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Validated Spectrofluorimetric and HPLC-UV Method for Determination of Memantine in Pharmaceutical Dosage Forms.

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ABSTRACT

Two simple and accurate methods were developed for the determination of memantine in bulk drug and pharmaceutical dosage forms. Memantine was derivatized using fluorescamine in borate buffer at pH 9.5. The derivatization reaction was optimized and the reaction product was determined by HPLC-UV method at 306 nm or spectrofluorimetric method at 475 nm (excitation at 395 nm). The chromatographic separation was achieved on a Nova-Pak CN column using a mixture of acetonitrile and water containing 0.07% TEA and 0.1% H₃PO₄ (40:60) as mobile phase. The calibration curves were linear over the concentration range of 1-80 µg/mL and 4-100 µg/mL for HPLC-UV and spectrofluorimetric method, respectively. The within-day and between-day coefficient of variations were less than 2 and 3% for HPLC-UV and spectrofluorimetric method, respectively. The proposed methods were successfully used for the determination of memantine in pharmaceutical dosage forms which showed acceptable results without any interference from the excipients.

Keywords: Memantine, Fluorescamine, Derivatization, HPLC-UV, Spectrofluorimetry

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INTRODUCTION

Memantine, 3, 5-dimethyladamantan-1-amine, is a tricyclic amine which is structurally related to amantadine. Memantine is a noncompetitive antagonist of N-methyl-D-aspartate (NMDA) receptor complex which is orally administered for the treatment of neurodegenerative diseases such as Alzheimer's disease, vascular dementia and parkinson's disease [1-3]. The tricyclic structure of memantine possesses no significant UV absorption or fluorescence properties which is necessary for suitable detection. Therefore it could not readily be determined by HPLC equipped with UV or fluorescence detector without derivatization. The primary amine of the memantine could bind to various derivatizing reagents and the reaction product could be determined by UV or fluorescence detection.

For determination of memantine in biological fluids, few HPLC methods using different derivatizing reagents such as anthraquinone-2-sulfonyl chloride, dansyl chloride, o-phthalaldehyde, 4-fluorenylmethyl chloroformate and 4-(4, 5-diphenyl-1H-imidazol-2-yl) benzoyl chloride [4-9] have been reported before. The derivatization time and chromatographic retention time for these methods were in the range of 1-45 min and 10-23 min, respectively.

Simultaneous determination of memantine and four other related compounds in bulk drug was also performed using 4-fluoro-7-nitro-2, 1, 3-benzoxadiazole as fluorescence reagent [10]. Derivatization time was short (5 min), but the retention time of memantine derivative is higher than 30 min. There is another report for determination of memantine in drug substance using 9-fluorenylmethyl chloroformate and UV detection [11]. The reaction time is 20 min and the retention time is about 8 min. LC/MS and LC-MS/MS methods were also utilized for determination of memantine [12-14].

Fluorescamine is a fluorescent reagent which reacts readily with most primary and secondary amino groups and has been used for determination of some drugs using HPLC [15-20] or spectrofluorimetry [21-24], before. In a recent study HPLC with fluorescence detection after derivatization with fluorescamine has been used for determination of memantine in human plasma [25]. The derivatization optimization was not discussed in detail and the chromatographic retention time was about 15 min.

In this study fluorescamine was used as derivatizing reagent for determination of memantine by HPLC-UV and also spectrofluorimetric method. The reaction conditions were optimized and the analytical methods were validated according to the International Conference on Harmonization (ICH) guidelines to be suitable for determination of memantine in quality control laboratories.

MATERIALS AND METHODS

Chemicals

Memantine hydrochloride (Batch 0740378) was from Fidia Farmaceutici S.P.A. (Milano, Italy). Fluorescamine was from Acros Organics (Belgium). All other analytical grade chemicals and HPLC grade solvents were purchased from Merck (Darmstadt, Germany). Ebixa[®] 10 mg tablets (Lundbeck, Denmark) were purchased from a local pharmacy. HPLC grade water was prepared using a Milli-Q purification system (Millipore, Milford, MA, USA).

