ORIGINAL RESEARCH ARTICLES

BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF MEMANTINE HYDROCHLORIDE BY SPECTROFLUORIMETRIC METHOD USING OPA β-MERCAPTOETHANOL AS DERIVATIZING AGENT

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(Received 22 May 2014) (Accepted 27 July 2014)

ABSTRACT

A new, simple, accurate, sensitive and specific bioanalytical spectrofluorimetric method was developed and validated for estimation of memantine hydrochloride (MEM) in human plasma. Estimation of MEM in human plasma was done by spiked human plasma studies. Extraction of MEM from human plasma was carried out using 5% trichloroacetic acid for protein precipitation followed by liquid-liquid extraction with 5% IPA in n-hexane. For spectrofluorimetric estimation delta value 65 applied in synchronous mode (medium sensitivity mode) and fluorescence intensity was measured at 420 nm. Developed method was found Linear to be in the concentration range of 50-300 ng/mL with correlation coefficient (R^2) 0.9951. Percentage recovery was found to be 77.85-83.68% for MEM. High recovery shows that the method is free from the interference from plasma constituents. Hence, proposed method can be used for estimation of MEM in routine quality control laboratories for quantitative determination of MEM in bulk as well as in human plasma. Further it can also be used to determine plasma MEM concentration in drug monitoring or in pharmacokinetic investigations.

Keywords: Memantine hydrochloride, Synchronous spectrofluorimetry, OPA β-Mercaptoethanol, Bioanalytical method.

INTRODUCTION

Memantine (MEM) is 1-amino-3,5-dimethyladamantan (Fig. 1) derivative used for treatment of dementia[12]. Recently MEM is approved for the treatment of various diseases like Alzheimer's disease, Parkinson's disease, spasticity, pervasive developmental disorders, schizophrenia, alcohol abuse and withdrawal[2,3,4]. Literature survey for MEM reveals that it lacks chromophoric group, hence primary amino group of MEM has been derivatized using reagents such as; o-phthalaldehyde (OPA)[5], 2-naphtoxyacetyl chloride[6,7], anthraquinone-2-sulfonyl chloride[8], 9-fluorenylmethyl chloroformate[9] and dansyl chloride[10,11] followed by quantitative estimation of MEM in various matrices. Other analytical methods like HPLC-MS, HPLC-MS-MS[12,13,14,15], GC-MS [16,17] capillary zone electrophoresis[18], micellar electrokinetic chromatography with laser induced fluorescence (MEKC-LIF)[19] and HPLC-MS[20] were also reported. OPA reacts with primary amines in presence of any thiol-containing compound like β-mercaptoethanol and gives alkylthiol-isoindole derivative under basic pH condition (above 9.0), which has fluorescent property showing excitation wavelength near 340 nm and emission wavelength near 455 nm (Fig. 2.) However, there is no method reported till now for the estimation of MEM by derivatizing with OPA-β-mercaptoethanol and quantification by spectrofluorimetric method in human plasma. The aim of this work was to develop a validated, sensitive, specific and simple and time saving spectrofluorimetric method for the determination of MEM in human plasma.

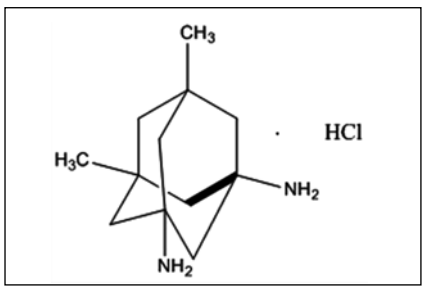

Figure 1: Structure of MEM

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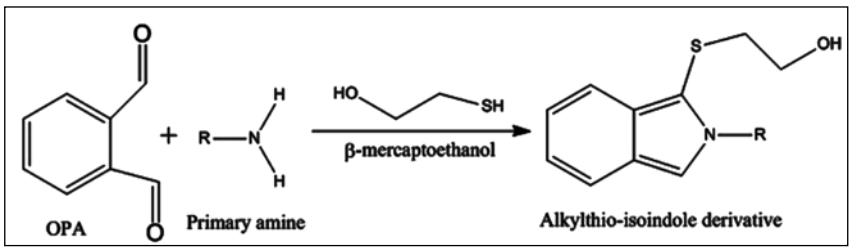

Fig. 2: Reaction Pathway of Derivatization of Primary Amine with OPA

MATERIALS AND METHODS

Instrument

Spectrofluorimeter Model: FP- 6500 PC series, Matched quartz cell (1cm), Wavelength range: 220.00 to 750.00 nm, JASCO Japan,

Chemicals and Reagents

MEM was kindly gifted by INTAS Pharmaceuticals, Ahmedabad, Gujarat. OPA, β – mercaptoethanol (AR Grade, Sisco Research Laboratories Pvt Ltd.), boric acid, potassium chloride, sodium hydroxide, trichloroacetic acid, perchloric acid, n-hexane, iso-propyl alcohol, (AR Grade, S.D.Fine Chemicals Ltd., Bombay, India) were used. Blank human plasma was kindly gifted by Prathma Blood Bank, Ahmedabad, Gujarat.

