Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2017; 9(5): 417-424
doi: 10.25258/ijpcr.v9i5.8606

ISSN- 0975 1556

Research Article

Analytical Method Development and Validation for the Analysis of Donepezil Hydrochloride and Its Related Substances Using Ultra Perfomance Liquid Chromatography

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Available Online: 25th May, 2017

ABSTRACT

A novel, economic and time-efficient reverse-phase ultra-performance liquid chromatographic (RP-UPLC) method has been developed for the analysis of Donepezil hydrochloride in the presence of both impurities and degradation products generated by forced degradation. When Donepezil hydrochloride was subjected to acid hydrolysis, oxidative, base hydrolysis, photolytic, and thermal stress, degradation was observed only in oxidative and base hydrolysis. The drug was found to be stable to other stress conditions. Successful chromatographic separation of the drug from impurities formed during synthesis and from degradation products formed under stress conditions was achieved on a Waters Acquity C18, 50 mm x 2.1mm, 1.7µ particle size column, UV detection at 286nm and a gradient elution of Trifluoroacetic acid, Acetonitrile and methanol as mobile phase. The method was validated for specificity, precision, linearity, accuracy, robustness and can be used in quality control during manufacture and for assessment of the stability samples of Donepezil hydrochloride. Total elution time was about 6 min and equilibration time of about 2 min which allowed analysis of more than 100 samples per day. The analytical method discussed in British Pharmacopeia was pH sensitive and not compatible to LC-MS analysis but the method reported in this study is more compatible to LC-MS which will be more suitable to perform LC-MS.

Keywords: Donepezil hydrochloride; Impurities; Degradation products; UPLC method Validation.

INTRODUCTION

Donepezil hydrochloride is a new anti-Alzheimer drug. Chemically it is 2, 3-Diydro-5, 6-dimethoxy-2-[[1-phenylmethyl0-4-piperidinyl0methyl0-1H-inden-1-one hydrochloride (The Merck Index, 2006) and it is also known as Aricept. Donepezil hydrochloride is used to increase the levels of a chemical (acetylcholine) in the brain involved in memory function. It does this by slowing down the destruction of acetylcholine. It belongs to a group of medicines called acetyl cholinesterase inhibitors. It is used to treat symptoms of dementia. 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 is in a class of medications called cholinesterase inhibitors. It improves mental function (such as memory, attention, and the ability to interact with others, speak, think clearly, and perform regular daily activities) by increasing the amount of a certain naturally occurring substance in the brain.

This paper describes a simple linear gradient reverse phase UPLC method which separates all the five impurities reported in United States Pharmacopoeia (USP 2010) as well as its possible impurities during its synthesis process developed in our laboratory. Analytical method discussed by Kaftala et al., 2008 was pH sensitive but method described in this study was not pH dependent. The structure of Donepezil hydrochloride and its impurities are illustrated in Figure 1. Organic impurities can arise during the manufacturing process and storage of the drug substances, the criteria for their acceptance up to certain limits are based on the pharmaceutical studies or known safety data defined in International Conferences on Harmonization (ICH) (ICH draft revised guidance on Impurities in New Drug Substances Q3A(R2).2006). Most of analytical methods for Donepezil Hydrochloride are not LC-MS compatible but the analytical method discussed in this study is compatible to LC-MS and not pH dependent with elution time of about 6.0 minutes and equilibration time of 2.0 minutes with total runtime of around 8.0 mins. The accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness of the method were determined in accordance with ICH guidelines (International Conferences on Harmonization.Q2(R) Validation of Analytical Procedures.1994). This article reports, for the first time a new, rapid, efficient, pH independent, simple and validated stability indicating UPLC method for separation of eight potential impurities and degradation products as 'one shot' analysis.

