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Validated stability indicating simultaneous estimation of Memantine and Donepezil in pharmaceutical formulation by RP-HPLC-DAD

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ABSTRACT

A simple, rapid, precise, accurate and low-cost stability-indicating HPLC method with diode array detection (DAD) has been developed for the simultaneous estimation of Memantine (MEM) and Donepezil (DPZ) in the presence of their degraded products. The chromatographic conditions used herein were inertsil ODS (4.6 x 250mm, 5µ) and acetonitrile with 0.1% orthophosphoric acid at a ratio of 10:90 as the mobile phase, with 271 nm as the wavelength of estimation, using DAD detector and 1.0 ml/min flow rate. Chromatographic separation of the drugs was achieved within 3.2 minutes from all the degradants. The linearity concentration for this method was found to be in the range of 10-50µg/ml for both drugs. The validation of the proposed method was performed as per the International Conference on Harmonization (ICH) guidelines for linearity, accuracy, precision, robustness, limit of detection and limit of quantification. The sample drugs were subjected to various forced degradation methods such as acid and base hydrolysis, oxidation, thermal, and photolytic degradation by sunlight. The drug substances underwent mild degradation irrespective of the induced stress. The proposed method can be used to quantify target drugs on a regular basis in tablet formulations.

Keywords: Memantine, Donepezil, HPLC-DAD, simultaneous, stability-indicating

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INTRODUCTION

Memantine (MEM), chemically 1-amino-3, 5-dimethyladamantane hydrochloride, is a tricyclic amine used in the treatment of Parkinson's disease, movement disorders [1], dementia syndrome [2], pervasive developmental disorders [3], alcoholism and its withdrawal [4]. Unlike other non-competitive NMDA antagonists such as phencyclidine and ketamine, MEM has few adverse effects like agitation, confusion and psychosis [5,6]. Likewise, donepezil (DPZ), chemically 2, 3-dihydro-5, 6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl] methyl]-1H-inden-1-one hydrochloride, is an *acetyl cholinesterase* inhibitor that acts by increasing the concentration of acetylcholine, leading to an increase in the cholinergic function. Therefore, it is well indicated for treating mild to moderate dementia in Alzheimer's patients [7,8].

Several analytical methods have been reported for quantifying MEM and DPZ, either as a single entity or in combined dosage forms using high-performance liquid chromatography (HPLC) with Mass/Ultraviolet-visible/fluorescence detectors for MEM. These detection techniques all require derivatization of the moiety either by using chromophores or fluorophores, which are tedious and time-consuming, limiting their use for regular

quantification of MEM [9-14]. The spectroscopy methods reported were also with derivatization procedures [15]. Simple HPLC with UV has also been reported in some literature [16, 17]. In the case of DPZ formulation, the quantification of DPZ from human plasma [18-20] using either HPLC or HPLC-MS, enantiomeric estimation of the DPZ by LC-MS [21], an electro-analytical method for DPZ[22] and single entity quantification from various dosage forms [23,24] were reported previously. Recently, the US FDA approved the combined dosage form of MEM and DPZ. Also some simultaneous estimation procedures were reported recently. Among them, Noetzli *et al* and Bhateria *et. al.*, reported simultaneous UPLC-MS/MS [25] and LC-MS/MS [26] methods of estimation for MEM and DPZ drugs. Some simultaneous HPLC methods were also reported for the simultaneous estimation using HPLC-RI detection and UV detection. Refractive Index (RI) detection by HPLC is a rarely used and expensive procedure [27]. Further, it is easily affected by RI modifying agents that may be used as additives in some formulations. In cases of HPLC reported using UV detection, acetonitrile was used as an ion pairing organic phase in high concentrations (70% to that of the total mobile phase), which is not cost effective or eco-friendly [28]. Further, the drugs to be estimated are moderately polar and could be easily eluted by low molar acids or low molar buffers that have not been studied yet. Use of pure acids or alkalies should be avoided due to the considerations of column life and performance. Hence, in this study, we aimed to develop a simple, rapid and validated RP-HPLC method using low molar phosphoric acid with acetonitrile at a low ratio as the mobile phase and DDA detection for accurate estimation.

