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Extractive-Spectrophotometric Methods for Determination of Alzheimer's Drug in Pharmaceutical Formulations and in Biological Samples Using Sulphonphthalein Acid Dyes

Hany A. Omara[1]* and Alaa S. Amin[2]

[1]*Chemistry Department, Preparatory Year Deanship, Al-Jouf University, Al Qurayyat, KSA*
[2]*Chemistry Department, Faculty of Science, Benha University, Benha, Egypt*

[*] *Corresponding author E- mail: hanyomara666@yahoo.com, Tel: 00966551469767*

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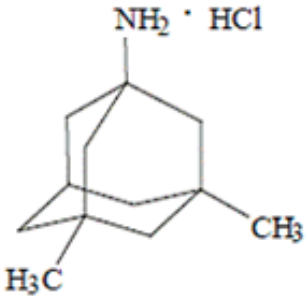
Abstract: A simple, accurate and highly sensitive spectrophotometric methods are proposed for the rapid and accurate determination of Memantine HCl (MEM) using Bromophenol blue (BPB), Bromocressol purple (BCP) and Methyl orange (MO). The developed methods involve formation of stable yellow colored chloroform extractable ion-associate complexes of the amino derivative (basic nitrogen) of the MEM with three sulphonphthalein acid dyes, namely; BPB, BCP and MO, in phthalate buffer of pH range (3.0–3.8). The ion-associates exhibit absorption maxima at 415, 410 and 422 nm for BPB, BCP and MO, respectively. MEM can be determined up to 1.1-12.5, 0.8-13.1 and 0.6-14 µg/mL, respectively. The effect of optimum conditions via pH, reagent concentration, time, and solvent were studied. The low relative standard deviation values indicate good precision and high recovery values. These methods have been successfully applied for the assay of MEM in pharmaceutical formulations. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

Keywords: Memantine HCl; Bromophenol blue; Bromocressol purple; Methyl orange; Ion-pair complex.

1. INTRODUCTION

Memantine hydrochloride [1,2] is an uncompetitive, moderate affinity N-methyl-D-aspartate (NMDA) receptor antagonist used for treating patients with moderate to severe Alzheimer's disease. The chemical name is 1-amino-3,5-dimethyladamantane hydrochloride (Scheme 1). It is believed that over stimulation of nerve cells by glutamate may be responsible for the degeneration of nerves in some neurological diseases such as Alzheimer's disease.

Stability indicating high performance liquid chromatographic method coupled with ultra violet detection has been applied in the determination of MEM in bulk [3] High performance liquid chromatographic methods coupled with fluorescence detection have been reported in the literature for the



Scheme 1: Structure of memantine hydrochloride (MEM).

quantification of MEM in rat plasma [4-6], human plasma [7,8]. Determination of memantine in human plasma by liquid chromatography with mass spectrometry (LC-MS) [9-11], gas chromatography with mass spectrometry (GC-MS) [11] and micellar electro kinetic chromatography (MEKC) [13], or Potentiometrically [14,15]. Though the above mentioned chromatographic methods are sensitive. The methods suffer from one or more drawbacks such as expensive instrumentation, tedious extraction procedures, time consumption, complex and derivatization of the drug with suitable chromophores or fluorophores.

Especially in developing countries, spectrophotometric method has generally been the method of choice for routine analysis in quality control laboratories. The spectrophotometric method is simpler, rapid, sensitive, selective and inexpensive. Spectrophotometric methods were reported [16,17] and by extraction [18,19].Bromocressol green (BCG), bromophenol blue (BPB) and bromothymol blue (BTB) are known to yield an ion pair complex, which are applied in the determination of many pharmaceutical compounds [20-36].

The present work aims to present a simple, rapid and sensitive method for the determination of MEM in pure form and in their pharmaceutical preparations and can be used for the quality control and assurance of the drug in industry. The methods are based on the formation of ion-associate between the cited drug and using three anionic dyes BPB, BCP or MO. These methods are very simple in applications and less expensive in comparison to the above mentioned techniques, whereas at the same time offering a high degree of accuracy and precision when compared to the pharmacopoeia method and could be used simply to determine the shelve stability time of the drug.