MATERIAL AND METHODS

Reagents and materials

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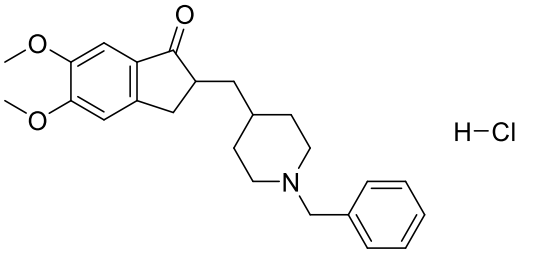
Trifluoroacetic purchased from Sigma-Aldrich, Hydrogen peroxide (30%) was bought from Tianjin Fuyu Fine Chemistry Engineering Co., Ltd (Tianjin, China). Methanol and acetonitrile were HPLC grade and others were analytical grade. HPLC-grade water was purified by a Milli-Q Reagent Water system (Millipore, Bedford, MA) and in preparing the aqueous solutions and the mobile phase throughout the experiments.

Sample of Donepezil hydrochloride and its eight impurities A-H (Figure 1) were synthesized and characterized by using MS, IR and NMR.

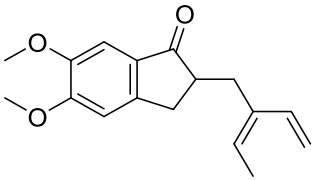
Instrumentation

Chromatographic analysis was performed on an AcquityTM UPLC system (Waters Corp., Milford, MA, USA), equipped with a binary pump solvent management

Donepezil Hydrochloride

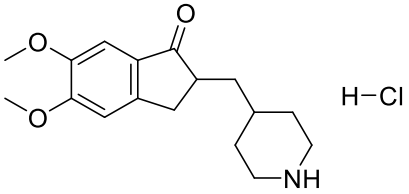


Impurity A



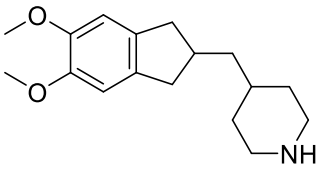
5,6-Dimethoxy-2-(4-pyridyl)methyl-indan-1-one

Impurity B



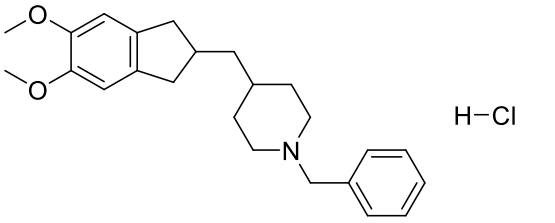
(2,3-Dihydro-5,6-dimethoxy-2-(4-piperidinyl)methyl-indan-1-one hydrochloride)

Impurity C



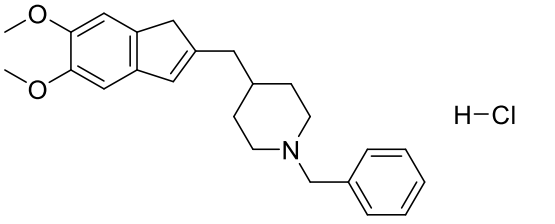
4-(5,6-Dimethoxy-indan-2-ylmethyl)-piperidine

Impurity D



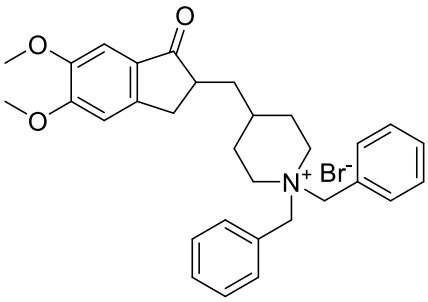
1-Benzyl-4-[(5,6-dimethoxy-2,3-dihydro-1Hinden-2yl)methyl]piperidine hydrochloride

Impurity E



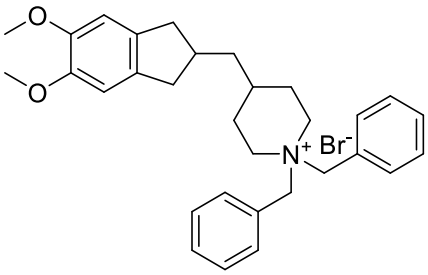
1-Benzyl-4-[(5,6-dimethoxy-1H-inden-2yl)methyl]piperidine hydrochloride

Impurity F



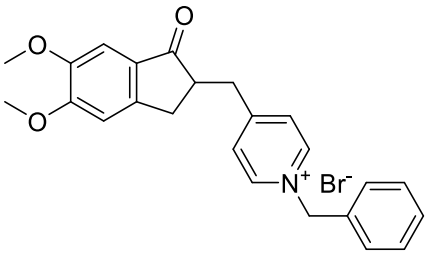
1,1-Dibenzyl-4-[(5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl]piperidinium bromide

Impurity G



1,1-Dibenzyl-4-(5,6-dimethoxy-indan-2ylmethyl)-piperidinium bromide

Impurity H



1-benzyl-4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)pyridinium bromide

Figure 1: Structure of Donepezil hydrochloride and its impurities.

