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Article

LC-PDA and LC-MS Studies of Donepezil Hydrochloride Degradation Behaviour in Forced Stress Conditions

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O objetivo deste trabalho foi estudar a estabilidade intrínseca da donepezila em diferentes condições de estresse forçado (meios ácido, alcalino e oxidante, exposição à luz e ao calor seco). A donepezila e seus produtos de degradação em meio ácido e alcalino foram separados e detectados usando cromatografia líquida de alta eficiência (HPLC) acoplada à espectrometria de massas e HPLC usando detector de arranjo de diodos. Após sete dias, a recuperação do fármaco em hidróxido de sódio 0,1 mol L^{-1} foi de aproximadamente 42%, sendo detectados três produtos de degradação, enquanto que em meio ácido clorídrico 0,1 mol L^{-1}, a recuperação da donepezila foi de aproximadamente 86%, sendo detectados três produtos de degradação. Deste modo, foi possível a proposição de um método indicativo da estabilidade rápido e seletivo usando HPLC em modo fase reversa para a análise de donepezila e seus produtos de degradação.

The aim of this work was to study the intrinsic stability of donepezil hydrochloride in conditions of forced degradation (acid stress, alkaline stress, oxidant stress, light exposure and dry heat). The degradation profile of donepezil was characterized by liquid chromatography-mass spectrometry (LC-MS) and high-performance liquid chromatography coupled with photodiode array detection (LC-PDA). According to the results, the degradation products were separated and detected in acid and alkaline solutions. After seven days at room temperature, the recovery of donepezil in alkaline solution (0.1 mol L^{-1} NaOH) was about 42%, and three degradation products were detected. In acid solution (0.1 mol L^{-1} HCl), the drug recovery was about 86%, and three degradation products were detected. Thus, it was possible to propose a rapid and selective stability-indicating assay method using reversed-phase liquid chromatography for analysis of donepezil and their degradation products.

Keywords: donepezil hydrochloride, intrinsic stability, degradation studies, liquid chromatography, mass spectrometry

Introduction

Acetylcholinesterase is an enzyme responsible for hydrolysing acetylcholine, and its inactivation can result in an excess of acetylcholine, and activation of the parasympathetic nervous system.[1] Donepezil (2-[(1-benzyl-4-piperidyl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one) is a drug classified as a selective reversible acetylcholinesterase inhibitor, being the most currently prescribed therapeutic agent for the treatment of Alzheimer's disease.[2] According to its chemical structure (Figure 1), donepezil is a lipophillic (log K_{ow} between 3.08 and 4.11) and basic drug with a tertiary amine group (pKa = 8.82). It is commercially available in immediate release formulations for oral use (tablets of 5 and 10 mg), but there is an interest in research of controlled released formulations including transdermal patches.[2-5]

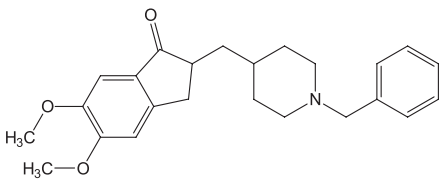

Figure 1. Chemical structure of donepezil. Molecular weight: 379.5 g mol^{-1}.

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The intrinsic stability of drugs can be evaluated by forced degradation studies in different stress conditions. For this, the degradation profile of drug must be characterised using a validated stability-indicating assay method. Stability-indicating assay methods have the selectivity as their main attribute in order to separate and detect the drug and its degradation products. These analytical methods can be applied to estimate and confirm the expiration date of pharmaceutical formulations in stability studies of commercial drugs (accelerated and long-term stability studies), and as an auxiliary tool in the design of new formulation.[6-8] In the routine analysis, the stability-indicating assay methods can be highlighted to analyse whether the drug is degraded during the manufacture process or during the storage. The amount of drug in pharmaceutical product, and the percentages of degradation products can be both evaluated by stability-indicating assay methods. Despite the importance of these assays, the development step of the analytical methods is not be a simple task, requiring several laboratory tests to ensure the separation and detection of the degradation products.[6,9,10]

