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Impurity Profiling for Donepzil Hydrochloride Tablet Formulations and Characterisation of Potential Degradant

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ABSTRACT

Compendia methods from the USP (United States pharmacopoeia) are widely used in Pharmaceutical drug product testing. However USP methods are under uninterrupted revision to improvement. Donepezil USP monograph is having two methods to quantify donepezil Related substance. The present research work is a single, simple and economic stability indicating impurity profiling method has been developed for scrutiny of Donepezil hydrochloride. Successfully Chromatographic separation has been achieved on an Inertsil C8 3v (150mm x 4.6mm) 3µm with buffered mobile phase consisting of solvent A (mixture of 0.1M phosphate (pH 2.8) buffer and methanol in the ratio 90: 10 (v/v); respectively) and Solvent B (mixture of 0.1 molar (M) phosphate (pH 2.8) buffer , Acetonitrile and methanol in the ratio 20:20: 60 (v/v); respectively) delivered at flow rate of 1.0 mL/min and the detection wavelength is 215 nm. The drug was subjected to the stress conditions. Donepezil hydrochloride was found to deteriorate significantly in basic, oxidative stress conditions and stable other degradation conditions. One degradant was observed in the stability studies, Which is crossing Identification threshold .the same was isolated and structural elucidation was carried out by H-NMR, Mass spectroscopy. The developed method was corroborated as per ICH guidelines.

Keywords: Impurity profile, Stability indicating, Development, HPLC, Mass spectroscopy, Impurities.

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INTRODUCTION

Donepezil hydrochloride is a new anti-Alzheimer drug. It is the robust acetylcholine esterase inhibitor [Barner and Grey. 1998)[1]. Chemically 2, 3-Dihydro-5, 6-dimethoxy-2-[[1-(phenylmethyl)-4piperidinyl*)* methyl)-1H-inden-1-one hydrochloride (also known as Aricept). It has an experimental formula of $C_{24}H_{29}NO_3HCl$ and molecular weight of 415.96. Donepezil hydrochloride is a white crystalline powder and is freely soluble in chloroform, soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and n-hexane. Donepezil hydrochloride was the first piperidine type capricious based inhibitor of the enzyme acetylcholinesterase (AChE). It has been approved for the emblematic treatment of tepid to moderate Alzheimer's disease[1]. In vitro studies have exhibited that donepezil hydrochloride has a significantly greater grade of selectivity of AChE in the central nervous system (CNS) than for butyryl cholinesterase (BuChE) in the brink. Clinical trials undertaken in USA and Europe have demonstrated that donepezil hydrochloride 5 mg, 10 mg and 23mg an interest in research of controlled released formulations including transdermal patches [2-6]. significantly improves cognitive and global function in patients with Alzheimer's disease. Furthermore, these studies have shown that donepezil hydrochloride is well endured and is not co-related with the hepatotoxicity that commonly seen with acridine based cholinesterase inhibitors, such as tacrine. Phase I studies conducted in USA have indiacated that donepezil hydrochloride pharmacokinetics are linear and dose proportional and are portrayed by slow plasma clearance and a long half-life (70-80 h). Although donepezil hydrochloride is metabolized primarily by the P-450 isoenzyme CYP-3A4 and to a lesser extent by CYP-2D6, negotiated hepatic function does not significantly affect its pharmacokinetic profile(Sugimoto *et al.*, 2002; Kosasa *et al.*, 1999; Roger *et al.*, 1998; Roger *et al.*, 1998) [7-10]. The different analytical techniques reported so far for analysis of this drug in biological samples and in pharmaceutical formulations include electrophoresis (Yeh *et al.*, 2008), UV-visible spectrophotometry [Sangshetti *et al.*, 2008)[11-12].. Several HPLC methods for assay and LC-MSMS method for analysis of donepezil hydrochloride have previously been published (Nakashima *et al.*, 2006; Radwan *et al.*, 2006; Lu *et al.*, 2004; Shah *et al.*, 2009; Barot and Patel, 2009)[13-17.].

MATERIALS AND METHOD

Chemicals and reagents

Donepezil Hydrochloride working standard, impurity standards and drug product were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade Acetonitrile, methanol, and

analytical grade potassium di hydrogen phosphate, potassium hydroxide, ammonium, Formic acid and ortho phosphoric acid were purchased from Merck, Darmstadt, Germany. Water used was obtained by using Millipore MilliQ Plus water purification system.

Equipment

Water HPLC with 2695 separation module equipped with 2996 PDA detector. The output signal was monitored and processed using empower2 software. Cintex digital water bath was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber. Thermal stability studies were performed in a dry air oven.

Chromatographic Conditions

The chromatographic column used was an Inertsil C8-3 150 x 4.6 mm, 3µm. The separation was achieved on a gradient method. Solvent A [mixture of 0.1 molar phosphate (pH 2.8) buffer and methanol in the ratio 90: 10 (v/v); respectively] and solvent B [mixture of 0.1 molar phosphate (pH 2.8) buffer, Methanol and Acetonitrile in the ratio 20: 60: 20 (v/v/v); respectively]. The flow rate was 1.0 mL min and the detection wavelength was 286 nm. The HPLC gradient program was set as: Time (minute)/% solution B: 0.0/20, 5/20, 35/35, 40/40, 45/55, 55/70, 57/20 and 65/20. The column temperature was maintained at 35° C. The injection volume was 25µl. Buffer (pH2.8) and Methanol in the ratio of 70:30 (v/v) used as diluent.

