Stability Indicating Methods for Determination of Donepezil Hydrochloride According to ICH Guidelines

Samah Sayed ABBAS,* Yasmin Mohamed FAYEZ, and Laila El-Sayed ABDEL FATTAH

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University; Kasr El-Aini St., 11562, Cairo, Egypt.
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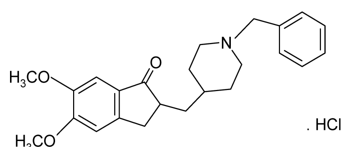
Stability indicating assays for determination of Donepezil Hydrochloride in presence of its oxidative degradate were developed and validated. The first three are spectrophotometric methods depending on using zero order (D^0), first order (D^1) and second order (D^2) spectra. The absorbance was measured at 315 nm for (D^0) while the amplitude was measured at 332.1nm for (D^1) and 340 nm for (D^2) using deionized water as a solvent. Donepezil Hydrochloride (I) can be determined in the presence of up to 70% of its oxidative degradate (II) using (D^0), 80% using (D^1) and 90% using (D^2). The linearity range was found to be 8—56 μg ml^{-1} for (D^0), (D^1) and (D^2). These methods were applied for the analysis of I in both powder and tablet form. Also, a spectrofluorimetric method depending on measuring the native fluorescence of I in deionized water using λ excitation 226 nm and λ emission 391 nm is suggested. The linearity range was found to be 0.32—3.20 μg ml^{-1} using this method, I was determined in the presence of up to 90% of II. The proposed method was applied for the analysis of I in tablet form as well as in human plasma. The last method depends on using TLC separation of I from its oxidative degradate II and I was then determined spectrodensitometrically. The mobile phase was methanol : chloroform : 25% ammonia (16 : 64 : 0.1 by volume). The linearity range was found to be 2—15 μg/spot. This method was applied to the analysis of I in both powder and tablet form using acetonitrile as a solvent.

Key words spectrophotometry; spectrofluorimetry; densitometry; donepezil hydrochloride; stability indicating assay

Donepezil Hydrochloride (Aricept; Memac) is 2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]-methyl]-1*H*-inden-1-one hydrochloride.[1)]

It is a reversibly inhibitor of acetylcholinesterase, indicated for the treatment of mild to moderate dementia of Alzheimer's type 1. The structural formula of Donepezil Hydrochloride is as follows:



A few methods have been reported for the determination of I including HPLC methods in tablets[2)] and human plasma.[3—7)] Isocratic HPLC method with fluorescence detection at 390 nm with an excitation at 325 nm for determination of donepezil in plasma and microdialysate samples was developed.[3)]

Analysis and enantioresolution of Donepezil was performed using capillary electrophoresis.[8)] Determination of enantiomers of Donepezil Hydrochloride in rat plasma by liquid chromatography with fluorimetric detection of I with excitation at 318 nm and emission at 390 nm was described by Haginaka and Seyama.[9)] Donepezil Hydrochloride was found to be stable in acid and alkaline medium, but liable to oxidative degradation using hydrogen peroxide.[2)] Reviewing the literature in hand, it is obvious that no spectroscopic study has been attempted to determine donepezil hydrochloride in the presence of its oxidative degradate.

Upon scanning the absorption spectra of each I and II, it was observed that I has λ_{max} at 315 nm, at this wavelength II has a very little constant absorbance. Trials to use zero order absorption at 315 nm for determination of I in presence of II was successful for up to 70% of II. Zero order solve the problem of overlapping bands of I and II to certain extent but derivative spectrophotometry is a well established technique that is able to enhance the resolution of overlapping bands so to increase the selectivity of the method. D^1 and D^2 methods were constructed by measuring I at 332.1 nm and 340 nm (zero crossing of II), respectively. Different parameters affecting the D^1 and D^2 spectra were studied; namely $\Delta\lambda$ interval and smoothing factor. The best condition was by using 4 nm and 8 nm $\Delta\lambda$ interval for D^1 and D^2, respectively, without smoothing.

The scientific novelty of the present work is that the methods used are simple, rapid, sensitive, and less expensive and less time consuming compared with other published LC methods. In addition, the aim of this work is to develop simple stability indicating methods for determination of I in presence of its oxidative degradate (II).

Experimental

Instrumentation A double beam UV-Visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm path length, connected to IBM compatible computer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2 nm and wavelength–scanning speed 2800 nm/min. A spectrofluorimeter (SHIMADZU, Japan) model RF-1501 with an Epson Lx-300+Printer. 1 cm quartz cell was used at low sensitivity and 2.5 nm bandwidth. TLC glass plates precoated with silica gel G F_{254} (E. Merck). 25-μl Hamilton syringe. Chromatographic tank 20×21×9 cm (Desaga). SHIMADZU Dual wavelength flying spot scanner, densitometer model CS9000.

