RESEARCH ARTICLE

Determination of Memantine Hydrochloride by Spectrophotometry using Anionic Dyes, Bromothymol Blue and Solochrome Black T, in Bulk and Tablet Dosage Forms

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Determination of Memantine Hydrochloride by Spectrophotometry using Anionic Dyes, Bromothymol Blue and Solochrome Black T, in Bulk and Tablet Dosage Forms

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Abstract

Three spectrophotometric methods are described for the determination of memantine hydrochloride (MTH) in bulk and tablet dosage forms. The methods are based on the formation of ion-pairs of the MTH with anionic dyes such as bromothymol blue (BTB) and solochrome black T (SBT), which are extracted into chloroform and have absorption maxima at 415 nm (BTB) and 510 nm (SBT). Regression analysis of the Beer's plots showed good correlation in the concentration ranges 2–20 and 5–25 µg/mL for BTB and SBT, respectively. The proposed methods were successfully applied to the tablet dosage forms containing the MTH. No interference from common excipients was observed.

Keywords: Memantine HCl; anionic dyes; Beer's law; molar absorptivity; validation.

1. Introduction

Memantine hydrochloride [1-5] 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 (Figure 1). Glutamate is the major excitatory neurotransmitter in the brain. 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. Glutamate is produced and released by nerve cells in the brain, travels to nearby nerve cells where it attaches to the NMDA receptor on the surface of the cells. Memantine blocks the receptor and thereby decreases the effects of glutamate. Thus memantine protects nerve cells from excess stimulation by glutamate.

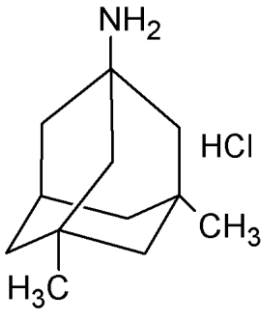

Figure 1: Structure of memantine HCl.

Stability indicating high performance liquid chromatographic method coupled with ultra violet detection has been applied in the determination of MTH in bulk [6]. High performance liquid chromatographic methods coupled with fluorescence detection have been reported in the literature for the quantification of MTH in rat plasma [7], human plasma [8-10] and vitreous humour [10]. Determination of memantine in human plasma by liquid chromatography with mass spectrometry (LC-MS) [11-14], gas chromatography with mass spectrometry (GC-MS) [15] and micellar electro kinetic chromatography (MEKC) [16] were reported in the literature. Though the above mentioned chromatographic methods are sensitive, they are not suitable for routine analysis of the MTH in

quality control laboratories. 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. Jagathi *et al.* have recommended two spectrophotometric methods for the estimation of MTH in bulk and pharmaceutical formulations [17]. The first method is based on the formation of blue colored complex with Folin-Ciocalteau reagent and the second method involves the condensation of the MTH with 1,2 napthoquinine-4-sulphonate. Two other spectrophotometric methods were reported by Michail *et al.* [18]. The methods are based on the derivatization of MTH with 4-chloro-7-nitro-2,1,3-benzoxadiazole or o-phthalaldehyde/N-acetyl-L-cysteine reagents in alkaline media. A spectrofluorimetric method has also been described based on the reaction of the MTH with 4-chloro-7-nitro-2,1,3-benzoxadiazole in alkaline buffer and the product formed is measured spectrofluorimetrically in acetone at 500 nm after excitation at 455 nm [18]. The reported spectrophotometric methods suffers from disadvantages like poor sensitivity, lack of selectivity, preparation of buffer, critical dependence on pH, heating step, long standing for color development, derivatization of the drug and measurement at shorter wavelength (340 nm).

In the present paper, we describe the development and validation of two simple, sensitive, accurate and precise spectrophotometric methods using two anionic dyes, bromothymol blue (BTB method) and solochrome black T (SBT method). The proposed methods are based on ion-pair complex formation between the MTH and dyes. The proposed methods were applied to the determination of MTH in tablets, and no interference from common excipients in tablets was observed in the assay.