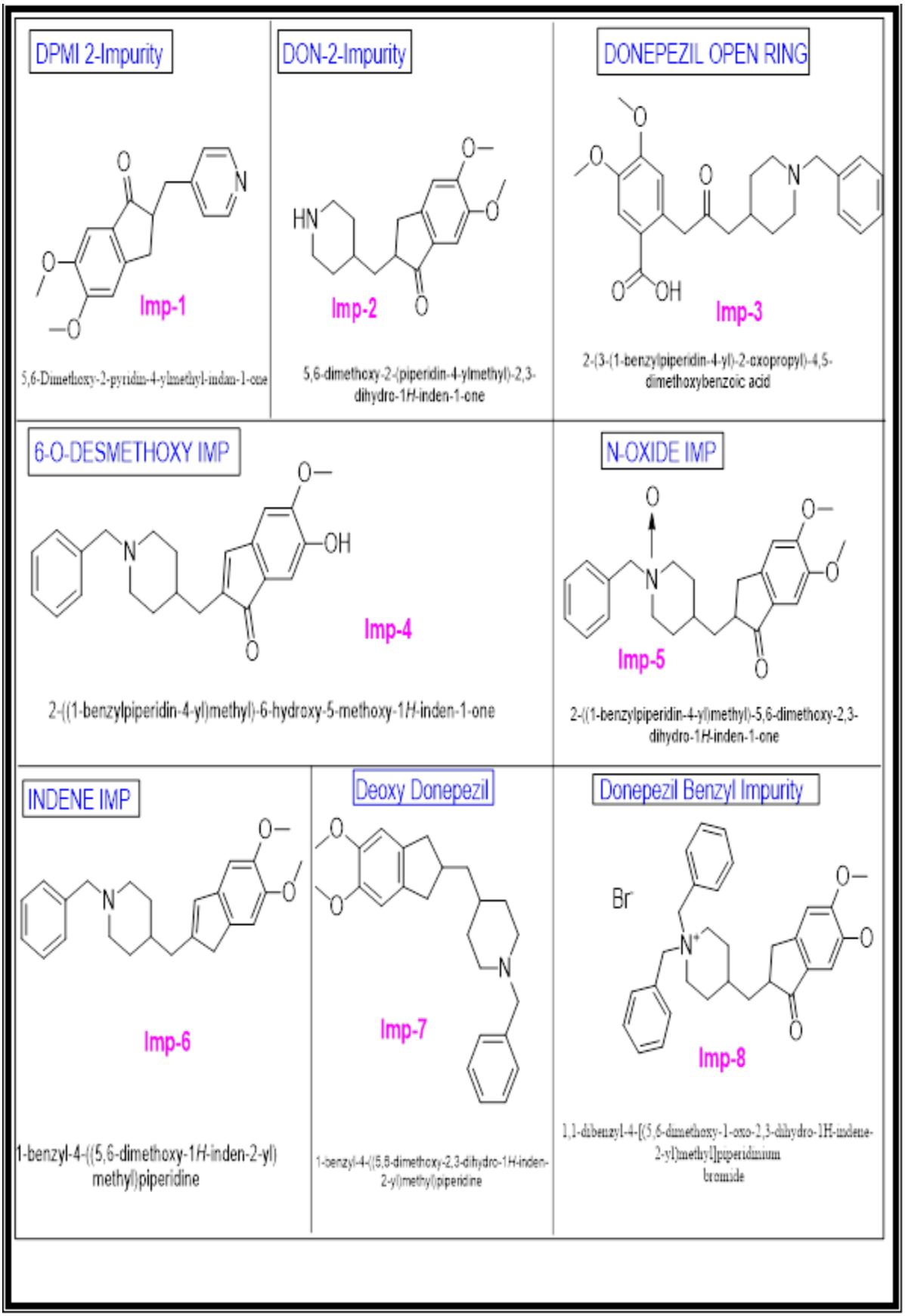

Figure 1: Chemical Structures of Donepezil and its related impurities

Preparation of system suitability solution:

A stock solution of Benzyl impurity and Deoxy impurity (0.1 mg mL) and Donepezil working standard (0.8mg/mL) was prepared by dissolving an appropriate amount in methanol and diluents(Figure 5,6) , (Table -2).

Preparation of diluted standard solution:

Prepared 0.0025 mg/mL by dissolving an appropriate amount of Donepezil Hydrochloride working standard in diluents .

Preparation of sample solution

A fine crushed tablets powder of 14mg and 23mg tablets equivalent to 75 mg drug was dissolved in 100 ml of volumetric flask with diluents, rotary shaking for 10 min and sonication for 15 min then centrifuged portion of solution at 5000rpm a clear solution containing 0.8 mg/mL. This solution was filtered through a 0.45 µm pore size Nylon 66 membrane filter.

LC–MS conditions

LC–MS system (Waters 2695 Alliance liquid chromatography was coupled with Quattro micro mass spectrometer with Mass Lynx software, Waters Corporation, Milford, USA) was used to determine the mass of unknown compound (Imp-5) formed during forced degradation and stability testing studies. The method was developed using Inertsil C8-3 150×4.6 mm, 3µm column as stationary phase. The mobile phase containing a gradient mixture of solvent-A and solvent-B. The solvent-A is a mixture 0.1% Formic acid buffer, adjusted pH 2.8 with ammonia solution and Methanol in the portion of 90:10(v/v). The solvent-B contains a mixture of 0.1%M formic acid buffer, adjusted pH 2.8 , Methanol and Acetonitrile in the proportion of 20:60:20 (v/v/v). The gradient program (T/%B) was set as 0/0, 12/20, 30/40, 47/40, 50/100, 53/100, 55/0, 65/0 respectively prior to use, the mobile phase was mixed thoroughly and degassed. The mobile phase pumped at 1.0 mL min^{-1}. The eluted compounds Donepezil, known and unknown impurities were monitored at 248 nm. The run time was 65 min. The column temperature was maintained at 30°C. The injection volumes were 60 µL. Capillary and cone voltages were 3.5 kV and 25 V, respectively. Source and dissolvation temperatures were 120 and 350°C, respectively. Dissolvation gas flow was 650 Lh^{-1}.

Enrichment and Isolation of potential degradant (Imp-5)

An unknown impurity at 1.26 relative retention time (RRT) with respect to Donepezil was observed during peroxide deterioration and stability study analysis. In order to enhance the impurity, API was subjected to oxidation in the presence of peroxide at room temperature around 2-3% of unknown peak was observed. The stressed sample was injected in to LCMS the mass of

unknown impurity (RRT1.26) observed as 396.6, which is 16 mass unit more than the Donepezil. From the mass data of LCMS it might be N-Oxide of Donepezil.