Materials and Reagents Donepezil Hydrochloride was kindly supplied by Pfizer-Egypt S.A.E Cairo, A.R.E and was certified to have a purity of 99.60±1.22%. Aricept tablet (Pfizer-Egypt S.A.E Cairo, A.R.E under authority of Pfizer INC., U.S.A) labelled to contain 5 mg of Donepezil Hydrochloride per tablet, batch number 3006. Acetonitrile, Methanol and chloroform (E. Merck) were of analytical grade. Ammonia, 25% solution, Hydrogen peroxide 30% (ADWIC). Plasma was purchased from VACSERA. Deionized water, 0.1 M NaOH, 0.1 M HCl, 0.1 M acetic acid, 0.05 M H_2SO_4 and phosphate buffer pH (2—8).

Standard Solutions. Donepezil Hydrochloride Stock Standard Solution Two hundred micrograms per milliliter in deionized water (for spectrophotometric and Spectrofluorimetric method) and 1 mg ml^{-1} in acetonitrile (for densitometric method).

* To whom correspondence should be addressed. e-mail: samah_abbas2005@yahoo.com
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Donepezil Hydrochloride Working Solutions Eighty micrograms per milliliter in deionized water (for spectrophotometric method), 8 μg ml^{-1} in deionized water (for spectrofluorimetric method). They were prepared by suitably diluting the stock standard solution with deionized water.

Donepezil Oxidative Degradate Standard Solution 200 μg ml^{-1} It was prepared by adding 10 ml of 30% H_2O_2 to 50 ml of standard stock solution of 200 μg ml^{-1} of I, refluxed for 4 h, evaporated to dryness and then the residue dissolved in 20 ml deionized water. The obtained solution was transferred into 50-ml volumetric flask and the volume was completed with suitable solvent to have a concentration of 200 μg ml^{-1}. Complete degradation was assured by the suggested TLC method, as indicated by the disappearance of I spot and appearance of one spot of II using methanol : chloroform : 25% ammonia. (16 : 64 : 0.1 by volume) as a mobile phase. Spotting of 5 μg at different successive times of reflux, showed complete degradation after 4 h.

Laboratory Prepared Mixtures Solutions containing different ratios of I and II were prepared to contain 10—90% of II.

Procedure. Construction of Standard Curve for Spectrophotometric Methods Aliquots of I working standard solution equivalent to 80—560 μg were accurately transferred into a series of 10 ml volumetric flasks, the volume was completed to the mark with deionized water. The absorbance was measured at 315 nm. A calibration curve was constructed representing the absorbance versus concentration and the regression equation was computed. D^1 and D^2 curves were computed at $\Delta\lambda$ 4 nm, 8 nm and scaling factor 100, respectively. The peak amplitude values of D^1 and D^2 were measured at 332.1 nm and 340 nm, respectively (zero crossing of II).

Construction of Calibration Graph for Spectrofluorimetric Method Aliquots of I working standard solution equivalent to 8—80 μg were accurately transferred into a series of 25 ml volumetric flasks, the volume was completed to the mark with deionized water. The fluorescence was measured at λ emission 391 nm using λ excitation 226 nm. A calibration curve was constructed representing the fluorescence intensity versus concentration and the regression equation was computed.

Construction of Calibration Graph for Densitometric Method Aliquots of donepezil hydrochloride stock solution (1 mg ml^{-1}) equivalent to 1—7.5 mg were accurately transferred into a series of 10 ml volumetric flasks; the volume was completed to the mark with acetonitrile. 20 μl of each of I, II and their laboratory prepared mixture solutions were applied to 10×20 cm (TLC) glass plates precoated with 0.25 mm silica gel G F_{254} using 25 μl Hamilton microsyringe. The plates were spotted 1.5 cm apart from each other and 1.5 cm apart from the bottom edge. The chromatographic chamber was pre-saturated with the developing solvent for one h. The plate was developed by ascending chromatography with methanol : chloroform : 25% ammonia (16 : 64 : 0.1 by volume) to a distance of about 7 cm and a developing time of 30 min approximately. The plates were air dried at room temperature and detected under UV lamp. Densitometric scanning was done at 315 nm under the following conditions:

Photo mode: reflection.
Scan mode: zigzag.
Swing width: 5.
Result output: chromatogram and peak area.