2. EXPERIMENTAL

2.1. Apparatus

All the spectral measurement were made using double-beam UV/Vis spectrophotometer (Biotech Engineering Ltd., UK), with wavelength range 190-1100 nm, spectral bandwidth 2.0 nm, with scanning speed 400 nm/min, equipped with 10 mm matched quartz cells. A thermostat water bath, Buchi 461 water bath, Schwiz (France) was used to carry out the temperature studies and Magnetic Mix. 100, Thermo

Scientific, UK. A centrifuge Model 90-1 with speed 50000 rpm (USA) was used to carry out for the spiked plasma samples. Hanna pH-meter instrument (Portogal) (HI: 9321) was used for checking the pH of phthalate buffer solutions pH ranges from 2.0 to 7.0.

2.2. Reagents and materials

All chemicals used were of analytical grade and all solutions were freshly prepared in doubly distilled water. Pure memantine HCl bulk powder, its purity was found to be 100.29±0.76 (n = 5) according to the HPLC procedures which obtained from Egyptian Organization for Control and Pharmaceutical Research - Egypt. Memantine HCl working solution prepared by dissolving 0.01 g of pure MEM in 50 mL of bidistilled water and complete to 100 mL with bidistilled water to obtain the working standard solution of concentration 100 μg/mL.

1. The acid dye reagents BCG, BPB and BTB were obtained from E. Merck Darmstdt F. R. Germany.
2. Stock solution of bromophenol blue (BPB), bromocresol purple (BCP) and methyl orange (MO) (1.0×10^{-3} M), were prepared by dissolving the appropriate weight, in 10 mL 96% ethanol and diluted to 100 mL with bidistilled water. These solutions are stable for at least 1 week if kept in the refrigerator, were stable for several weeks.
3. 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 by following the standard methods [37]. Potassium hydrogen phthalate–HCl (pH 2.0–7.0) was prepared by dissolving 1.28 g potassium hydrogen phthalate in water and completed to 50 mL with water and adjusting pH by addition of 0.2 M hydrochloric acid. Freshly prepared solutions were always employed.

2.3. General Procedure

Into a 50 mL separating funnel, a volume of memantine HCl (100 μg/mL) 2.0, 1.7 and 1.5 mL (1×10^{-3} M) of BPB, BCP and MO respectively, at pH = 3.1, 3.8 and 3.5 of potassium hydrogen phthalate buffer in the case of BPB, BCP and MO, respectively. The formed ion-associates were extracted with 4.0 mL chloroform after shaking for 2.0 min, the volume was made up to 10 mL with bidistilled water. The reaction mixture was allowed to separate into two phases. The absorbance of the extracts was measured at the recommended maximum wavelength (415, 410 and 422 nm for BPB, BCP and MO, respectively), against a reagent blank prepared in the same manner without the addition to the drug. All measurements were carried out at room temperature (25 ± 2 ^{o}C).

2.4. Determination of memantine HC1 in tablets

Twenty tablets from (Alzmenda 10 mg per tablet, Marcyrl Pharmaceutical Industries, El-Obour City, Egypt or Memexa 10 mg per tablet, Copad Egypt for Trade and Pharmaceutical Industries "Copad Pharma", Egypt) were carefully evacuated; their contents were weighed and finely powdered. An accurately weighed quantity of the capsule contents equivalent to 10 mg of MEM was transferred into a

