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RESEARCH ARTICLE

A NEW VALIDATED, STABILITY-INDICATING, RP-UPLC METHOD FOR DETERMINATION OF DONEPEZIL HYDROCHLORIDE ASSAY AND IMPURITIES CONTENT IN BULK DRUG

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ABSTRACT

A simple, economic, and time-efficient stability-indicating, reverse-phase ultra-performance liquid chromatographic (RP-UPLC) method has been developed for analysis of donepezil hydrochloride in the presence of both impurities and degradation products generated by forced degradation. When donepezil hydrochloride was subjected to acid hydrolytic, oxidative, base hydrolysis, photolytic, and thermal stress, degradation was observed after 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 286nm and a gradient elution of trifluoro acetic acid, acetonitrile and methanol as mobile phase.

The method was validated for specificity, precision, linearity, accuracy and robustness and can be used for quality control during manufacture and for assessment of the stability of samples of donepezil hydrochloride. To the best of our knowledge, a validated stability indicating UPLC method which separates all the eight impurities disclosed in this investigation has not been published elsewhere. Total elution time was about 8 min which allowed quantification of more than 100 samples per day.

INTRODUCTION

Donepezil hydrochloride is a new anti-Alzheimer drug. It is the potent acetylcholine esterase inhibitor [Barner and Grey., 1998; Martindale., 2002). Chemically 2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4piperidinyl*)*methyl) -1H-inden-1-one hydrochloride [Merck Index, 2006*)* (also known as Aricept). It has an empirical formula of $C_{24}H_{29}NO_3HCl$ and molecular weight of 415.96. Donepezil hydrochloride was the first piperidine type reversible based inhibitor of the enzyme acetylcholinesterase (AChE). It has been approved for the symptomatic treatment of mild to moderate Alzheimer's disease. In vitro studies have demonstrated that donepezil hydrochloride has a significantly greater degree of selectivity of AChE in the central nervous system (CNS) than for butyrylcholinesterase (BuChE) in the periphery. Clinical trials undertaken in USA and Europe have demonstrated that donepezil hydrochloride (5 mg or 10 mg, once daily) significantly improves cognitive and global function in patients with Alzheimer's disease. Further more, these studies have shown that donepezil hydrochloride is well tolerated and is not associated with the hepatotoxicity that commonly seen with acridine based cholinesterase inhibitors, such as tacrine.

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Phase I studies conducted in USA have demonstrated that donepezil hydrochloride pharmacokinetics are linear and dose proportional and are characterized 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, compromised hepatic function does not significantly affect its pharmacokinetic profile. 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 (Sugimoto *et al.,* 2002; Kosasa *et al.,* 1999; Roger *et al.,* 1998; Roger *et al.,* 1998)

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). Several HPLC methods for assay and LC-MS-MS 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).

This paper describes a simple linear gradient reverse phase UPLC method which separates all the impurities reported in (USP., 2010) and partially reported in (Kafkala

et al., 2008), and degradation products. Although one of the impurity described in the earlier publication (Kafkala *et al.,* 2008), was not related to process being followed, separation of donepezil hydrochloride with all the other impurities has been achieved. The structures of donepezil hydrochloride and its impurities are illustrated in the

Time (min)	0.01	4.0	6.0	6.10	8.0
Mobile phase-A	80	50	20	80	80
Mobile phase-B	20	50	80	20	20

Fig.1. Organic impurities can arise during the manufacturing process and storage of the drug substances and criteria for their acceptance up to certain limits are based on the pharmaceutical studies or known safety data (ICH., 2006). In accordance with regulatory guidelines, pharmaceutical studies using a sample of the isolated impurities can be used for safety assessment. It is therefore, essential to isolate and characterize unidentified impurities present in the drug.

Because a process for synthesis of donepezil hydrochloride has recently been developed in our laboratory, an RP-UPLC method was developed for analysis of donepezil hydrochloride and its impurities in the synthetic product. Eight impurities were identified. Five were those reported in (USP., 2010).The analytical method discussed by Kafkala *et al.,* 2008 was pH sensitive but the method discussed in this study is pH independent. The accuracy, precision, limit of detection(LOD),limit of quantification(LOQ) and robustness of the method were determined in accordance with ICH guidelines (ICH., 1994).This paper reports, for the first time a new, a rapid, efficient, pH independent, simple and validated stability indicating UPLC method for separation of eight potential impurities and degradation products as 'one shot' analysis

EXPERIMENTAL

Chemicals

Sample of donepezil hydrochloride and its eight impurities A-H (Fig.1) were synthesized in our laboratory and characterized by using LC-MS, IR and NMR. All the reagents used were of analytical reagent grade unless and otherwise stated. HPLC grade acetonitrile, HPLC grade methanol and HPLC grade trifluoroacetic acid were purchased from Merck(Germany).

