuous variation of equimolar solutions was employed: a $1.0 \times 10^{-3}$mol $L^{-1}$ standard solution of MCN and $1.0 \times 10^{-3}$ mol $L^{-1}$ solution of BCP, BPB or MO, respectively were used. A series of solutions was prepared in which the total volume of MCN and reagent was kept at 2.0 mL. The absorbance was measured at the optimum wavelengths.

The molar ratio of the ion pair complexes (MCN/dye) was determined by the continuous variations and molar ratio methods (Figures 5 and 6). The results indicate that 1:1 (MCN/dye) ion-pairs are formed through the electrostatic attraction between positive protonated $VARD^+$ and negative $BCP^-$, $BPB^-$, and $MO^-$. The extraction equilibrium can be represented as follows:

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

Where $MCN^+$ and $D^-$ represent the protonated MCN and the anion of the dye, respectively, and the subscript (aq) and (org) refer to the aqueous and organic phases, respectively (Scheme 1).

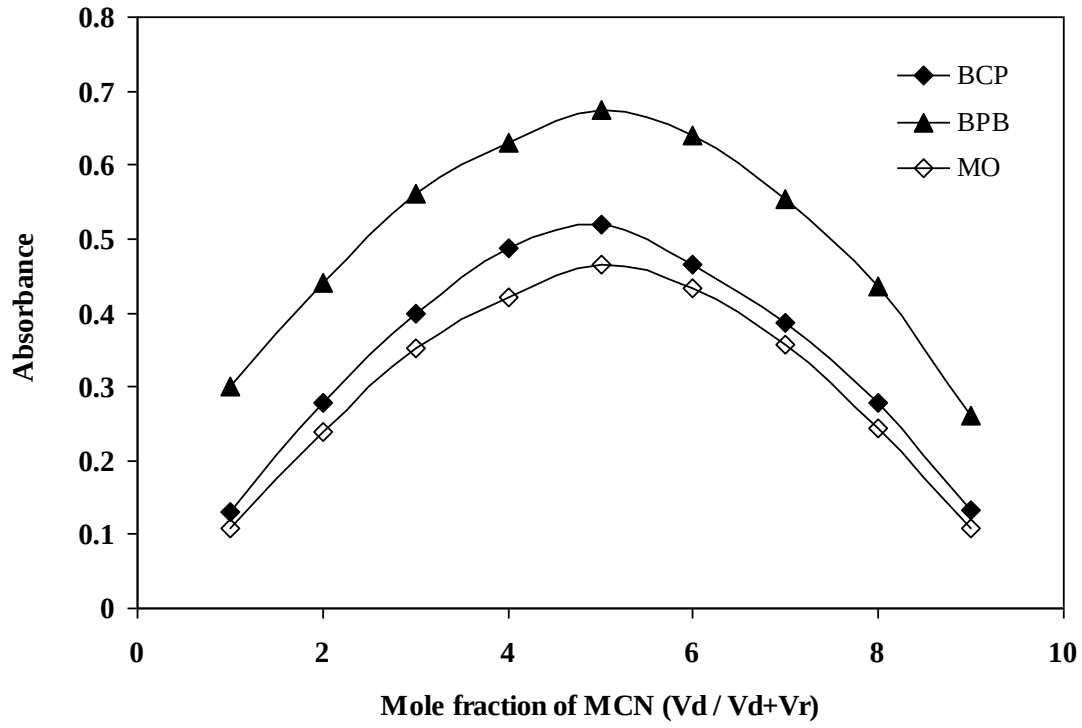



Figure (5) Job's method of continuous variation graph for the reaction of MCN with dyes; BCP, BPB and MO, [drug] = [dye] = $1.0 \times 10^{-4}$ mol $L^{-1}$.