Further reaction conditions were explored to enrich the unknown compound, mCPBA was added to the solution of Donepezil at 0 to 5˚C and stirred at room temperature for 24h.

The reaction mass was given Aq. sodium bicarbonate solution wash followed by water wash. The organic layer was concentrated under vacuum and crude purified by column chromatography to yield desired compound (Figure 2).

The chromatographic purity of isolated degradation compound was found to be 98.9% and structure was confirmed by LC-MS and NMR analytical techniques.

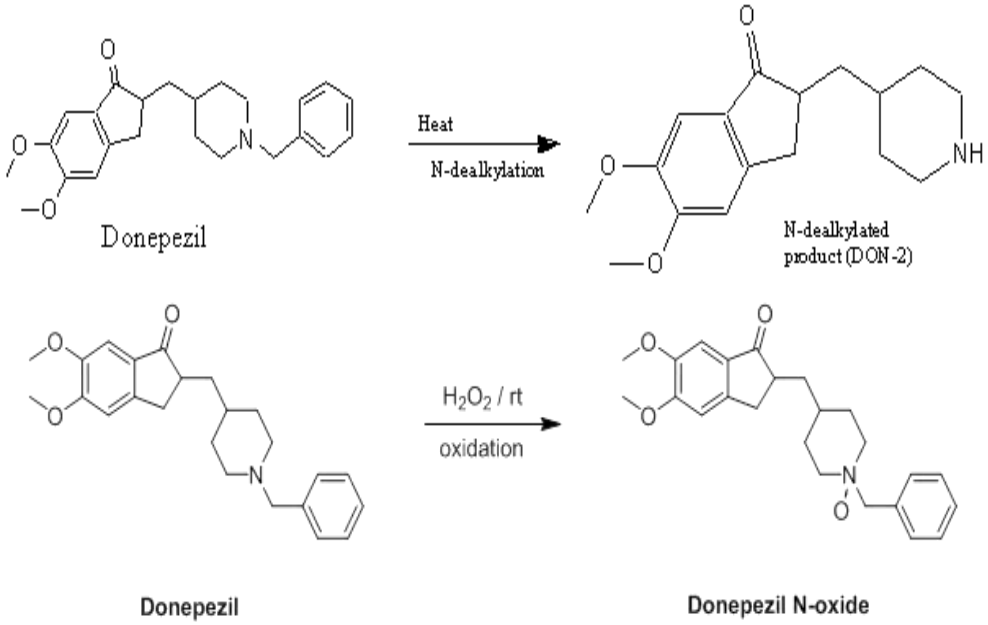

Figure 2: Degradation pathways of donepezil hydrochloride

RESULTS AND DISCUSSION

Structure elucidation of potential degradant Impurity-5 (Donepezil N-Oxide).

ESI-MS mass spectral analysis (positive mode) (Figure 3) impurity showed a molecular ion at m/z 396.6 amu [M+H]+ which was 16 amu more than that of Donepezil (379.5). This data indicated the presence of additional ‘O’ functionality in impurity.

To get the structural information, impurity was further subjected to 1H NMR study (Figure 4). The number of proton resonances is the same as that in Donepezil. However, the 1H NMR chemical shifts of the methylene groups attached to the nitrogen atom in the piperidine ring are deshielded when compared to those of Donepezil. Variations were observed in the ppm values (shifted slightly toward downfield) for the hydrogens present on piperidine ring and the aliphatic side chain

attached to nitrogen atom. This observation lends support to the formation of N-Oxide in the piperidine ring.