MATERIALS AND METHODS

Chemicals and reagents

Memantine HCL (Sigma Aldrich), Donepezil HCL (Sigma Aldrich), Potassium dihydrogen orthophosphate, HPCL grade Acetonitrile, Millipore water.

Methodology

Preparation of mobile phase

1 mL of orthophosphoric acid was ipette into 1000 mL of HPLC grade water and mixed well with a magnetic stirrer to give 0.1% v/v orthophosphoric acid. The prepared solution was degassed using an ultrasonicator for 5 minutes. Then, a respective quantity of the solution was mixed with acetonitrile and filtered through a 0.45 µ Whatman cellulose acetate membrane filter.

Preparation of Memantine HCl and Donepezil HCl standard stock solution

10 mg each of the MEM and DPZ working standards were accurately weighed and transferred into a 10 mL clean dry volumetric flask, and mobile phase was added as the diluent. The solution was sonicated to dissolve it completely then the volume was brought up to the mark with the diluent and used as the standard stock solution. From the above solution, 3.0 mL was pipetted into a 10 mL volumetric flask and brought up to the mark with the diluent. Again 3.0 mL of the above solution was pipetted into another 10.0 mL volumetric flask and diluted up to the mark with the diluent, and used for analysis.

Instrumentation and chromatographic conditions

The high-performance liquid chromatography instrument was equipped with a diode array detection (HPLC-DAD) system (Waters, Alliance autosampler 2695 series, USA) Diode Array Detector (2487 series). The HPLC system is featured with a thermostated column compartment fitted with a 100 µL sample loop. The analytes were separated with an Inertsil ODS (4.6 X 250mm, 5µm) column. Ambient temperature was maintained in the column oven. The injection volume was 20 µL with 1.0 ml/min as flow rate.

Linearity and Range

The linearity was determined to establish the working concentration ranges with the proportional relationship with the detectors' response. Five increasing concentration ranges from 10 to 50µg/mL were prepared as per the USP and ICH guidelines [29, 30]. The minimum specific range for the assay was from 80 – 120% of the target concentration. The prepared solutions were subjected to linearity measurements by injecting each solution with increasing concentration. The acceptance criteria for the linearity data is generally derived from the correlation coefficient, which was found through regression correlation.

LOD and LOQ

The detection limit (LOD) is the lowest absolute concentration of an analyte in a sample that may be detected but not quantified by the proposed method. LOD can be calculated from the signal to noise ratio obtained from the

baseline noise of the blank solution and the signal obtained from the respective LOD solutions of MEM and DPZ. Likewise, the limit of quantitation (LOQ) is the lowest target (drug of estimation) concentration in a sample that can be assayed with satisfactory precision and accuracy under the proposed conditions.

System suitability

The system suitability parameter is useful for verifying whether the accuracy and precision of a system are acceptable for the established method. Theoretical plate count, tailing factor, and resolution were the parameters measured and compared with standard specifications as per the ICH guidelines. In this study, system suitability studies were conducted with 3 µg/mL solution of MEM and DPZ.

Specificity

In establishing an HPLC method for analysis of pharmaceutical active ingredients, it is crucial to understand the sensitivity of the drug to degradation. Probably, degradants that interfere with the established assay method and the interference of the chemical entities generated during sample preparation or from the excipients of the formulation. Specificity can also be measured in the presence of intentionally produced degradants that may deter the assay method. The degradants generated by the induced stress are discussed in the later part of this work. To determine the specificity of the method, reference standard materials of both MEM and DPZ were injected to demonstrate the ideal separation from potential interference. Further, this interference in the assay was examined by injecting placebo prepared with the common excipients stated in the USP.