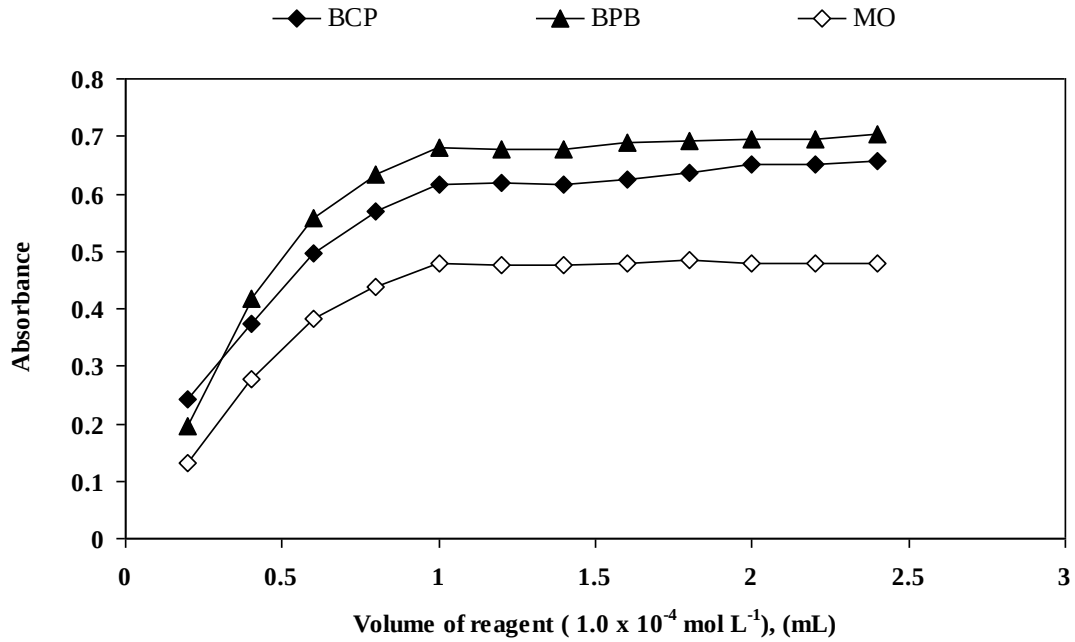
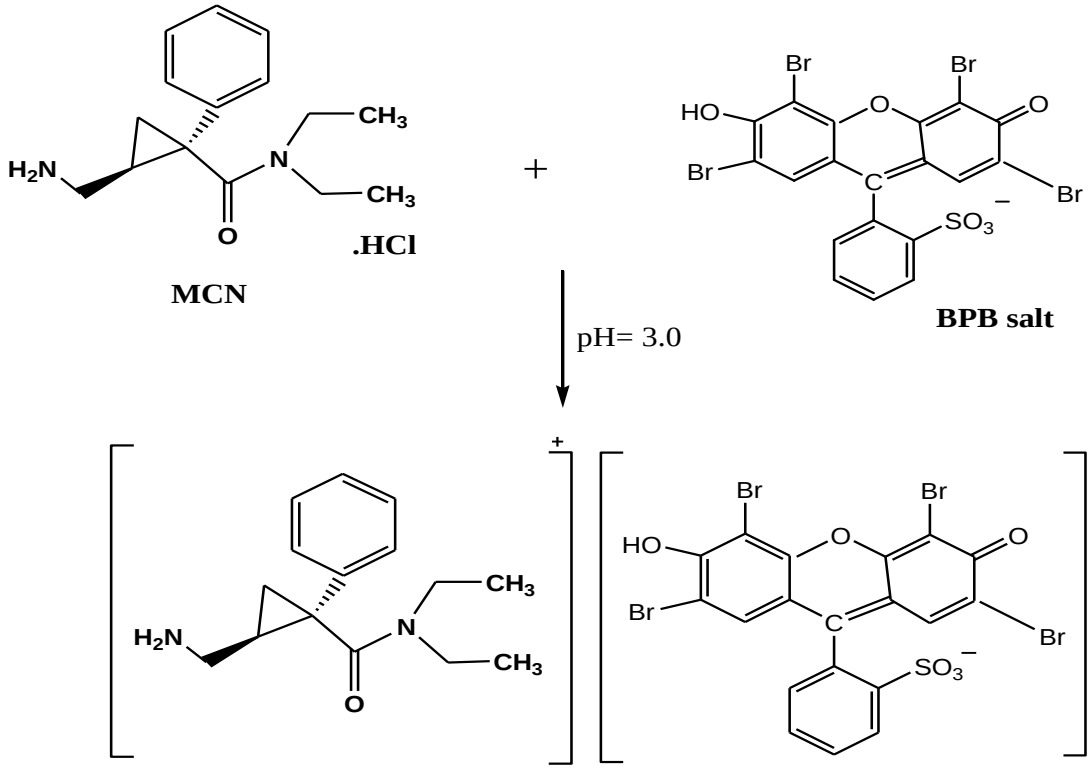