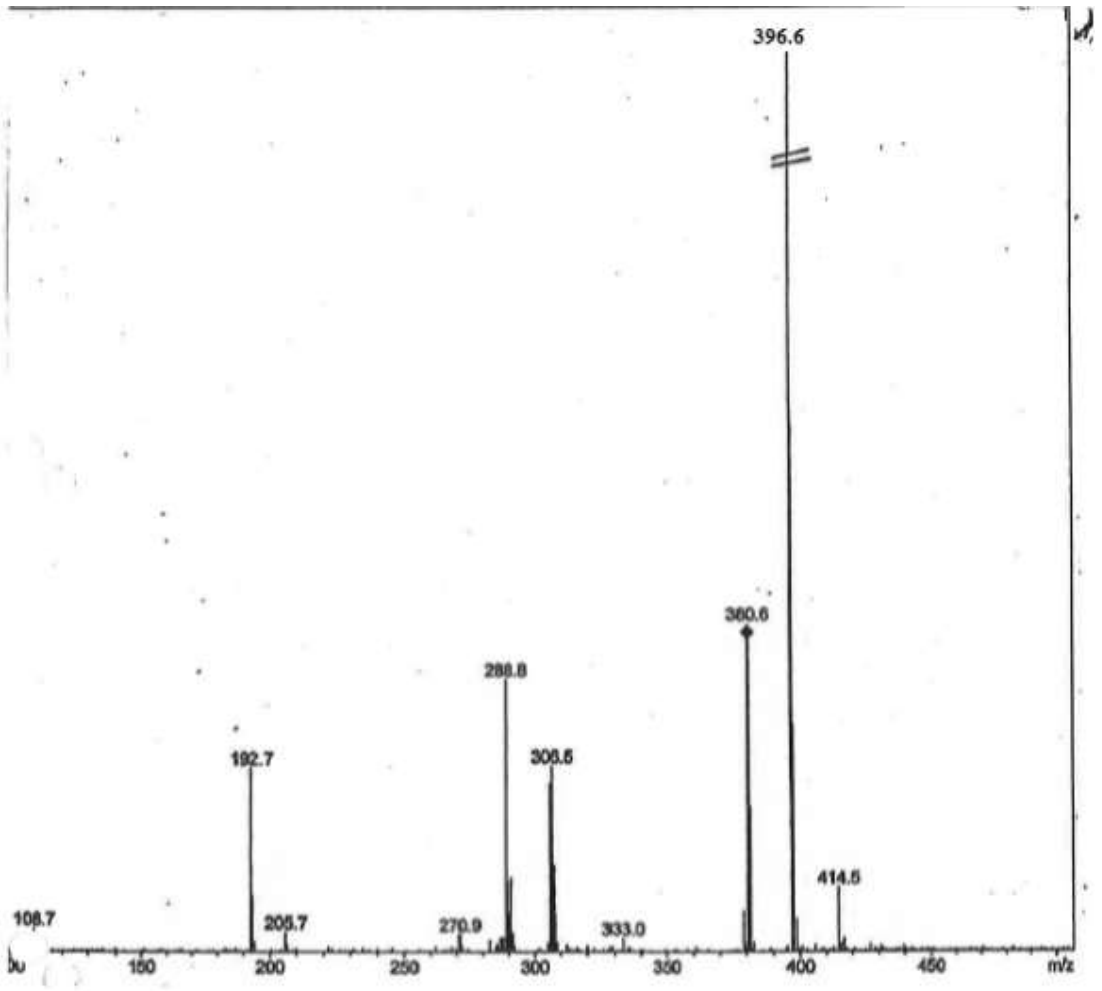

Figure 3: Mass spectra in positive ionization mode Donepezil Impurity-5

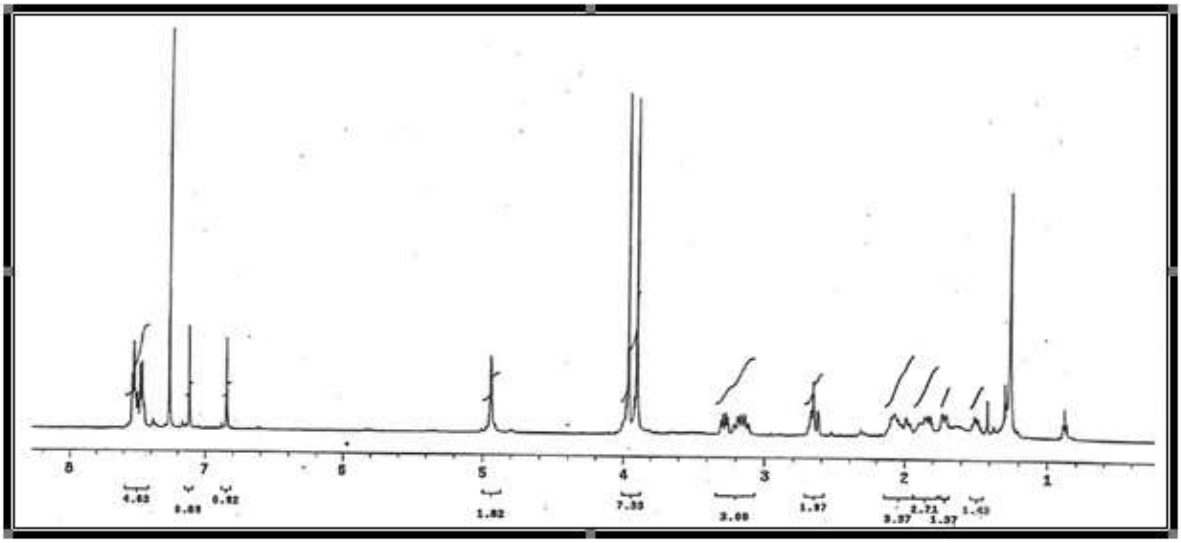

Figure 4: NMR spectra of Impurity-5

Method development and optimization

Donepezil hydrochloride Drug substance and Tablets are official in the USP. Related substances methods is mentioned in the USP monograph of Donepezil Tablets [26]. USP methods for the drug product is having two methods for polar and non-polar impurities. Objective is to develop a single related substances method for polar and non-polar impurities of the Donepezil drug substance and Tablets with multiple strengths (5, 10 and 23mg).

These formulations of Donepezil Tablets (5,10 and 23mg) contains minor quantity (about 2.5% to 11%) of Donepezil and major quantity of Eudragit and cellulose polymer (about 45%) in the drug product. These cellulose polymers are rate controlling polymer for extended release formulation. These polymers are not soluble and swell in the aqueous diluents.

The official compendia method and published methods showing the placebo interference at the confinement time of specified impurity and recovery of Donepezil and its related impurities from cellulose polymer was not achieved.

Based on the solubility of Donepezil, Donepezil related impurities, major excipients like cellulose polymer, methanol selected as a diluent for extraction of the Donepezil and its related impurities from sample matrix. Because of the 100% organic content in the diluent, Donepezil and its impurities peak shapes was not symmetrical. To get the symmetric peak shape, extracted sample was further diluted with mixture of Milli-Q water, Acetonitrile and hydrochloric acid in the proportion of 90:10:0.5 (v/v/v); respectively. The selected diluent given stability for the prepared solution for 24h.

Hence all the impurities having wavelength maxima at 215 nm, to increases the sensitivity of the method, wavelength chosen at 215nm instead of 250 nm as per USP method[26] .The relative response factor for all the eight impurities has been established with respect to Donepezil hydrochloride at 215 nm. (Table 1).

Table 1: Donepezil and its related impurities

S.NO	Compound	RRT [a]	MS Analysis(M+H) [+]	RRF [b]	Nature
1	Donepezil	1.0	379.5	1	Drug product
2	Impurity-1	0.26	283.12	1.68	Process related
3	Impurity-2	0.31	289.17	1.34	Degradant
4	Impurity-3	0.59	411.20	1.20	Process related
5	Impurity-4	0.79	363.18	1.27	Process related
6	Impurity-5	1.27	396.6	0.54	Degradant
7	Impurity-6	1.38	363.50	1.48	Process related
8	Impurity-7	1.47	365.24	0.62	Process related
9	Impurity-8	1.50	549.19	0.49	Process related

[a] Relative retention times (RRT) were calculated against the retention time (RT) of Donepezil Hydrochloride.

[b]Relative response factor were calculated against the response factor of Donepezil Hydrochloride.

