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LIQUID CHROMATOGRAPHY AND LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF DONEPEZIL DEGRADATION PRODUCTS

Article Highlights
- Forced degradation study was performed on Donepezil hydrochloride in solution
- Acid and alkali hydrolysis, oxidation mediated by hydrogen peroxide and thermal treatment were used
- Parent HPLC method was converted to LC-MS compatible method
- Degradation products observed were monitored by LC-MS

Abstract

This study describes the investigation of degradation products of donepezil (DP) using a stability-indicating RP-HPLC method for determination of donepezil, which is a centrally acting reversible acetylcholinesterase inhibitor. In order to investigate the stability of the drug and formed degradation products, a forced degradation study of the drug sample and finished product under different forced degradation conditions was conducted. Donepezil hydrochloride and donepezil tablets were subjected to stress degradation conditions recommended by the International Conference on Harmonization (ICH). Donepezil hydrochloride solutions were subjected to acid and alkali hydrolysis, chemical oxidation and thermal degradation. Significant degradation was observed under alkali hydrolysis and oxidative degradation conditions. Additional degradation products were observed under the conditions of oxidative degradation. The degradation products observed during forced degradation studies were monitored using the developed high performance liquid chromatography (HPLC) method. The parent method was modified in order to obtain LC-MS compatible method, which was used to identify the degradation products from forced degradation samples using high resolution mass spectrometry. The mass spectrum provided the precise mass from which the molecular formula of the drug substance and formed degradation products was derived, and proved the specificity of the method unambiguously.

Keywords: donepezil hydrochloride; forced degradation; stability-indicating HPLC method; liquid chromatography-mass spectrometry.

Donepezil hydrochloride is a new anti-Alzheimer drug. It is a potent acetylcholine esterase inhibitor, chemically known as 2,3-dihydro-5,6-dimethoxy-2-[[1--(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one hydrochloride (also known as aricept). It has an empirical formula of $C_{24}H_{29}NO_3 \cdot HCl$ and molecular weight of 415.96. Donepezil hydrochloride was the first piperidine type reversible based inhibitor of the enzyme acetylcholinesterase (AChE). Its main therapeutic use is in the treatment of Alzheimer's disease where it is used to increase cortical acetylcholine. Donepezil is postulated to exert its therapeutic effect by enhancing cholinergic function. *In vitro* studies have demonstrated that donepezil hydrochloride has a significantly greater degree of selectivity of AChE in the central nervous system than for butyrylcholinesterase in the periphery [1-3]. The molecular structure of donepezil is given in Figure 1.
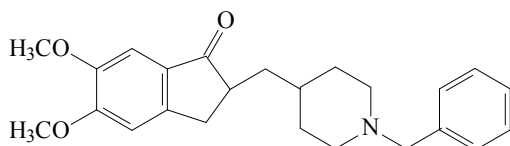
Figure 1. The structure of donepezil.

The different analytical techniques reported so far for analysis of this drug in biological samples and in pharmaceutical formulations include electrophoresis [4] and UV-Visible spectrophotometry [5]. Several HPLC methods for assay and LC-MS method for analysis of donepezil hydrochloride have been previously published [6-9].

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. Organic impurities can arise during the manufacturing process and storage of the drug substances and drug products under the influence of various environmental factors and criteria for their acceptance up to certain limits are based on the pharmaceutical studies or known safety data. The identification and qualification of degradation products in pharmaceuticals is important since impurities may cause the undesirable effects on the patients and on the other hand may have influence on quality, safety and efficacy of the drug product [10,11].

Knowledge of the stability of a molecule helps in selecting proper formulation and packaging, as well as providing proper storage conditions and shelf life, which is essential for registration of pharmaceutical for human use.

HPLC methods should be able to separate, detect, and quantify the drug-related degradants that can form on storage or during manufacture of drug product, plus detect and quantify any drug-related impurities that may be introduced during drug synthesis [12]. Forced degradation is a process that involves degradation of drug products and drug substances under more severe conditions than those used in accelerated stability studies, and thus generate degradation products that can be studied to determine the stability of the molecule [13].

Many authors have reported the studies on degradation of donepezil [14-22]. Pappa *et al.* observed degradation of donepezil in 1 mol/L HCl, 1 mol/L NaOH and 30% of H_2O_2 under reflux. Degradation products were not characterized [17].

Dutt *et al.* studied degradation of donepezil hydrochloride under acid/base and oxidation conditions. They observed 9.3% of degradation under used base

conditions and no significant degradation under acid or oxidation conditions [18].