Assay of formulation

About 20 tablets were weighed accurately and powdered well. From the powder, the weight equivalent to 10 mg each of both drugs was weighed and transferred into a 10 mL clean dry volumetric flask. 7.0 mL of the mobile phase was added and sonicated to aid complete solubility then the volume was topped with the diluent and used as the stock solution. From the above sample stock solution of MEM and DPZ, 3.0 mL was pipetted into a 10 mL volumetric flask and brought up to the mark with the diluent. Again, 3.0 mL of the above sample stock solution was pipetted into another 10.0 mL volumetric flask and diluted up to the mark with the diluent then used for analysis. The results of the assay were compared with previously reported method[28]to determine its suitability and adaptability.

Precision

Precision is the parameter used to measure the degree of repeatability for the developed analytical method under a normal condition, which is indicated as the percent relative standard deviation (%RSD). Ideally, three different levels are used for measuring the precision. Repeatability results are obtained from repeating the method with the same conditions over a short duration of time (intra–day assay precision). In this study, the standard solution was injected five times and the areas for all five replicates were calculated for the %RSD.

Accuracy

Accuracy is used to check whether the method yields results close to the true value or not. As far as the assay method is concerned, the triplicate numbers for the spiked samples were prepared at three different levels between 50% and 150%. The percentage of recovery was calculated from the data.

Ruggedness

Ruggedness or intermediate precision tests whether similar results for the established method are obtained within the same laboratory with mild variations, for example, analysis on different days, changing either analysts or column or instruments. The complete experimental setup is employed in the ruggedness measurement in order to monitor the effects of individual variables if any. The intra-assay precision criteria for an assay method should be $\leq$ 2%, instrument precision for the impurity assay should be $\leq$ 5% and the intra-assay precision should be $\leq$ 10%. The intermediate precision for this present study was determined by using a different make of the columns with the same dimensional parameters and evaluating the relative percent purity. 30 µg/mL of the solution was injected five times and the %RSD was calculated.

Robustness

Robustness is used to measure the reliability of the method i.e., to ensure whether the developed method remains unaffected by slight, deliberate variations in the method parameters. Robustness is measured in one of three different ways: by making deliberate changes to the method parameters, by using multivariate statistical experimental design

to control method variables or by using software like theoretical modeling to predict the robustness. In this study, 30 µg/mL solutions of MEM and DPZ were prepared and injected by changing method parameters such as by altering the flow rate to ± 10% and altering the mobile phase compositions. The plate count and the tailing factor were calculated as the parameter criteria for robustness.

Placebo interference

Placebo interference evaluates whether the intended assay is free from the common excipients that are used in formulating oral dosage forms. A placebo will be formulated with the common excipients listed in the United States Pharmacopoeia such as talc, magnesium stearate, and starch. Solutions were prepared with the placebo as per the assay procedure and injected into the HPLC system for six replicates.

Stress-induced degradation of MEM and DPZ

In order to elucidate the inherent stability of the drug entities, forced degradation was carried out as per the International Conference on Harmonization (ICH)[29]. In this accord, stress degradation studies were performed on the MEM and DPZ using the proposed HPLC method. From the standard stock solution, 1.0 mL each of MEM and DPZ was pipetted individually into a 10 mL volumetric flask and was diluted up to the mark with the diluent. The mixture was then subjected to various stress conditions as described below.

Hydrolytic degradation under acidic condition

From the stock solution of individual MEM and DPZ solutions as well as the mixture, 3.0 mL was pipetted into a 10 mL volumetric flask and 3 mL of 0.1N HCL was added. This mixture was kept at 60ºC for 6 hours, neutralized with 0.1N NaOH and brought up to 10 mL volume with the diluent. Then the solution was filtered into vials with a 0.45 µ syringe filter and used for injection into the HPLC system.

Hydrolytic degradation under basic condition

The same procedure as in the acidic degradation study was followed for the basic condition by replacing 0.1N HCL with 0.1N NaOH.

Oxidative degradation

From the stock solutions of MEM and DPZ and the mixture, 3.0 mL was pipetted into 10 mL volumetric flasks to which 1 mL of 3% w/v of H_2O_2 was added and brought up to the volume with diluent. The solutions were left undisturbed at room temperature for 15 mins. The solution was filtered with 0.45 µ syringe filters and kept in vials for HPLC measurements.