The chromatographic column used was an Inertsil C8-3 150 x 4.6 mm, 3µm. The separation was achieved on a gradient method. Solvent A [mixture of 0.1 molar phosphate (pH 2.8) buffer and methanol in the ratio of 90: 10 (v/v); respectively] and solvent B [mixture of 0.1 molar phosphate (pH 2.8) buffer, Methanol and Acetonitrile in the ratio of 20: 60: 20 (v/v/v); respectively]. The flow rate was 1.0 mL min and the detection wavelength was 286 nm. The HPLC gradient program was set as: Time (minute)/% solution B: 0.0/20, 5/20, 35/35, 40/40, 45/55, 55/70, 57/20 and 65/20. The column temperature was maintained at 35° C.

While performing the stability samples analysis, observed that one of the unknown impurity which is exceeding identification threshold (0.2%), this impurity observed in oxidative stress study. This degradant was isolated and characterized by NMR, Mass Spectroscopy.

Table 2: System suitability Results

System suitability parameters	Observed value	Acceptance limit
The resolution between Benzyl Impurity and Deoxy impurity peaks.	3.4	NLT 2.0
The peak area ration of two standard preparation for Donepezil Hydrochloride.	0.99	Between 0.9 To 1.1
The Tailing factor for Donepezil Hydrochloride peak in Standard preparation.	1.1	NMT 2.0

Validation of the method

Specificity and forced degradation studies

The specificity of a method is its suitability for analysis of a substance in presence of potential impurities [18-22]. Stress testing of a drug substance can help identify likely degradation products, which can helps to establish degradation pathways and the intrinsic

Stability of the molecule. It can also be used to validate stability-indicating power [23-25] of the analytical procedures used. The specificity of the LC method for Donepezil hydrochloride has been determined in the presence of impurities and degradation products. The stress conditions used for the degradation study include light (conducted as stipulated in ICH Q1B), heat (105 ˚C), acid hydrolysis (2 M HCL at 60 ˚C for 4 h), basic hydrolysis (2 M NaOH at 60 ◦C for 30 min), aqueous hydrolysis at 60 ◦C for 8 h, and oxidation (5% H2O2 at RT for 1 h 30 min). For studies of the effects of light the study period was 10 days where as for heat, acidic, basic, and aqueous hydrolysis and oxidation it was 3 h. Peak purity has been checked for the Donepezil hydrochloride

peak by using PDA detector in stress samples. Assay of stressed samples has been performed by comparison with reference standard and the mass balance (% assay + % impurities + % degradation products) was calculated. There was no peak found at the retention time of Donepezil hydrochloride and it's all eight impurities in blank and placebo blend chromatograms proves no interference from blank and placebo. Degradation was not observed when Donepezil hydrochloride has been subjected to water hydrolysis. Degradation was observed when the drug has been subjected to acidic, base hydrolysis, photo, humidity and heat and peroxide oxidation (Figure 8, 9, 10, 11). Donepezil hydrochloride were sensitive to base and Oxidative stress conditions .This was confirmed by co-injection with all impurity standards. Donepezil hydrochloride was degraded into Imp-5 (major degradation product), by oxidation with 5% hydrogen peroxide. Donepezil hydrochloride was degraded into Imp-5 (major degradation product), by Sunlight, UV light and dry heat. Peak-purity test results from PDA detector confirmed the Donepezil hydrochloride peak obtained from all stress samples analyzed was homogeneous and pure. Peak purity results from the PDA detector for the peaks produced by degradation of Donepezil hydrochloride confirmed that all these peaks were homogeneous and pure for all the stress samples analyzed (Table 3). The mass balance for the stressed samples was close to 99% (Table 3). Assay of Donepezil hydrochloride was unaffected by presence of the impurities/degradation products, confirming the stability-indicating power of the method.

Table 3: Results of Specificity Studies

Stress Conditions	Total % Degradation	Purity angle	Purity threshold	Purity Flag	% mass Balance
Acid Hydrolysis	0.1	0.173	0.297	No	99.6
Base Hydrolysis	3.3	0.165	0.295	No	99.3
Peroxide degradation	0.28	0.177	0.299	No	99.4
Water Hydrolysis	0.1	0.177	0.303	No	99.5
Sunlight Degradation	0.1	0.551	1.008	No	99.8
UV light Degradation	0.5	0.503	1.012	No	99.4
Thermal degradation	0.36	0.189	0.305	No	99.6
Humidity degradation	0.04	0.179	0.304	No	99.7