Al-Zoman *et al.* found that around 8% of the DP enantiomers was degraded under acid stress for 48 h. Whereas, under alkaline stress conditions 14% degradation of DP enantiomers was observed. The complete degradation of the DP enantiomers under oxidative degradation treatment using 30% H_2O_2 was achieved and 28% when using 3% H_2O_2 for 48 h. It was found that under heat stress conditions at 60 °C DP enantiomers were largely unstable and degradation of 60% was observed [19].

Chothe *et al.* were investigated stability of donepezil hydrochloride using stability indicating high performance liquid chromatography. Stability study was performed in 1 mol/L HCl, 2 mol/L NaOH, 6% H_2O_2, neutral, photolytic and dry heat conditions. The authors observed fast hydrolysis in alkaline condition in comparison to oxidative and neutral conditions [20].

Abbas *et al.* performed forced degradation study according to the ICH guidelines for acidic, alkaline and oxidative conditions and photo degradation. It was found that donepezil hydrochloride is stable under acidic and alkaline conditions but liable to degradation with hydrogen peroxide [21].

In addition, the oxidative ability of donepezil hydrochloride, was examined at a gold electrode in pH 3 phosphate buffer solution using cyclic linear sweep voltammetry. Electrochemical degradation was performed for predicting its oxidative stability as well as for recognition of the involvement of the oxidative process in the formation of the hydroxyl derivative of donepezil. The identification and characterization of the major product formed during the potential cycling and isolated by preparative high performance liquid chromatography, was performed by high resolution mass spectrometry and 1D and 2D nuclear magnetic resonance spectroscopy. Hydroxy donepezil derivative formed by hydroxylation reaction is identified as the major electrochemical oxidation product from donepezil [22].

The probable degradation products of drug substance can be produced using certain stress conditions like hydrolytic (acidic, basic, *etc.*), oxidative, thermal, photolytic, *etc*. The ICH guideline states that stress testing is intended to identify the likely degradation products, which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used [23,24].

The aim of this work is the investigation of forced degradation behavior of donepezil hydrochloride under various stress conditions using reversed

phase liquid chromatography and liquid chromatography coupled with high resolution mass spectrometry technique. To our present knowledge, no forced degradation study is reported which includes analysis of degradation products of donepezil hydrochloride using HPLC method coupled with high resolution mass spectrometry technique.

EXPERIMENTAL

Materials and methods

Donepezil hydrochloride and donepezil hydrochloride chemical reference standard (CRS), were kindly provided from Hemofarm A.D. (Vršac, Serbia). HPLC grade of methanol and acetonitrile were purchased from J.T. Baker, potassium dihydrogen orthophosphate, orthophosphoric acid, ammonium acetate, hydrochloric acid, hydrogen peroxide were purchased from Merck. Sodium hydroxide was purchased from J.T. Baker. All chemical used were of analytical grade. For preparing solution of ammonium acetate purified water was used.

Apparatus

HPLC analysis were performed using a HPLC system consists of an Agilent 1100 series vacuum degasser, G1311A quaternary pump, G1329A auto sampler with column oven and G1316A PDA Detector. Separation was achieved on C18 250 mm×4.6 mm, 5 μm column (Wakosil II 5C 18RS) at the flow rate of 1.0 mL/min. Column temperature was 25 °C. The mobile phase consists of A (57%) and B (43%). The mobile phase A was phosphate buffer (2.72 g of potassium dihydrogen orthophosphate with 5 mL triethylamine in 1000 mL water adjusted to pH 5.0±0.05 using 20% aqueous orthophosphoric acid) and mobile phase B was pure methanol. The injection volume was set to 20 μL. These chromatographic conditions were employed for testing of donepezil HCl degradation products in bulk electrolyte at detection wavelength 230 nm.

LC-MS Analysis

For LC-MS analysis of the selected degraded samples, the parent method was modified in order to have LC-MS compatible method. Potassium dihydrogen orthophosphate was replaced with 10 mmol ammonium acetate solution in the aqueous component of the mobile phase. Aqueous phase and methanol proportion was kept same, *i.e.*, 57:43 and apparent pH adjusted at 5.0 with glacial acetic acid. The mobile phase was run at a flow rate of 1.0 mL/min on the same column. These chromatographic conditions eluted the drug as a sharp peak at 18 min

and also resolved the degradation products formed under varied forced conditions.