Instrumentation

A Waters HPLC system consisted of a 515 isocratic pump, 710 plus autosampler, a 486 UV detector and a multi-channel Chrom&Spec software (version 1.5x) was used for separation and data processing. A RF-5000 spectrofluorimeter (Shimadzu, Japan) with 1cm quartz cells was used for fluorimetric determinations. The fluorescence intensity was measured at excitation wavelength of 395 nm and emission wavelength of 475 nm. The slit widths of both monochromators were set at 10.0 nm.

Standard solutions

Memantine hydrochloride stock solution of 200 µg/mL was prepared by dissolving appropriate accurately weighed of drug substance in distilled water. Standard calibration solutions (1, 2, 4, 10, 20, 40, 60,

80, and 100 µg/mL) were prepared using the stock solution by subsequent dilution of stock solution with distilled water. Fluorescamine solution (0.4 mg/mL) was freshly prepared in acetone. Borate buffer (0.25 M, pH 9.5) was prepared by dissolving appropriate amounts of H₃BO₃ and KCl in distilled water and adjusting the pH value.

Chromatographic condition

A Nova-Pak CN HP column, 4 µm (3.9 mm×150 mm, Waters, Milford, USA) and an isocratic mobile phase consisted of acetonitrile and water containing 0.07% TEA and 0.1% H₃PO₄ (40: 60, v/v) at a flow rate of 1 mL/min was used for chromatographic separation. The mobile phase degassed by filtration through a 0.22 µm Teflon membrane filter (Millipore, Milford, MA, USA) and also sonication. The detection was performed at 306 nm and ambient temperature.

General sample preparation

For the spectrofluorimetric method. Standard solutions of memantine hydrochloride (0.5 mL) were added to 10 mL volumetric flasks. Then 0.5 mL of 0.25 M borate buffer and 0.5 mL of fluorescamine solution (0.4 mg/mL) were added respectively. After mixing, the solutions were kept at room temperature for 5 min and completed to volume by water. The fluorescence intensity of the resulting solution was measured at excitation wavelength of 395 nm and emission wavelength of 475 nm.

For the HPLC method. Standard solutions of memantine hydrochloride (0.5 mL) were transferred to a test tube. Then 0.5 mL of 0.25 M borate buffer and 0.5 mL of fluorescamine solution (0.4 mg/mL) were added and the mixture vortexed for 10 seconds. After 5 min, 20 µL of the resulting solution was injected into the HPLC system.

Optimization of the reaction conditions

The effect of experimental parameters such as borate buffer pH, reagent volume and reaction time were studied to find the optimum reaction conditions. The optimum derivatization conditions were studied by varying one parameter at a time while the other parameters kept constant.

To find out the stability of the reaction product, the reaction product was left at room temperature for 8 h and different samples were determined at various time intervals.

Method validation

The linearity of the developed method was investigated by the analysis of six series of calibration standard solutions in the range of 1-80 µg/mL and 4-100 µg/mL for HPLC-UV and spectrofluorimetric method, respectively. The calibration curves were constructed and the statistical data were calculated.

The within-day and between-day accuracy and precision of the method were also evaluated by preparation and determination of three memantine hydrochloride solutions at three different concentration levels in one day and three separate days. Different chromatographic conditions with small changes in mobile phase composition were used to find out the robustness of the proposed method.

Application of the method for the analysis of dosage forms

Accurately weighed amount of fine powder of grinded 20 tablets equivalent to one tablet was transferred to a 50 mL volumetric flask. After addition of 40 mL of distilled water, the mixture was sonicated for 20 min. The resulting solution was cooled to room temperature and diluted to volume with water. After filtration through a 0.45 µm Millipore filter and five times dilution, the solution was subjected to the general sample preparation and determined by HPLC-UV or spectrofluorimetric method. The results compared with a standard solution of memantine hydrochloride at the same concentration value.