The International Conference of Harmonization (ICH) defines some key points to perform stability studies.[11,12] The guidance Q1A discusses on stability testing for new drugs and formulations, emphasizing the changes that could happen during storage and can modify the quality, safety and efficacy. In this way, it is recommended that the forced degradation studies include various stress conditions such as extreme pH values, oxidative stress, thermal stress and light exposure. Stress tests can help to meet the most likely routes of degradation for each drug. The guidance Q3B refers to impurities in pharmaceutical products, and limits in which degradation products should be reported, identified and qualified, according to the daily dose of the active pharmaceutical ingredient are recommended. Recently in Brazil, the National Health Surveillance Agency (Anvisa) published the resolution RDC No. 58 that defines guidelines on conducting of the forced degradation studies.[13]

Analytical methods have been described for donepezil analysis.[14-17] Impurities originating from the steps of synthesis of donepezil were already well characterized, but the method validation was not described.[18] Preliminary studies on the stability of donepezil in forced degradation studies have been reported.[19,20] However, the drug degradation profile was not characterised in these works, and a complete separation and detection of the degradation products was not performed.

In this study, we investigated the intrinsic stability of donepezil hydrochloride using a developed and validated stability-indicating assay method. For this, the drug was subjected to forced stress conditions and the samples were analysed by high performance liquid chromatography (HPLC) using a diode array detector (LC-PDA) and liquid chromatography coupled to mass spectrometry (LC-MS). In this way, unknown degradation products of donepezil were separated and detected in different stress conditions.

Experimental

Chemical, standards and reagents

All reagents were of analytical grade. Acetic acid, ammonium hydroxide, disodium hydrogen phosphate, hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), phosphoric acid, potassium chloride, sodium chloride, sodium dihydrogen phosphate, sodium hydroxide (NaOH), and triethylamine were purchased from Vetec (Rio de Janeiro, Brazil). Donepezil hydrochloride standard (Lot G0K230, 99.8%) was purchased from United States Pharmacopeia (Rockville, USA). Donepezil hydrochloride in raw material was supplied by Megafine Pharma Limited (Maharashtra, India). Methanol for liquid chromatography was acquired from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade water was prepared by Milli-Q reverse osmosis (Millipore, Billerica, MA, USA).

Instrumental

Samples were analysed by a series LC-10A HPLC from Shimadzu (Kyoto, Japan), consisting of a LC-20AD pump, a CTO 20-A column oven, a DGU 14-A degasser, a SPD-M10AVP diode array detector, a SIL 20-A HT auto-sampler, and a SCL-10 AVP controller. LC-MS studies were carried out on a Shimadzu LCMS-8030 mass spectrometer coupled to a LC-10A HPLC from Shimadzu consisting of a LC-20AD pump, a CTO 20-A pump, a DGU 14-A degasser, a SIL 20-A HT auto-sampler, a SPD 20-A ultraviolet detector, and a SCL-10 AVP controller.

LC-PDA analysis

The UV spectrum in the range of 190-370 nm was investigated in order to determine the similarity index of donepezil peak in samples of forced degradation in comparison with a standard solution. The assay was performed using a Phenomenex (Torrance, CA, USA) reversed-phase C_{18} Acqua end-capping column 150.0 × 4.6 (i.d.) with particles of 4 μm. Phenomenex pre-column C_{18} Acqua 125 4.0 × 3.0 mm (i.d.) was also used. The mobile phase was a mixture of monobasic potassium phosphate buffer (0.5 mmol L^{-1}) pH 3.0 with 0.5% of triethylamine and methanol (55:45). The mobile phase flow

rate was 1.0 mL min^{-1}, the injection volume was 50 µL and UV detection of donepezil and their degradation products was carried out at 268 nm. The samples were filtered using a 0.45-µm syringe filter composed of hydrophilic polytetrafluoroethylene (PTFE). The retention time of the donepezil was approximately 7.9 min. The run time was 20 min.

LC-MS analysis

Forced stress samples were analysed by LC-MS in positive electrospray ionization mode (ESI +). Data acquisition was performed in the total ion current mode (TIC). The mass-to-charge ratio (*m/z*) was investigated in the range of 100-500. The assay was performed in order to investigate the molar mass of the unknown degradation products. A Phenomenex (Torrance, CA,USA) reversed-phase C_{18} Acqua end-capping column 150.0 × 4.6 (i.d.) with particles of 4 µm, and a Phenomenex pre-column C_{18} Acqua 125 4.0 × 3.0 mm (i.d.) were used in the system. The mobile phase was a mixture of 0.2% acetic acid pH 3.2 and methanol (60:40). The mobile phase flow rate of was 0.4 mL min^{-1} and the injection volume was 5 µL. The samples were filtered using a 0.45-µm syringe filter composed of hydrophilic PTFE. The retention time of the donepezil was approximately 18.0 min. The run time was 50 min in order to evaluate possible strongly retained compounds.