Equipment

UPLC system was equipped with binary gradient pumps with auto sampler and auto injector (Model Acquity UPLC from Waters, USA) connected with a photo diode array detector (PDA) controlled with Empower software(Waters).

Preparation of Standard and sample solutions

A standard preparation consisting of 0.0015mg mL^{-1} concentration of all impurities along with 0.001mg mL^{-1} concentration of donepezil hydrochloride was prepared for related substances. A sample solution consisting of donepezil hydrochloride 1.0mg mL^{-1} spiked with all impurities at 0.15% level (0.0015 mg mL^{-1}) was prepared for related substances method .The standard and sample concentration for the assay method was 0.10mg/mL.

Chromatographic conditions

Mobile phase-A consisted of 0.1% trifluoroacetic acid in water. Mobile phase-B consisted of 0.1% trifluroacetic acid in a mixture of acetonitrile and methanol 70:30. Before use the mobile phase was filtered through a 0.2µm PTFE filter (make: Millipore) and degassed by ultrasonication. The system was equilibrated for 15 min and the analysis was carried out under linear gradient condition using a flow rate of 0.4mL min^{-1} at 40°C and Waters Acquity C18, 50mm,2.1mm,1.7µm column was used for separation. Chromatograms were recorded at 286nm. The injection volume was 1.0µL and the following linear gradient programme was used for the separation:

Validation of related substance and assay method

System suitability

For related substances method, standard solution was injected in six replicate and relative standard deviation (RSD) for the area of all impurities and donepezil hydrochloride peaks were calculated. The resolution between Impurity-D and Impurity-F was calculated. The USP plate count for the donepezil hydrochloride peak in the standard solution was calculated.

For the related substances method, the resolution between Impurity-D and Impurity-F from the system suitability preparation should be more than 2.0, RSD for the area of donepezil hydrochloride peak and all the impurities from the replicate injections of standard preparation should not be more than 10.0% and USP plate count for donepezil hydrochloride peak in the standard preparation should not be less than 25,000.

For the assay method, 0.1mg mL^{-1} concentration of donepezil hydrochloride standard was prepared in duplicate namely standard solution-1 and standard solution-2. Standard solution-1 was injected in six replicates and standard solution-2 was injected in duplicate. RSD was calculated for six replicate injections for standard prepration-1, similarity factor was calculated between standard preparation-1 and standard preparation-2 and peak asymmetry was calculated for the first injection of standard preparation-1.

For the assay method, the tailing factor for the donepezil hydrochloride standard peak from the first injection of the standard preparation-1 should be less than 2.0, The relative standard deviation for the mean area calculated for donepezil hydrochloride peak from the six replicate injections of standard preparation - 1 should be less than 1.0 % and the similarity factor calculated between standard preparation -1 and standard preparation-2 should be within 0.98 to 1.02.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. During specificity study, donepezil hydrochloride, Impurity-A to Impurity-H were injected separately. Also donepezil hydrochloride sample preparation (1.0 mg mL^{-1}) spiked with impurities at 1% level (mixture of all impurities at 0.010 mg mL^{-1}) were injected. The spectra and purity plots were extracted through diode array detector for each ingredient in the spiked sample.

For assay method

During specificity study, sample solution was spiked with all impurities at 1% level. Three such spiked sample preparation were made and difference in the assay of spiked sample solution and unspiked sample solution (from Method precision) was calculated. Peak purity was calculated from the donepezil hydrochloride peak in the spiked sample solution.

Table 1. System suitability data
System suitability results in related substance method

Parameter	Resolution between Impurity-D and Impurity-F	USP plate count for donepezil hydrochloride peak
Specificity, Repeatability	4.3	48852
Forced Degradation	4.05	50234
Linearity	4.5	54825
Solution Stability	4.0	58899

System suitability results in the Assay method

Parameter	RSD for the area of donepezil hydrochloride	USP Tailing factor for donepezil hydrochloride	Similarity factor*
Specificity	0.19	1.3	1.01
Forced Degradation	0.51	1.4	1.00
Linearity	0.38	1.3	1.01
Precision	0.26	1.3	0.99
Solution Stability	0.66	1.4	1.00

*(Mean Area of Std prep-1 x Conc. on Standard prep.-2)
(MeanArea of Std prep-2 x Conc. on Standard prep.-1)

Table 2. Result of forced degradation

Control sample (No treatment)	Peak purity	
	Purity angle	Purity Threshold
	0.075	0.333

STRESS CONDITIONS

Sample	Condition	% Degradation w.r.t. Control	Peak Purity	
			Purity angle	Purity threshold
Acid Degradation	5ml 6N.HCl /30mins	-	0.248	0.498
Alkali Degradation	2ml 10N NaOH/90°C/ 60 mins	7.5	0.133	0.719
Peroxide Degradation	1ml 30% H_2O_2/ 60°c/4 Hrs	8.9	0.136	0.317
Thermal Degradation	80°C/72Hrs	-	0.162	0.573
Humidity Degradation	25°C/95%RH/ 72Hrs	-	0.158	0.571
UV light solid(Shorter wave length)	72 Hrs	-	0.145	0.569
UV light Solution(Shorter wave length)	72 Hrs	-	0.173	0.572
White light Solid	72 Hrs	-	0.166	0.586
White light -Solution	72 Hrs	-	0.157	0.601

