ASIJ Pharm

Research Article

A Safranin Dye-Based Luminescent 'Signal-On' Complex for Highly Sensitive Detection of Memantine Hydrochloride in Tablets and Human Plasma

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Citation: Abdel Hamid, M.A.; Mabrouk, M.M.; Ahmed, H.M.; Samy, B.; Batakoushy, H.A.
A Safranin Dye-Based Luminescent 'Signal-On' Complex for Highly Sensitive Detection of Memantine Hydrochloride in Tablets and Human Plasma.
AlSalam International Journal of Pharmacy, 2024

Received: [2025-02-05**]**
Accepted: [2025-03-01**]**

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Abstract

A novel, rapid, accurate, and sensitive spectrofluorimetric assay for detecting memantine hydrochloride (Mem-HCl) a none chromophoric drug utilized for treatment of Alzheimer's disease was developed. This method relies on the complexation of Mem-HCl with safranin, forming a highly fluorescent complex. The developed complex was extracted using chloroform under optimized conditions, and the fluorescence intensity was measured at 549 nm following excitation at 520 nm. The assay effectively measured memantine hydrochloride concentrations in the range of 3.00-50.00 µg/ml with good accuracy, with detection and quantification limits of 0.86 and 2.61 µg/ml, respectively. The method was validated according to International Council for Harmonization (ICH) guidelines and was successfully applied to analyze Mem-HCl in both pharmaceutical dosage forms and spiked human plasma samples, showcasing its potential for use in clinical and quality control laboratories. The proposed assay offers a high degree of sensitivity, making it an excellent alternative for the routine analysis of Mem-HCl in various applications, including therapeutic monitoring and quality assurance in pharmaceutical formulations. This technique is efficient, cost-effective, and well-suited for routine clinical and industrial use.

Keywords:

Memantine hydrochloride; safranin; complex; spectrofluorimetry; human plasma

1. Introduction

About 30% of the population suffers from depression symptoms and 40% of the community affected by Alzheimer's disease. [1]. According to FDA recommendations ,memantine was recently found to be effective in the treatment of Alzheimer's disease [2]. It is also effective on vascular dementia [3]. Mem-HCl is chemically antagonized N-methyl-D-aspartate receptors (Fig.S1). It is available in the market as Ebixa® tablets. Because the saturated tricyclic amine has no UV or fluorescence characteristics, it cannot be analyzed using these methods without a chemical change [4]. Despite the fact that memantine has been extensively investigated in the literature, there are surprisingly few publications on the drug's sensitive detection in biological matrices [5]. There are some analytical techniques were reported for assay of studied drug such as high performance liquid chromatography (HPLC) [6-12], gas chromatography-mass spectroscopy (GC-MS) [13], capillary zone electrophoresis [14] and potentiometry [15, 16].

However, these methods have disadvantages either expensive, have tedious steps or low sensitivity. Furthermore, utilizing derivatizing agent such as 9-fluorenyl methyl chloroformate [17], dansyl chloride [18], 7-nitro-4-fluoro-2,1,3-benzoxadiazole (NBD-F) [19], o-Phthalaldehyde (OPA) [19], 1, 2-Naphthoquinone-4-sulfonic NQS [20], eosin [21] and 2, 2-dihydroxyindane-1, 3-dione [22] which takes a long time and exhausted steps. Spectrofluorimetry is a well-known, easy to use, sensitive and cheap technique that is used for quantitative analysis [23, 24]. A new simple, sensitive, and cost-effective spectrofluorimetric approach for Mem-HCl measurement in the dosage form as well as human plasma was necessary. As a result, using safranin as a fluorophore is a good idea. The reaction of the basic component of safranin with the amine group on memantine to generate a highly fluorescent complex is the basis for this approach. The chloroform-extracted compound has a 520nm excitation wavelength and a 549nm emission wavelength. The goal of this study was to develop a reliable spectrofluorimetric method for measuring Mem-HCl in commercial (Ebixa®) tablets and biological samples.

2. Experiment

2.1. Instruments

A JASCO Spectro-fluorimeter, FP 6300 (Japan, Tokyo) installed with xenon lamp and equipped with Spectra Manager software (V1.53.01) was employed to conduct all fluorescence measurements. The speed of the scanning were1000 nm/min, the excitation and emission slit widths were both set to 10 nm. Adwa pH meter (model AD1030) and an analytical digital balance (Switzerland) have been used.