Forced degradation studies

Different conditions of forced degradation were evaluated according to ICH Q1A guideline.[11] Donepezil solutions, in the concentration of 100.0 µg mL^{-1}, were subjected to thermal stress (70 °C) in ultrapure water, 2 mol L^{-1} HCl and 2 mol L^{-1} NaOH for 48 h to give a higher degradation rate. Donepezil solutions in the same theoretical concentration were also subjected to alkaline stress (0.1 mol L^{-1} NaOH), acid stress (0.1 mol L^{-1} HCl), ultrapure water, light exposure (directly on the daylight) and oxidative stress (3% H_2O_2) for seven days at room temperature to give a lower degradation rate. Samples of the raw material were subjected to dry heat (oven at 85 °C for seven days).

The formation of degradation products in alkaline (2 mol L^{-1} NaOH) and acid (2 mol L^{-1} HCl) conditions at 70 °C was studied at different times (0.5, 1, 2, 3 and 6 h). The conditions were selected due to detection of the degradation products. The samples were neutralised, and analysed by LC-PDA and LC-MS.

The stability of the drug in aqueous solution (isotonic phosphate buffer 20 mmol L^{-1}, pH 7.4) at 37 °C simulating physiological conditions was also evaluated. The samples were analysed at different times (up to 150 h) in three replicates.

The percentage of recovery of the drug (R) was determined in all forced degradation studies according to equation 1:

$$R = \frac{Experimental\ concentration}{Theoretical\ concentration} \quad (1)$$

The percentage of each degradation product in the samples was determined by the ratio of the areas between the peak of the donepezil degradation product in the sample solution and the area of donepezil peak in standard solution.

Validation studies

The validation assay was performed according to recommendations of the ICH Q2B guidance.[21] The samples were prepared in a mixture of methanol and water (1:1).

Selectivity assay was evaluated using standard solutions and samples from forced degradation studies. All solutions in theoretical concentration of 100.0 µg mL^{-1} were injected into the chromatographic system. The purity of chromatographic peaks of donepezil was determined by comparison of ultraviolet spectral data (190-370 nm) with spectral data of donepezil standard. Diluents were analysed in the absence of drug to assess possible interferences in the retention time of donepezil.

Linearity was evaluated to demonstrate the proportionality relationship between the concentration of the standard solutions of donepezil and the detector response (peak area). For this, a standard stock solution of donepezil in methanol (10.0 mg mL^{-1}) was prepared and successive dilutions were performed so as to obtain standard solutions at concentrations of 0.5, 1, 5, 10, 50, 100, and 200 µg mL^{-1}. The assay was performed in three replicates and the correlation coefficient (*r*) and the equation line (y = ax + b) were calculated, which *a* corresponds to the slope and *b* is the linear coefficient (intercept).

Precision was established using six replicates of donepezil standard solutions at three concentration levels (5, 50, and 200 µg mL^{-1}). Analyses were performed on three consecutive days. The intraday and interday precision were established as the dispersion of the measurements around the average value and expressed mathematically by the relative standard deviation (RSD), determined by equation 2:

$$RSD = \frac{Standard\ deviation}{Mean\ value} \times 100 \quad (2)$$

Accuracy was determined using three replicates of standard solutions of donepezil at three concentration levels (5, 50, and 200 μg mL^{-1}). Analyses were performed on three consecutive days. The intraday and interday accuracy were presented as relative error (RE) and were calculated by equation 3, as follows:

$$RE = \frac{\textit{Experimental concentration} - \textit{Theoretical concentration}}{\textit{Theoretical concentration}} \times 100 \quad (3)$$

The limit of quantification (LOQ) was determined as the lowest concentration that showed accuracy and precision within the linear range, being determined by injecting standard solutions of decreasing concentrations from 1.0 to 0.01 μg mL^{-1}. The limit of detection (LOD) of donepezil was determined as the minor concentration level that was detected.