Thermal degradation

For the thermally induced degradation study, around 10 mg portions of MEM and DPZ drug powders were placed in a petri dish and kept in a Hot Air Oven at 110ºC for 24 h. After the completion of thermally induced degradation, a stock solution (100 µg mL^{-1}) of each drug was prepared by quantitative transfer into a 50 mL volumetric flask and diluted well with the diluent. Around 1 mL of the above stock solutions of MEM and DPZ, individually or combined, were transferred into 10 mL volumetric flasks and diluted to the mark with the diluent for HPLC measurements. All the above degradation studies were carried out in the dark to avoid the possible degradation effect of the light.

Photostability

Photodegradation study was performed by exposing the 10 mL volumetric flasks containing the stock solution (a) of MEM and DPZ individually or combined to the sunlight for 3 days. Later, the solution was subjected to HPLC measurements and the samples protected from light were used as a control.

Statistical analysis

Statistical analysis such as students ‘t’ test, %RSD and regression were calculated using Originpro-8 (OriginLab Corporation, MA, United States) and SPSS version 10.0 (SPSS inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Method development and optimization

The ideal chromatographic condition for the elution of samples from the closely related degradants was developed. Various trials were conducted to optimize the solvent system, flow rate and wave length detection. Since both drugs are soluble in water, they were tested with 100% HPLC grade water as the mobile phase. However, elutions were detected very early in the chromatogram, which may be due to the fast elution of the drugs by water. Since the primary goal of our present work is to use the low ion pairing organic solvents for fast and accurate detection of the target components, we tested various combinations of phosphoric acid and acetonitrile/methanol as the binary solvent system and found that 0.1% v/v orthophosphoric acid and acetonitrile with the ratios of 90:10 gave ideal results. Hence, finally, the above said solvent combination was used for further analysis of the targeted moieties. The chromatogram with optimized conditions Fig 1.a and the blank (solvent only) chromatogram Fig 1.b were given.

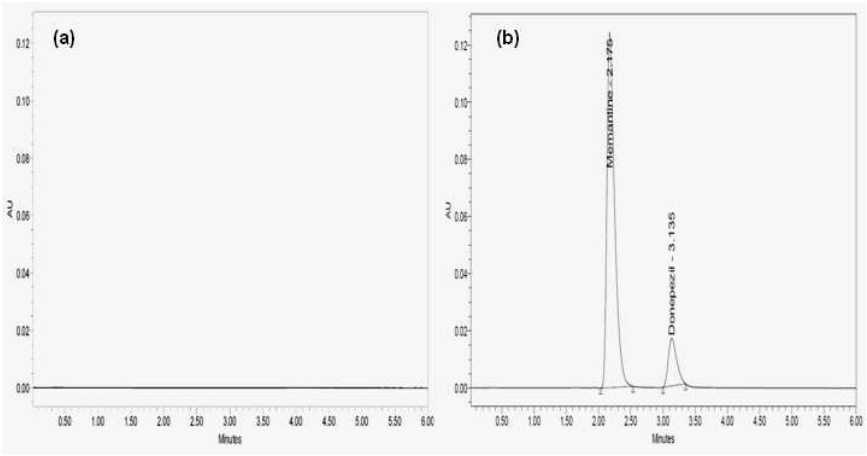

Fig 1: Chromatogram of (a) Blank and (b) standard MEM and DPZ

Method validation

Linearity, LOD, and LOQ

In the present study, both drugs satisfied the linearity requirements in the concentration range of 10-50 µg/ml with r^2 = 0.999. The linearity parameters for MEM and DPZ are given in Figure

2 and summarized in Table 1. LOD and LOQ were also very low as compared to a previously reported method [28] Table 1, which confirms the high sensitivity of the method.