Table 3. Precision and Accuracy results Precision and recovery result for related substance method

Validation step	Parameter	Impurities								Total Impurities
		A	B	C	D	E	F	G	H	
Method precision	RSD	0.00	2.72	3.44	2.72	2.72	5.02	4.21	2.72	2.02
Intermediate precision	RSD	2.72	3.44	3.51	3.78	4.21	4.21	3.44	2.76	2.79
	Overall RSD	2.0	3.0	3.3	3.3	3.4	4.5	3.8	2.9	2.3
Accuracy (50%, 100% & 120%)	Average percent recovery	93.65	95.34	96.70	96.32	95.62	94.55	92.89	95.39	Not applicable
	RSD for Percent recovery	4.58	5.36	4.03	2.54	5.10	6.31	4.49	3.97	

Precision result for Assay method

Validation step	Parameter	Result
Method precision		
	Mean	99.5% w/w
	RSD	0.21%
Intermediate precision	Mean	98.60% w/w
	RSD	0.19%
	Overall Mean,	99.06% w/w
	Overall RSD	0.51%

Table 4. Linearity, LOD and LOQ of Related substances and Assay method

Component	Concentration range	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
Assay method	80.26-120.38	y = 2033x + 1873	0.99913	Not applicable	

Acceptance criteria $R^2 > 0.995$

Table 5. Robustness of related substances and assay method

Method parameter	Impurities content in % w/w								Total impurities	Assay of Donepezil HCl % w/w
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Imp-G	Imp-H		
Flow 0.36mL/min	0.15	0.16	0.13	0.15	0.15	0.14	0.15	0.15	1.18	98.9
Flow 0.44mL/mim	0.17	0.17	0.15	0.16	0.16	0.16	0.17	0.15	1.29	98.2
Column temperature :25°C	0.14	0.14	0.13	0.14	0.14	0.14	0.14	0.17	1.15	98.3
Column temperature-:30°C	0.15	0.16	0.14	0.15	0.15	0.16	0.16	0.17	1.24	99.3
281nm	0.15	0.15	0.13	0.14	0.15	0.14	0.15	0.15	1.16	99.6
291nm	0.15	0.15	0.14	0.14	0.15	0.16	0.14	0.15	1.18	98.8
Initial organic :18%B Conc.	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.15	1.21	99.7
Initial organic :22%B Conc.	0.16	0.16	0.14	0.15	0.15	0.17	0.16	0.15	1.24	100.3
As per method	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	1.20	99.5
% RSD	5.5	3.3	7.2	6.5	5.7	3.7	3.3	7.2	6.5	0.69

Table 6. Solution stability of related substance and assay method

	Parameter	Area of Donepezil and Impurities								
		Donepezil	A	B	C	D	E	F	G	H
Standard solution stability in related substance method	Cumulative RSD between initial to 24hrs	0.77	0.44	0.55	1.77	3.18	0.67	0.73	0.85	0.53
Sample solution stability in assay method	Initial and final assay values & Cumulative RSD between initial to 24hrs	Initial Assay 99.3% w/w			After 24 Hrs 99.4% w/w			Cumulative RSD 0.45% w/w		

Forced degradation

Donepezil hydrochloride was deliberately subjected to stress to establish the stability-indicating nature of the method. The compound was exposed to UV light (254 nm), heat (80°C), acid (6.0 N HCl, 90°C), alkali (10.0 N NaOH, 90°C), oxidation (30.0 % H_2O_2 for 4 Hrs), and humidity (95% RH,25°C) to evaluate the ability of the method to separate donepezil hydrochloride from its degradation products[18*)*. For heat and light stress the study period was 72 h; for acidic 30 min, alkaline 30 min, and oxidative stress it was 4 h respectively. Peak purity was determined using PDA detector.

Precision

System precision

The system precision was examined by analyzing standard solution in six replicates. For assay method, standard solution-1 was injected in six replicates prepared in system suitability preparation.

Method precision

For related substances method, method precision was examined by analyzing donepezil hydrochloride six preparations of sample solution (since sample does not have any impurities, sample solution was spiked with mixture of impurities at specification limit concentration) against standard preparation containing donepezil hydrochloride and mixture of impurities. Calculated the RSD for the individual impurity and total impurity values. For assay method, method precision was examined by analyzing six preparations of sample solution against donepezil hydrochloride standard solution . RSD was calculated on the assay values.