Figure (6) Mole ratio plots for the ion-association complexes of MCN (1.0 x $10^{-4}$ mol $L^{-1}$) with constant concentration of reagents solution (1.0 mL of 1.0 x $10^{-4}$ mol $L^{-1}$) at the optimum conditions.




Scheme (1) Proposed mechanism of the reaction between MCN and BPB salt.

### 3.3. Method validation

#### 3.3.1. Linearity

At described experimental conditions for MCN determination, standard calibration curves with reagents were constructed by plotting absorbance vs. concentration. The statistical parameters were given in the regression equation calculated from the calibration graphs. 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 were also calculated and recorded in Table 2. The molar absorptivity of MCN complexes are arranged in the following order BCP > BPB> MO as illustrated in Table 2.

**3.3.2. Sensitivity**

The limits of detection (LOD) and quantitation (LOQ) for the proposed methods were calculated using the following equation (31, 32):

$$LOD = 3s / k \text{ and}$$
$$LOQ = 10\ s / k$$

Where s is the standard deviation of the intercept of regression lines and *k* is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the limits of detection and quantitation were found in Table (2).

**Table (2**) Statistical analysis and analytical data in the determination of MCN using the proposed methods.

| Parameters | BCP | BPB | MO |
|---|---|---|---|
| **Wavelengths, $\lambda_{max}$ (nm)** | 408 | 413 | 423 |
| **Linearity (µg mL$^{-1}$)** | 1.0-14 | 2.0-20 | 1.0-18 |
| **Molar absorptivity ε, (L/mol$^{-1}$ cm$^{-1}$) x 10$^4$** | 1.1644 | 0.9241 | 0.7475 |
| **Sandal's sensitivity (ng cm$^{-2}$)** | 24.29 | 30.60 | 37.83 |
| **Regression Equation** [a] | | | |
| **Intercept (*a*)** | 0.0036 | -0.0005 | 0.0005 |
| **Sa** [b] | 0.0027 | 0.0045 | 0.0024 |
| **Slope (b)** | 0.0402 | 0.0327 | 0.0257 |
| **$S_b$** [b] | 0.0003 | 0.0003 | 0.0003 |
| **Sy/x** [b] | 0.0039 | 0.0059 | 0.0044 |
| **Correlation coefficient (r)** | 0.9996 | 0.9996 | 0.9995 |
| **Mean ± SD** [b] | 99.50±0.72 | 99.30±0.54 | 99.60±0.65 |
| **RSD%** [b] | 0.72 | 0.54 | 0.65 |
| **RE%** [b] | 0.76 | 0.57 | 0.68 |
| **LOD (µg mL$^{-1}$)** [c] | 0.22 | 0.45 | 0.30 |
| **LOQ (µg mL$^{-1}$)** [c] | 0.74 | 1.51 | 1.0 |
| **t-test** [d] | 0.68 | 1.29 | 0.48 |
| **F- test** [d] | 1.12 | 1.59 | 1.09 |

[a] $A = a + bC$, where $C$ is the concentration in µg/ml, $A$ is the absorbance units, $a$ is the intercept, $b$ is the slope.
[b] Sa, standard error of intercept; Sb, standard error of slope; Sy/x, residual standard deviation of the regression line; SD, standard deviation; RSD%, percentage relative standard deviation; RE%, percentage relative error.
[c] LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity.
[d] The theoretical values of *t* and *F* at P= 0.05 are 2.571and 5.05, respectively, at confidence limit at 95% confidence level and five degrees of freedom (*p*= 0.05).
99.80 ± 0.68%.