Also, the dissolution of memantine tablets was determined using an Erweka dissolution apparatus (Heusenstomm, Germany). The dissolution medium was 900 mL of 0.1 M HCl (pH 1.2) (containing 2 g NaCl in

one liter) and paddle apparatus at 50 rpm was used at 37 ± 1 °C. The samples were drawn at 10, 20, 30 and 45 min and analyzed according to the general procedure after neutralization with appropriate amount of sodium hydroxide. The resulted peak area or fluorescence intensity was compared with a standard solution to calculate the percent drug released.

Relative recovery

The recovery of memantine hydrochloride from tablet dosage forms was evaluated using standard addition method. Standard solution of memantine hydrochloride was added to a weighed amount of powdered tablet equivalent to 10 mg of memantine hydrochloride in a 50 ml volumetric flask. The assay method was performed and memantine hydrochloride concentration was determined. The relative recovery was determined by comparing the peak area or fluorescence intensity of this solution with a standard solution at the same concentration level.

Specificity

According to the results of Narola et al. [11] degradation of memantine was observed under 5 M HCl, 1 M NaOH and 30% H₂O₂. To find out the specificity of the proposed method, a standard solution of memantine (1 mg/mL) was exposed to the above mentioned conditions in a 10 mL volumetric flask. Then, 500 µL of the resulting solution transferred to a 10 mL volumetric flask and adjusted to the volume by distilled water after appropriate neutralization. The solutions were derivatized analyzed by HPLC system.

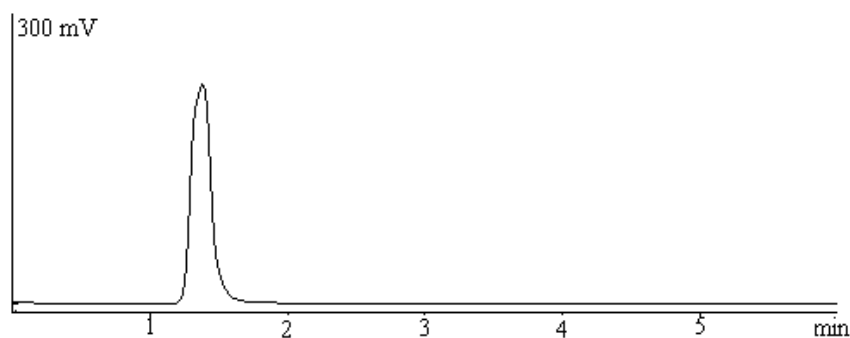
Stoichiometry of the reaction

To evaluate the stoichiometry of the derivatization reaction, two sets of reaction solutions were used. The first set was carried out using increasing concentration of memantine hydrochloride (8×10^{-5} - 2×10^{-4} M) at fixed concentration of fluorecamine (2×10^{-4} M). The second set carried out using increasing concentrations of fluorecamine (4×10^{-3} - 1×10^{-2} M) at fixed concentration of memantine hydrochloride (2×10^{-3} M). The derivatization reaction was performed according to the general procedure and the fluorescence intensity of the product was measured after appropriate dilution. The logarithms of the fluorescence values were plotted against the concentration of memantine hydrochloride.