HPLC system (Agilent 1200 Series, Agilent Technologies) equipped with degasser, autosampler, Wakosil II 5C 18RS column (250 mm×4.6 mm i.d., 5 μm particle size) and DAD detector coupled with 6210 time-of-flight LC-MS systems (Agilent Technologies) was used. An isocratic MS compatible method and the mobile phase consisted of 2.3 g/L of ammonium acetate solution-methanol (57:43, *V*/*V*) was used. The flow rate was maintained at 1.0 mL/min. The injection volume was 20 μL and the column temperature was maintained at 25 °C. The chromatograms were monitored at 230 nm. A personal computer system running Mass Hunter Workstation software was used for data acquisition and processing.

The positively charged molecular ions were obtained with electrospray ionization (ESI) at atmospheric pressure: the eluted compounds were mixed with nitrogen in the heated nebulizing interface, and polarity was tuned to positive with the following ESI parameters: capillary voltage 4.0 kV, gas temperature 350 °C, drying gas flow rate 12 L/min, nebulizer pressure 45 psig (310.26 Pa), and fragmentor voltage 70 V. Mass spectra were acquired over an *m*/*z* range of 100-1500.

Procedure for forced degradation study

Preparation of sample solution

Thermal degradation. Investigation of the degradation of donepezil hydrochloride under the influence of the heat is carried out by the following procedure:

Accurately weighed 100 mg of donepezil hydrochloride (or equivalent of finely powdered donepezil tablets) was heated for 72 h at a temperature of 105 °C on the heated plate. Then, accurately weighed 25 mg of thermal treated sample of donepezil hydrochloride (or equivalent of finely powdered donepezil tablets) was transferred into volumetric flask and diluted to 50 mL with mobile phase.

Acid hydrolysis. Investigation of the degradation of donepezil hydrochloride under acidic conditions in solution was performed using the following procedure:

Accurately weighed 25 mg of donepezil hydrochloride (or equivalent of finely powdered donepezil tablets) was transferred into 50 mL volumetric flask, 5 mL of 5 mol/L HCl was added and the solution was heated for 60 min at 80 °C. The solution was thereafter neutralized with 5 mL of 5 mol/L NaOH. The solution was cooled to room temperature and diluted to 50 mL with mobile phase.

Alkaline hydrolysis. Investigation of the degradation of donepezil hydrochloride under alkaline conditions in solution was performed using the following procedure:

Accurately weighed 25 mg of donepezil hydrochloride (or equivalent of finely powdered donepezil tablets) was transferred into a 50 mL volumetric flask. In the first experiment, 5 mL of 0.1 mol/L NaOH was added and the solution was stored at room temperature for 8 h. Thereafter the solution was neutralized with 5 mL of 0.1 mol/L HCl. The second experiment was carried out using 5 mL of 5 mol/L NaOH and the solution was heated for 30 minu at 80 °C. The solution was thereafter neutralized with 5 mL of 5 mol/L HCl. For LC-MS analysis donepezil hydrochloride was treated with 5 mL of 5 mol/L NaOH and the solution was heated for 1 h at 80 °C, after which the solution was neutralized with 5 mL of 5 mol/L HCl.

In third experiment donepezil hydrochloride was treated with 5 mL of 5 mol/L NaOH at reflux for 8 h. Thereafter the solution was neutralized with 5 mL of 5 mol/L HCl.

After neutralization, all solutions were cooled to room temperature and diluted to 50 mL with mobile phase.

Oxidative degradation. Investigation of the oxidative degradation of donepezil hydrochloride through the use of hydrogen peroxide in the solution is carried out by the following experiments: accurately weighed 25 mg of donepezil hydrochloride (or equivalent of finely powdered donepezil tablets) was transferred into a 50 mL volumetric flask. In the first experiment, 5 mL of 3.0% H_2O_2 was added, and the solution was heated for 30 min at 80 °C. The solution was cooled to room temperature and diluted to 50 mL with mobile phase. For LC-MS analysis, donepezil hydrochloride was treated with 3.0% H_2O_2 and the solution was heated for 1 h at 80 °C and then diluted to 50 mL with mobile phase.

In second experiment donepezil hydrochloride was treated with 30% H_2O_2 at reflux for 8 h, after which the solution was diluted to 50 mL with mobile phase.

All solutions were filtered through a 0.45 µm membrane filter RC discarding the first 2 mL of filtrate before injecting into HPLC system.