**3.3.3. Accuracy and precision**

In order to evaluate the accuracy and precision of the proposed methods, solutions containing three different concentrations of MCN covering the range of each analytical method 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 repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision). The analytical results of intra-day and inter-day precision and accuracy were summarized in Tables (3). The low values of percentage relative standard deviation (RSD %) and percentage relative error (RE %) indicates that the developed methods are precise and accurate. The percentage relative error was calculated using the following equation:

$$RE\ \% = [(founded - added) / added] \times 100$$

**Table (3)** Intra-day and Inter-day precision and accuracy data for MCN obtained by the proposed methods.

| Method | Added concentration | Intra-day | | | | Inter-day | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Recovery** | **Precision** | **Accuracy** | **Confidence** | **Recovery** | **Precision** | **Accuracy** | **Confidence** |

| | (μg mL$^{-1}$) | % | RSD % [a] | RE % | limit [b] | % | RSD % [a] | RE % | limit [b] |
|---|---|---|---|---|---|---|---|---|---|
| BCP | 4.0 | 100.60 | 0.47 | 0.60 | 4.024 ± 0.019 | 99.30 | 0.51 | -0.70 | 3.988 ± 0.02 |
| | 8.0 | 99.20 | 0.72 | -0.80 | 7.936 ± 0.057 | 99.70 | 0.70 | -0.30 | 7.976 ± 0.056 |
| | 12 | 100.20 | 1.17 | 0.20 | 12.024±0.141 | 100.30 | 1.10 | 0.30 | 12.036±0.132 |
| BPB | 5.0 | 100.10 | 0.49 | 0.10 | 5.005 ± 0.025 | 99.60 | 0.62 | -0.40 | 4.98 ± 0.031 |
| | 10 | 99.80 | 0.79 | -0.20 | 9.98 ± 0.079 | 99.40 | 0.87 | -0.60 | 9.94 ± 0.086 |
| | 15 | 99.70 | 1.01 | -0.30 | 14.955±0.151 | 100.20 | 1.24 | 0.20 | 15.03 ± 0.23 |
| MO | 5.0 | 99.60 | 0.65 | -0.40 | 4.98 ± 0.032 | 99.70 | 0.54 | -0.30 | 4.985 ± 0.027 |
| | 10 | 100.10 | 0.88 | 0.10 | 10.01 ± 0.088 | 99.30 | 0.75 | -0.70 | 9.93 ± 0.074 |
| | 15 | 100.40 | 0.93 | 0.40 | 15.06 ± 0.140 | 100.20 | 1.08 | 0.20 | 15.03 ± 0.162 |

[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).*

### 3.3.4. Robustness and ruggedness

For the evaluation of the method robustness, some parameters were subjected to minimum deliberate changes, e.g., pH and dye volume. Small values of RSD% reveal that the methods' capacity remains unaffected by small deliberate variations in experimental conditions. Methods' ruggedness was expressed as RSD % of the same procedure applied by two analysts and using two different instruments on different days. The results showed no significant statistical differences between different analysts and instruments suggesting that the developed methods were rugged (Table 4).