Intermediate precision - Ruggedness

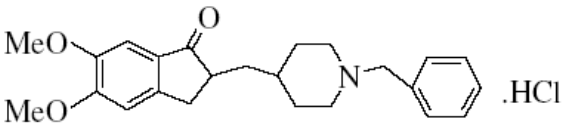
Precision was repeated using different analyst, on different day, on different instrument and using column of different lot. Overall RSD was calculated for the individual impurity and total impurities for related substances method and overall RSD for assay was calculated.

Linearity

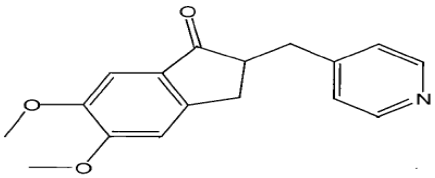
The Linearity of the method was determined by using different concentration of mixture of donepezil hydrochloride and impurities prepared and analysed in triplicate from LOQ to 200% of the specification limit concentration(0.0015 mg mL^{-1}).The peak response verses concentration data was treated by linear regression analysis for each ingredient was performed.

Limit of detection (LOD)and Limit of Quantification(LOQ)

The LOD and LOQ were determined by software from the standard preparation containing a mixture of donepezil hydrochloride and impurities using signal to noise ratio

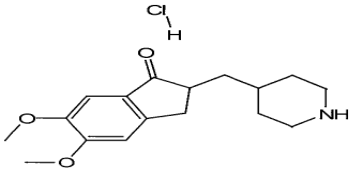


Donepezil hydrochloride



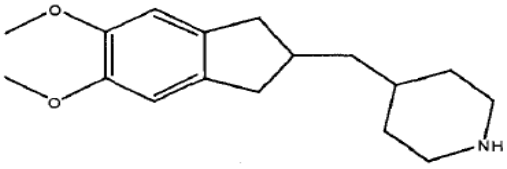
Impurity-A

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



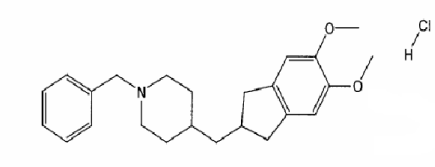
Impurity-B

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



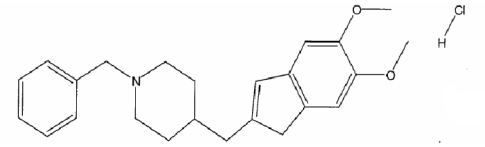
Impurity-C

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



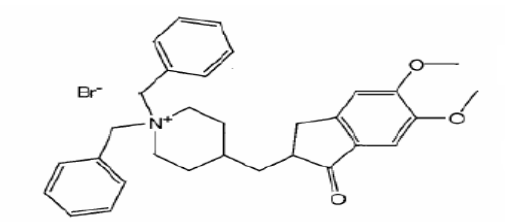
Impurity-D

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



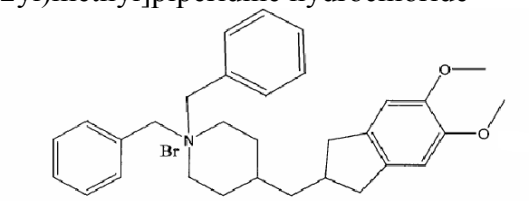
Impurity-E

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



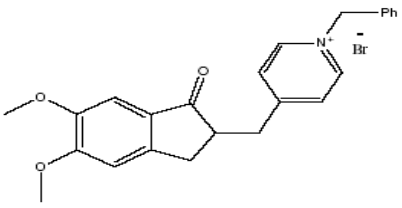
Impurity-F

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

Fig 1 : Structure of donepezil hydrochloride and its impurities

method by comparing the baseline noise with height of the peak of the corresponding impurity. The minimum concentration of the analyte at which 3:1 signal to noise ratio obtained was considered as detection limit. The minimum concentration of the analyte at which 10:1 signal to noise ratio obtained was considered as quantification limit. A solution containing donepezil hydrochloride and impurities was prepared around their LOD and LOQ concentration and analyzed.

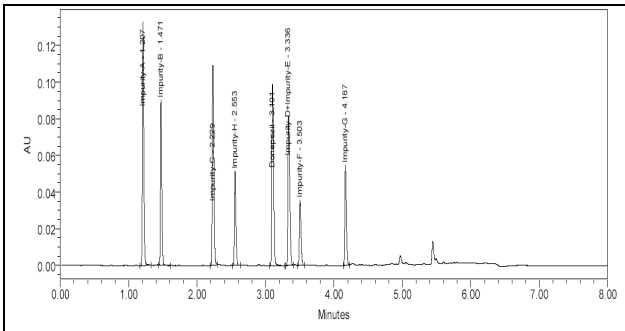
Accuracy

The accuracy study was carried out in triplicate sample preparation of donepezil hydrochloride spiked with impurities at 50%, 100% and 120% levels. The percentage of recoveries were calculated from the respective known concentrations.