RESULTS AND DISCUSSION

Investigation of forced degradation study of donepezil hydrochloride and donepezil tablets was originally performed using parent HPLC method. Results from chromatographic peak purity testing

obtained by HPLC analysis of the all forced degradation samples showed spectral homogeneity of drug substance peak, proving that is spectrophotometrically pure and well resolved from all of its degradation products thus confirming the stability indicating nature of the method.

Besides HPLC analysis of donepezil hydrochloride degradation products under stress conditions, LC-MS analysis was performed. In our preliminary experiment, forced degradation study was carried out by subjecting donepezil hydrochloride to hydrolytic degradation under acidic and alkali conditions, chemical oxidation and thermal degradation in order to achieve the complete degradation of donepezil hydrochloride.

Hydrolytic degradation in acid conditions in 5 mol/L HCl at 80 °C for 1 h was performed and showed that donepezil hydrochloride was stable under the applied conditions. No significant degradation (less than 1% of degradation) of donepezil and donepezil finished product was observed when donepezil hydrochloride was exposed to acid hydrolysis conditions. When donepezil was exposed to acid, there was no significant change in the peak area for donepezil and no significant additional peaks were detected. The degradation of donepezil and donepezil tablets samples was negligible in acid hydrolysis condition and only in acid hydrolysis of donepezil tablets sample two degradation products as minute peaks were observed. Significant degradation of donepezil hydrochloride and donepezil finished product was observed when exposed to alkali hydrolysis and hydrogen peroxide.

Hydrolytic degradation in alkali conditions in 0.1 mol/L NaOH performed at room temperature for 8 h showed that donepezil hydrochloride was stable under the applied conditions. But the degradation was achieved at more drastic conditions. Donepezil drug was found to undergo degradation under alkali condition (5 mol/L NaOH). About 1 to 1.5% of donepezil was degraded after exposure of donepezil and donepezil tablets in alkali hydrolysis condition with formation of a few major products. For LC-MS analysis donepezil hydrochloride was subjected to hydrolytic degradation under alkali conditions (5 mol/L NaOH) at 80 °C for 1 h. The obtained HPLC chromatogram is shown in Figure 2b. It was recorded that 3% of donepezil hydrochloride was degraded. In order to obtain the maximum degree of degradation donepezil hydrochloride was conducted to 5 mol/L NaOH at reflux for 8 h. The degree of obtained degradation of donepezil hydrochloride was about 15%.