2. Methods

2.1. Apparatus

All spectrophotometric measurements were carried out using a Systronics (Ahmedabad, India), model Visiscan-167, digital spectrophotometer. The cells used for absorbance measurements were 1-cm matched quartz cells. Samples were weighed by using Shimazdu electronic weighing balance (Tokyo, Japan), BL 220 H model.

2.2. Reagents

All the chemicals used were of analytical reagent grade and used as received. All the solutions were prepared afresh daily in double distilled water. Aqueous solutions 0.2 % BTB (SD Fine-Chem Limited, Mumbai, India) and 0.5% SBT (Qualigens Fine Chemicals Limited, Mumbai, India), 0.1 N HCl (Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India) were prepared. Spectrophotometric grade chloroform (Merck Specialities Pvt. Ltd, Mumbai, India) was used for the extraction of the ion-pair complex.

2.3. Preparation of stock standard solution

Pharmaceutical grade MTH was obtained as a gift sample from Matrix Laboratories, Hyderabad, India. Stock standard solution of MTH (1 mg/mL) was prepared by dissolving 100 mg of drug in water and then diluting to the mark in a 100 mL volumetric flask. The working standard solution of MTH containing 100 µg/mL was prepared by dilution of the stock solution (1 mg/mL) with water. Commercial dosage forms of MTH such as admenta-5 and 10 mg tablets (Sun Pharmaceutical Industries Ltd., Mumbai, India) were obtained from a local pharmacy store.

2.4. General procedure

Into a series of 50 mL separating funnels, aliquots of working MTH [0.2-2.0 mL (BTB method) or 0.5 to 2.5 mL (SBT method)] solution were transferred. The volume in each separating funnel was adjusted to 2.5 mL with distilled water. Then, to each separating funnel 1 mL of 0.1 N HCl and 1.0 mL of dye solution [0.2% bromothyol blue (BTB method) or 0.5 % solochrome black T (SBT method)] were transferred and mixed well. The funnels were shaken vigorously with 5 mL of chloroform for 2 min. The funnels were allowed to stand for the clear separation of the two phases. The separated chloroform phase was transferred into a 10 mL volumetric flask, made up to the mark with chloroform and mixed well. The absorbance of the chloroform phase was measured at 415 nm and 510 nm against reagent blank for methods BTB and SBT, respectively. The amount of the drug in the unknown sample solution was computed either from the corresponding calibration graph or from the regression equation.

2.5. Procedure for tablets

Twenty tablets were accurately weighed and finely powdered. A powdered amount equivalent to 50 mg was dissolved in 25 mL of water by sonication for 10 minutes. The solution was filtered using Whatmann No. 1 filter paper. The filtrate was transferred into a 50 mL volumetric flask and made up to volume with water. This solution was further diluted appropriately to get 100 µg/mL concentrations for analysis by BTB and SBT methods.

3. Results and Discussion

3.1. Method development

The ion-pair complex is a special form of molecular complex resulting from two oppositely charged ions extractable into organic solvents from aqueous phase at suitable pH [19-21]. Initially, the field of physical chemistry has investigated the ion pair complex formation which is now being applied widely for the chemical as well as pharmaceutical analyses [22]. By using an anionic dye as a reagent and organic solvent as an extractant the quantification of several pharmaceutical compounds that possess basic moieties (secondary or tertiary amino group) is done by the ion-pair extractive spectrophotometry. As bromothymol blue [23-26] and solochrome black T [27, 28] are anionic dyes; they involve in the formation of ion pair complexes. Because of this nature, they contribute to simple and rapid spectrophotometric determination of many organic compounds of pharmaceutical significance.

The results obtained in the proposed methods were based on the tendency of the MTH to form chloroform extractable ion-pair complex with anionic dyes, bromothymol blue (BTB method) or solochrome black T (SBT method), under experimental conditions. The positively charged primary nitrogen of MTH in acid medium is likely to attract the negatively charged part of the anionic dyes, bromothymol blue (BTB method) or solochrome black T (SBT method), and form an ion-pair complex held together through electrostatic attraction. Absorption spectra of the colored MTH-BTB and MTH-SBT ion-pair complexes are shown in Figures 2 and 3. MTH-BTB and MTH-SBT ion-pair complexes showed maximum absorbance at 415 and 510 nm, respectively. The MTH-BTB and MTH-SBT ion-pair complexes were stable for 1 hour at room temperature. The proposed reaction schemes are presented in Figures 4 and 5.