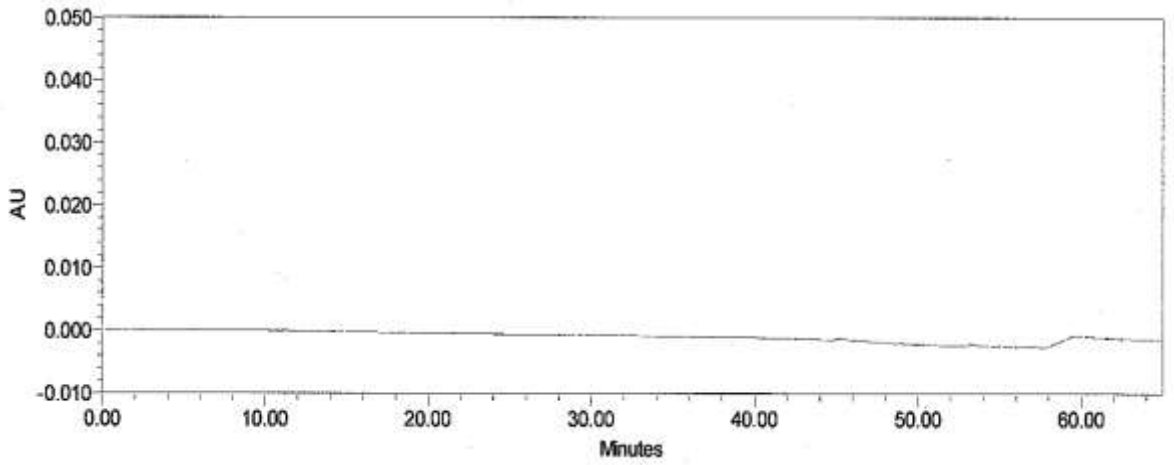

Figure 5: Typical HPLC Chromatogram of Diluent

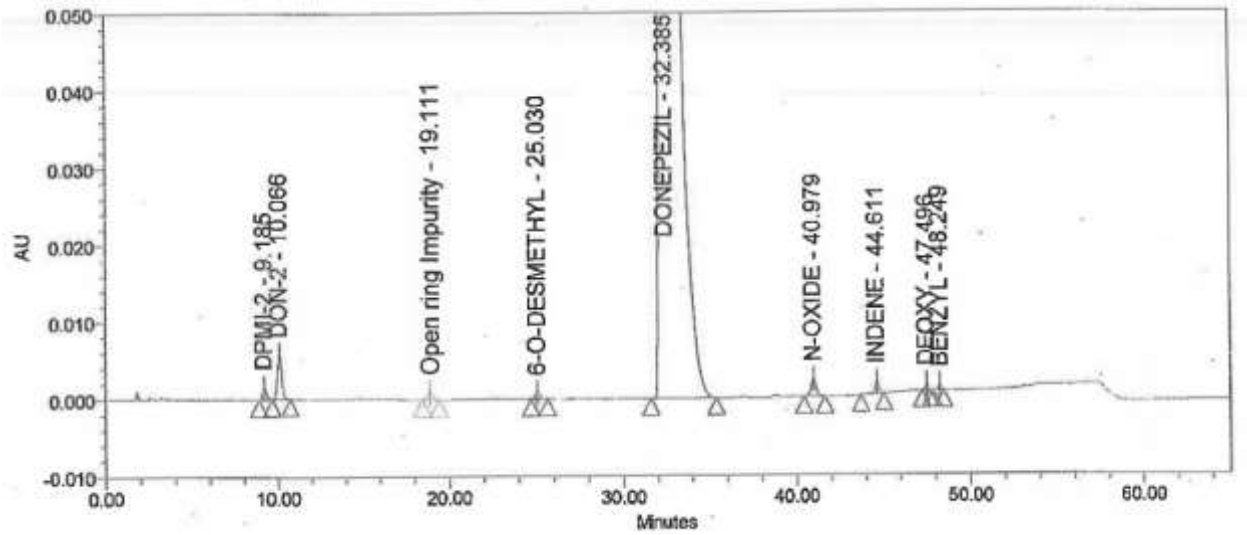

Figure 6: Typical HPLC Chromatogram of Impurities Blend Solution

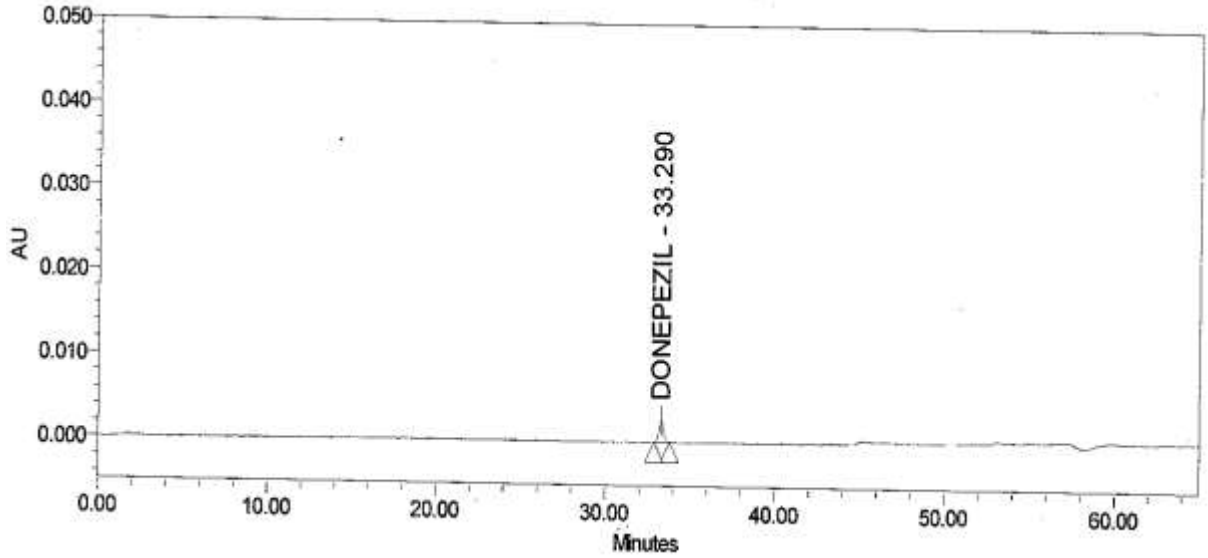

Figure 7: Typical HPLC Chromatogram of Standard

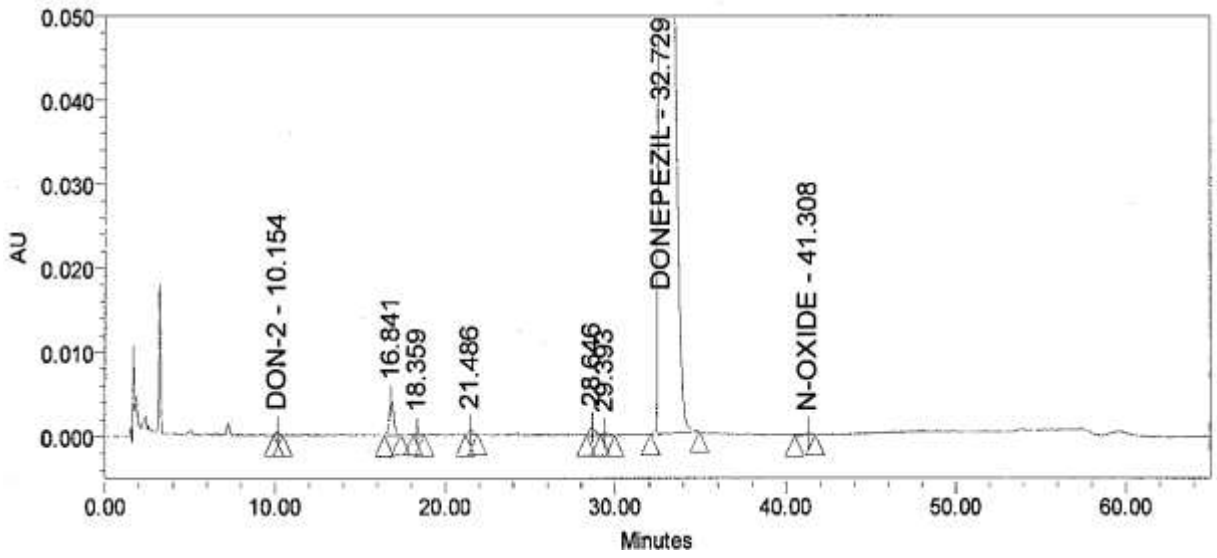

Figure 8: Typical HPLC Chromatogram of Acid Degradation

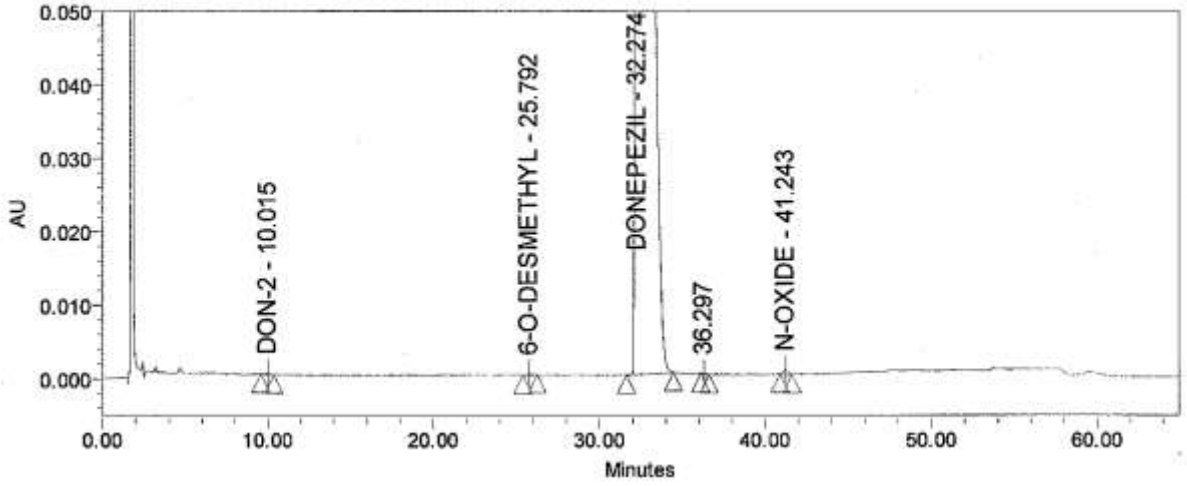

Figure 9: Typical HPLC Chromatogram of Oxidation Degradation

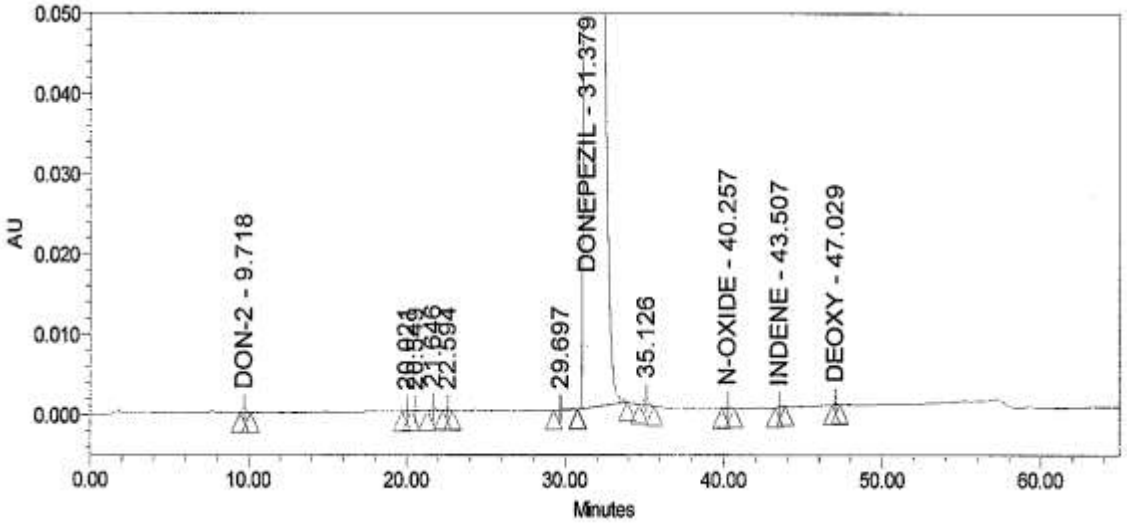

Figure 10: Typical HPLC Chromatogram of UV light Stressed Degradation

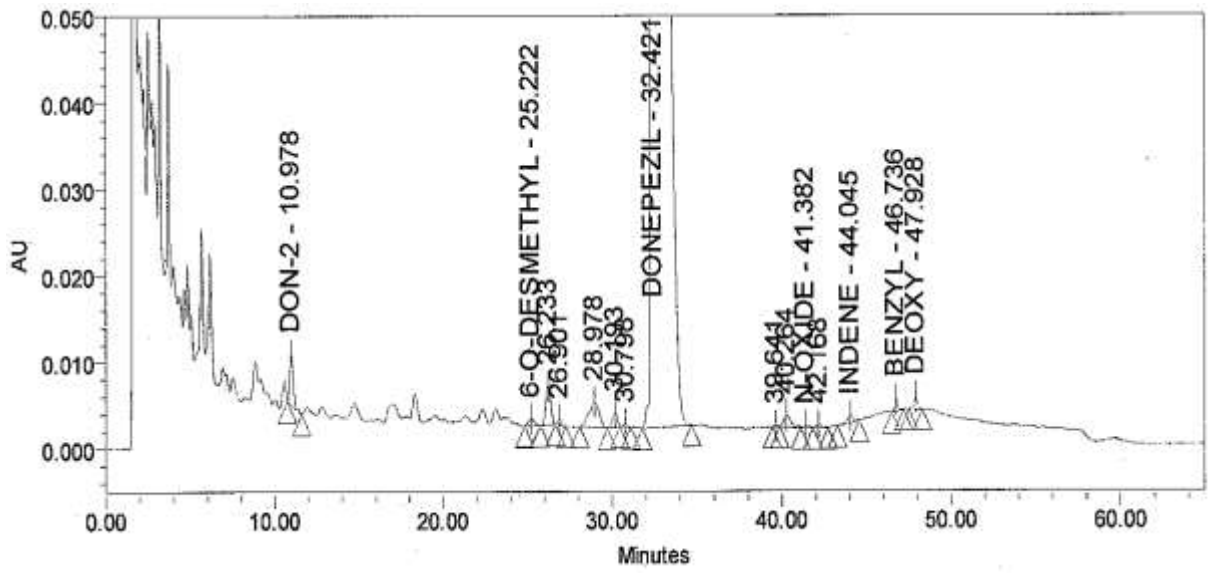

Figure 11: Typical HPLC Chromatogram of Base Stressed Degradation

Limits of detection and quantification

LOD and LOQ for the eight impurities and Donepezil hydrochloride were estimated as amounts for which the signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. Precision was also determined at the LOQ level by analysis of six individual preparations of the eight impurities and calculating the RSD (%) of the peak area for each impurity (Table 4).

.Linearity

Solutions for testing linearity for related substances were prepared by diluting the impurity stock solution to five different concentrations from LOQ to 150 percent of the permitted maximum level of the impurity (i.e. the LOQ to 0.5% for Donepezil hydrochloride, Impurities) for an analyte concentration of 200 µg mL^{-1}).

Table 4: Percentage RSD of impurities in precision study

Parameter	DPMI-2 Impurity	DON-2 Impurity	6-O-Desmethyl impurity	N-Oxide Impurity	Indene Impurity	Deoxy Impurity	Benzyl impurity
LOD(µgmL¯1)	0.002	0.003	0.004	0.009	0.002	0.004	0.006
LOQ(µgmL¯1)	0.006	0.009	0.014	0.029	0.006	0.014	0.019
Correlation coefficient(r)	0.9999	0.9999	0.9999	0.9999	0.9998	0.9992	0.9984
Precision(% RSD)a	1.9	1.1	3.4	2.5	2.9	4.0	4.5
Intermediate precision (% RSD)a	0.5	0.3	0.9	0.7	0.8	0.9	1.2