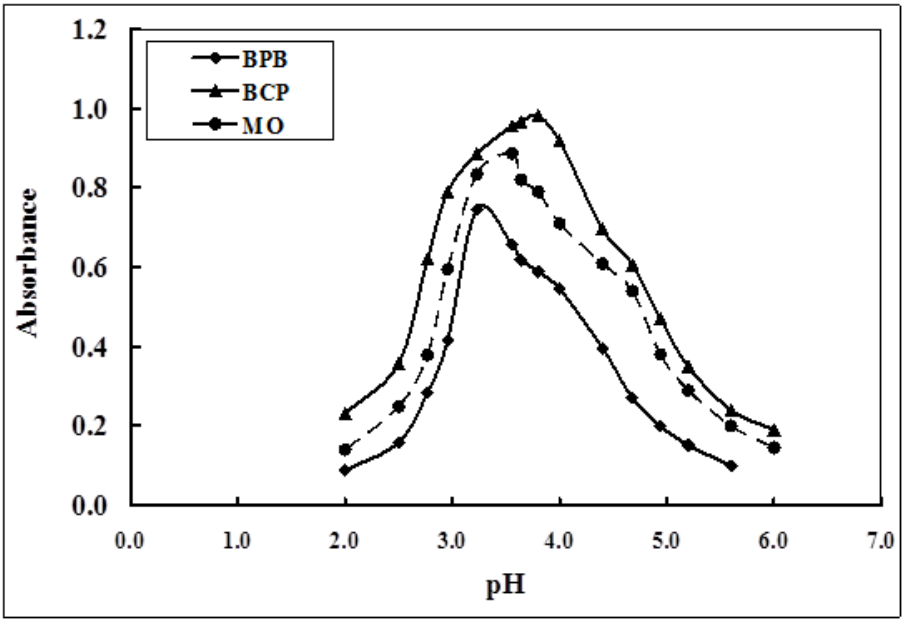


Figure 1. Effect of pH of potassium hydrogen phthalate buffer solution on the MEM–dye ion-pair

100 mL calibrated flask, and dissolved in about 40 ml of distilled water. The contents of the flask were swirled, sonicated for 5.0 min, and then completed to volume with water. The contents were mixed well and filtered rejecting the first portion of the filtrate. The prepared solution was diluted quantitatively with distilled water to obtain a suitable concentration for the analysis.

2.5 Procedure for spiked plasma samples

Aliquots of 1.0 mL of plasma were spiked with different concentration levels of MEM. The spiked plasma samples were treated with 0.1 mL of 70% perchloric acid and vortexed for 1.0 min. The samples were centrifuged for 20 min at 13000 rpm. The supernatants were transferred into test tubes and neutralized with 1.0 mol/L NaOH solution.

3. RESULTS AND DISCUSSION

Several parameters such as buffer, reagent concentration, and sequence of addition and effect of extracting solvent were optimized to achieve high sensitivity, stability, low blank reading and reproducible results.

3.1. Effects of pH on the ion-pair formation

The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as 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). It was noticed that the maximum color intensity and highest absorbance value were observed in potassium hydrogen phthalate–HCl buffer of pH 3.2, 3.8 and 3.5 of potassium hydrogen phthalate buffer using (BPB, BCP and MO), respectively. In addition to the stability of the color without affecting the absorbance at the optimum pH values (Fig. 1). Further, 2.0 mL potassium hydrogen phthalate buffers gave maximum absorbance and reproducible results.

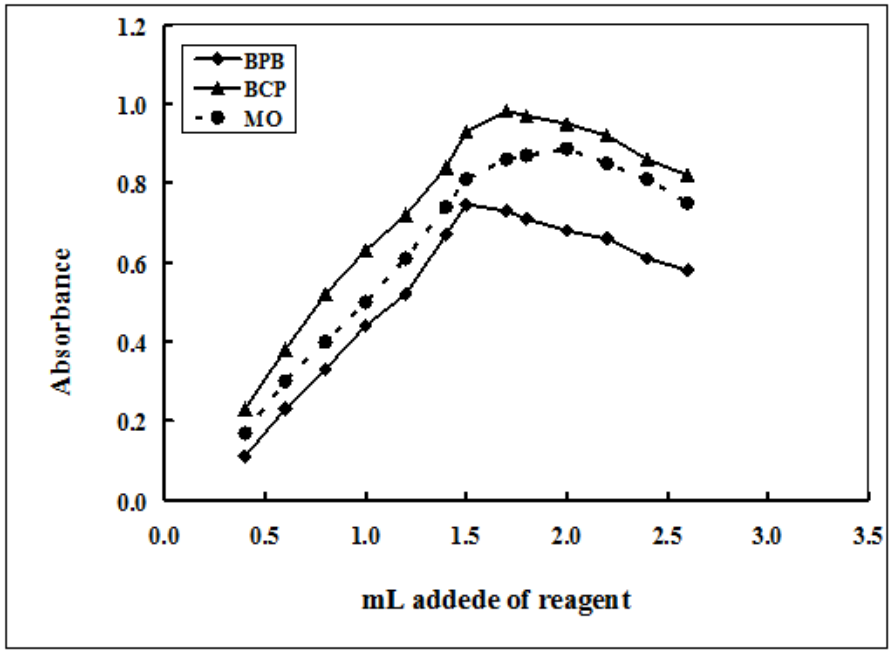


Figure 2. Effect of mL added of reagent (1.0×10^{-3} M)