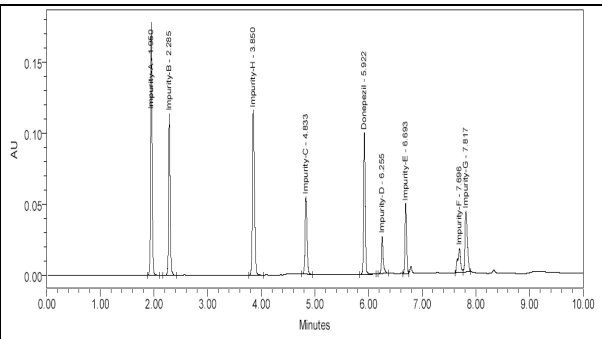
was studied by varying the wavelength of -5 to +5nm. For related substances method, the RSD for the individual impurity and total impurities were calculated. For assay method, RSD for the assay value was calculated.

Solution stability

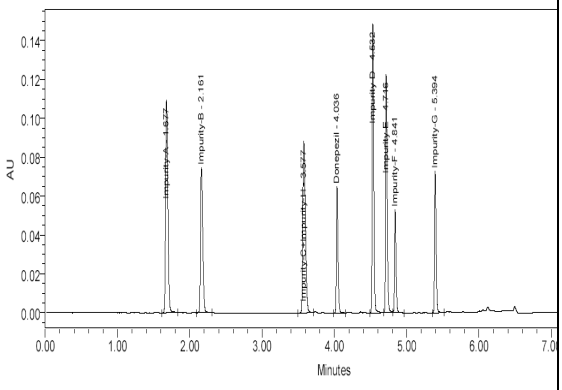
For related substances, standard solution and sample solution was injected at different time interval for about 24 h stored at 25±2°C. The cumulative RSD was calculated for area of impurities and donepezil hydrochloride peak in the standard solution and area of impurities in sample solution. For assay method, sample solution was injected at different time intervals for about 24 h. Cumulative RSD for the assay value was calculated.



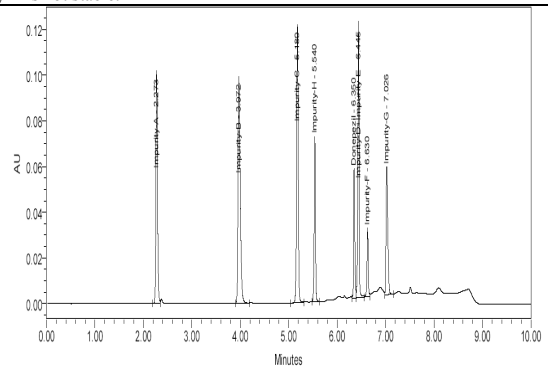
Mobile phase-A: 0.1% phosphoric acid, pH adjusted to 6.0 with triethyl amine
Mobile phase-B: Acetonotrile.
Column : Acquity C8(100x 2.1mm,1.7µ)
Initial B conc 20%, increased to 50% in 3.0 min, further to 80% at 5.0 min, maintained up to 6.0 min with 80%. Revert back to initial conc at 6.1 min and maintained for 2 min Column flow 0.5mL/min, column temp at 30°C and 1.00µL injection volume.
Result : Impurity-D and Impurity-E were co-eluting.



Mobile phase-A: 0.1%Phosphoric acid in water
Mobile phase-B: Acetonotrile.
Column : Acquity C8(100x 2.1mm,1.7µ)
Initial B conc 25%,increased to 60% in 5.0 min, further to 80% at 7.0 min, maintained up to 8.0 min with 80%. Revert back to initial conc at 8.1 min and maintained for 2 min. Column flow 0.4mL/min, column temp as 30°C and 1.00µL injection volume.
Result: Although all impurities and donepezil hydrochloride are separating well, resolution between Impurity-F and Impurity-G is poor and Impurity-F is not stable.



Mobile phase-A: 0.02M Monobasic potassium phosphate
Mobile phase-B: Acetonotrile.
Column :Acquity C18(50x 2.1mm,1.7µ)
Initial B conc 10%,increased to 40% in 4.0 min, further to 75% at 5.0 min, maintained up to 6.0 min with 75%. Revert back to initial conc at 6.1 min and maintained for 2 min.
Column flow : 0.3mL/min, column temp at 30°C and 1.00µL injection volume.
Result: Impurity-C and Impurity-H were co-eluting.



Mobilephase-A:.0.02M Ammonium acetate in water
Mobile phase-B: Acetonotrile:Methanol(50:50).
Column :Acquity C18(50x 2.1mm,1.7µ)
Initial B conc 15% to 40% in 5.0 min, further to 80% at 8.0 min, . Revert back to initial conc at 8.1 min and maintained for 2 min.
Column flow 0.4mL/min, column temp as 30°C and 1.00µL injection volume.
Result: Impurity-D and Impurity-E were co-eluting.