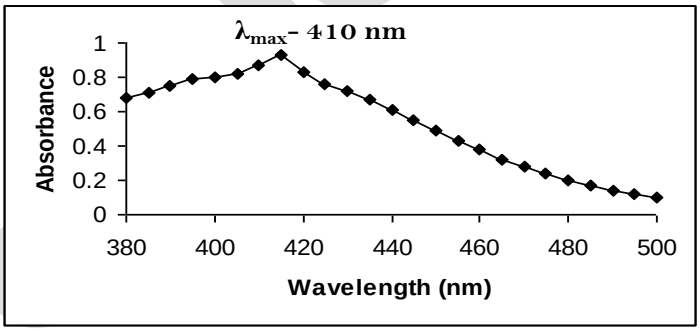

Figure 2: Absorption spectra of MTH-BTB ion-pair complex.

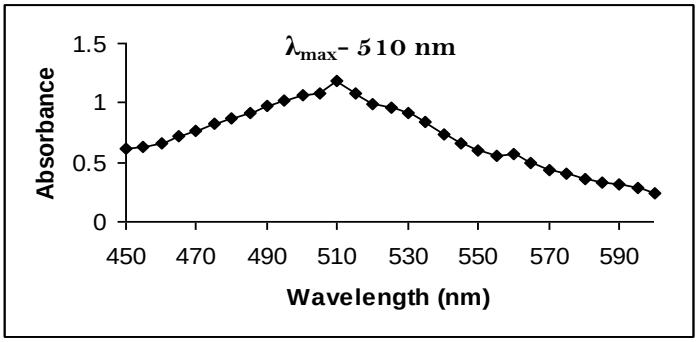

Figure 3: Absorption spectra of MTH-SBT ion-pair complex.

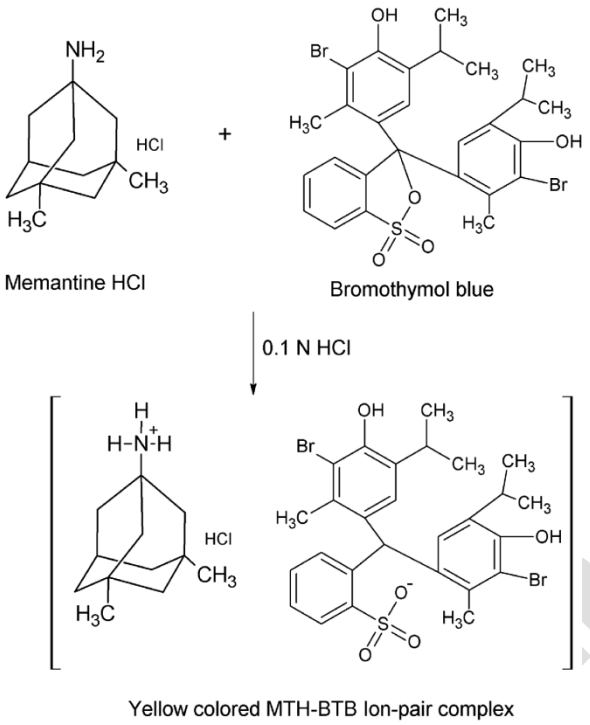

Figure 4: Formation of MTH-BTB ion-pair complex.