3.2. Effect of the reagent concentration

The effect of reagent concentration was tested by using amounts (0.1-2.0 mL) of 1.0×10^{-3} M solution of each reagent with 8.0 µg/mL of MEM. The results showed that 2.0, 1.7 and 1.5 mL of BPB, BCP and MO, respectively (Fig. 2), were sufficient for the production of maximum and reproducible color intensity.

3.3. Effect of sequence of mixing

The effect of sequence of additions was studied by measuring the absorbance of solution prepared by different sequence of additions (reagent-drug-buffer, reagent- buffer-drug, buffer-drug-reagent, buffer-reagent-drug, drug-buffer-reagent and drug-reagent-buffer) at λ_{max} against a blank solution prepared in the same manner. Experiments showed that (reagent-drug-buffer) gave the best results, i.e highest color intensity and the shortest time for developing maximum absorbance, while the other sequence require longer time and produce lower absorbance values.

3.4. Effect of shaking time and temperature

The effect of shaking time and temperature on the formation and stability of the ion-associates was studied by measuring the absorbance of the extracted ion-associates at increasing time intervals, the results showed that the ion-associates were formed almost instantaneously in all cases at room temperature (25 ± 2 °C) with 2.0 min shaking time (Fig. 3). The developed color remained stable (i.e. no effect on the absorbance of the formed complexes) for 24, 18 and 12 h using BPB, BCP and MO, receptively. After these intervals, a slight decrease in color intensity occurred.

