Bioanalytical method development and validation of Memantin Hydrochloride and Donepezil Hydrochloride by Spectrofluorimetry

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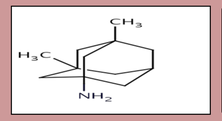
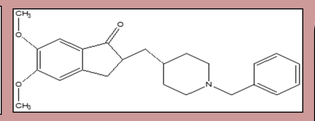
Introduction

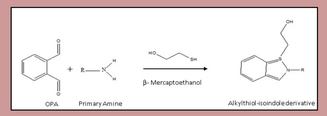
Alzheimer's disease is a progressive neurologic disease of the brain leading to the irreversible loss of neurons. Few drugs are available to slow down the progress of disease, mainly cholinesterase inhibitors (Donepezil Hydrochloride) and NMDA antagonists (Memantine Hydrochloride).



MEMANTINE



DONEPEZIL



Reaction pathway of derivatization of primary amine with OPA

Aim & Objective

Development and validation of Bioanalytical Method for estimation of Memantin HCl (MEM) and Donepezil HCl (DH) in human plasma.

Derivatization of MEM with OPA β-Mercaptoethanol

MEM is lacking of chromophores or auxochromes, thus it shows no distinct absorption in UV-VIS region. Therefore, primary amino group of MEM was derivatized using OPA β-Mercaptoethanol as derivatizing agent for quantitative estimation of MEM in plasma.

EXPERIMENTAL WORK

Method Optimization

Dilution Solvent: Borate Buffer (pH 9.6)

Delta value: 65 applied in synchronous mode

Sensitivity: Medium sensitivity

Detection Wavelength: Fluorescence intensity was measured at 420 & 389 nm for MEM and DH, Respectively.

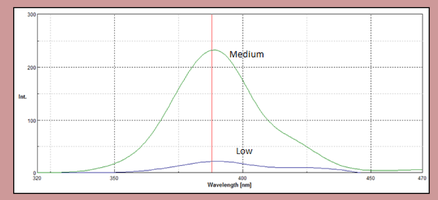
Extraction Solvent: 5% IPA in Hexane

Extraction Procedure

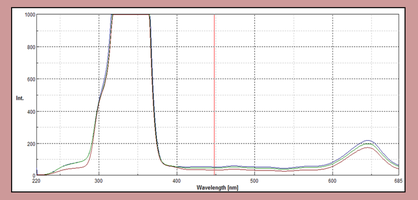
For estimation of MEM & DH in human plasma, extraction was carried out using 5% trichloroacetic acid for protein precipitation followed by liquid-liquid extraction with 5% IPA in n-Hexane.

Derivatization of Extracted MEM:
Extracted MEM from plasma was taken in dried volumetric flask. 1 mL borate buffer & 0.1 mL of derivatization solution was added and kept at water bath for 10 min at 70^0C.

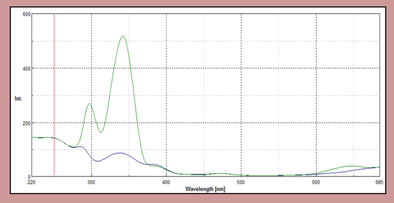
After 10 minutes volume was made with borate buffer and solution were kept at room temperature for next 10 minutes. After that solution were scanned and measurement were done



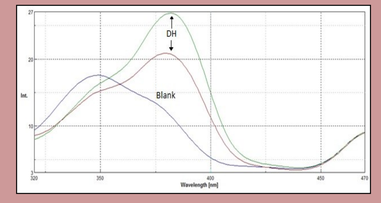
Overlay Synchronous spectra of DH 1 µg/mL solution in medium and low sensitivity



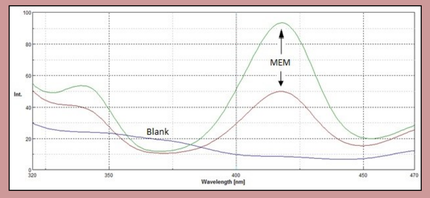
Synchronous spectra of DH in borate buffer after extraction from plasma by TCA



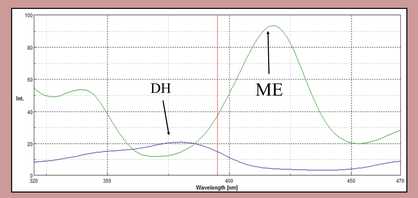
Synchronous spectra of DH in borate buffer using DCM as extraction solvent



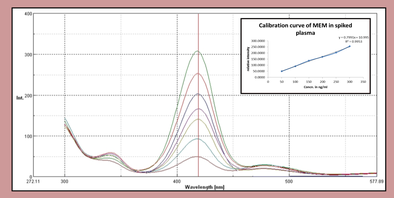
Synchronous spectra of DH in borate buffer after extraction using 5% IPA in n- Hexane as extraction solvent



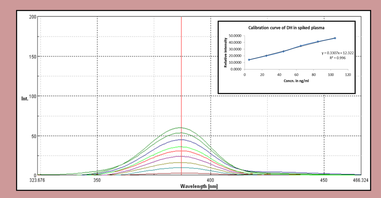
Synchronous spectra of MEM in borate buffer after extraction using 5% IPA in n- Hexane as extraction solvent



Overlay synchronous spectra (medium sensitivity) of DH 25 ng/mL and MEM 100 ng/mL in borate buffer (pH 9.6)



Overlay synchronous spectra of MEM in spiked plasma (50-300 ng/mL)



Overlay synchronous spectra of DH in spiked human plasma (5-105 ng/mL)

Summary of Validation Parameter

Parameter	MEM	DH
Detection Wavelength	420 nm	389 nm
Linear range (ng/ml)	50-300	5-105
Regression equation	y = 0 .0009x - 0.001	y = 0.003x - 0.0017
Correlation coefficient	0.9953	0.996
LOD (ng/ml)	13.65	4.84
LOQ (ng/ml)	41.37	14.68
Precision		
Intra day (% RSD)	2.33-4.76	6.14-6.54
Inter Day (% RSD)	2.31-4.61	6.28-7.31
Accuracy study	77-83 %	75-85 %

Result

All the parameter of the developed bioanalytical method for the determination of MEM & DH in human plasma was validated as per USFDA guidelines as shown in table. Validation parameters of the proposed method were found to be satisfactory with acceptable recovery and precision. LOD indicates sensitivity of the method. High recovery show that the method is free from the interference from plasma constituents.

Conclusion

The proposed spectrofluorimetric method for estimation of DH and MEM in human plasma is simple, precise and sensitive. Hence, it can be successful applied for the estimation of MEM and DH in human plasma as well as for pharmacokinetic study. The developed and validated method can be routinely useful for estimation of MEM as very limited methods are available for its estimation, as MEM does not have any chromophore in it.

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