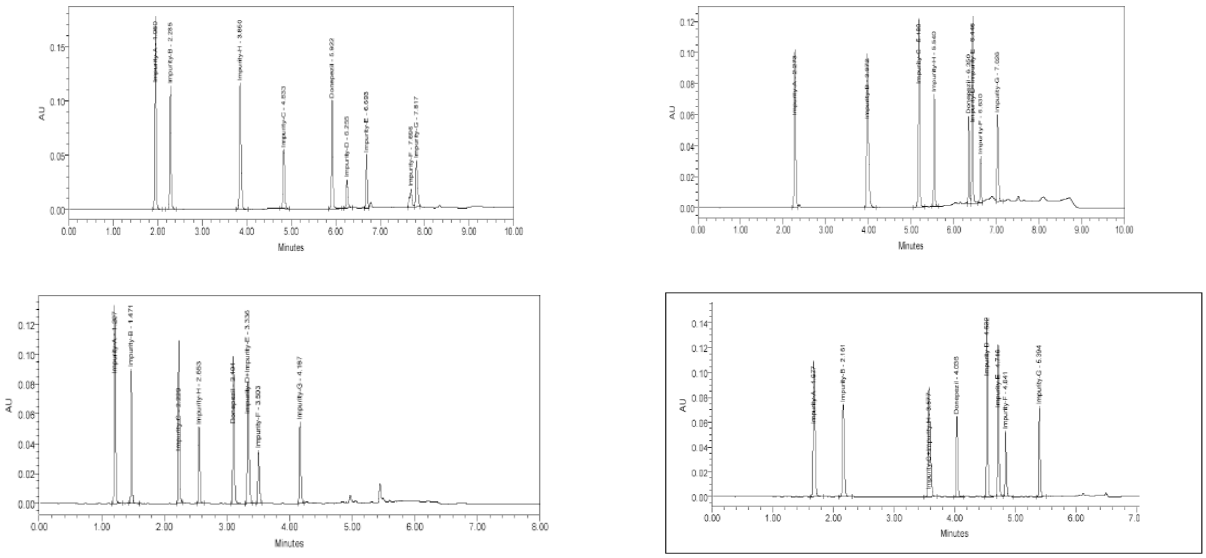
system, micro degasser, an autoplate-sampler, and thermostatic column compartment. Chromatographic separation was carried out on a Waters Acquity C18 50 mm x 2.1mm, 1.7µ particle size column with an in-line filter (0.22 µm) prior to the column. DK-S26 water baths were equipped with MV controller, electro-thermostatic blast oven (DHG-9146A, Shanghai Jinghong Experimental Equipment Co., Ltd. Shanghai, China), Hundred Thousandth Balance (AUW120D, SHIMADZU, Japan). A 50-W clear xenon lamp was employed as the light source for estimating the photolytic experiment (CEL-HXB F300, Beijing Zhongjiao Jinyuan technology co., Ltd. Beijing, China).