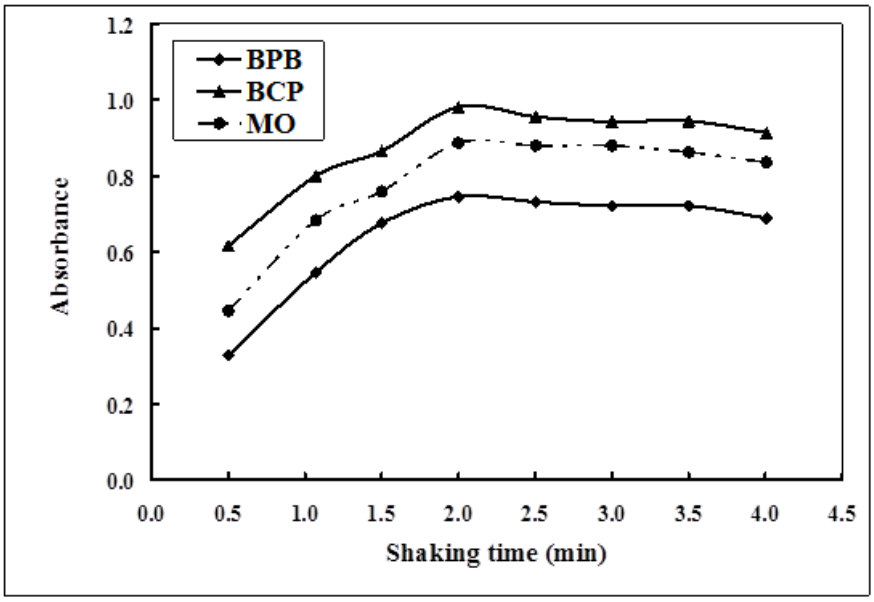


Figure 3. Effect of shaking time on the ion-pair complexes

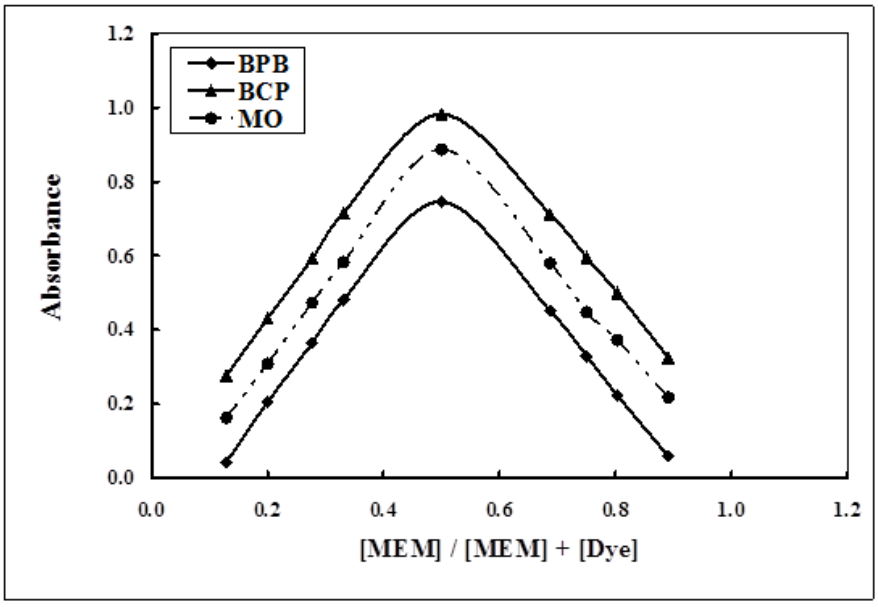


Figure 4. Job's method of continuous variation graph for the reaction of MEM with acid-dyes BPB, BCP and MO, in chloroform [drug] = [dye] = 1.0×10^{-3} M

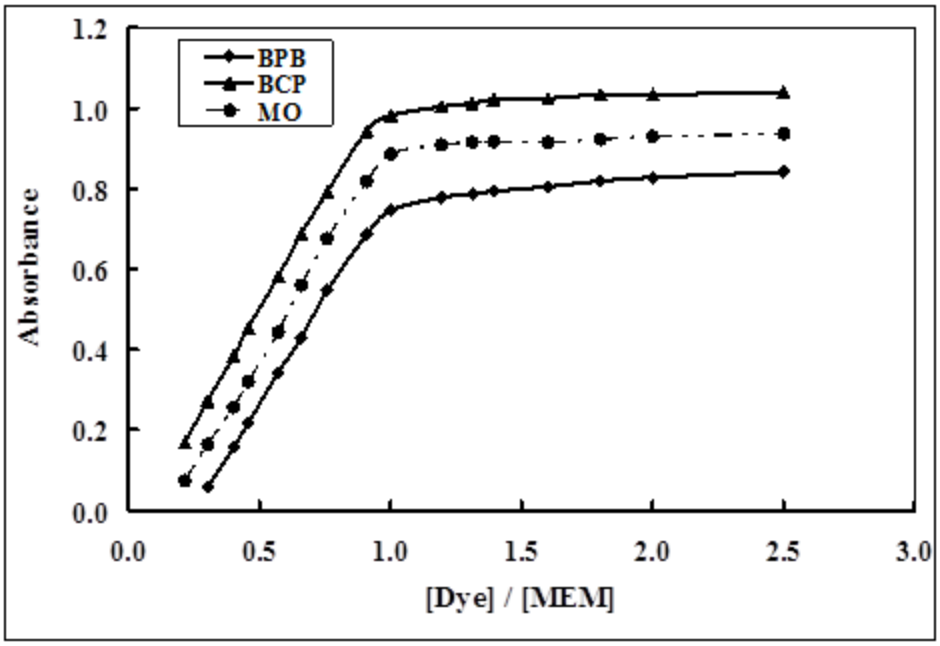


Figure 5. Molar ratio plots for the reaction of MEM with acid-dyes BPB, BCP and MO, in chloroform [drug] = [dye] = 1.0×10^{-3} M