A calibration curve was constructed representing the relationship between the peak area and amount of I in μg/spot and the regression equation was computed.

Application to Pharmaceutical Formulation Film coat of ten tablets were removed with the help of a filter paper moistened with ethanol, and then were weighed, crushed, finely powdered and mixed well. A weight equivalent to 20 mg of pure I was transferred into 100 ml volumetric flask; 20 ml acetonitrile were added and shaked vigorously. The volume was completed with acetonitrile and mixed well. The obtained solution was centrifuged for 5 min at 3000 rpm. Twenty milliliters of the centrifugate were transferred into 100-ml beaker and evaporated to dryness on hotplate. After cooled, it was dissolved in 20 ml deionized water accurately transferred to 100-ml volumetric flask and the volume was completed with deionized water to obtain concentration 80 μg ml^{-1}. Aliquots were transferred to 10-ml flasks and diluted with deionized water to obtain a concentration of 16 μg ml^{-1}. The general procedure under spectrophotometric methods was followed. For fluorimetry, the final solution obtained was diluted with deionized water to obtain a concentration of 0.64 μg ml^{-1} and the fluorescence intensity was measured. For densitometry, the previous procedure was followed for the extraction of I from Aricept tablets. The final solution obtained was diluted with acetonitrile to have a concentration of 0.4 mg ml^{-1}, 4 ml of this solution was diluted to 10 ml with acetonitrile. The general procedure under densitometric method was followed.

Application of Fluorimetric Method to Human Plasma In a series of 10-ml measuring flasks, 1 ml blank (drug-free) plasma sample was spiked with different concentrations of Donepezil Hydrochloride, the volume was completed to the mark with acetonitrile to provide final concentrations from 2.4—24 μg ml^{-1}. The samples were centrifuged at 2300 rpm for 15 min. Two milliliters of the protein free supernatant were transferred to test tube then evaporated to dryness, cooled and 10 ml deionized water was added. The suggested method was applied as described under construction of calibration graph for spectrofluorimetric method. The concentration of I was calculated from regression equation.

Results and Discussion

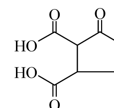
The stability of I was studied according to the ICH guidelines for:

(a) Stress, Acid and Alkaline: 0.1 N HCl/0.1 N NaOH for 8 h, 1 N HCl/1 N NaOH for 12 h, 2 N HCl/2 N NaOH for 24 h finally 6 N HCl/6 N NaOH for 24 h.

(b) Oxidative Condition: 3% H_2O_2 for 6 h, 3% H_2O_2 for 24 h, 10% H_2O_2 for 24 h finally 30% H_2O_2 for 24 h.

(c) Photodegradation: 2×10^5 lux h and 6×10^5 lux h.

The degradation process under the previously mentioned conditions was followed using TLC and the compound was found to be stable under acidic, and alkaline condition but it is liable to degradation with hydrogen peroxide which is confirmed with a previous study on stability of donepezil Hydrochloride.[2)] It is a single component which confirmed by TLC method as indicated by appearance of one spot of oxidative degradate after complete degradation and also confirmed by IR. The suggested degradate is:



Degradation product not interfered with donepezil hydrochloride quantification. No evidence of interactive degradation product.[2)]

The present work is concerned with determination of Donepezil Hydrochloride in presence of its oxidative degradate.

In this work simple, sensitive and rapid methods are described for the determination of I in presence of its oxidative degradate (II). The first three are spectrophotometric methods are based on direct measurement of I at its λ_{max} 315 nm in presence of its oxidative degradate which does not show appreciable absorption at this wavelength as shown in Fig. 1. A linear correlation was obtained between absorbance and concentration of the drug in a concentration range 8—56 μg ml^{-1} with mean percentage recovery of 100.02±0.77.

The following regression equation was computed, $A=0.0255C+0.0067$ where A is absorbance, C is concentration in μg ml^{-1}.

The selectivity of the method was determined by analyzing mixtures of I and II at different ratios. The results showed that I can be determined in presence of up to 70% of II with mean recovery % of 100.70±1.24.

To increase the selectivity, D^1 and D^2 methods were constructed by measuring I at 332.1 nm and 340 nm (zero crossing of II), respectively, as shown in Fig. 2.

A linear correlation was obtained between amplitude and concentration of the drug in a concentration range 8—56 μg ml^{-1} for D^1 and D^2 with mean percentage recovery of

99.71±0.94 for D^1 and 100.80±1.43 for D^2.