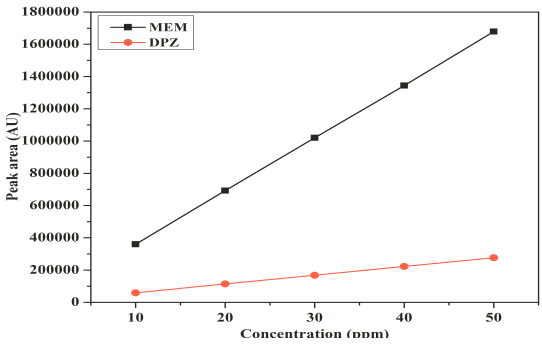

Fig 2: Linearity of MEM and DPZ

Table 1: Optical properties of MEM and DPZ

Parameters	MEM	DPZ
Linearity (ppm)	10-50	10-50
Wavelength of detection (nm)	270	270
Detection technique	DAD	DAD
Regression equation	y=mx+c	y=mx+c
Correlation co-efficient (r^2) *	0.999	0.999
Slope (m) *	32889.83	5420.78
Standard Error on Slope	100.163	16.934
Intercept (c) *	32275.1	5382.4
Standard error on intercept	3322.0292	561.64
LOD (µg/mL)	0.216	0.072
LOQ (µg/mL)	0.238	0.244
USP tailing factor (limit <2)*	1.67	1.45
Plate count (limit not<2000)*	2524.84	3177.99

** Average of six values (n=6)*

System suitability

The data obtained for system suitability parameters shows that the tailing factor (T) and plate count (N) for MEM and DPZ were within the recommended acceptance criteria (Table 1).

Specificity and placebo interference

From the results obtained by injecting the standard MEM and DPZ at specific concentrations, the chromatogram (Figure 3.a) confirmed that the drugs were not affected by any interfering materials, as individual peaks were eluted from each standard. Further, the chromatogram (Figure 3.b) depicts the placebo chromatogram obtained from the placebo prepared using the common excipients in the formulation of tablets, demonstrating that the method was not affected by any of the excipients.

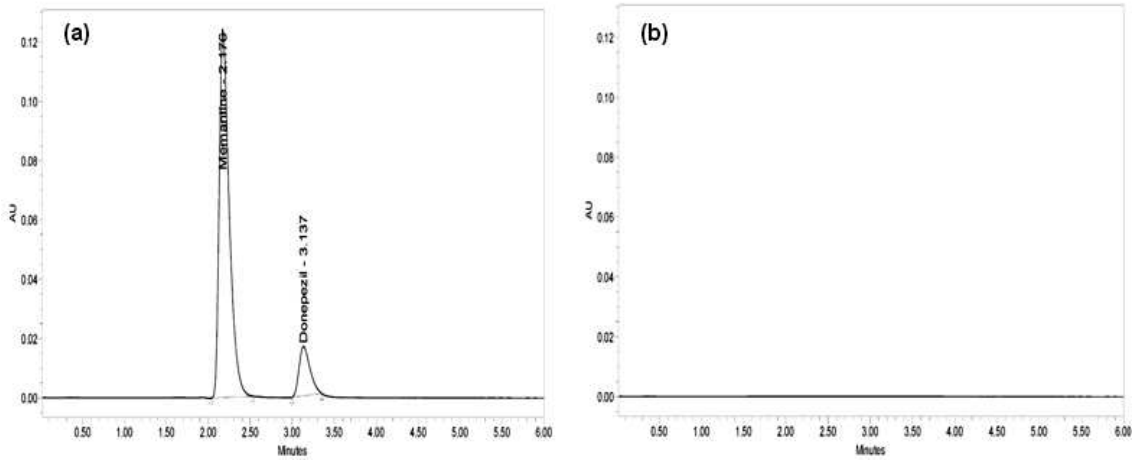

Fig 3: Chromatogram of specificity solution (a) standard (b) placebo

Table 2: Assay results of MEM and DPZ

Label Claim	Assay (%)		% RSD*		Statistical confidence**	
	Present	Reported	Present	Reported	t-test	F-test
10 mg MEM/tab	98.96±0.42	98.82±0.56	0.542	0.586	0.322	1.078
10 mg DPZ/tab	99.62±0.89	98.67±0.45	0.785	0.886	0.365	1.016

**Mean of 6 determinations*

*** Student's t-test and the F-test were values at 95% confidence level and the theoretical values for the same was 2.36 and 4.96 respectively*

Application of method to formulation (Assay)

From the results (Table 2) of the assay using the proposed and reported HPLC with UV detector method [28], it was evident that the method proposed herein was also suitable for the regular analysis of the targeted drugs in their solid

formulations. The results of the student's 't' test and 'F' test showed that there is no significant difference between the calculated and theoretical values at the 95% confidence level.