2.2. Materials and reagents

All utilized solvents and reagents were of analytical grade. Safranin (3.00 % w/v solution, Sigma-Aldrich, Germany) was diluted with distilled water to obtain a 0.004% w/v solution. Chloroform and anhydrous sodium sulfate were purchased from (Merck Company, Kenilworth, and NJ 07033 U.S.A). Mem-HCl (99.80 % purity) was obtained from National Organization for Drug Control and Research, (NODCAR), Giza, Egypt. Ebixa® tablets contain 10.00 mg of Mem-HCl per tablet. It was purchased from the local market.
The pH range of Teorell and Stenhagen buffer solution was (6-10) [25]. Teorell and Stenhagen buffer composition (phosphoric, citric acid, 1.00 M sodium hydroxide and reached to the desired pH by 0.10 M hydrochloric acid). Human plasma samples were kindly provided from the blood bank (Menoufia University Hospital, Menoufia, Egypt) and stored at -20ºC until analysis and thawed in a 38 ºC water bath with gentle shaking before use.

2.3. Standard solutions

A particular weight (10.00 mg) of Mem-HCl weighted accurately and dissolved in distilled water in 100.00ml volumetric. Further dilutions were done with distilled water to get a working solution covering the concentration range (3.00-50.00 µg/ml). Freshly prepared solutions are required.

2.4. General analytical procedures

1.00 mL Teorell and Stenhagen buffer solution at pH 8, an accurate volume of standard Mem-HCl within the concentration range, and 1.00 mL safranin solution (0.004 % w/v) were added to the separating funnel. The obtained mixture was extracted for 3 minutes with three separate portions of chloroform, each measuring 3.00 ml.
Anhydrous sodium sulphate was used to dry the chloroform extract. Then, filtered into a 10.00 mL volumetric flask and completed to the mark. The extract fluorescence intensity was measured at 549 nm, after excitation at 520 nm. The same procedures were used to conduct a blank experiment. The fluorescence intensity was plotted against drug concentrations to present the calibration curve.

2.5. Procedure for the commercial tablets

The content of ten Ebixa® tablets were weighed, finely powdered, and mixed thoroughly. an amount of the powder equivalent to 25.00 mg of Mem-HCl transferred to 25 ml volumetric flask, then 20.00 ml distilled water was added. Each extraction and sonication finished in 20 min. The final volume was completed to 25.00 ml with distilled water and filtered. Further dilutions were applied to get the concentration range with the same solvent. The general analytical procedures were followed. Percent recoveries were calculated from regression equation.

2.6. Spiked human plasma sample procedure

In 10.00 ml centrifuge tube an accurate volume of 100.00 µl Mem-HCl standard stock solution was quantitatively added to 1.00 ml of plasma free from drug and 3.00 ml acetonitrile. Then centrifugation occur at 5000 rpm for 20 min. Different volumes of the supernatant were withdrawn to obtain the working concentration range. Then the general analytical procedures were followed.

3. Results and discussion

The main objective of this study is to perform a sensitive and reliable spectrofluorimetric approach for analysis of Mem-HCl in its authentic, dosage form and biological sample. The suggested analytical method is based on the reaction of Mem-HCl with safranin to produce a highly fluorescent complex. The electron donor moiety (amine group) [26] of Mem-HCl having loan pair of electron interacts with acceptor moiety, quaternary ammonium on safranin (cationic ammonium group) to produce luminescent complex (Fig.1). The obtained complex was extracted easily with chloroform and assayed at fluorescence intensity 549 nm with excitation wavelength at 520 nm (Fig. 2).