Results and Discussion

The development of the analytical method was based on chromatographic conditions proposed by Park *et al.*,[3] wherein the organic solvent used in the mobile phase was changed from acetonitrile to methanol. Other parameters such as wavelength, injection volume and preparation of buffer for mobile phase were subsequently adjusted to optimise the method. The phosphate buffer pH 3.0 in the mobile phase was selected in order to analyse the basic drug in the ionised specie (pKa = 8.82). Donepezil is a lipophilic drug (log $K_{ow} > 3$) and analysing the ionised drug in the reversed-phase columns can decrease the retention time, since the polarity of the molecule is increased and the retention is decreased. The addition of triethylamine in the mobile phase was performed to reduce the interactions of tertiary amine grouping with ionised residual silanols from the stationary phase. This strategy can improve the asymmetry factor of peaks.[22] The parameters of the system suitability of the chromatographic method were shown in Table 1. System suitability parameters are important in order to verify if the chromatographic system are adequate for the analysis to be done.[23]

Table 1. System suitability results according to the United States Pharmacopeia,[23] $n = 6$

Parameter	Result
Retention factor (k')	4.5
Tailing factor	1.127
Theoretical plates number (N)	2198
Retention time / min	7.9

Forced degradation studies are an important tool in determining the selectivity of the analytical method. These studies allow obtaining information about the intrinsic stability of the drug, as well as the stability of pharmaceutical products. During the development and storage of these formulations, the drug can be susceptible to temperature variations, hydrolysis, oxidation or other chemical reactions. These factors can compromise the stability of the pharmaceutical product. Physical interactions or even chemical reactions with excipients and packaging materials can modify the stability of the formulation, compromising its efficacy and safety.[8,10,24]

The forced degradation studies were firstly conducted at 70 °C, at three conditions: water, 2 mol L^{-1} HCl, and 2 mol L^{-1} NaOH. According to the results (Table 2), donepezil remained stable in water at 70 °C, but drug degradation was observed in acid and alkaline conditions. Donepezil showed a lower stability in the alkaline condition. The peaks associated with degradation products could be separated from the main peak of donepezil (Figure 2). Additionally, the peak purity of donepezil was assured in forced stress conditions by similarity index using LC-PDA.

Table 2. Recovery of donepezil amounts in forced stress conditions, $n = 3$

Stress condition	Recovery ± SD[a] / %	Similarity index[b]
Water[c]	104.07 ± 4.55	0.993021
2 mol L^{-1} HCl[c]	80.72 ± 1.25	0.999194
0.1 mol L^{-1} HCl[d]	86.27 ± 4.95	0.998484
2 mol L^{-1} NaOH[c]	29.27 ± 2.14	0.999967
0.1 mol L^{-1} NaOH[d]	41.95 ± 1.82	0.999901
3% Hydrogen peroxyde[d]	90.22 ± 3.09	0.997127
Light exposure[d]	101.05 ± 4.38	0.999920

[a]SD, standard deviation; [b]determined by LC-DAD; [c]recovery of donepezil after 48 h at 70 °C; [d]recovery of donepezil after seven days at 25 °C.

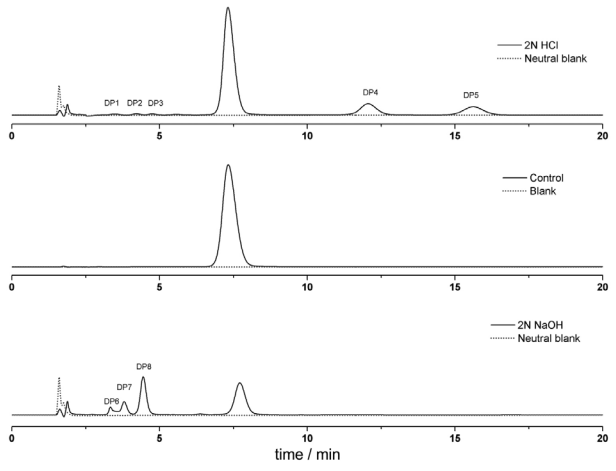

Figure 2. Chromatograms of donepezil at forced degradation studies at 70 °C for 48 h by LC-DAD. Results were shown in comparison with standard solution (control) at 100.0 μg mL^{-1}.