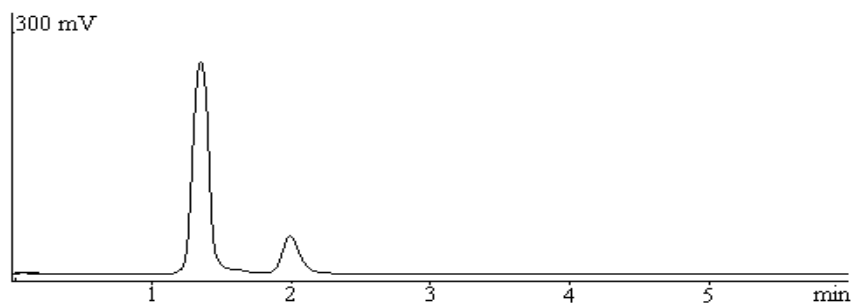
RESULTS AND DISCUSSION

Chromatographic conditions

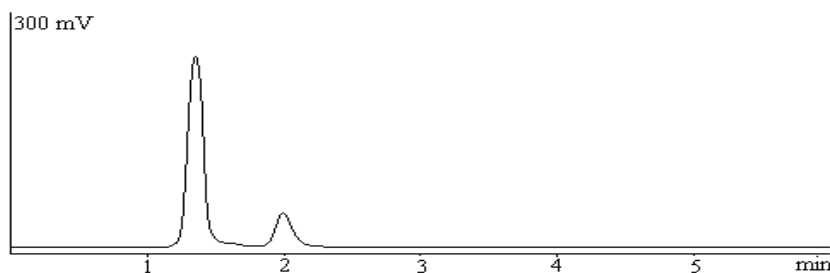
A sharp and symmetrical peak was obtained for the reaction product of memantine and fluorecamine using a Nova-Pak CN column and a mixture of acetonitrile and water containing 0.07% TEA and 0.1% H₃PO₄ (40: 60, v/v) as mobile phase. The chromatogram was clear and without any interference from the excess reagent or pharmaceutical dosage from excipients. Under the optimized conditions, the retention time of the derivatized memantine was about 2 min. Typical chromatograms are shown in figure no. 1.



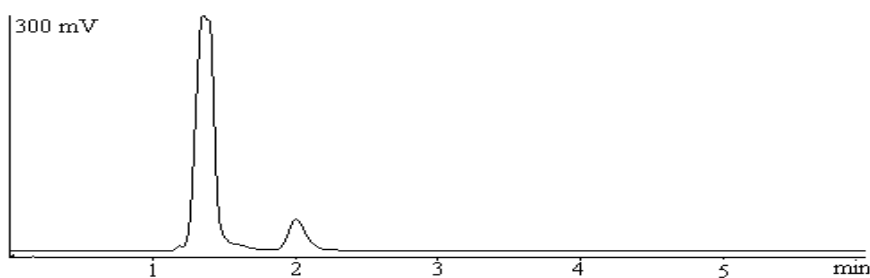
(A)



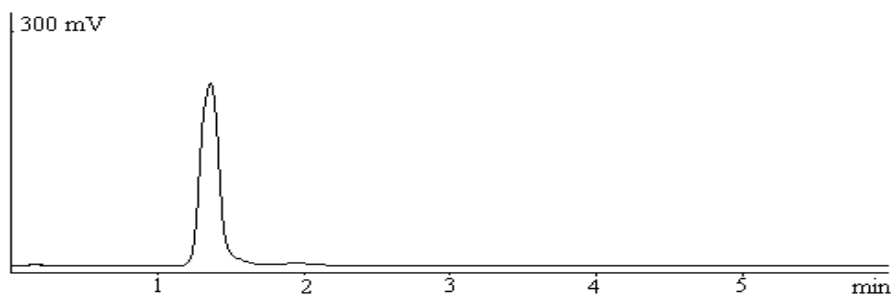
(B)



(C)



(D)



(E)

Figure 1: Typical HPLC chromatograms of memantine-fluorescamine product. (A) Fluorescamine reagent (B) Memantine standard solution (50 µg/mL); (C) Memantine in 5 M HCl Solution after 5 min in room temperature; (D) Memantine in 1 M NaOH solution after 5 min in room temperature; (E) Memantine in 15% H₂O₂ solution after 5 h at 80°C.

The system suitability of the chromatographic method was checked by injecting six replicates of a reaction product from a standard solution of memantine hydrochloride (50 µg/mL). The number of USP theoretical plates ($N > 2900$) and USP tailing factor ($T < 1.2$) were within the acceptable criteria. Also low variability in the peak area ($RSD = 0.43\%$) and retention time ($RSD = 0.30\%$) were observed after replicate injections.

Excitation and emission spectra

The derivatization product exhibited maximum fluorescence intensity at 475 nm (λ_{em}) after excitation at 395 nm (λ_{ex}).