**Table (4)** Results of method robustness and ruggedness (all values in RSD%) studies for MCN (n=3).

| Methods | Nominal concentration (μg mL$^{-1}$) | RSD% | | | |
|---|---|---|---|---|---|
| | | **Robustness** | | **Ruggedness** | |
| | | **Variable alerted [a]** | | | |
| | | **pH** | **Dye volume** | **Different analysts** | **Different instruments** |
| BCP | 4.0 | 0.43 | 0.50 | 0.80 | 0.45 |
| | 8.0 | 0.70 | 0.85 | 0.67 | 0.51 |
| | 12 | 1.10 | 1.30 | 1.60 | 1.20 |
| BPB | 5.0 | 0.50 | 0.30 | 1.0 | 0.40 |
| | 10 | 0.90 | 0.50 | 1.50 | 0.60 |
| | 15 | 1.40 | 1.06 | 1.90 | 1.45 |
| MO | 5.0 | 0.70 | 0.52 | 0.36 | 0.29 |
| | 10 | 1.0 | 0.60 | 0.70 | 0.80 |
| | 15 | 1.60 | 0.90 | 1.25 | 1.50 |

[a] pH (±0.2) and dye volume (±0.2) were used.

### Effects of interference

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

### 3.4. Analysis of pharmaceutical formulations

The proposed methods have been successfully applied to the determination of MCN in pharmaceutical formulations (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). There was no significant difference between the slopes of calibration curves and standard addition methods. Therefore, it is concluded that the excipients in dosage forms of MCN did not cause any interference in the analysis of MCN. The results were compared with those obtained using the reported method for MCN (15). Statistical analysis of the results did not detect any significant difference between the proposed methods and he reported methods in pharmaceutical formulations with respect to accuracy and precision as revealed by the Student's t-value and variance ratio F-value at 95% confidence level (32). The results show that the Student's t- and F-values at 95 % confidence level did not exceed the theoretical values (Table (6).

**Table (5)** Results of recovery experiments by standard addition method for the determination of MCN in tablets using the proposed methods.

| Method | Taken drug (µg mL$^{-1}$) | Pure drug added (µg mL$^{-1}$) | Averomilan®tablets | |
|---|---|---|---|---|
| | | | **Total found (µg mL$^{-1}$)** | **Recovery [a] (%) ± SD** |
| BCP | 4.0 | 2.0 | 5.964 | 99.40 ± 0.54 |
| | | 4.0 | 7.976 | 99.70 ± 0.80 |
| | | 8.0 | 11.892 | 99.10 ± 1.10 |
| BPB | 6.0 | 3.0 | 8.919 | 99.10 ± 0.43 |
| | | 6.0 | 11.916 | 99.30 ± 0.74 |
| | | 9.0 | 15.03 | 100.20 ± 1.25 |
| MO | 4.0 | 2.0 | 5.970 | 99.50±0.57 |
| | | 4.0 | 7.936 | 99.20 ± 0.90 |
| | | 8.0 | 12.08 | 100.40 ± 1.35 |

[a] Average of six determinations.

Table (6) Results of analysis of tablets by the proposed methods for the determination of MCN and statistical comparison with the reported method (15).

| Samples | Recovery [a] (%) ± SD | | | |
|---|---|---|---|---|
| | **Proposed Methods** | | | **Reported method (15)** |
| | **BCP** | **BPB** | **MO** | |
| Averomilan®tablets | 99.40±0.30 | 99.53±0.59 | 99.70±0.62 | 99.20±0.40 |
| ***t-value*** [b] | 0.90 | 1.03 | 1.52 | |
| ***F-value*** [b] | 1.78 | 2.18 | 2.40 | |

[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).

## IV. Conclusion

This work describes the application of extractive ion–pair complexation reaction with dyes for the quantification of anti-depression drug; milnacipran HCl (MCN) in pure form and pharmaceutical formulations. Compared with the existing spectrophotometric method, the proposed methods are relatively simple, rapid, cost-effective and more sensitive for determination of MCN. Moreover, the proposed methods are free from tedious experimental steps such as heating unlike the previously reported method. The most attractive feature of these methods is the relative freedom from interference, by the usual diluents and excipients in amounts higher than their normal existence in pharmaceutical formulations. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. Therefore, the validated methods could be useful for routine quality control assay of MCN in raw material and pharmaceutical formulations.

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