The following regression equations were calculated, $A=0.1317C-0.0007$ for D^1 and $A=0.0083C+0.0063$ for D^2, where A is peak amplitude, C is concentration in $\mu g\,ml^{-1}$.

The selectivity of the D^1 and D^2 methods were determined by analyzing mixtures of I and II at different ratios and it is increased up to 80% for D^1 and 90% for D^2 with mean recovery % of 99.98±1.79 and 100.18±2.20 for D^1 and D^2 methods, respectively.

A spectrofluorimetric method suggested is based on the fact that I has intense fluorescence while II has no fluorescence. The λ emission and λ excitation were found to be 391 and 226 nm, respectively.

λ excitation was chosen to be 226 nm, as the results obtained using this λ have the highest sensitivity and precision as demonstrated in the regression equation by the highest slope and lowest RSD.

The effect of different diluting solvents namely water, 0.1 M NaOH, 0.1 M HCl, 0.1 M acetic acid, 0.05 M H_2SO_4, methanol, ethanol, acetonitrile and buffers with different pH (2—8) on the fluorescence intensity of I is done. It was found that dilution with water gave the highest fluorescence intensity. Thus water was used as the chosen solvent.

A linear correlation was obtained between fluorescence intensity and concentration of the drug in a concentration range 0.32—3.20 $\mu g\,ml^{-1}$ with mean percentage recovery of 99.47±1.49. The following regression equation was calculated $I_f=41.739C+1.7826$ where I_f is fluorescence intensity at 391 nm, C is concentration in $\mu g\,ml^{-1}$.

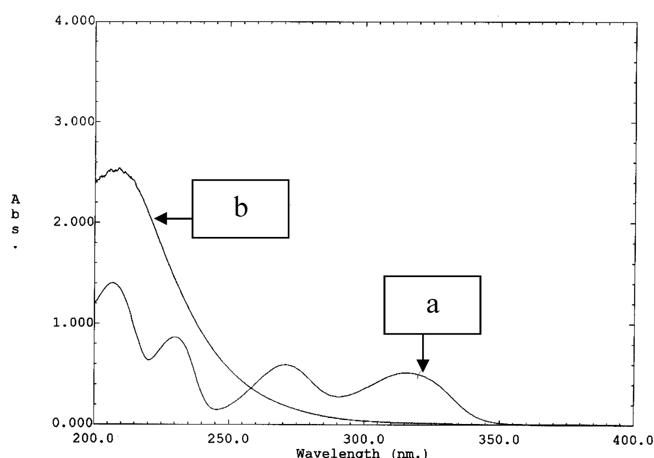


Fig. 1. Absorption Spectra of a Solution of Donepezil Hydrochloride 20 μg/ml (a) and Its Oxidative Degradate 20 μg/ml (b) in Water

To assess the validity of proposed fluorimetric method as stability indicating method, it was applied on laboratory prepared mixtures of intact drug and its oxidative degradate in different ratios. The results showed that, the drug could be determined in presence of up to 90% of its oxidative degradate with mean recovery % of 99.79±1.35.

The last method is a TLC-densitometric one depends on measurement of I after chromatographic separation from II at 315 nm as it is λ_{max} of I. The Rf values obtained were 0.78 for I and 0.5 for II upon using methanol : chloroform : 25% ammonia (16 : 64 : 0.1 by volume) as a mobile phase. Carrying out several trials showed that the best separation was achieved upon using the above mentioned mobile phase. A linear correlation was obtained between peak area and concentration of the drug in a linear range 2—15 μg/spot with mean percentage recovery of 99.79±0.92. The following regression equation was calculated and found to be: $A=11850C+57010$ where A is peak area, C is concentration in μg/spot.

The selectivity of the densitometric method upon analyzing mixture of I and II was determined and the results showed that I could be determined in presence of II up to 80% with mean recovery % of 98.93±1.37.

The proposed methods were successfully applied to the determination of donepezil hydrochloride in its pharmaceutical dosage form, and validated by standard addition technique which found to be 98.51±0.46, 98.14±0.45 and 99.67±0.46 for D^0, D^1 and D^2 methods, respectively.

Application of standard addition technique to the analysis of donepezil hydrochloride in tablets and recovery % were calculated from the corresponding regression equations and were found to be 97.79±1.50 and 97.87±1.01 for fluorimetric and densitometric methods, respectively. The excellent recoveries of the standard addition technique indicate good accuracy of the proposed methods and there is no interference from the excipients.