Optimization of the derivatization conditions

Fluorescamine is a nonfluorescent derivatizing reagent which reacts with primary amines in alkaline medium rapidly and yields a fluorescent derivative. Due to the absence of a fluorogenic structure of memantine, derivatization with fluorescamine was performed. The primary amino group of memantine readily reacts with fluorescamine in borate buffer solution at ambient temperature. The reaction is rapid and the product is both chromaphoric and also fluorogenic which could be determined by HPLC-UV or spectrofluorimetric method. The derivatization conditions were optimized by HPLC-UV method to reach a higher sensitivity.

The effect of pH on the reaction yield was studied by using borate buffer at different pH values. It was shown that the reaction between memantine and fluorescamine proceed at room temperature with best results at the pH value of 9.5 (figure no. 2). Higher pH values did not increase the reaction yield.

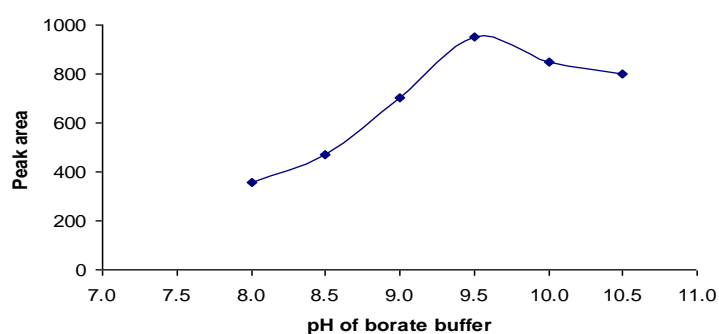


Figure 2: The effect of borate buffer pH on the derivatization reaction of fluorescamine and memantine (40 μ g/mL) (n=3).

To find out the effect of fluorescamine amount on derivatization, the reaction was proceeded using varying volumes of the reagent (100-800 μ L) in the presence of borate buffer (pH 9.5). It was demonstrated that the peak area of the reaction product increased steadily as the fluorescamine amount increased up to the volume of 500 μ L (figure no. 3). Higher amounts of the reagent had no effect on the peak area of the reaction product.

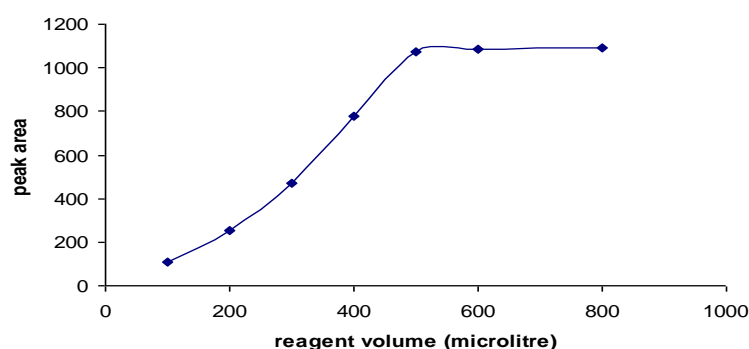


Figure 3: The effect of reagent amount on the derivatization reaction of fluorescamine and memantine (40 μ g/mL) (n=3).

It was also demonstrated that the derivative formed immediately and the peak area reached to maximum value after 5 min.

Stability

Stock standard solutions of memantine were relatively stable for at least 7 days at 4°C. The reaction product of memantine and fluorecamine was also stable at least for 5 h (recovery>98%).

Linearity

Calibration curves were constructed for both HPLC-UV and spectrofluorimetric method under specified optimum reaction conditions in six series. Linear relationship of concentration versus peak area or fluorescence intensity was observed with an acceptable correlation coefficient. The statistical data are summarized in Table 1.