Donepezil hydrochloride also undergoes degradation in oxidative conditions with 3% hydrogen
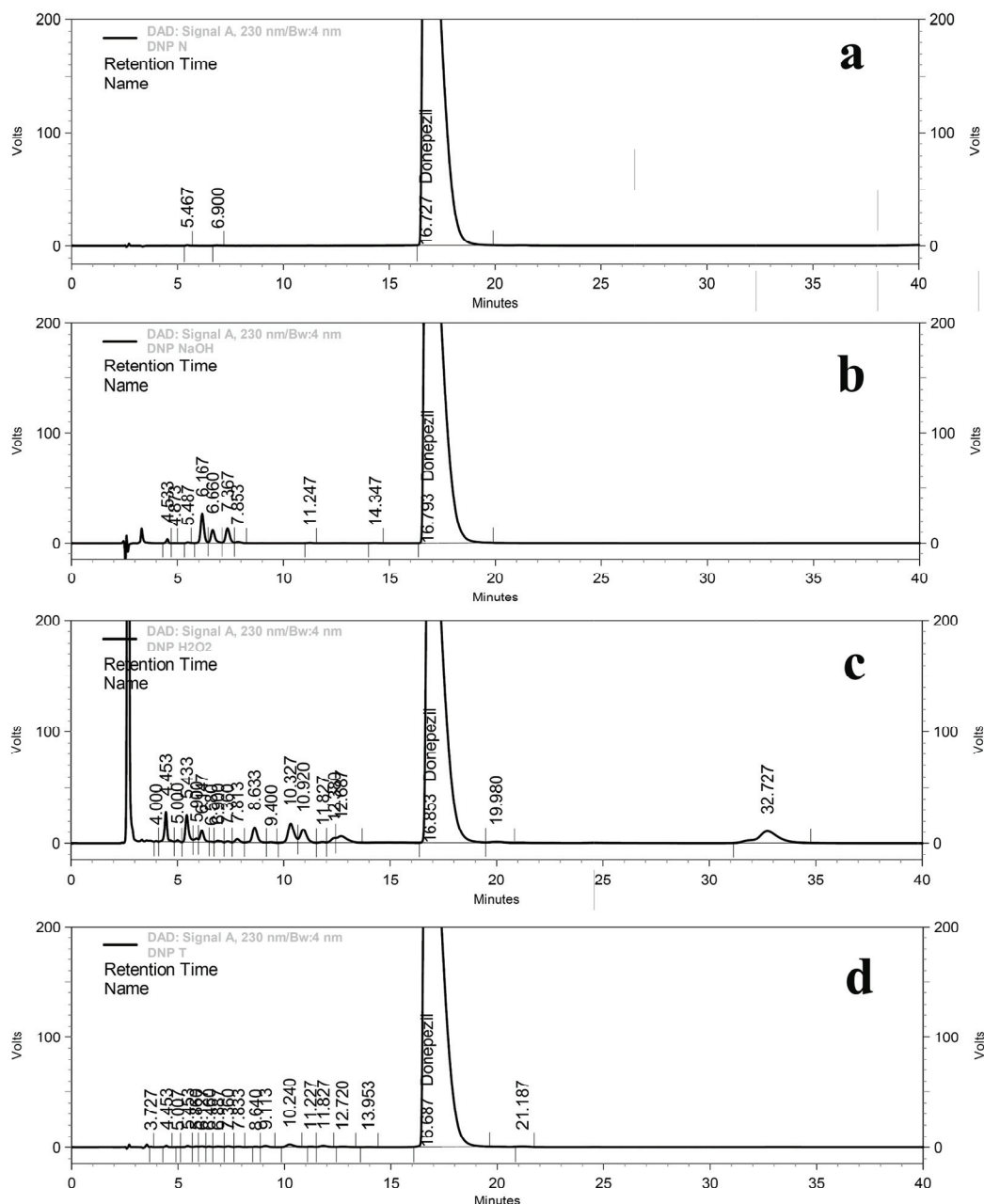
Figure 2. Chromatograms of a, b, c and d samples obtained from untreated, alkali, peroxide and thermal stress testing of donepezil hydrochloride, respectively, using LC-MS compatible method.

peroxide. Donepezil was very sensitive towards oxidation degradation mediated with hydrogen peroxide. When oxidation degradation of donepezil and donepezil tablets was performed in 3% hydrogen peroxide solution at 80 °C, about 1.5 and 3.5% of degradation occurred, respectively. For LC-MS analysis oxidative degradation with 3% H_2O_2 of donepezil hydrochloride performed at 80 °C for 1 h showed about 12% donepezil degradation (Figure 2c). In order to obtain maximum degree of degradation sample was conducted to 30% H_2O_2 at reflux for 8 h. The degree of obtained degradation of donepezil hydrochloride was 57%.

Thermal degradation of donepezil hydrochloride was performed at 105 °C for 72 h (Figure 2d). In thermal degradation samples degradation of donepezil was also negligible and several degradation products as minute peaks were detected in thermal treated donepezil hydrochloride and in thermally treated donepezil tablets. The degree of obtained degradation of donepezil hydrochloride was about 1%.

Based on the investigation of forced degradation behavior in our work, donepezil was found to be stable under mild acidic condition and alkaline condition, but sensitive to degradation with hydrogen per-

oxide, which is also reported in earlier studies on stability of donepezil hydrochloride [17-21]. Even if it appears stable under mild conditions, in our experiment degradation was achieved in alkaline solution under more severe conditions, using high molarity alkaline solution and reflux. Similar results were reported in earlier studies where degradation under alkaline conditions was performed using reflux [17] or prolonged time of treatment with higher alkaline concentration [18-20]. Also, in our study donepezil hydrochloride showed lower degree of degradation under applied conditions for thermal stress, but it is reported that under prolonged exposure to high temperature considerable degradation was induced [19].

Characterization of degradation products by LC-MS

For LC-MS analysis of the selected degraded samples, the parent HPLC method was modified in order to have LC-MS compatible method. LC-MS procedure included alkaline, oxidative and thermal degradation of donepezil hydrochloride. The results are given in Figure 2 and Tables 1-3.