Stress degradation studies

Stress degradation studies of Donepezil hydrochloride were carried out under hydrolysis (acid and base), oxidation, photolytic and thermal forced conditions. The stock solutions of Donepezil hydrochloride were prepared at the concentration of 1.0 mgml−1 by dissolving accurately weighted 100 milligrams in 100 ml volumetric flask by using dissolving solvent. The tests of acidic and basic hydrolysis were carried out in 5 ml of 6.0 N hydrochloric acid solution (6 mol·L -1) and 10.0N sodium hydroxide solution (10 mol·L -1), respectively and the hydrolysis processes were conducted at 90℃ for 2h and controlled the concentration of each analyte as 1.0mg/mL for related substances. The oxidative degradation study was carried out in 1 ml of 30% of hydrogen peroxide at 60 for 4 hours and the concentration of each analyte was maintained at 1.0 mgml−1. In the tests of photolytic and thermal studies, few milligrams of Donepezil hydrochloride was put on the watch glasses and kept at 80℃ for the thermal experiment and similarly Donepezil hydrochloride was exposed to UV light (254 nm) for about 72 hrs. The blank samples were prepared without adding the analytes in each stress condition.

Preparation of sample solutions

The solutions obtained in the acidic and basic hydrolysis tests were cooled down to room temperature and neutralized with sodium hydroxide solution (6 mol·L -1) and hydrochloric acid solution (10 mol·L -1), respectively and then diluted to the mark with dissolving solvent. The solutions obtained in the test of oxidative degradation were diluted to the volume of 1.0 mg/mL with dissolving solvent and similarly for UV light exposure and thermal



Mobile phase-A: 0.02M Monobasic potassium phosphate
Mobile phase-B: Acetonitrile
Column : Acquity C18 (100x 2.1mm,1.7µ)
Initial B conc 10%, increased to 40% in 4.0 min, further to 75% at 5.0 min, maintained upto 6.0 min with 75%Revert back to initial conc at 6.10 min and maintained for 2.0 min.
Column flow : 0.3mL/min,
Column temp at 30°C and 1.00µL injection volume.
Result: Resolution between Impurity C and Impurity H were coeluted

Mobile phase-A: Water
Mobile phase-B: Acetonitrile
Column : Acquity C18 (100x 2.1mm,1.7µ)
Initial B conc 5%, increased to 40% in 3.0 min, further to 75% at 5.0 min, maintained upto 6.0 min with 75%Revert back to initial conc at 6.10 min and maintained for 1.0 min.
Column flow : 0.4mL/min,
Column temp at 40°C and 1.00µL injection volume.
Result: Resolution between Impurity D and Impurity E were coeluted

Figure 2: Various trials and conditions for method development.

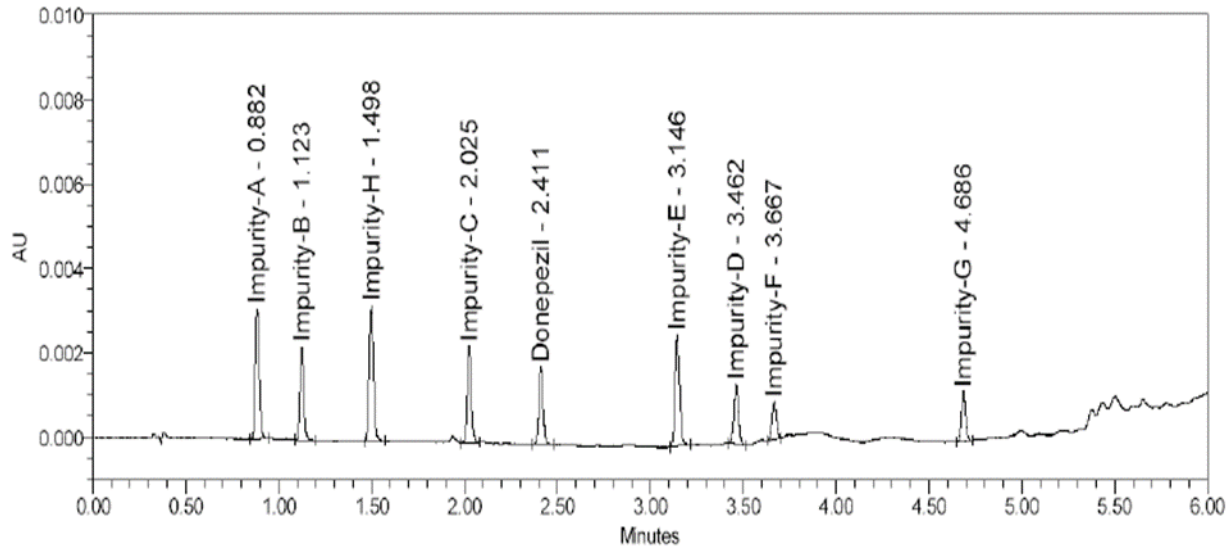


Figure 3: Chromatographic separation of Donepezil hydrochloride and its impurities.

degradation. All the solutions were filtered by 0.22 µm membrane filters and kept in refrigerator at 4°C before UPLC analysis.