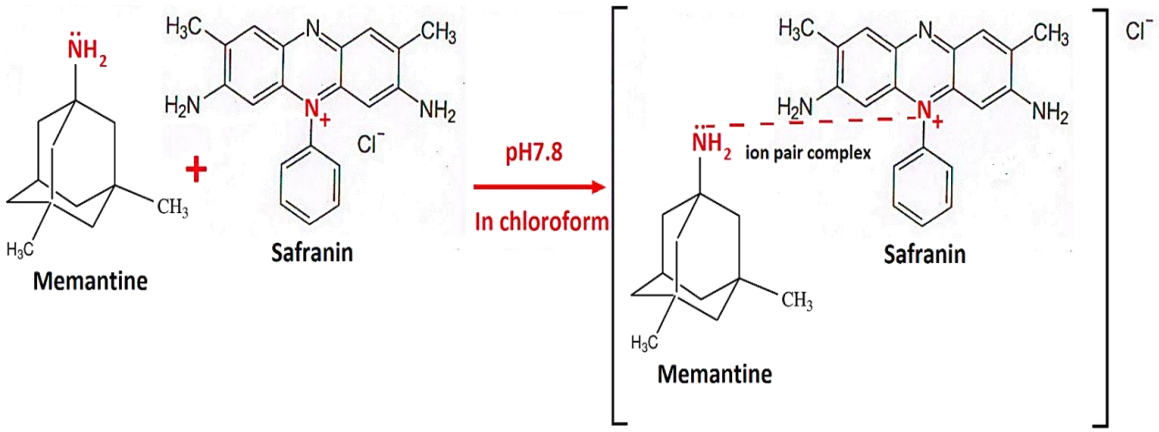

Fig.1: The proposed reaction mechanism between memantine and safranin

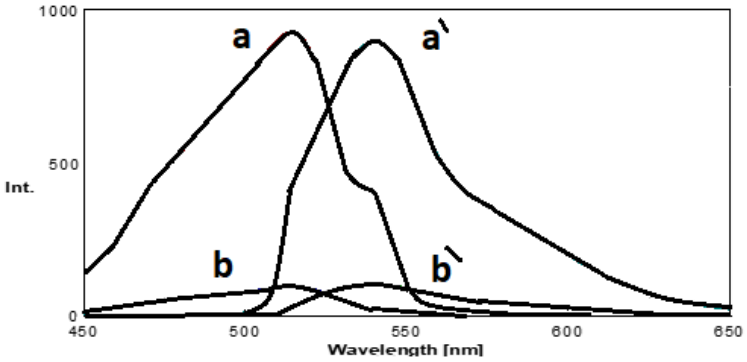

Fig. 2: Excitation, emission spectra (b, b$^-$) of the reagent blank and excitation, emission spectra of reaction product (a, a$^-$) between safranin (0.004%) and memantine (40.00 µg/ml).

3.1. Method Optimization

3.1.1. The pH Effect

The process was carried out at pH (6-10) to determine the best pH for complex formation. The relative fluorescence intensity reached the maximum value from pH (7-8.5) then decreased with further pH increasing. The optimum pH was found to be pH 8 ± 0.20 as shown in Fig.S2.

3.1.2. Effect of Volume of the Buffer:

To maintain pH of the reaction, Teorell and Stenhagen buffer solution were used. Different volumes were used (0-2.00 ml) at pH 8. The selected buffer solution (1.00 ± 0.20 ml) which indicated a good result as shown in **(Fig.S3)**. It was observed that each increase or decrease the volume will affect fluorescence intensity.

3.1.3. Effect of safranin volume

In this study, the safranin volume (0.004 % w/v) affects the relative fluorescence intensity (RFI). So, a different safranin volume was examined (0.25-2.00ml). Also, The best RFI at (1.00 ± 0.20 ml) of safranin solution **(Fig. S4)**.

3.1.4. Effect of reaction time

The reaction mixture was applied at different time intervals before extraction to investigate the influence of reaction time. The obtained fluorescent complex was measured directly and remained stable for 2-10 minutes. The resulting data was presented in (**Fig.S5).**

3.1.5. Effect of stability of complex

The stability of complex was examined by measuring RFI after extraction with chloroform at different time intervals. It showed that each complex and RFI remain stable and unaffected for at least 30 min then started to decrease **(Fig. S6).**

3.1.6. Effect of extraction solvent

To choose the suitable solvent for extraction different solvent have been used such as methanol, ethanol, methylene chloride and acetonitrile. It was found that the highest RFI observed with chloroform compared with other solvents.

3.2. The stoichiometry of the reaction

under the optimum condition, the stoichiometry of the reaction between Mem-HCl and safranin was determined by Job's method [27]. The obtained results showed that the molar ratio was 1:1 between them **(Fig. 3).**

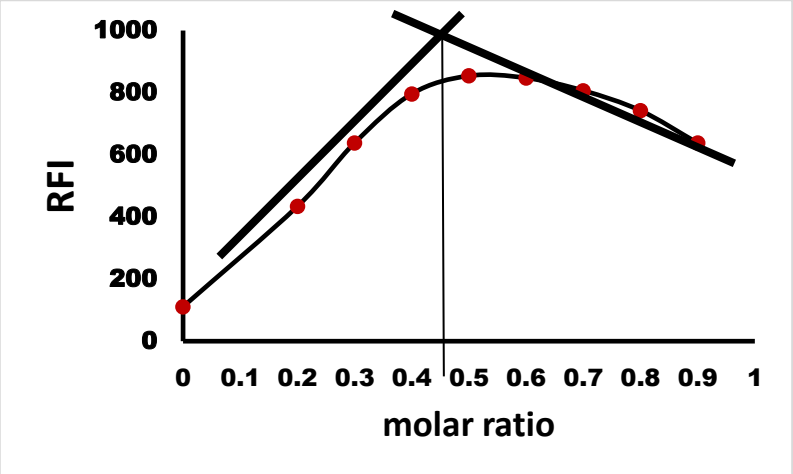

Fig. 3: Job's plot for molar ratio determination between safranin and memantine.

3.3. Method Validation

The validation process for the suggested method were examined as ICH guidelines. [28]. The validation parameters were linearity range, accuracy, precision, limits of detection and quantitation and robustness.