In the next step, the degradation of the drug was evaluated at room temperature after seven days. The samples were analysed, and the recovery of donepezil was determined (Table 2). The chromatograms obtained by LC-PDA in forced stress conditions at room temperature were shown in Figure 3. Degradation products were detected in the acid (0.1 mol L^{-1} HCl) and alkaline (0.1 mol L^{-1} NaOH) conditions. In the H_2O_2 3% solution, the recovery of the drug was 90.22%, but degradation products were not detected. These results can suggest that minor amounts of degradation products can be formed in oxidative stress. The drug solution was stable when exposed to daylight at room temperature (recovery of 101.05% ± 4.38), and also when the raw material of donepezil hydrochloride was directly exposed to dry heat at 85 °C (recovery of 99.89% ± 1.21).

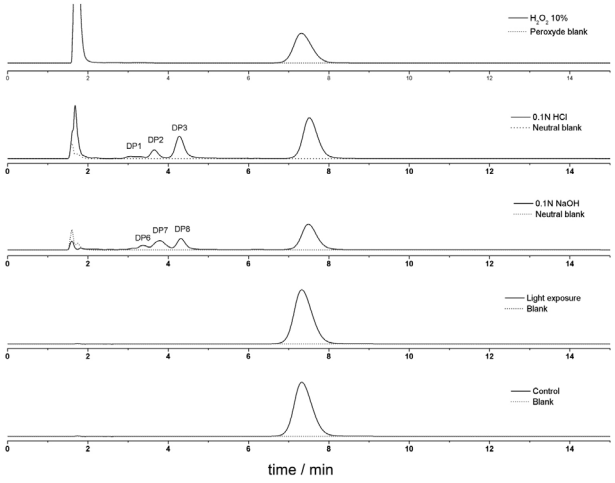

Figure 3. Chromatograms of donepezil at forced degradation studies, at room temperature for seven days, by liquid chromatography using a photo diode array. Results were shown in comparison with standard solution (control) at 100.0 μg mL^{-1}.

The acid stress condition at room temperature led to detection of three major degradation products (denominated as DP1, DP2, and DP3) that were eluted before the retention time of the major peak (donepezil), suggesting that these compounds have a higher polarity than the donepezil (Figures 2 and 3). Meanwhile, in the acid stress conditions (2 mol L^{-1} HCl at 70 °C or 0.1 mol L^{-1} HCl at room temperature), three degradation products were detected before the major peak. However, two additional degradation products (denominated as DP4 and DP5) were detected in the 2 mol L^{-1} HCl at 70 °C after the retention time of the major peak. Probably, DP4 and DP5 have a minor polar character than donepezil. No differences were observed in the chromatographic profile of the samples in alkaline stress conditions (2 mol L^{-1} NaOH at 70 °C or 0.1 mol L^{-1} NaOH at room temperature). Three major degradation products were detected, and they are denominated as DP6, DP7, and DP8 (Figure 2 and 3).

The analysis of donepezil samples in forced stress conditions was also conducted by LC-MS (ESI +) in TIC mode (Table 3). In this regard, studies by LC-MS were carried out using ESI + due to the major drug ionisation. Donepezil has a basic character containing a tertiary amine group, and it is in cationic form in the acidic pH of the mobile phase. The column used in LC-MS assay was the same employed in LC-PDA studies in order to maintain the same elution order of the sample compounds. The donepezil has a molar mass 379 g mol^{-1}, and in its cationic form has a molar mass of 380 g mol^{-1} (Table 3). In the same way, the molar mass of the degradation products corresponds to their cationic forms. The chromatograms obtained by LC-MS (ESI +) in TIC mode for acid and alkaline conditions were shown in Figure 4.

Table 3. Characterisation of degradation products by LC-MS (ESI + in TIC mode) in forced degradation studies: acid solution (0.1 mol L^{-1} HCl) and alkaline solution (0.1 mol L^{-1} NaOH) at room temperature after seven days

Acid solution (0.1 mol L^{-1} HCl)			Alkaline solution (0.1 mol L^{-1} NaOH)		
Peak	RRT[a]	*m/z*[b]	Peak	RRT[a]	*m/z*[b]
DP1[c]	0.42	248	DP6[c]	0.45	410
DP2[c]	0.49	396	DP7[c]	0.51	396
DP3[c]	0.57	412	DP8[c]	0.57	412
Donepezil	1.00	380	Donepezil	1.00	380
DP4[c]	1.62	414	–	–	–
DP5[c]	2.08	428	–	–	–

[a]Relative retention time; [b]mass-to-charge ratio of molecular ion peak; [c]degradation product.