Precision @LOQ(% RSD)a	7.7	7.7	8.4	5.4	6.0	5.1	6.2

The correlation coefficients, slopes, and y-intercepts of the calibration plots are reported. These calibration plots for the 8 related substances were linear over the ranges tested. The correlation coefficients were more than 0.998 for all components (Table 4). These results show there was an excellent correlation between the peak area and concentration for the impurities.

Precision

The precision of the method was verified by repeatability and by intermediate precision. Repeatability was checked by (Waters HPLC system with PDA detector, Milford, USA) injecting 6 individual preparations of Donepezil hydrochloride samples (12 mg and 23 mg tablets) spiked with 0.50% of its eight impurities (0.50% of impurities with respect to 0.2 mg mL^{-1} Donepezil hydrochloride). The intermediate precision of the method was also evaluated using different analysts and different types of instruments (Waters HPLC system with tunable ultraviolet detector, Milford, USA), and performing analysis on different days. %RSD of area for each impurity was calculated for both precision as well as intermediate precision and was found within 10.0%. These results confirmed the precision and ruggedness of the method (Table 4).

Accuracy

For the impurities, recovery was determined in triplicate for 0.1, 0.25, 0.50, 0.62, and 0.75% of the analyte concentration (200 µg mL^{-1}) for Donepezil hydrochloride and recovery of the impurities was calculated (Table 5). An HPLC chromatogram obtained from a sample of Donepezil hydrochloride spiked with all impurities at the 050% level is shown in (Fig.6).

Table 5: Evaluation of accuracy

Amount Spiked	DPMI-2 Impurity	DON-2 Impurity	6-O-Desmethyl impurity	N-Oxide Impurity	Indene Impurity	Deoxy Impurity	Benzyl impurity
LOQ	104.6	102.6	109.8	102.4	107.0	105.6	109.5
50%	107.1	104.7	102.2	107.0	106.1	102.3	98.1
100%	107.2	105.3	103.2	107.3	105.5	103.6	111.6
150%	100.0	107.4	103.1	110.1	107.4	100.0	111.0