Robustness

To determine the robustness of the analytical method, experimental conditions were deliberately altered in order to determine the robustness of the method. To study the effect of flow rate, flow was changed by 0.04 units from 0.36 to 0.44 mL min-1. The effect of the column temperature was studied at 25 and 30^{o} C and wavelength

RESULTS AND DISCUSSION

Method development

Several fast LC methods aiming for shorter run time and high throughput were tried for the separation of eight impurities and donepezil hydrochloride from each other. These includes different stationary phase, column dimension and buffers. Various trials, its conditions and

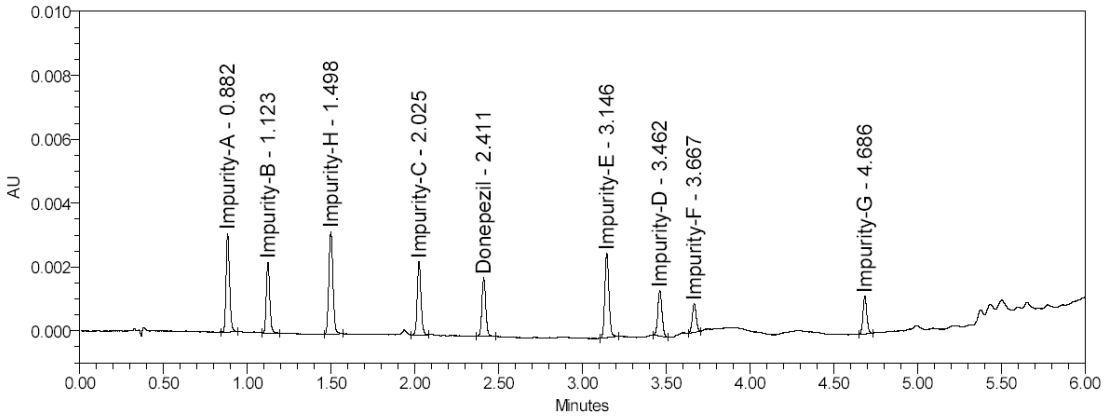


Fig.2. Different trial conditions and corresponding chromatograms of method development

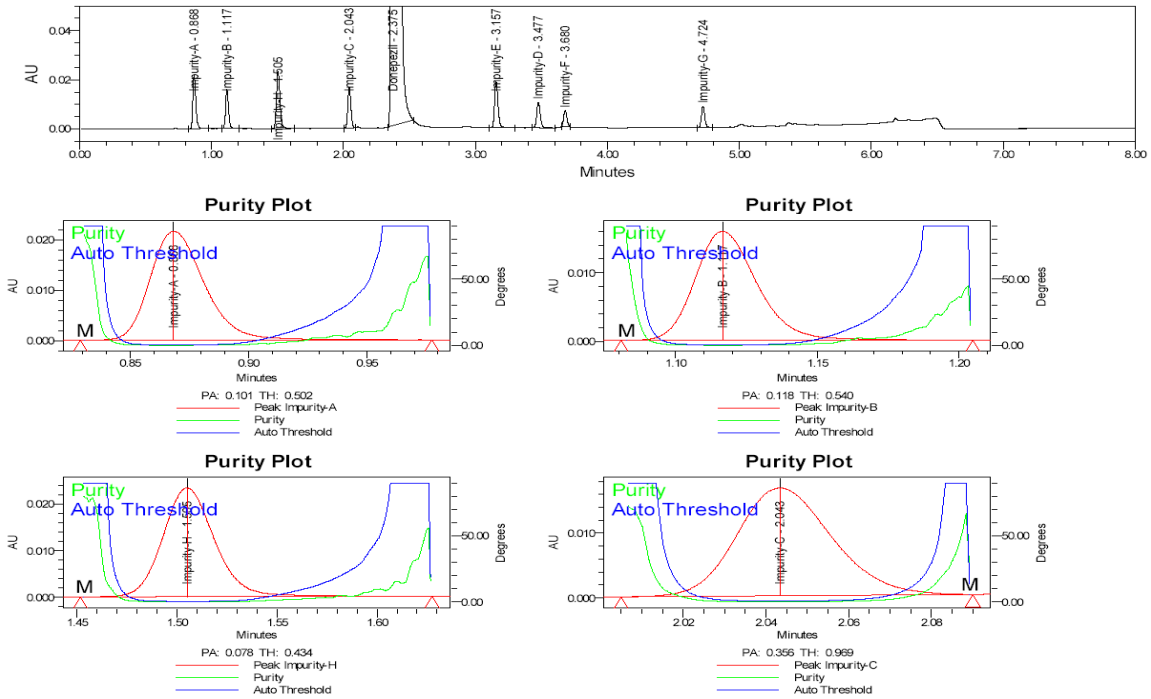


Fig. 3. Chromatographic separation of the donepezil hydrochloride and its impurities