According to the results (Table 3 and Figure 4), DP1 (in the acidic condition) and DP6 (in alkaline condition) were the first eluted compounds in each condition. The molecular ion peaks and relative relation times for DP1 and DP6 were different. These results suggest that different compounds are formed depending of the solution pH. However, DP2 and DP7 are compounds formed in different conditions, but with the same molecular ion peak and with similar relative retention times. The same behavior was observed to DP3 and DP8, suggesting that these degradations products could be the same molecules or related compounds.

The evaluation of the degradation products by LC-MS allows the determination of the mass of molecular ion peak of the degradation products of donepezil. If the degradation products were isolated, the structural elucidation of these molecules can be achieved using other techniques as nuclear magnetic resonance, infrared spectroscopy, and the analysis

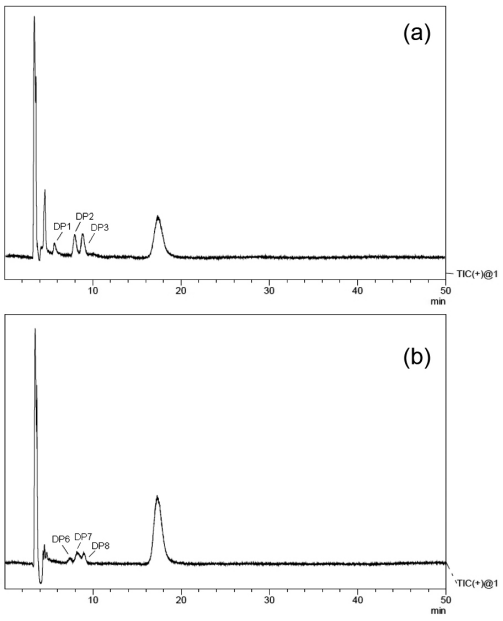

Figure 4. Total ion current (TIC) chromatograms of donepezil at forced degradation studies at room temperature for seven days by liquid chromatography coupled to mass spectrometry analysis (LC-MS). Results of samples in (a) 0.1 mol L^{-1} HCl and (b) 0.1 mol L^{-1} NaOH were shown.

of fragmentation pattern by mass spectrometry.[10] However, in this work, the main objective was to develop an analytical method to assess the intrinsic stability of the drug. In this case, the method should have the property to detect the formation of the degradation products in order to evaluate the drug stability. In this way, the analytical method showed satisfactory separation and detection of donepezil in the presence of their degradation products, and it demonstrates that can be a useful tool in the evaluation of the stability of pharmaceutical products containing donepezil.

Pappa *et al.*[19] evaluated the donepezil intrinsic stability in 1 mol L^{-1} HCl, 1 mol L^{-1} NaOH and 30% H_2O_2 for 4 h under reflux conditions (thermal stress). The authors also evaluated the drug stability to daylight exposure for 24 h. In alkaline condition and 30% H_2O_2 there was around 50% of drug degradation. In other conditions, there was not drug degradation. Similar results were obtained by Kafkala *et al.*, with major donepezil degradation under alkaline (1 mol L^{-1} NaOH for 1 h) and oxidative stress (30% H_2O_2 for 24 h)[20]. The lower stability of donepezil in alkaline solutions is in agreement with the results found in our experiments. However, donepezil was more stable under oxidative conditions in our assays while drug degradation was found in other works.[19,20] These degradations could be associated to the oxidative stress under a higher concentration of hydrogen peroxide, increasing the degradation rate of the drug. Stress conditions with a higher degradation rate are only interesting to perform a screening of the drug stability, because the degradation products could be degraded again in these conditions. In this way, the degradation products formed under lower degradation rates are more interesting, since that during stability studies are expected to find major amounts of primary degradation products.