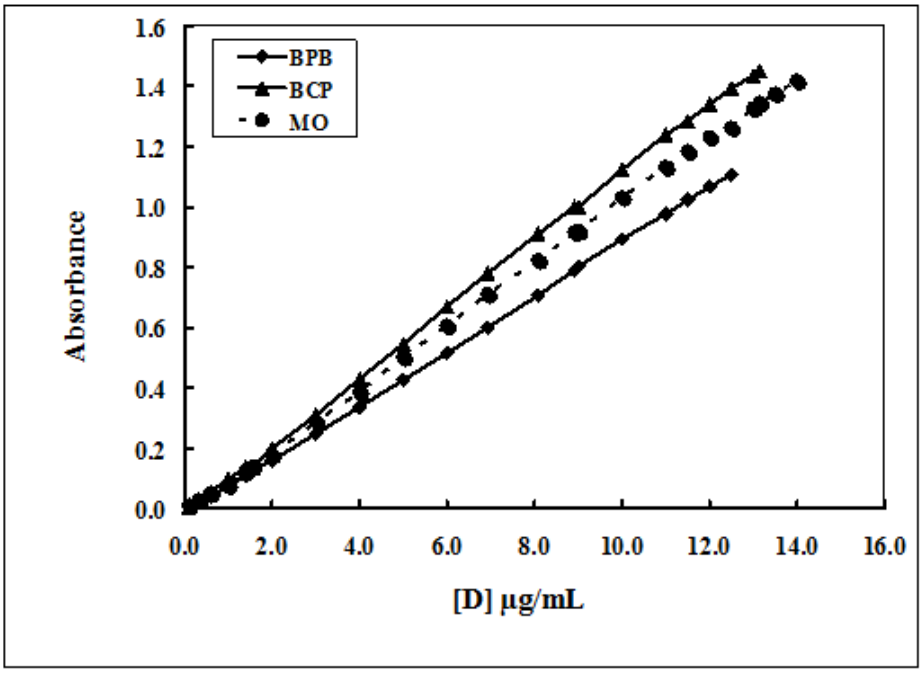


Figure 6. Calibration curve (Linearity) of MEM using BPB, BCP and MO

Table 1. Statistical analysis of calibration graphs and analytical data in the determination of the MEM using the proposed methods (n=6)

Parameters	BPB	BCP	MO
λ_{max} (nm)	415	412	414
Stability /(h)	24	18	12
pH	3.2	3.8	3.5
Shaking time /(min.)	2		
Beer's law limits /(µg/mL)	1.1–12.5	0.9–13.1	1.2–14.0
Ringbom limits /(µg/mL)	1.9–11.4	1.3–12.5	2.0–12.8
Molar absorptivity /(L mol^{-1} cm^{-1})	1.93×10^4	2.13×10^4	1.90×10^4
Sandell sensitivity (ng/cm^2)	11.19	10.12	11.34
Detection limits (µg/mL)	0.097	0.073	0.084
Quantitation limits (µg/mL)	0.323	0.243	0.280
Regression equation [a]			
Slope (b)	0.0894	0.0988	0.0882
RSD% of slope	0.0061	0.0041	0.0071
Intercept (a)	0.0019	-0.0063	-0.0012
RSD% of intercept	0.0040	0.0032	0.0075
Correlation coefficient (r)	0.9998	0.9997	0.9999
Stoichiometric ratio	1:1	1:1	1:1
Range of error	0.67	0.60	0.58
RSD %	1.02	0.96	0.47

Calculated t–values (2.57) [b]	0.47	0.96	0.77
Calculated F- test (5.05) [b]	2.27	1.93	1.49

[a] *With respect to A = a + bC where C is concentration of drug and A is absorbance*
[b] *Values in parentheses are the theoretical values for t- and F- values at 95% confidence limits and five degrees of freedom*

3.5. Effect of extracting solvent

The polarity of the solvents affects both extraction efficiency and absorptivity of the ion associates. Several water-immiscible organic solvents including benzene, toluene, xylene, carbon tetrachloride, chloroform, methylene chloride, 1,2-dichloroethane and nitrobenzene were tried. The most convenient solvent found to produce the highest absorbance, extraction power and stability of color of the formed ion-associates were chloroform for BPB, BCP and MO. The study repealed that a volume ratio of 3:2 (aqueous: organic) was the most suitable (which give highest absorbance) for the ion- associate extraction.

3.6. The Stoichiometric ratio of the ion-associate

The stoichiometry of the ion-associates formed between the drug and the reagents was investigated by applying the continuous variation [38], (Fig. 4) and the molar ratio methods [39] (Fig. 5), at the wavelengths of maximum absorbance. The results obtained showed that the stoichiometric ratio of the ion-associates is 1:1 (reagent: drug) in all cases.