The high sensitivity attained by the proposed fluorimetric method allowed the determination of the drug in plasma. A

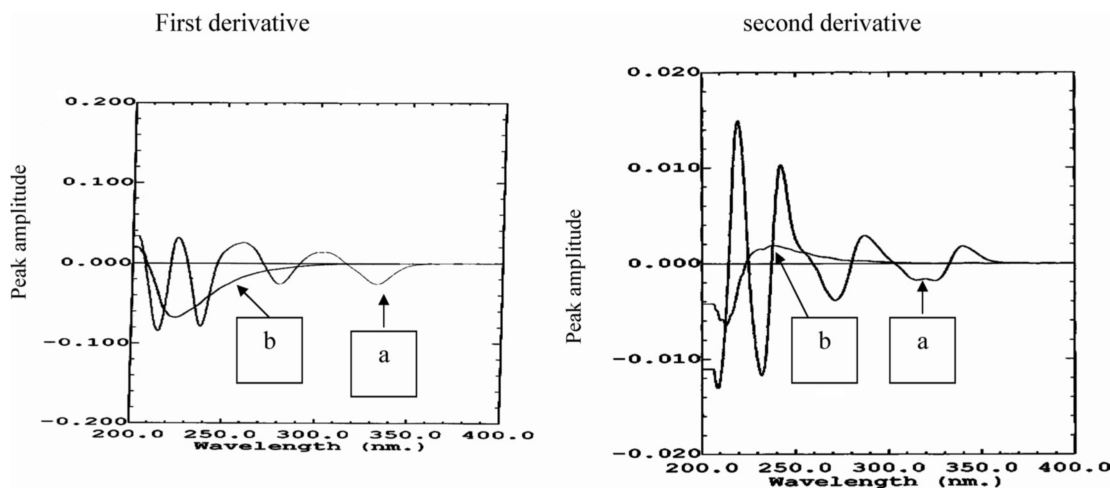


Fig. 2. First and Second Derivative Spectra of a Solution of Donepezil Hydrochloride 20 μg/ml (a) and Degradate 20 μg/ml (b) in Water

linear range was found to be 0.48—4.8 μg ml^{-1} with mean percentage recovery of 100.03±2.69 as shown in Table 1.

Method validation was performed according to USP[10] for all the proposed methods. Table 2 shows the results of accuracy, repeatability and reproducibility of the methods.

Also, the methods were compared by a reference method,[11] no significant difference was found for accuracy and precision upon statistical analysis.

Conclusion

Donepezil Hydrochloride could be determined by simple, sensitive and accurate stability indicating methods, economic and more selective which suggested their suitability for the routine analysis and quality control of pharmaceutical formulation.The proposed fluorimetric method shows the highest sensitivity of all the proposed methods which allows its use to determine the drug in human plasma at low concentrations.

Table 1. Results of Accuracy for Fluorimetric Assay of Donepezil Hydrochloride in Plasma[a]

Taken (μg ml^{-1})	Recovery (%)
0.48	100.00
1.12	99.11
1.60	105.00
2.72	97.06
4	100.25
4.8	98.75
Mean	100.03
S.D.	2.69
RSD%	2.69

a) Regression equation 31.465x−0.9349. Mean±S.D., n=6.

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11) Personal Communications with Pfizer Company.

Table 2. Assay Validation Sheet of the Proposed Methods for the Determination of Donepezil Hydrochloride

Parameter	D^0 at 315 nm for (I)	D^1 at 332.1 nm for (I)	D^2 at 340 nm for (I)	Fluorimetric method for (I)	Densitometric method for (I)
Accuracy (mean±S.D.)	100.02±0.77	99.71±0.94	100.80±1.43	99.47±1.49	99.79±0.92
Specificity	100.70±1.24	99.98±1.79	100.18±2.20	99.79±1.35	98.93±1.37
Precision					
Repeatability[a]	100.33±0.73	99.55±1.04	103.08±1.78	99.73±0.93	98.38±1.25
Intermediate precision[b]	100.60±0.78	98.94±0.54	101.35±0.36	99.06±1.91	100.40±1.25
Linearity					
Slope	0.0255	0.1317	0.0083	41.739	11850
Intercept	0.0067	−0.0007	0.0063	1.7826	57010
Correlation coefficient	0.9998	0.9998	0.9995	0.9993	0.9995
Range	8—56 μg ml^{-1}	8—56 μg ml^{-1}	8—56 μg ml^{-1}	0.32—3.2 μg ml^{-1}	2—15 μg/spot

a) The intraday (n=3), average of three different concentrations repeated three times within day. *b*) The interday (n=3), average of one concentration repeated three times in three successive days.