Forced degradation studies of donepezil were performed at different time points (30 min to 6 h) in acid and alkaline stress condition (2 mol L^{-1} HCl and 2 mol L^{-1} NaOH) at 70 °C. The results (Table 4) show that in values of extreme pH (acid or alkaline) were firstly formed two degradation products. Donepezil shows less stability in alkaline condition according to the previous results (Table 2). The amount of DP7 initially found in alkaline condition was 0.41% in 30 min, with consequent decrease to 0.31% at 1 h and 0.18% at 2 h. These results suggest that this degradation product exhibits a lower stability, and may be associated with the formation of secondary degradation products. Increasing amounts of DP8 were determined in alkaline condition. At the time of 30 min, the amount of DP8 was 1.76% and reach to 6.48% in 6 h. These results may suggest that DP8 is a primary degradation product and it is quickly formed in significant amounts during forced degradation studies. This degradation probably could be found in stability studies. In the acidic condition, the formation of degradation products was observed only after 3 h. Lower amounts of degradation products were observed in acidic condition (DP2 and DP3) in relationship to alkaline condition (DP7 and DP8).

The analytical validation assays showed satisfactory results for determination of donepezil.[21] The linearity

Table 4. Donepezil degradation in alkaline stress (2 mol L^{-1} NaOH) and acid stress (2 mol L^{-1} HCl) at 70 °C in different times

time / h	Alkaline stress			Acid stress		
	R^a / %	$DP7^b$ (RRT^c = 0.51)	$DP8^b$ (RRT^c = 0.57)	R^a / %	$DP2^b$ (RRT^c = 0.49)	$DP3^b$ (RRT^c = 0.57)
0.5	97.83	0.41	1.76	100.24	ND[d]	ND[d]
1.0	96.39	0.32	3.29	99.01	ND[d]	ND[d]
2.0	95.31	0.18	4.51	99.05	ND[d]	ND[d]
3.0	94.71	0.22	5.07	99.18	ND[d]	0.34
6.0	93.30	0.22	6.48	98.95	0.02	0.14

[a]Recovery; [b]degradation product; [c]relative retention time, [d]not detected.

of the method was carried out in the range from 0.5 to 200.0 μg mL^{-1}. The determined equation was 93928.50719 x + 8805.17207 and the correlation coefficient was equal to 0.999983.

The LOQ was established as 0.1 μg mL^{-1}, with accuracy and intraday precision (n = 6) of 102.04% and 1.89% respectively. The results of accuracy and interday precision for the LOQ in three different days (n = 6, for each day) were 102.91% and 1.29% respectively. The LOD was experimentally determined as 0.01 μg mL^{-1} by successive injections of diluted standard solutions.

The results of the accuracy and precision of the analytical method were presented in Table 5. The results were satisfactory according to ICH recommendations.[21]

Table 5. Precison and accuracy of analytical method by LC-PDA, n = 6

Theoretical concentration / (μg mL^{-1})	Accuracy (R[a])		Precision (RSD[b])	
	Intraday	Interday	Intraday	Interday
5.1	97.40	96.95	1.45	0.22
50.6	100.30	100.51	0.63	0.21
202.4	100.43	100.81	0.37	0.13

[a]Recovery; [b]relative standard deviation.

Donepezil was stable for up to 150 h in isotonic phosphate buffer (pH 7.4) at 37 °C simulating physiologic conditions. The results (Table 6) were not significantly different to the values determined at initial time (Student's test, P > 0.05). This assay is important during *in vitro* release studies, in which drug is exposed to simulated biological fluids.

Table 6. Stability of donepezil in isotonic phosphate buffer (pH 7.4) at 37 °C, n = 3

	Initial solution	24 h	96 h	150 h
Mean / %	100.00	100.25	99.87	98.89
RSD[a]	0.05	0.03	0.13	0.01

[a]Relative standard deviation (%).

Conclusions

The intrinsic stability of donepezil hydrochloride was assessed by a developed method using LC-PDA and LC-MS. The analytical method using reversed-phase liquid chromatography allowed the rapid determination of donepezil, even in the presence of their degradation products formed in forced degradation studies. Degradation products were detected only in the acidic and alkaline conditions. The drug was less stable in the alkaline stress condition, with a minor drug recovery. In this way, a stability-indicating assay method for donepezil was developed in this work. The analytical method proved to be a useful tool for the determination of the drug, allowing the separation and detection of their degradation products, which is of great importance in stability studies and in the research of new formulations containing donepezil.

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