Table 1 shows the results of LC-MS analysis of donepezil hydrochloride after hydrolytic degradation under alkali conditions (5 mol/L NaOH) at 80 °C for 1 h. Several degradation product peaks were noted in the HPLC chromatogram as main degradation products of donepezil after degradation under alkali conditions, at relative retention times (*RRT*) 0.38, 0.41 and 0.43 (Figure 2b). The compounds were detected at *m/z* 410.1948, 396.2162 and 412.2109 (corresponding to theirs exact molecular masses of 409.1875, 395.2089 and 411.2036) with molecular formula $C_{24}H_{27}NO_5$, $C_{24}H_{29}NO_4$ and $C_{24}H_{29}NO_5$, respectively. The molecular mass, found value of *m/z* and proposed molecular formula of all potential donepezil hydrochloride degradation products are given in Table 1.

The results of LC-MS analysis of donepezil hydrochloride under oxidative degradation are given in Table 2. The oxidative experiment indicated degradation. Based on liquid chromatography results 12% of donepezil degradation was achieved. Main degradation product was observed at relative retention time 1.79. The compound was detected at *m/z* 396.2162 (corresponding to its exact molecular mass of 395.2089) with molecular formula $C_{24}H_{29}NO_4$. Several more degradation products were found at relative retention times 0.30, 0.34, 0.48, 0.58, 0.61, 0.69, 0.71 and 1.74 (Figure 2c). The compounds were detected at *m/z* 290.1736, 412.2103, 396.2157, 366.2051, 396.2158, 412.2108, 366.2053 and 396.2162 (corresponding to theirs exact molecular masses of 289.1664, 411.2033, 395.2085, 365.1978, 395.2085, 411.2035, 365.1980 and 395.2089) with molecular formula $C_{17}H_{23}NO_3$, $C_{24}H_{29}NO_5$, $C_{24}H_{29}NO_4$, $C_{23}H_{27}NO_3$, $C_{24}H_{29}NO_4$, $C_{24}H_{29}NO_5$, $C_{23}H_{27}NO_3$ and $C_{24}H_{29}NO_4$, respectively.

The results of LC-MS analysis of donepezil hydrochloride under thermal degradation (at 105 °C for 72 h) are summarized in Table 3. The degradation was negligible under thermal treatment with just several degradation products but as minute peaks. About 1% of drug was degraded after exposure of donepezil hydrochloride to thermal conditions. Degradation products were found at relative retention times 0.51, 0.58 and 0.71 (Figure 2d). The compounds were detected at *m/z* 396.2161, 396.2160 and 366.2053 (corresponding to theirs exact molecular mass of 395.2088, 395.2087 and 365.1980) with molecular formula $C_{24}H_{29}NO_4$, $C_{24}H_{29}NO_4$ and $C_{23}H_{27}NO_3$, respectively.

Forced degradation of donepezil hydrochloride under oxidative conditions showed the presence of compound at relative retention time 1.79 with molecular formula $C_{24}H_{29}NO_4$, corresponding to donepezil

Table 1. Results of LC-MS analysis of donepezil hydrochloride in alkali degradation (5 mol/L NaOH) at 80 °C for 1 h. Proposed molecular formula for degradation products was determined based on high precision measured masses; λ_{max} values from obtained UV spectral data for compounds

No.	*RRT*	λ_{max} / nm	Experimental *m/z* values of important ions	Molecular mass and molecular formula
1	0.20	206; 258; 290	234.1465; 256.1282	233.1392; $C_{14}H_{19}NO_2$
2	0.30	208; 230; 272; 316	290.1736; 312.1558; 328.1311	289.1664; $C_{17}H_{23}NO_3$
3	0.34	208; 226sh; 256; 294	412.2103; 434.1920; 450.1648; 845.3975; 861.3715	411.2033; $C_{24}H_{29}NO_5$
4	0.38	218; 258; 300	410.1948; 432.1795; 448.1525	409.1875; $C_{24}H_{27}NO_5$
5	0.41	208; 232; 276; 320	396.2162; 418.1980; 434.1728	395.2089; $C_{24}H_{29}NO_4$
6	0.43	208; 234; 276; 322	412.2109; 434.1940	411.2036; $C_{24}H_{29}NO_5$
7	0.58	208; 232; 272; 318	366.2051	365.1978; $C_{23}H_{27}NO_6$
8	0.61	210sh; 228; 272; 316	396.2158; 418.1981; 434.1770	395.2085; $C_{24}H_{29}NO_4$
9	0.71	208; 232; 276; 320	366.2053	365.1980; $C_{23}H_{27}NO_3$
10	1.00	208; 230; 270; 316	380.2216; 402.2026; 418.1777	379.2143; $C_{24}H_{29}NO_3$ – donepezil