Glassware used in each procedure was soaked overnight in a chromic mixture ($K_2Cr_2O_7$+concentrated H_2SO_4), rinsed thoroughly with double distilled water and dried in dust-free air. Whatman filter paper No. 42 was used to filter the MEM solution to separate it from the precipitated proteins and debris.

EXPERIMENTAL

Preparation of Standard Stock Solution of MEM

MEM (25 mg) was accurately weighed and transferred to a 25 mL volumetric flasks, dissolved in 10 mL double-distilled water, sonicated for 10 min and volume made up to mark with double-distilled water to obtain standard stock solution having concentration 1000 µg/mL. From the stock solution, 5 mL aliquot was transferred to the 50 mL volumetric flask and volume made up with double distilled water to obtain working standard stock solution of 100 µg/mL.

Preparation of Borate Buffer pH (9.6)

Boric acid (3.09 g) and potassium chloride (3.73 g) were accurately weighed and transferred to 250 mL volumetric flask. Volume was made up with distilled water. Then, solution was transferred to 1000 mL beaker and pH of solution was adjusted to 9.6 by addition of 0.2 M of sodium hydroxide solution and volume was made up to 1000mL with distilled water.

Preparation Of Derivatization Reagent

OPA (50 mg) was transferred into a 25 mL amber coloured volumetric flask, 5 mL methanol was added and sonicated for 5 minutes. β-Mercaptoethanol (20 µL) was added to it and finally volume was made up to mark with borate buffer pH 9.6 and stored at a temperature below 10^0C.

Extraction Procedure

500µL of plasma sample was taken in 2mL microcentrifuge tube, then 50 µL of drug solution was spiked and 500 µL of 5% trichloroacetic acid (TCA) solution was added. It was centrifuged for 3 min at 5000 rpm, supernatant was transferred into another 2 mL microcentrifuge tube and 100 µL of 0.1 N NaOH solution was added to make the supernatant alkaline. To this microcentrifuge tube, 1 mL of 5% IPA in n-hexane solution was added and centrifuged for 3 min at 8000 rpm and upper organic phase was separated and remaining aqueous phase was again centrifuged with 1 mL 5% IPA in n- hexane solution and upper organic phase was separated and both organic phase were taken in 10 mL volumetric flask and evaporated to dryness. Then it was reconstituted with borate buffer to made up volume 10 mL and analysed with the help of spectrofluorimeter.

Derivatization Method of MEM.

After extraction of MEM from plasma as discussed above, in 10 mL dried volumetric flask containing MEM was added 1 mL borate buffer and 0.1 mL of derivatization solution and kept in water bath for 10 min at 70^0C. After 10 minutes, volume was made up with borate buffer and solution were kept at room temp. After that, solutions were scanned within 10 minutes.

Optimization of Experimental Conditions Selection of Analytical Wavelength

From the working standard solution, 1 µg/mL solutions were prepared by appropriate dilution using diluent and scanned in the synchronous mode with delta value 65 (medium sensitivity mode) from 220 to 680 nm. From the overlain synchronous spectra of MEM, 420 nm wavelength was selected for measurement of fluorescence intensity of MEM.

METHOD VALIDATION[21, 22]

Linearity

For generation of calibration curve of MEM, 500 µL of blank plasma was spiked with 50 µL of drug solution of concentration of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL and 60 µg/mL, to get the final concentration of 50 ng/mL, 100 ng/mL, 150 ng/mL, 200 ng/mL, 250 ng/mL and 300 ng/mL, respectively. Then, extraction, followed by derivatization, was performed as discussed above. Then, fluorescence intensity of prepared solutions was measured at emission wavelength of 420 nm.

Precision

Intra-day and inter-day precision was determined by measuring the fluorescence intensity of sample three times within a day and on three consecutive days, respectively. For intraday and interday precisions studies 100, 200 and 300 ng/mL concentrations were selected. 500 µL of blank plasma was spiked with 50 µL of drug solution of concentration of 20 µg/mL, 40 µg/mL and 60 µg/mL to get the final concentration of 100 ng/mL, 200 ng/mL and 300 ng/mL respectively. Extraction was performed as discussed earlier, further MEM was derivatized as described earlier. The fluorescence intensity of samples was measured using spectrofluorimeter.

Recovery

Recovery study of the method was performed by spiking the plasma with standard MEM solution as described in linearity range. Then extraction was performed as discussed earlier, further, MEM was derivatized as described earlier. The fluorescence intensity of MEM was measured using spectrofluorimeter. Amount of recovered MEM was calculated in terms of % RSD.