The LOQ and LOD values were determined according to the following equations:

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

Table 1: Statistical data of calibration curves of memantine in standard solutions (n = 6)

Parameters	HPLC-UV	Spectrofluorimetry
Linearity	1.00-80.00/ $\mu\text{g mL}$	4.00-100.00/ $\mu\text{g mL}$
Regression equation	$y = 20.62x + 5.04$	$y = 6.41x + 13.81$
SD of slope	0.21	0.11
RSD of slope (%)	1.02	1.72
SD of intercept	2.22	2.57
Correlation coefficient	0.9996	0.9989

Where σ is the standard deviation of the intercept and s is the slope of the regression line. The LOQ and LOD values were 1.08 and 0.32 $\mu\text{g/mL}$ for HPLC-UV method or 4.01 and 1.20 $\mu\text{g/mL}$ for spectrofluorimetric method, respectively.

Precision and accuracy

The within-day and between-day precision and accuracy results for both methods are illustrated in Table 2. The low coefficient of variations and error values revealed the repeatability of the HPLC-UV and spectrofluorimetric method.

Table 2: Precision and accuracy of method for determination of memantine in standard solutions (n=9; 3 sets for 3 days)

Concentration added ($\mu\text{g/mL}$)	Within-day (n = 3)			Between-day (n = 9)		
	Found ($\mu\text{g/mL}$)	CV (%)	Error (%)	Found ($\mu\text{g/mL}$)	CV (%)	Error (%)
HPLC-UV						
2.00	1.97 ± 0.02	1.02	-1.50	1.98 ± 0.03	1.51	-1.00
20.00	20.10 ± 0.07	0.35	0.05	20.38 ± 0.22	1.08	1.90
80.00	81.46 ± 0.89	1.09	1.83	80.80 ± 0.14	0.17	1.00
Spectrofluorimetry						
4.00	3.98 ± 0.10	2.51	-0.50	4.03 ± 0.12	2.97	0.75
40.00	40.46 ± 0.51	1.26	1.15	40.52 ± 0.75	1.85	1.30
100.00	101.05 ± 1.87	1.85	1.05	100.53 ± 1.77	1.76	0.53

The proposed methods were applied for determination of a standard solution of memantine hydrochloride by two analysts in different days to find out the ruggedness of the procedure. The RSD values did not exceed 2%.

Robustness

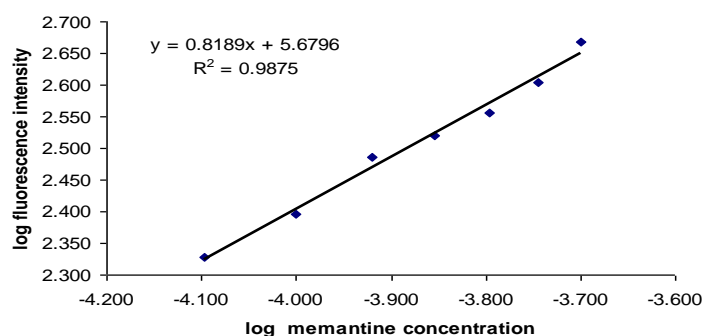
Robustness of the derivatization procedure was quantified by evaluating the influence of the small variations in derivatization conditions such as fluorescamine amount ($\pm 10\%$), reaction time (5 ± 1 min) and the pH value of the reaction medium (9.5 ± 0.1). These minor changes did not show any significant effect on derivatization yield which shows the reliability of the proposed derivatization method for routine analysis. The robustness of the chromatographic condition was also checked by 10% variation in the TEA and H_3PO_4 composition in the mobile phase. No significant influence on the peak area values ($CV < 1.2\%$) and selectivity was observed which illustrated the robustness of the method.