3.3.1. Linearity range

As a general analytical procedure, the fluorescence intensity was estimated using a series of Mem-HCl solutions. The Mem-HCl concentration range was (3.00-50.00µg/ml) and correlation coefficient was 0.9997. That indicated good results as showed in **Table 1**.

Table 1: Statistical data and quantitative parameters for Mem-HCl determination by spectrofluorimetry for the proposed method.

Parameter	Value
λex (nm)	520
λem (nm)	549
Linearity Range (µg/ mL)	3.00-50.00
Intercept (a)	394.86
SD of Intercept (Sa)	2.60
Slope (b)	9.980
SD of Slope (Sb)	0.092
Correlation coefficient (r)	0.9997
Coefficient of determination (r2)	0.9996
SD of residual (Sy/x)	4.3237
Limit of detection (µg /mL)	0.03
Limit of quantitation (µg /mL)	2.61

[a] standard deviation.

3.3.2. Limits of detection (LOD) and quantitation (LOQ)

The detection and quantification limits (LOD, LOQ) were calculated by this formula: (LOD =3.3 σ / S), (LOQ =10 σ / S), Where S is the slope of calibration curve and σ is standard deviation of intercept. The resulting data were shown in Table 1. LOD and LOQ were of 0.86 and 2.61 µg/ml, respectively, which showed an excellent method sensitivity.

3.3.3. Precision and accuracy

Five concentrations of Mem-HCl were used to test the accuracy of the proposed approach within the concentration range (3.00-50.00 µg/ml). Also, each concentration was tested three times, as indicated in **Table S1**. It was observed that a close agreement of data with true value which showed good accuracy of the proposed analytical method. Three different concentrations were triplicated to determine reproducibility and repeatability (**Table S2**). The resulting data demonstrated excellent precision.

3.3.4. Robustness

The robustness was estimated by checking the minor variations in the experimental condition and observing the effect of fluorescence intensity. These changes; pH, safranin volume and the volume of buffer solution. As indicated in **Table S3,** there's no significant differences for the small changes in the suggested method condition.

3.3.5. Selectivity

The proposed method showed good selectivity for Mem-HCl determination. To ensure that the percent recovery was calculated on the pharmaceutical dosage form. No interference detected from the tablet excipients such as microcrystalline cellulose, magnesium stearate, colloidal anhydrous silica, and titanium dioxide, so the proposed analytical method is practically valuable for Mem-HCl analysis.

4. Applications

4.1. Application to Mem-HCl tablets

The proposed method was applied to commercial dosage form. The results were compared statistically with reported method [29]. According to calculated t-tests and F-tests, no significance difference between the proposed and reference was showed with confidence level of 95% in respect of accuracy and precision. **(Table 2)**.

4.3. Mem-HCl assay in biological fluid

A drug free human plasma was obtained from Menoufia University Clinics which stored at -18ºC. Then thawed immediately before use. One milliliter of human plasma was transferred into a centrifuge tubes and spiked with concentration of Mem-HCl within the range (3.00-50.00 µg/ml). Then addition of 1.00 mL acetonitrile to precipitate plasma proteins, followed by centrifugation at 5000 rpm for 20 min. The results obtained were accurate, precise and the percent recovery was 99.6% as shown in **Table 3**.

Table 2: Application of the proposed spectrofluorimetric method for assay of Mem-HCl in tablet dosage form.

Parameter	Ebixa® 10mg tablets	
	Proposed method	Reported method
% Recovery	99.74	98.43
Standard deviation (SD)	0.50	0.30
Number of determinations	5.00	3.00
t-Value[a]	0.116	
f-Value[a]	2.985	

[a] tabulated value at 95% confidence limit; t= 2.306 and F= 6.338.

Table 3: Application of the proposed spectrofluorimetric method for assay of Mem-HCl in spiked human plasma.

Parameter	Added Conc. (µg /mL)	Found[a] (µg /mL)	% Recovery ± SD
Spiked human plasma	10.00	10.10	101 ± 0.25
	20.00	19.60	98 ± 0.94
	30.00	29.51	98.4 ± 0.59
	40.00	40.50	101.2 ± 0.10

[a] Mean of five determinations, SD: standard deviation

5. Conclusion

The suggested spectrofluorimetric assay has a great value as designed a validated method that fast, accurate, and economic approach. This method has no need for tedious pretreatment or equipment. The present study was successively performed for analysis of Mem-HCl in both dosage form and spiked human plasma with good recovery and precision. The proposed method was characterized by high sensitivity and selectivity. For those reasons, the developed method can be applied for routine analysis and in quality control laboratories as well as clinical laboratories.

Conflict of interest

The authors declare that they have no conflict of interest.

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