Chromatographic conditions

Analysis was performed on a Waters Acquity UPLC® equipped with diode array detector (DAD). Analysis was carried out at 286nm. Separation was achieved using Waters Acquity C18 50 mm x 2.1mm, 1.7µ particle size fast LC column. The data acquired via Waters Empower2 software. Mobile phase-A was 0.1% trifluoroacetic acid in water. Mobile phase-B consist of a mixture of acetonitrile, methanol and trifluoroacetic acid in the ratio 700:300:1. The gradient time program was (Tmin/A: B; T0.01/80:20; T4.0/50:50; T6.0/20:80 ;) thus the analysis time is 6.0 min and the initial eluent composition was

Table 1: System Suitability Data a) %RSD for the area of Donepezil hydrochloride and its impurities in related substance validation.

Parameters	Donepezil Hydrochloride	Impurities							
		A	B	C	D	E	F	G	H
Forced degradation	4.4	3.1	2.6	3.8	4.3	3.4	2.9	2.6	4.2
Repeatability	4.1	3.2	2.4	3.6	4.1	3.2	2.7	2.9	4.1
Linearity	4.3	3.1	2.8	3.4	4.2	3.3	2.6	2.8	4.2
Accuracy	4.4	3.3	2.7	3.5	4.2	3.6	2.5	3.0	4.1
Ruggedness	4.2	3.3	2.8	3.4	4.3	3.6	2.8	3.1	4.2
Robustness	4.6	3.2	2.9	3.5	4.3	3.6	2.8	3.1	4.2

b) Resolution between impurity-D and impurity F

Parameters	Impurity-D and Impurity-F
Forced degradation	4.1
Repeatability	4.3
Linearity	4.4
Accuracy	4.3
Ruggedness	4.1
Robustness	4.2

Table 2: Result of forced degradation.

Control sample (No treatment)			Peak purity	
			Purity angle	Purity Threshold
			0.075	0.333
Stress Study				
Samples	Condition	% Degradation	Peak Purity	
			Purity angle	Purity Threshold
Acid Degradation	5ml 6N.HCl/ 30mins	-	0.114	0.526
Alkali Degradation	2ml 10N NaOH/ 90°C/60 mins	8.3	0.133	0.686
Peroxide Degradation	1ml 30% H_2O_2/ 60°C/4hrs	7.6	0.108	0.371
Thermal Degradation	80°C/72Hrs	0	0.162	0.573
Humidity Degradation	25°C/95%RH/ 72Hrs	0	0.148	0.586
UV light solid (Shorter wavelength)	72 Hrs	-	0.121	0.542
UV light Solution (Shorter wavelength)	72 Hrs	-	0.133	0.528
White light - Solid	72 Hrs	-	0.181	0.574
White light - Solution	72 Hrs	-	0.163	0.591

restored at 8.0 min (80:20) and maintained further for 2.0 mins.

The flow rate was set at 0.40 mL min^{-1}, the column temperature was maintained at 40°C and the injection volume was 1.00 µL. A mixture of water: acetonitrile (90:10) was used as a dissolving solvent for the preparation of standard and sample solutions. Both mobile phase and diluent were filtered through a nylon membrane filter (pore size 0.2µm).

A standard consisting of 0.0015 mg/mL of all impurities along with Donepezil hydrochloride was prepared. A sample solution consisting of Donepezil hydrochloride 1.0mg/mL was prepared.

Method validation results and tables

System suitability

The criteria of resolution between impurity D and Impurity F peak from the system suitability preparation was more than 1.5.RSD for the area of Donepezil hydrochloride peak and all the impurities from the replicate injections of standard preparations was less than 10.0%, all the parameters were met during the course of entire validation (Table1).