3.7. Analytical data

Beer's law (Linearity) was verified up to (1.1 - 12.5), (0.8 - 13.1) and (0.6 - 14.0) µg/mL of MEM with BPB, BCP and MO, respectively (Fig. 6). The molar absorptivity (Є) calculated and found to be 1.93×10^4, 2.13×10^4 and 1.90×10^4 L mol^{-1} cm^{-1}, with BPB, BCPB and MO, respectively, indicating high sensitivity of the reagents under investigations for the determination of MEM. The limits of detection (K=3) and quantitation (K=10) were established according to IUPAC definitions [40] are recorded in Table 1. The regression equations (A = a + bC) where A = absorbance, a = intercept, b = slope and C = concentration in µg/mL), calculated from the calibration graph, were evaluated and recorded in (Table 1). The intercept of the lines were very small indicating that there is no systematic difference between determined and expected concentration within the investigated rang using the present method, for more accurate results, Ringbom concentration range was determined by plotting log [drug] in µg/mL against % transmittance from which the linear portion of the curve gave accurate range for the determination of the drug under investigation (Table 1).

In order to determine the accuracy and precision of the present method, solutions containing five different concentrations of drug were prepared and six replicate determinations, converting the usable concentration range, were carried out for the pure form and the pharmaceutical of the drugs under investigation. The recovery values almost reach 100% recovery, revealing a high accuracy of the results (Table 2).

Table 2. Evaluation of the accuracy and precision of the proposed procedure

Reagent	Taken	Recovery %	Precision	Accuracy	Confidence limits [c]
	µg/mL		RSD[a] %	RE[b] %	
	4.00	101.25	0.39	0.29	4.05 ± 0.0116
BPB	8.00	100.25	0.28	0.22	8.02 ± 0.0178
	12.0	98.83	0.25	0.17	11.98 ± 0.0207
	4.00	99.25	0.89	1.01	3.97 ± 0.0402
BCP	8.00	99.38	0.64	0.62	7.95 ± 0.0490
	13.0	99.85	0.51	0.48	12.98 ± 0.0625
	5.00	100.4	0.64	0.70	5.02 ± 0.0349
MO	9.00	98.78	0.54	0.66	8.89 ± 0.0589
	14.0	100.07	0.47	0.40	14.01 ± 0.0566

[a] *Relative standard deviation for six determinations*
[b] *RE %, percentage relative error*
[c] *95 % confidence limits and five degrees of freedom*

The calculated standard deviations are compared with those obtained by the pharmacopoeia method of memantine HCl [41]. The proposed method was successfully applied to determine MEM in its dosage forms and in spiked serum plasma. The accuracy of the proposed methods is evaluated by applying standard addition technique, in which variable amounts of the drug were added to the previously analyzed portion of pharmaceutical preparations and in spiked serum plasma. The results recorded in Table 3, were compared statistically with the official method by Student's *t*-test (for accuracy), and variance ratio *F*-test (for precision) [42] at 95% confidence level as recorded in Table 4. Such comparison showed that there is no significant difference, at 25% confidence level, between the values obtained by the proposed and the pharmacopoeia method. This indicates the high accuracy and precision of the present method.

Table 3. Determination of MEM in pharmaceutical formulations using Standard Addition Technique (Taken 8.0 µg/mL)

Samples	Added µg/mL	BPB		BCP		MO	
		Found* µg/mL	Recovery %	Found* µg/mL	Recovery %	Found* µg/mL	Recovery %
	0.0	8.01	100.1	7.96	99.50	7.98	99.75
Alzmenda	1.0	9.01	100.1	8.93	99.22	9.02	100.2
10 mg/Tablet [1]	2.0	9.96	99.60	9.97	99. 07	9.99	99.90
	3.0	10.99	99.91	10.89	99.00	11.01	100.1
	0.0	7.97	99.63	7.91	98.88	7.98	99.75
Memexa	1.0	8.98	99.66	9.01	100.1	8.96	99.56
10 mg/Tablet [2]	2.0	9.97	99.78	9.92	99.20	9.95	99.50
	3.0	11.02	100.2	10.96	99.64	11.01	100.1
Spiked plasma pimple	0.0	7.96	99.50	7.97	99.63	8.02	100.3
	1.0	9.02	100.2	8.90	98.89	9.01	100.1
	2.0	9.97	99.70	9.99	99.90	9.94	99.40
	3.0	11.01	100.1	10.94	99.45	11.02	100.2