Table 2. Results of LC-MS analysis of donepezil hydrochloride in oxidative degradation (3% H_2O_2) at 80 °C for 1 h. Proposed molecular formula for degradation products was determined based on high precision measured masses; λ_{max} values from obtained UV spectral data for compounds; sh - shoulder

No.	RRT	λ_{max} / nm	Experimental *m/z* values of important ions	Molecular mass and molecular formula
1	0.19	206; 226sh; 260; 290	306.1676; 328.1496; 344.1254; 633.3133	305.1604; $C_{17}H_{23}NO_4$
2	0.19	206; 260	318.1682	317.1609; $C_{18}H_{23}NO_4$
3	0.19	206; 260	320.1834	319.1761; $C_{18}H_{25}NO_4$
4	0.20	206; 258; 290	234.1465; 256.1282	233.1392; $C_{14}H_{19}NO_2$
5	0.21	210; 248	304.1887	303.1814; $C_{18}H_{25}NO_3$
6	0.22	208; 262	322.1628; 344.1488; 643.3214; 665.3031; 681.2760	321.1558; $C_{17}H_{23}NO_5$
7	0.22	208; 262	334.1993	333.1921; $C_{19}H_{27}NO_4$
8	0.22	208; 262	250.1418; 272.1277; 499.2783; 521.2605; 537.2375	249.1349; $C_{14}H_{19}NO_3$
9	0.30	208; 230; 272; 316	290.1736; 312.1558; 328.1311	289.1664; $C_{17}H_{23}NO_3$
10	0.34	208; 226sh; 256; 294	412.2103; 434.1920; 450.1648; 845.3975; 861.3715	411.2033; $C_{24}H_{29}NO_5$
11	0.38	218; 258; 300	410.1948; 432.1795; 448.1525	409.1875; $C_{24}H_{27}NO_5$
12	0.42	208; 260; 318	426.1905	425.2187; $C_{24}H_{27}NO_6$
13	0.43	208; 234; 276; 322	412.2109; 434.1940	411.2036; $C_{24}H_{29}NO_5$
14	0.48	210sh; 230; 272; 316	396.2157; 418.1979; 434.1747	395.2085; $C_{24}H_{29}NO_4$
15	0.58	208; 232; 272; 318	366.2051	365.1978; $C_{23}H_{27}NO_3$
16	0.61	210sh; 228; 272; 316	396.2158; 418.1981; 434.1770	395.2085; $C_{24}H_{29}NO_4$
17	0.69	208; 234; 274; 310	412.2108; 434.1934; 823.4182	411.2035; $C_{24}H_{29}NO_5$
18	0.71	208; 232; 276; 320	366.2053	365.1980; $C_{23}H_{27}NO_3$
19	1.00	208; 230; 270; 316	380.2216; 402.2026; 418.1777	379.2143; $C_{24}H_{29}NO_3$ – donepezil
20	1.74	208; 230; 270; 316	396.2162; 418.1974; 434.1712	395.2089; $C_{24}H_{29}NO_4$
21	1.79	208; 230; 270; 316	396.2162; 418.1974; 434.1712	395.2089; $C_{24}H_{29}NO_4$

Table 3. Results of LC-MS analysis of donepezil hydrochloride in thermal degradation (at 105 °C for 72 h). Proposed molecular formula for degradation products was determined based on high precision measured masses; λ_{max} values from obtained UV spectral data for compounds; sh - shoulder

No.	RRT	λ_{max} / nm	Experimental *m/z* values of important ions	Molecular mass and molecular formula
1	0.20	206; 258; 290	234.1465; 256.1282	233.1392; $C_{14}H_{19}NO_2$
2	0.26	226; 286; 334	398.2304	397.2231; $C_{24}H_{31}NO_4$
3	0.30	208; 230; 272; 316	290.1736; 312.1558; 328.1311	289.1664; $C_{17}H_{23}NO_3$
4	0.34	208; 226sh; 256; 294	412.2103; 434.1920; 450.1648; 845.3975; 861.3715	411.2033; $C_{24}H_{29}NO_5$
5	0.38	258; 318	388.2086; 410.1951	387.2058; $C_{22}H_{29}NO_5$
6	0.41	208; 232; 276; 320	396.2162; 418.1980; 434.1728	395.2089; $C_{24}H_{29}NO_4$
7	0.43	208; 234; 276; 322	412.2109; 434.1940	411.2036; $C_{24}H_{29}NO_5$
8	0.51	208; 232; 274; 310	396.2161; 418.1973	395.2088; $C_{24}H_{29}NO_4$
9	0.58	208; 232; 272; 312	396.2160; 418.1982	395.2087; $C_{24}H_{29}NO_4$
10	0.71	208; 232; 276; 320	366.2053	365.1980; $C_{23}H_{27}NO_3$
11	1.00	208; 230; 270; 316	380.2216; 402.2026; 418.1777	379.2143; $C_{24}H_{29}NO_3$ – donepezil