Specificity

As shown in the Figure 3, Donepezil hydrochloride peak was well separated from each other impurities. No blank peak interference was observed at the retention time of known peaks. The purity angle was less than purity threshold for the Donepezil hydrochloride peak in the spiked sample. Hence the method was selective and specific. Furthermore, specificity of the method was confirmed through forced degradation studies. Donepezil

Table 3: Linearity, Limit of detection (LOD) and Limit of quantification (LOQ).

Component	Concentration range (µg/mL)	Regression equation	R^2	LOQ (µg/mL)	LOD(µg/mL)
Donepezil	0.172-2.996	y = 2141x - 91	0.99839	0.172	0.052
Impurity-A	0.112-3.006	y = 3330x-199	0.99803	0.112	0.034
Impurity-B	0.143-3.008	y = 2301x-122	0.99845	0.143	0.043
Impurity-C	0.133-3.061	y = 2571x-66	0.99821	0.133	0.040
Impurity-D	0.233-3.061	y = 1791x-130	0.99816	0.233	0.070
Impurity-E	0.121-3.010	y = 3338x-200	0.99809	0.121	0.036
Impurity-F	0.306-3.018	y = 1146x-107	0.99749	0.306	0.092
Impurity-G	0.255-3.039	y=1403x-97	0.99701	0.255	0.077
Impurity-H	0.100-3.018	y=3988x-181	0.99799	0.172	0.052

Linearity results (n=3), Acceptance criteria $R^2 > 0.98$

Table 4: Precision and Accuracy results.

Validation step	Parameter	Impurities							
		A	B	C	D	E	F	G	H
Method precision	RSD	2.1	2.3	2.2	2.4	2.1	2.6	2.4	2.3
Intermediate precision	RSD	2.6	2.1	2.4	2.2	2.4	2.2	2.3	2.2
Accuracy (50%, 100% & 120%)	Average (% recovery)	102.4	106.8	99.3	103.4	106.9	98.3	97.3	98.4
	RSD (% recovery)	3.6	3.4	4.3	2.8	4.1	3.2	3.7	2.3

Table 5: Robustness.

Parameter	Impurities
Overall RSD for individual	

hydrochloride showed degradation products during alkali hydrolysis and oxidation. Since peak purity angle was less than the purity threshold for Donepezil hydrochloride peak in all the above degradation samples, the method was stability indicating for the determination of impurities in Donepezil hydrochloride. The results from forced degradation studies are summarized in Table 2.

Linearity, limit of detection (LOD) and limit of quantification for related substance method

Linear regression analysis for each ingredient showed that the calibration curves were linear over the concentration range shown in Table 3. Limit of detection and quantification were also presented in the same table.

Precision-repeatability

RSD for the individual and total impurities were found to be below the acceptance value (Table 4).

Intermediate precision-ruggedness

The RSD of individual and total impurities were calculated and found to be less than 15.0%. The overall RSD between method precision and intermediate precision were less than 15.0%, which demonstrates good precision of the method. Data presented in Table 4.

Accuracy

The recovery of three sample preparations at three different levels were examined, the range was from 97.3% to 106.9%. Results are summarized in Table4.

Robustness

The results obtained from the robustness study were well within the limit for related substance method (RSD NMT 15.0%). Data incorporated in Table 5.

Solution stability

Cumulative RSD was calculated for the individual impurity and total impurities in the standard solution and was found to be less than 10.0%. Results are summarized in Table 6.

Experimental

System suitability

Standard solution containing Donepezil hydrochloride and mixture of impurities at specification limit concentration was injected in six replicate and RSD for the area of all impurities and Donepezil hydrochloride peaks were calculated. The resolution between impurity D and Impurity F was calculated.

Specificity

During specificity study, Donepezil hydrochloride and impurity-A to impurity-H were injected separately. Donepezil hydrochloride sample preparation (1.0mg/mL) spiked with impurities at 0.15% level (mixture of all impurities at 0.0015mg/mL) were also injected. The spectra and purity plots were extracted through diode array detector for each ingredient in the spiked sample.