* *Average of six determinations*
[1] *Marcyrl Pharmaceutical Industries, El-Obour City, Egypt*
[2] *Copad Egypt for Trade and Pharmaceutical Industries (Copad Pharma), Egypt*

3.8. Robustness and ruggedness

The ruggedness of the proposed methods was assessed by applying the procedures using two different instruments in two different laboratories at different elapsed time. Results obtained from lab-to-lab and day-to-day variation was found to be reproducible as RSD did not exceed 1.02%. Robustness of the methods was assessed by evaluating the influence of small variation of experimental variables: concentrations reagent, buffer, temperature and reaction time, on the analytical performance of

Table 4. Determination of MEM in Tablet (10 mg/Tablet) and spiked plasma by the proposed and official method [PB, 2007]

Parameter	Alzmenda 10 mg/Tablet [1]			
	BPB	BCP	MO	Official Method
Recovery % [a]	99.6 ± 1.4	99.8 ± 1.3	100.1 ± 1.5	99.5 ± 1.9
± Standard Deviation	0.69	0.75	1.12	1.39
Variance	0.88	0.76	1.02	1.38
t-test [b]	1.09	0.99	1.16	1.28
F-value [b]	2.48	2.23	2.09	2.79
	Memexa 10 mg/Tablet [2]			
Recovery % [a]	99.8 ± 0.9	99.7 ± 1.3	99.8 ± 0.7	100.2 ± 1.8
± Standard Deviation	0.80	0.91	0.85	1.15
Variance	1.07	0.92	1.17	1.35
t-test [b]	0.76	0.93	1.08	1.11
F-value [b]	1.17	2.36	2.20	2.51
	Spiked Plasma Pimples			
Mean recovery % [a]	100.5 ± 0.6	101.2 ± 1.0	100.1 ± 1.2	100.3 ± 1.7
± Standard Deviation	1.29	0.90	1.18	1.39
Variance	1.22	0.98	1.20	1.53
t-test [b]	1.33	1.19	1.25	1.62
F-value [b]	2.63	2.51	2.31	2.77

[a] Average values of six determinations (Number of experiments = 6) were used for the official and the proposed methods, respectively
[b] Theoretical values for t and F at 95% confidence limit are 2.57 and 5.05, respectively
[1] Marcyrl Pharmaceutical Industries, El-Obour City, Egypt
[2] Copad Egypt for Trade and Pharmaceutical Industries (Copad Pharma), Egypt

the method. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results; recovery percentages were 99.6–100.5, 99.7–101.2 and 99.8-100.1% for BPB, BCP and MO, respectively. This provided an indication for the reliability of the proposed methods during routine work.

3.9. Interference

A systematic qualitative study was under taken by measuring the absorbance of solutions containing 1.0 mL of 100 µg/mL MEM together with varying excess of different additives and excipients which may be present in the pharmaceutical preparations using the recommended methods of such reagents. No significant interference was observed from the excipients commonly used such as glucose, micro-crystalline cellulose, lactose, fructose, starch and magnesium stearate, this shows that the method is applicable in the case of pharmaceutical preparations of the drug.

4. CONCLUSION

Unlike GC and HPLC procedures, the spectrophotometer is simple and is not of high cost. The proposed method for the estimation of memantine HCl using bromophenol blue, bromocresol purple and methyl orange are advantageous over many of the reported methods due to its sensitivity, rapidity and good agreement with the pharmacopoeia methods. The high recovery percentage and lows relative standard deviation reflect the high accuracy and precision of the proposed method. Moreover, the method is easy, applicable to wide ranges of concentration, beside less time consuming and depend on simple reagents which are available. This offering economic and acceptable method for the routine determination of the cited drug, Beer's law up to 1.1, 0.8 and 0.6 µg/mL uses BPB, BCP and MO, respectively. So it is recommended for the routine determination in pure samples and in their pharmaceutical formulations.

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The authors declare no conflict of interest

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