Limit of Detection (LOD) and Quantification (LOQ)

The sensitivity of the analytical method was evaluated by determining the LOD and LOQ. LOD & LOQ were calculated and reported using following equation:

LOD= 3.3X σ/s and LOQ= 10 X σ/s

Where, σ= standard deviation (SD) of the intercept and s = slope of calibration curve.

RESULTS AND DISCUSSION

Optimization of Experimental Conditions.

Initially, extraction of MEM was carried out with the help of protein precipitation by TCA. But their synchronous spectrum was exceeding the limit of fluorescence intensity (1000 unit). Therefore, extraction of MEM by only protein precipitation was not sufficient for proposed method. So, extraction of MEM by protein precipitation followed by liquid-liquid extraction using 5% IPA in n-hexane as extraction solvent was carried out. After this extraction procedure, good results were obtained for MEM and no interference was observed from blank for estimation of MEM. (Fig. 3) Additionally, it also showed good recovery of drug from plasma.

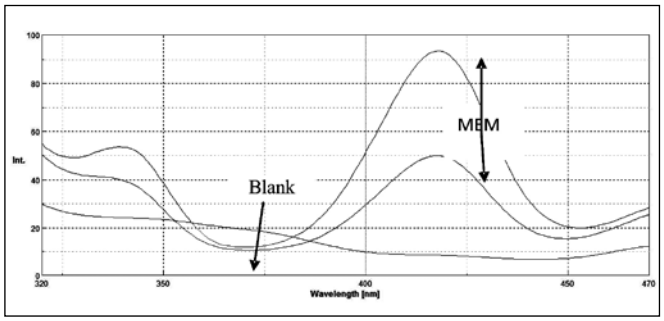

Fig.3: Synchronous spectra of MEM in borate buffer after extraction using 5% IPA in n-hexane as extraction solvent

Linearity

Linearity curve shows linearity in the range of 50 – 300 ng/mL for MEM. (Fig.4) The correlation coefficient (r^2) was found to be 0.9951 for MEM. Linearity study of MEM is summarized in Table I.

Table I: Calibration Curve Data of MEM in Spiked Human Plasma

Conc.(ng/mL)	Intensity	SD (n=6)	%RSD
50	49.59	3.82	7.72
100	91.40	4.79	5.24
150	136.90	6.59	4.82
200	167.89	6.44	3.84
250	203.24	13.33	6.56
300	255.95	9.15	3.58

Precision

Intra-day precision and Inter-day precision for MEM was performed by analysing three different concentrations (µg/mL) within linearity ranges and % RSD less than 10. Results of intra-day and inter-day precision studies are presented in Table II and III, respectively.

Table II: Intraday Precision Data of MEM on Spiked Human Plasma

Wave-length	Conc. ng/mL	Mean Intensity ± S.D.*	Mean conc. ± S.D*	% RSD
420 nm	100	90.56± 3.78	98.42 ± 4.68	4.76
	200	168.56± 5.68	194.88 ± 7.03	3.61
	300	249.45± 5.56	294.92 ± 6.88	2.33

**n=3*

Table III: Interday Precision Data of MEM in Spiked Human Plasma

Wave-length	Conc. ng/mL	Mean Intensity ± S.D.*	Mean conc. ± S.D*	% RSD
420 nm	100	89.23± 3.60	97.54 ± 4.49	4.61
	200	168.23± 6.11	196.01 ± 7.61	3.89
	300	249.12± 5.50	296.85 ± 6.86	2.31

**n=3*

Recovery

Recovery study was performed by comparing the response of drug extracted from spiked plasma with pure drug response and % RSD was calculated. Summary of recovery study is depicted in Table IV.

Summary of validation parameters for the developed method is shown in Table V.

Table IV: Recovery of MEM

Conc. ng/mL	% Recovery	% RSD (n= 3)
50	77.85	6.94
100	78.96	3.11
150	83.68	6.98
200	82.56	6.20
250	79.71	3.47
300	82.65	4.34

Table V: Summary of Validation Parameters

Parameters	Observation
Detection wavelength	420 nm
Linear Range (ng/mL)	50-300
Correlation coefficient R^2	0.9951
Intraday precision % RSD(n=3)	≤ 10
Interday precision % RSD(n=3)	≤ 10
Recovery (%)	77 – 83
LOD (ng/ml)	13.65
LOQ (ng/ml)	41.37

CONCLUSION

The proposed spectrofluorimetric method for estimation of MEM in human plasma was found to be simple, precise, accurate and specific. High recovery shows that the method is free from the interference from plasma constituents. Hence, it can be successfully applied for the estimation of MEM, in human plasma. The developed and validated method can be routinely useful for estimation of MEM, as very limited methods are available for its estimation due to absence of a chromophore in MEM.

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