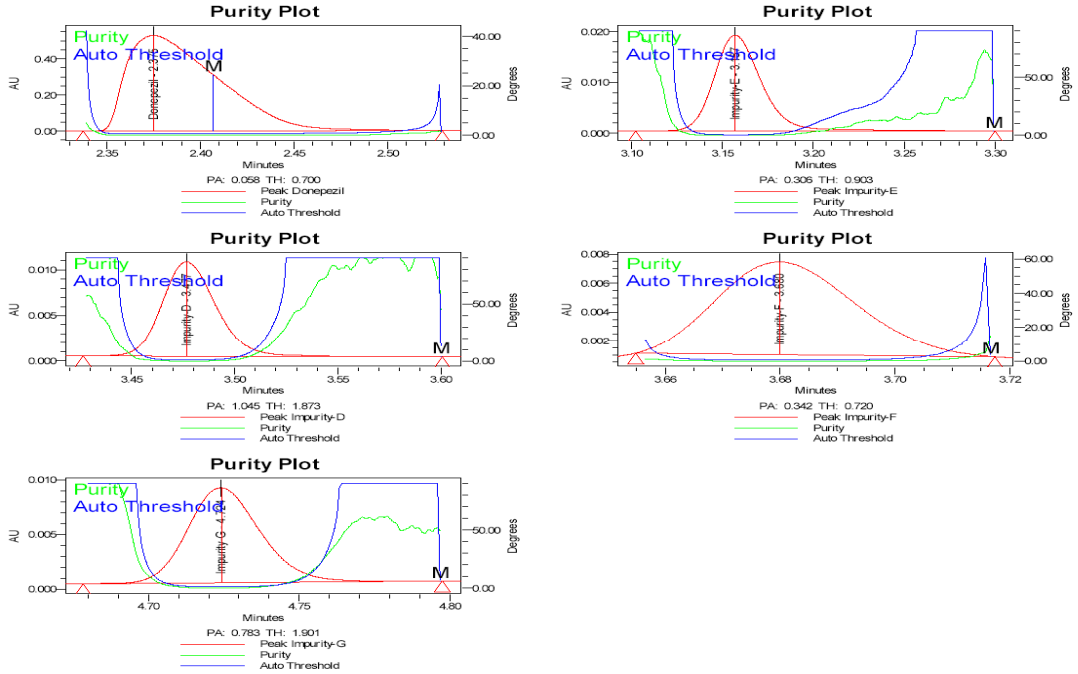
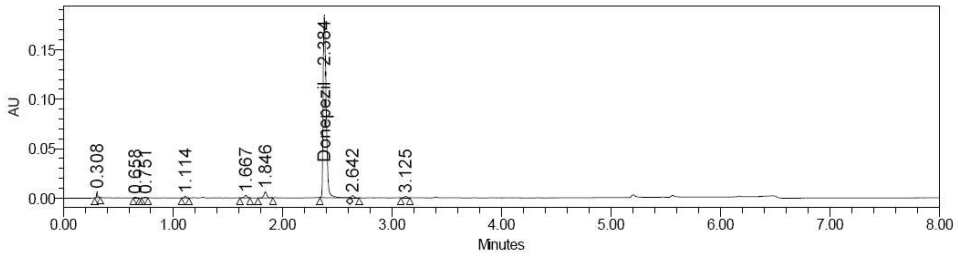
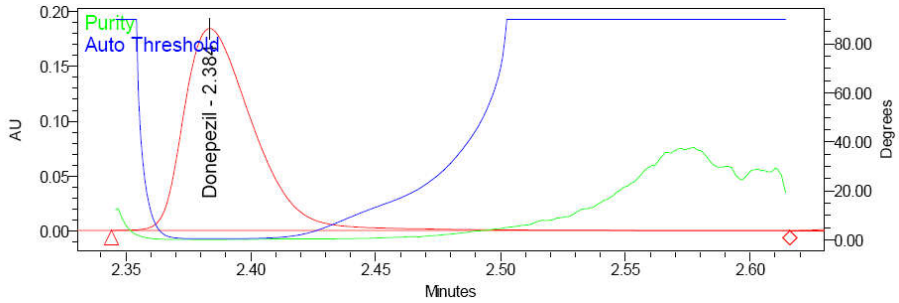