Precision

From the data for the results of intra-day precision, it was found that the % RSDs for MEM and DPZ were low and within the limits of intra-day assay precision for both drugs (RSD should be <2 % RSD) as per the ICH [29] and USP [30] guidelines for method validation (Table 3).

Table 3. Precision and accuracy of the method

Conc. (µg/ml)	Precision (Intra-day)		Accuracy		
	Amt. Found (µg/ml)*	% RSD*	Spike level (%)	(%) Recovery	(%) RSD*
30	29.8±0.78	0.561	50	103.66±0.56	0.351
			100	99.80±0.61	0.522
			150	97.43±0.52	0.781
30	31.1±0.36	0.623	50	100.59±0.32	0.623
			100	99.60±0.45	0.454
			150	97.28±0.61	0.360

** Mean SD of five replicates*

Accuracy

Accuracy is used to check whether the method yields results close to the true value or not. As for as the assay method is concerned, triplicates of the spiked samples were prepared at three different levels between 50% and 150%. The percentage of the recovery was then calculated from the data (Table 3) and found to be within the limit.

Ruggedness

From the results of ruggedness, the % RSD for five consecutive injections of MEM and DPZ was found to be 0.612 and 0.502, respectively, which are within the limits of the acceptance criteria of ruggedness for the method.

Robustness

Table 4 presents the results of the plate count and USP tailing factor calculated after deliberate changes in flow rate and mobile phase composition as discussed in the materials and methods section. From the image, it is evident that the method portrayed herein is robust and was not affected by the deliberate changes. In both changes, the plate count and tailing factors observed were with the acceptance criteria for the robustness as given by ICH guidelines (plate count should be more than 2000 and tailing factor should not be less than 2).

Table 4: Results of Robustness

Variables		MEM		DPZ	
		Plate count	Tailing factor	Plate count	Tailing factor
Flow rate (ml/min)	0.9	2680.73	1.60	3200.8	1.70
	1.0	2524.84	1.62	3177.99	1.41
	1.1	2124.40	1.55	2973.70	1.40
Mobile phase composition	10%less ACN	2573.86	1.66	3579.64	1.30
	Actual	2524.84	1.63	3177.99	1.42
	10% more ACN	2124. 41	1.50	2973.78	1.40

Stress-induced degradations

With reference to the ICH guidelines [29] for stability indicating assays, the target drug should not be degraded by more than 20% during the assay period for the bulk as well as for the formulation used in the assay. From the stability results depicted in Table 5, both the bulk and formulation samples were not degraded by more than 20%. Further, there were no co-eluting peaks observed near the main peaks under the stipulated stress conditions selected here. This suggests that the proposed method is highly specific for the estimation of drug targets in the presence of their degradation products and excipients.

Table 5: Results of degradation

Degradation Techniques	Bulk drug (% degradation)		Formulation (% degradation)	
	MEM	DPZ	MEM	DPZ
Acid	8.68	9.71	10.8	12.71
Base	9.01	10.01	12.41	11.95
Peroxide	2.11	3.85	2.33	4.05
Thermal	3.02	2.78	3.0	2.9
Photo	2.89	3.96	3.19	4.06

CONCLUSION

From the results of the study, it was concluded that the developed HPLC method for the stability indicating assay of memantine and donepezil in bulk or in a formulation is rapid, precise and accurate. Further, the method portrayed here was fully validated with reference to the parameters listed in ICH guidelines. Hence, we concluded that the method developed here can be used for the assay of pharmaceuticals containing memantine and donepezil without any interference. It can also be used for the analysis of stability samples.

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