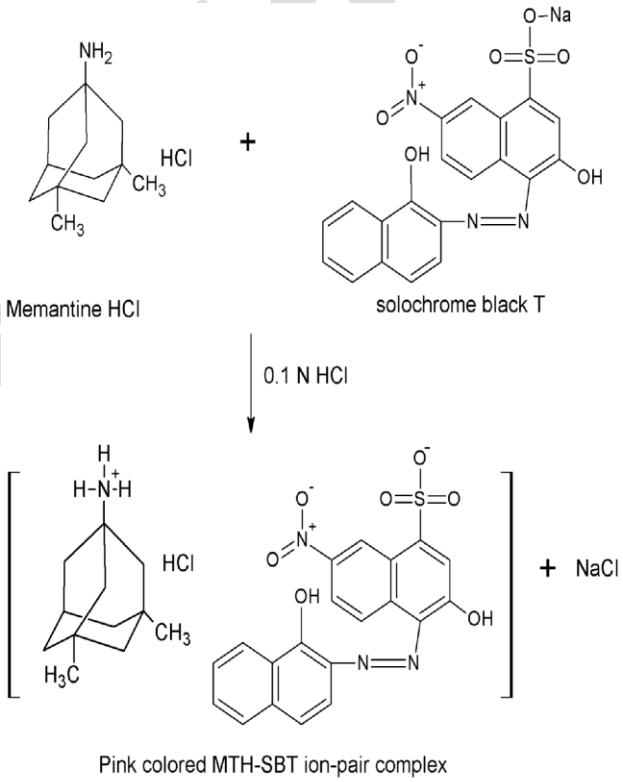

Figure 5: Formation of MTH-SBT ion-pair complex.

The different experimental parameters affecting the formation of colored MTH-BTB and MTH-SBT ion-pair complexes were studied and optimized to obtain the maximum color intensity. The effect of bromothymol blue (BTB method) or solochrome black T (SBT method) concentration was studied by adding different volumes (0.2–2 mL) of 0.2 % solution of BTB or 0.5% solution of SBT to a constant concentration of MTH (10 µg/mL). Maximum absorbance of MTH-BTB and MTH-SBT ion-pair complexes was found at 1.0 mL of 0.2 % solution of BTB and 0.5% SBT, respectively (Figure 6). Beyond this value the absorbance decreases. Hence, 1.0 mL of the dye solution was used throughout this study.

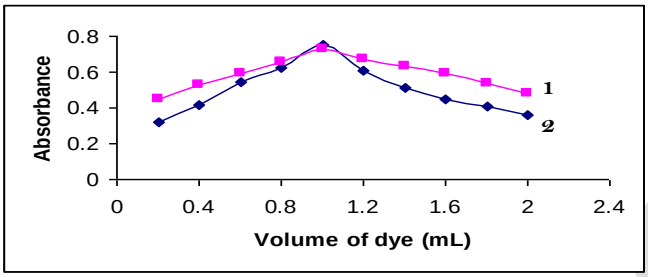

Figure 6: Effect of concentration of dye: 1. BTB method, 0.2 % BTB solution, λmax-415 nm; 2. SBT method, 0.5 % SBT solution, λmax-510 nm.

The influence of acidity on the development of colored MTH-BTB and MTH-SBT ion-pair complexes using different volumes of 0.1 N HCl (0.2–2 mL) to a fixed concentration of MTH (10 µg/mL) were tested in this study. The maximum color intensity was observed with 1 mL of 0.1 N HCl (Figure 7). Therefore 1 mL of 0.1 N HCl was used throughout the experiment.

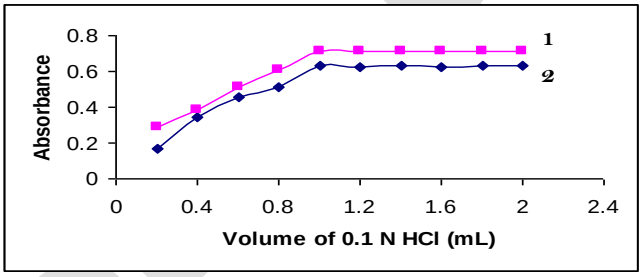

Figure 7: Effect of acidity: 1. BTB method, λmax-415 nm; 2. SBT method, λmax-510 nm.