Fig. 4. Specificity

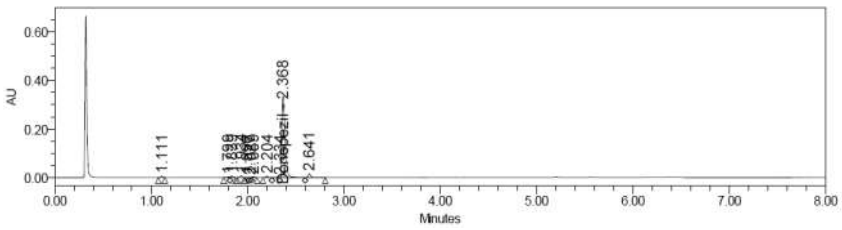
(a) Alkali degradation



(b) Alkali degradation peak purity



(c) Peroxide degradation



(d) Peroxide degradation peak purity

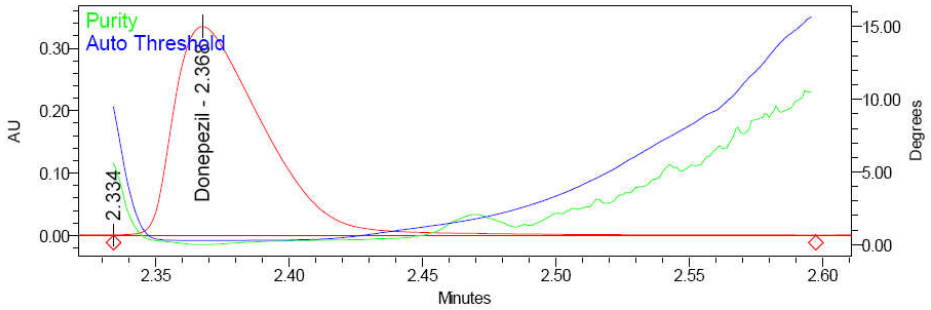


Fig.5 Chromatograms of **a** and **c** samples obtained from alkali and peroxide stress testing, **b** and **d** peak purity of alkali and peroxide stress testing.

corresponding chromatograms are given in Fig-2. Finally, 0.1% trifluoroacetic acid in water as mobile phase-A and 0.1% trifluoroacetic acid in 70:30 mixture of acetonitrile and methanol as mobile phase-B was tried and gradient was optimized so that all the impurities and donepezil peak were well separated from each other. No blank peak interference at the retention time of known peak was obtained as shown in Fig-3.

System suitability

For the related substances method, the resolution between Impurity-D and Impurity-F from the system suitability preparation is 4.5, RSD for the area of donepezil hydrochloride peak and all the impurities from the replicate injections of standard preparation was 3.3% and USP plate count for donepezil hydrochloride peak in the standard preparation was 58899.The above three system suitability parameters were met during the course of entire validation . For the assay method, The tailing factor for the donepezil hydrochloride standard peak from the first injection of the standard preparation-1 was 1.4, The relative standard deviation for the mean area calculated for donepezil hydrochloride peak from the six replicate injections of standard preparation - 1 was 0.66 % and the similarity factor calculated between standard preparation - 1 and standard preparation-2 was within 1.01. The above three system suitability parameters were met during the course of entire validation (Table 1).

Specificity

As shown in the Fig 4, donepezil hydrochloride peak was well separated from each other impurities; also no blank peak interference at the retention time of known peaks, the purity angle is less than purity threshold for the donepezil hydrochloride peak in the spiked sample, the method is selective and specific. Fig 4 demonstrates the specificity of the method.

Forced Degradation

Degradation was not observed when donepezil hydrochloride exposed to acid hydrolysis, thermal, UV

and white light. Donepezil hydrochloride was degraded when exposed to alkali and peroxide (Fig.5). Results from peak purity testing confirmed the main compound peak obtained by analysis of all the stress samples was homogenous and pure and unaffected by the presence of its degradation products, confirming the stability-indicating nature of the method. The results from forced degradation studies are summarized in Table 2.

Precision

The precision and intermediated precision were successfully demonstrated and RSD for the individual and total impurity values were found to be below the acceptance value.

For related substance method the RSD of individual impurity and total impurities were calculated and found less than 10% for impurities. The overall RSD between method precision and intermediate precision values are of less than 10% demonstrates good precision of the method. The assay of donepezil hydrochloride was determined as per the method of analysis using two columns of different lots, different UPLC instrument on two different days and two different chemist. Calculated overall RSD for the assay values. Results were summarized in Table 3.

Linearity, Limit of detection (LOD) and Limit of quantification (LOQ)

Linear regression analysis for each ingredient showed that the calibration curves were linear over the concentration range shown in Table 4. Limits of quantification and detection are also presented in the same table.

Linearity results (n=3)

Accuracy

The recovery of three sample preparation at each level was examined and ranged from 92.89% to 96.70%. Results are summarized in Table 3.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature, wavelength and gradient) the results obtained were well within the limit for related substance method(RSD NMT 10%) and assay method.(RSD NMT 2%). The validation data has been incorporated in Table 5.

Solution stability

No significant changes were observed in the content of impurities in the solution stability studies conducted after 24 h. The cumulative RSD was calculated for the individual impurities and total impurities in the standard solution for the related substance method and is less than 10%. In the assay method, cumulative RSD for the assay value from zero hour of preparation up to 24 h were calculated and found to be less than 2%. Results are summarized in Table 6.

Conclusion

The UPLC method developed for the determination of assay and related substances of donepezil hydrochloride in 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 control of donepezil hydrochloride in bulk pharmaceuticals. Moreover, the lower solvent consumption along with the short analytical run time of 8.0 min leads to cost effective chromatographic method.

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