Furthermore, forced degradation studies were conducted in order to prove the stability indicating nature of the method. Sample solution was subjected to acid and base hydrolysis, oxidation using 30% H_2O_2, exposure to white light, UV light (254 nm), humidity(95%) and thermal(105°C). Peak purity was determined using PDA detector.

Linearity, limit of detection (LOD) and limit of quantitation (LOQ)

Six different concentrations of linearity standard solutions

Table 6: Solution stability (stored at 25°C ± 2°C).

	Parameter	Area of Donepezil and its Impurities								
		Donepezil	A	B	C	D	E	F	G	H
Sample solution stability	Cumulative RSD between initial to 24hrs	3.3	3.6	4.2	4.8	3.1	2.9	3.2	3.6	3.4

were prepared with Donepezil hydrochloride and mixture of impurities from LOQ to 200% of the specification limit concentration. Each linearity standard solution was injected in triplicate and linear regression analysis for each ingredient was performed.

System precision-repeatability-standard solution

The system precision was examined by analyzing standard solution containing Donepezil hydrochloride and its impurities at 0.0015 mg/mL concentration in six replicates.

Method precision-repeatability-sample solution

Method precision was examined by analyzing Donepezil hydrochloride sample in six preparations and calculated the RSD for the individual and total impurities value.

Ruggedness- intermediate precision

Precision was repeated using different analyst, on different day, on different instrument and using column of different lot. Over all RSD was calculated for the individual and total impurities values.

Accuracy

Triplicate sample preparation of Donepezil hydrochloride spiked with impurities at 50% level, 100% level and 120% level were analysed.

Robustness

Several below parameters of the method were purposely altered in order to determine the robustness of the method. Standard solution containing Donepezil hydrochloride and mixture of impurities at specification limit concentration was injected in six replicate and RSD for the area of all impurities and Donepezil hydrochloride peaks was found to be less than 10.0%. The resolution between impurity D and Impurity F was found to be more than 1.5.

Variation in flow rate ± 10%

Variation in column oven temperature ± 5ºC

Variation in wavelength ± 5 nm

Solution stability

Sample solution was injected at different time intervals for about 24Hrs kept at 25±2°C by spiking impurities at 0.15% level. The cumulative RSD was calculated for the area of impurities and Donepezil hydrochloride peak in the standard solution and area for individual and total impurities in sample solution.

RESULTS AND DISCUSSION

Several LC methods with shorter run time and high throughput were tried for the separation of sixteen impurities along with Donepezil hydrochloride. These includes different stationary phase, column dimension and buffers. Various trials and their conditions were given in Figure 2. Finally, the method was optimized with waters acquity C18 50 mm x 2.1mm, 1.7µ and initial mobile phase gradient condition of 80% solvent A and 20% solvent B where A is 0.1% trifluoroacetic acid in water and B is 0.1% trifluoroacetic acid in (70:30) (acetonitrile: Methanol). The gradient time program was, initial (80: 20), increased to (50:50) up to 4 min, and further altered to (20:80) up to 6.0 min. Thus, the run time is 6.0 min. The initial eluent composition was restored at 6.10 min and maintained for 2.0 mins. The flow rate was maintained at 0.4mL/min, the column temperature was maintained at 40°C and the injection volume was 1.00µL. Sample cooler is kept at 5°C. A mixture of water: acetonitrile (90:10) was used as a diluent for the preparation of standard and sample solutions. All the impurities and Donepezil hydrochloride peak were well separated from each other and no interference was observed in blank at the retention time of known peaks. The LC-PDA studies were carried out to check the purity of prototype and each degradation product peak resolved in the UPLC-DAD chromatograms.

CONCLUSION

The UPLC method developed for the determination of impurities in Donepezil hydrochloride an active pharmaceutical ingredient is precise, accurate and specific. The method has been validated and satisfactory results were observed for all the tested validation parameters. The developed method can be conveniently used for determining the quality of Donepezil hydrochloride in bulk pharmaceuticals. The lower solvent consumption due to short analytical run time of 6.0 min leads to cost effective chromatographic method and greener chemistry.

ACKNOWLEDGEMENTS

The authors would like to thank department of chemistry-Vel's university for supporting this work.

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