The effect of extraction solvents such as chloroform, benzene, dichloromethane and butanol was investigated for effective extraction of the colored MTH-BTB and MTH-SBT ion-pair complexes. It was found that chloroform was suitable for the quantitative extraction of ion-pair complex, whilst only partial extraction of the ion-pair complex was achieved with other solvents (Figure 8).

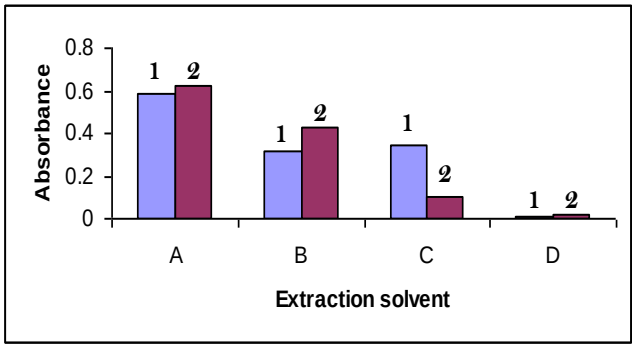

Figure 8: Effect of extraction solvent: 1. BTB method, λmax-415 nm; 2. SBT method, λmax-510 nm; A. Chloroform, B. Benzene, C. Dichloromethane, D. Butanol.

3.2. Validation

Method validation was performed by following the International Conference on Harmonization (ICH) guideline [29] for analytical method validation. Under the experimental conditions described, the Beer's law was obeyed over the concentration ranges of 2-20 and 5-25 µg/mL for BTB and SBT methods, respectively. The linear regression analysis using the method of least square was made to assess slope, intercept and regression coefficient (Table 1). High values of the regression coefficient and the small values of the intercepts of the regression equations proved the linearity of the calibration curves.

The sensitivity parameters such as molar absorptivity, Sandell's sensitivity, limit of detection and limit of quantification were calculated and are presented in Table 1. The high values of molar absorptivity and low values of Sandell's sensitivity, limit of detection, and limit of quantification reveal the high sensitivity of the proposed methods.

Table 1: Linearity and sensitivity data.

Parameters	BTB method	SBT method
Beer's Limit (µg/mL)	2-20	5-25
Regression equation (Y= mX + c)[$$]		
Slope (m)	0.743	0.702
Intercept (c)	0.006	0.006
Regression coefficient (r^2)	0.9990	0.9990
Molar Absorptivity (L mol-1cm-1)	1.349×10^5	1.299×10^5
Sandell's sensitivity (µg cm^{-2}/0.001 Absorbance unit)	0.01320	0.0137
LOD (µg/mL)	0.011	0.022
LOQ (µg/mL)	0.033	0.066

[$$]Y = mX + c, where Y is the absorbance, X is concentration in µg/mL, c is intercept and m is slope.

The accuracy and precision of the proposed methods (BTB and SBT methods) were assessed by determining the concentration of MTH at three different concentration levels (low, medium and high) within one day and on five consecutive days. The intra day and inter day assays were performed by performing six independent analyses at the 3, 12 and 18 µg/mL concentration levels using BTB method. The intra and inter day assays were also performed for SBT method at the concentration levels: 5, 15 and 25 µg/mL. The standard deviations, relative standard deviation, percentage of error and mean recoveries obtained by intraday and inter day assays for BTB and SBT methods were calculated and are summarized in Table 2. The results of the assays are acceptable and can be considered to be very reasonable.

Table 2: Precision and accuracy data.

Method	MTH taken (µg/mL)	MTH found (µg/mL) ± SD[$]	% RSD	% Recovery	% Error
BTB	**Intra day**				
	3	2.99±0.009	0.301	99.66	0.33
	12	11.96±0.006	0.050	99.66	0.33
	18	17.96±0.014	0.077	99.77	0.23
	Inter day				
	3	2.97±0.010	0.336	99.00	1.00
	12	11.95±0.090	0.753	99.58	0.42
	18	17.97±0.020	0.111	99.83	0.17
SBT	**Intra day**				
	5	5.01±0.006	0.119	100.20	0.20
	15	14.99±0.002	0.013	99.93	0.07
	25	25.03±0.012	0.047	100.12	0.12
	Inter day				
	5	4.98±0.02	0.401	99.60	0.40
	15	14.97±0.03	0.200	99.80	0.20
	25	24.96±0.04	0.160	99.84	0.16