Specificity

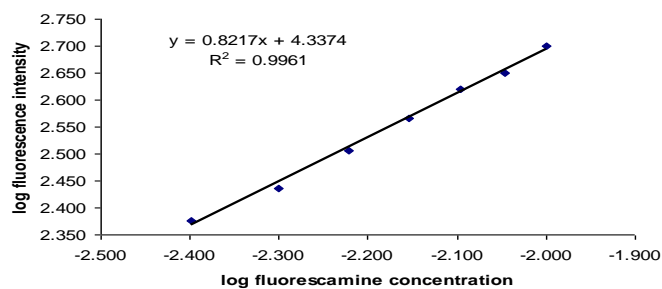
To find out the specificity of the proposed HPLC-UV method, the peak area of a standard solution of memantine was measured in the presence of tablet excipients using standard addition method. Acceptable relative recovery ($99.0 \pm 0.5\%$) and no interfering peak from excipients was indicated the specificity of the method. Using the same procedure, relative recovery of $100.6 \pm 1.0\%$ was also observed for the spectrofluorimetric method. Since the results of Narola et al. [11] indicated that memantine is degraded under acidic, alkaline and oxidative conditions, forced degradation studies were also performed in the same conditions. Significant degradation was observed under 5M HCl at room temperature (23% after 5 min), 1 M NaOH at room temperature (30% after 5 min) and 15% H_2O_2 at $80^\circ C$ (90% after 5 min). No interfering peaks were observed which showed the specificity of the HPLV-UV method in the presence of degradation products (figure no. 1).

Stoichiometry of the reaction

Using the limiting logarithmic method, the plots of log fluorescence over log fluorescamine concentration and log memantine concentration were constructed (figure no. 4). The slope values were 0.822 and 0.819, respectively which confirms the 1:1 (0.822/0.819) ratio for the derivatization reaction. Based on the molar reactivity of the derivatization reaction, the schematic reaction pathway presented in figure no. 5 could be proposed.



(A)



(B)

Figure 4: Limiting logarithmic plots for the molar reactivity of memantine with fluorescamine: (A) fluorescence versus log [memantine] with [fluorescamine] kept at 2×10^{-4} M; (B) log fluorescence versus log [fluorescamine] with [memantine] kept at 2×10^{-3} M.

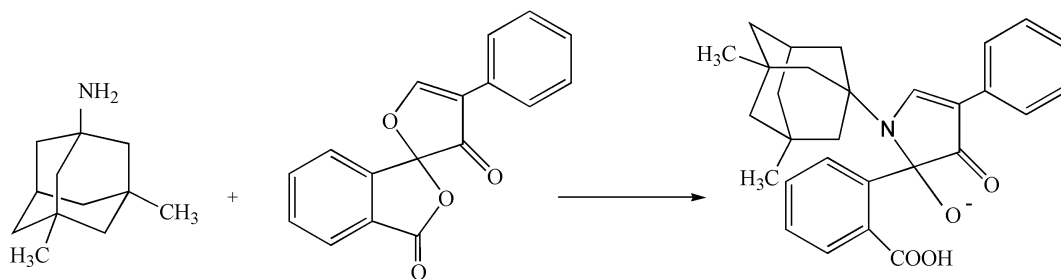


Figure 5: The proposed reaction pathway of memantine and fluorescamine.

Application

Both HPLC-UV and spectrofluorimetric methods were used for determination of memantine in tablet dosage forms. Good agreement was observed (9.92 ± 0.05 mg and 9.99 ± 0.24 mg for HPLC-UV and spectrofluorimetric method, respectively) with the label claimed amount (10.00 mg) of memantine using both methods. Also the proposed methods were used for determination of memantine in dissolution medium. The dissolution profile of the memantine showed 80% release within the first 10 min. No interferences from excipients were observed in both methods.

CONCLUSION

Memantine is not an official drug of USP or BP, and development of simple and reliable methods are essential for its determination in raw material and pharmaceutical dosage forms. Fluorescamine was used for derivatization of memantine to produce a chromophoric and also fluorogenic product. The derivatization reaction of memantine and fluorescamine is performed at room temperature in about 5 min and the sample preparation is relatively simple and fast. The derivatization product is relatively stable and the chromatographic retention time is about 2 min. The spectrofluorimetric method is also relatively simple and highly reliable and practical for determination of memantine in pharmaceutical dosage forms.

The results showed applicability of the methods for determination of memantine in pharmaceutical dosage forms. The proposed methods could be used for in vitro quality control methods (assay, dissolution) with a good precision.

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