Robustness

To determine robustness of the method the experimental conditions were deliberately changed and resolution of Donepezil hydrochloride and the eight impurities was evaluated. To study the effect of flow rate on resolution it was changed to 0.8 mL and 1.2 mL min^{-1}. The effect of pH was studied at pH 2.6 and 3.0. The effect of column temperature was studied at 30 and 40°C. In all these experiments the mobile phase components were not changed. The effect of the percent organic strength on resolution was studied by varying Acetonitrile and methanol by -10 to +10%

while other mobile phase components were held constant as stated in Section 2.3. In all the deliberate varied chromatographic conditions, the selectivity as well as performance of method were unchanged proves the robustness of the method.

Stability in solution and in the mobile phase

No significant changes in the amounts of the impurities were observed during solution stability and mobile phase stability experiments when performed using the related substances Method. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and sample were stable for up to 24 h during determination of related substances. The mobile phase was stable up to 48 h.

CONCLUSION

The rapid gradient RP-HPLC method developed for quantitative analysis of impurities of Donepezil hydrochloride present in pharmaceutical dosage forms is precise, accurate, linear, robust, rugged and specific. Satisfactory results were obtained from validation of the method. The method is stability-indicating and can be used for routine analysis of the production samples and to check the stability of the Donepezil hydrochloride dosage forms. The developed LC–MS method can be used for identification of m/z ratio of unknown impurities as well as conformation of known impurities or degradant's formed during stability testing.

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