[$]Average of five determinations

The accuracy and validity of the proposed methods were also checked by performing recovery experiments through standard addition technique. For this purpose, a known amount of MTH was added to pre-analyzed dosage forms at three different percentage levels (50%, 100% and 150% of that in tablet) and then determined by the proposed methods. The results (Table 3) showed that no interference from the common excipients was observed and establishes some degree of selectivity of the proposed methods.

Table 3: Results of recovery experiments.

Method	Labeled claim (mg)	Pure drug added (mg)	Found ± SD[$]	% RSD	% Recovery
BTB	5	2.5	7.48±0.021	0.280	99.73
	5	5	9.98±0.014	0.140	99.80
	5	7.5	11.97±0.043	0.359	95.76
SBT	5	2.5	7.49±0.017	0.226	99.86
	5	5	9.89±0.042	0.424	98.90
	5	7.5	12.64±0.064	0.506	101.12

[$]Average of five determinations

For the assessment of method robustness, some experimental parameters were interchanged; dye concentration (1±0.1 mL) and HCl concentration (1±0.1 mL). The analysis was performed at the deliberately varied experimental conditions by taking two different concentrations of MTH (BTB method - 3 and 18 µg/mL; SBT method - 6 and 24 µg/mL). The ability remained unaffected by small deliberate variations. The results, presented in Table 4, indicate acceptable robustness of the proposed methods.

Table 4: Robustness.

Method	Parameter	MTH taken (µg/mL)	MTH found (µg/mL) ± SD[$]	% RSD	% Recovery	% Error
BTB	BTB[a]	3	2.96±0.021	0.709	98.66	1.34
		18	17.95±0.064	0.356	99.72	0.28
	HCl[b]	3	2.98±0.037	1.254	99.33	0.67
		18	17.94±0.046	0.256	99.66	0.34
SBT	SBT[c]	6	5.96±0.048	0.805	99.33	0.77
		24	23.94±0.064	0.267	99.75	0.25
	HCl[d]	6	5.97±0.032	0.536	99.50	0.50
		24	24.13±0.044	0.176	100.54	0.54

[a] Volume of 0.2% BTB: 0.9, 1.0 and 1.0 mL
[b] Volume of 0.1 N HCl: 0.9, 1.0 and 1.0 mL
[c] Volume of 0.5 % SBT: 0.9, 1.0 and 1.0 mL
[$] Average of three determinations

3.3. Application of the proposed methods to tablet dosage forms

The proposed methods were applied to the quantification of MTH in tablet dosage forms purchased from a local pharmacy store. The results, shown in Table 5, suggest that the method is suitable for the determination of MTH with good accuracy and precision. The excipients in the dosage forms do not interfere in the assay procedure.

Table 5: Evaluation of memantine hydrochloride in tablet dosage forms.

Formulation	Method	Labeled Claim (mg)	Found ± SD[$$]	% RSD	% Recovery
Admenta[$]	BTB	5	5.02±0.024	0.478	100.40
		10	10.15±0.046	0.453	101.50
	SBT	5	4.98±0.021	0.421	99.60
		10	9.89±0.032	0.323	98.90

[$]Sun Pharmaceutical Industries Ltd. Mumbai, India
[$$]Average of five determinations

4. Conclusion

Two spectrophotometric methods were developed for the quantification of MTH hydrochloride using two anionic dyes, bromothymol blue and solochrome black T. The developed methods are validated as per ICH guidelines. It was observed that all validation parameters such as linearity, precision, accuracy, selectivity, and robustness convene the predetermined acceptance criteria. Thus, it has been concluded that the proposed methods are validated for the routine analysis of the drug in pure and tablet dosage form.

Competing Interests

None declared.

Authors' Contributions

APR and CBS designed the concept and experiments. The method development and validation was carried out by SB and CNL.

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