N-oxide derivate. The degradation of the molecule is ascribed to the oxidation of the tertiary amine group to amino oxide. The literature has revealed that the tertiary amines undergo oxidation to form *N*-oxide while the aromatic nucleus is susceptible to free radical catalyzed hydroxylation [25-27]. Also, it is indicative based on the proposed metabolic pathways of donepezil that donepezil molecule is susceptible to

reactions of *O*-demethylation at each ethoxy group of the dimethoxyindan moiety, *N*-dealkylation at the piperidine ring, *N*-oxidation at the piperidine ring and aromatic hydroxylation [28,29].

As we mentioned earlier, no forced degradation studies of donepezil hydrochloride using HPLC-MS method was reported so far. This integrated approach is employed to obtain molecular masses of degradation products and in order to complete MS analysis, LC preparative isolation and NMR spectroscopy measurements of compounds obtained by forced degradation of donepezil hydrochloride should be carried out to fully and accurately characterize their molecular structure. This will be the subject of our further investigation.

CONCLUSION

In this work, *in-vitro*-generated forced degradation products of donepezil hydrochloride were studied. Donepezil hydrochloride is stable to ICH recommended conditions of hydrolysis, oxidation and dry heat. Only a small degradation (~0.2%) is noted at the investigated acid hydrolytic condition and about 1% degradation in samples under selected conditions of dry heat. On the other hand, significant degradation of about 3% degradation under specific alkali hydrolytic condition and about 12% degradation in oxidative degradation mediated by hydrogen peroxide with formation of several degradation products was noted.

Combined information from retention behavior in reversed-phase HPLC and mass spectrometry using high resolution mass measurements revealed degradation products in forced degradation samples. Degradation products were characterized through proposed molecular formulas based on precise mass measurements obtained through LC-MS analysis. Moreover, the peak purity index of donepezil found confirmed that donepezil peak is spectrally pure in all samples under various degradation conditions and based on the above results the method can be used for quantitative determination of donepezil hydrochloride in donepezil hydrochloride tablets in presence of his degradation products. The method proved to be specific and stability indicating.

Acknowledgements

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NAUČNI RAD

ANALIZA DEGRADACIONIH PROIZVODA DONEPEZILA PRIMENOM TEČNE HROMATO-GRAFIJE I TEČNE HROMATOGRAFIJE-MASENE SPEKTROMETRIJE

Proučavanje degradacionih proizvoda donepezila je izvršeno korišćenjem RP-HPLC metode za određivanje stabilnosti donepezila, leka koji pripada grupi reverzibilnih inhibitora acetilholinesteraze. U cilju ispitivanja stabilnosti leka i njegovih degradacionih proizvoda sprovedena je studija forsirane degradacije čiste supstance kao i farmaceutskog proizvoda pod različitim uslovima. Donepezil-hidrohlorid i donepezil tablete su podvrgnuti različitim uslovima degradacije prema preporukama Internacionalne konferencije za harmonizaciju. Rastvori donepezil-hidrohlorida su podvrgnuti kiseloj i baznoj hidrolizi, hemijskoj oksidaciji i termalnoj degradaciji. Pri baznoj hidrolizi i hemijskoj oksidaciji uočena je značajna degradacija polaznog molekula. Oksidativnom degradacijom nastaju i proizvodi koji nisu uočeni kod ostalih postupaka forsirane degradacije. Nastali proizvodi analizirani su novorazvijenom HPLC metodom. Osnovna metoda je modifikovana u cilju dobijanja LC-MS kompatibilne metode kako bi se identifikovali nastali degradacioni proizvodi. Na osnovu rezultata dobijenih masenom spektrometrijom dobijene su tačne mase proizvoda degradacije, čime je omogućeno određivanje njihovih molekulskih formula.

Ključne reči: donepezil-hidrohlorid, forsirana degradacija, HPLC metoda za određivanje stabilnosti, tečna hromatografija-masena spektrometrija.