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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DONEPEZIL HYDROCHLORIDE TABELTS BY RP-HPLC AND UV

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Abstract

A new gradient HPLC and UV methods has been developed and validated for the determination of assay of Donepezil hydrochloride in oral pharmaceutical formulation. Different kinds of columns and gradient elution programs were tested in order to achieve satisfactory separation between the active substance, and an interfering excipients used in the formulation. The best results were obtained using a Wakosil C-18 column 250 mm X 4.6 mm, 5 µ, UV detection at 230 nm and a isocratic elution of phosphate buffer (0.02 M, pH 3.67) and Acetonitrile as the mobile phase. Both methods were validated with respect to linearity, precision, accuracy, specificity and robustness LOQ and LOD. It was also found to be stability indicating, and therefore suitable for the routine analysis of Donepezil hydrochloride in the pharmaceutical formulation.

Key words: Donepezil hydrochloride, HPLC, UV, Method Development.

1. Introduction

Donepezil hydrochloride is a reversible inhibitor of the enzyme acetylcholinesterase, known chemically as (±)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4- piperidinyl]methyl]-1*H*-inden-1-one hydrochloride[1]. Current theories on the pathogenesis of the cognitive signs and symptoms of Alzheimers disease attribute some of them to a deficiency of cholinergic neurotransmission. Donepezil hydrochloride is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetylcholinesterase. If this proposed mechanism of action is

correct, Donepezil hydrochloride's effect may lessen as the disease process advances and fewer cholinergic neurons remain functionally intact[2]. There is no evidence that Donepezil hydrochloride alters the course of the underlying dementing process, however several studies have shown modest benefits in cognition and/or behavior [3].

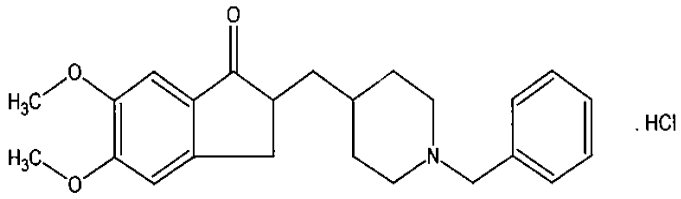

Fig-I: Structure of Donepezil hydrochloride [4]

Few works have been reported with HPLC determination of Donepezil hydrochloride estimation were shown various results [5-8]. The present work has been reported to describe the simultaneous determination of Donepezil assay with HPLC and by UV. This paper describes the development and validation of new precise, simple and reliable methods with isocratic elution and UV detection for the simultaneous determination of Donepezil hydrochloride assay content. The Methods have been proven to be suitable for bulk, final product release and stability testing in oral pharmaceutical formulations.

2. Experimental

2.1. *Materials and reagents*

The Working standard of Donepezil hydrochloride which were purchased from NATCO and the structure was shown in Fig-1 Solvents (water, acetonitrile) was HPLC grade, supplied by TKA Purification system and Merck respectively. Trial formulations of Donepezil hydrochloride consisting of a number of excipients including aspartame were tested. Aricept Evess orally

Disintegrating tablets labeled to contain 10 mg of Donepezil hydrochloride were also tested (Pfizer-Eisai, Germany, LOT: 6114504).

2.2. Chromatographic system and conditions

HPLC analysis was performed on a Agilent system equipped with a 2996 photodiode array detector. The diode array detector was used for the spectrum and peak purity extraction while the analysis was carried out at 230

nm. The separation was achieved using a Wakosil C18 stationary phase (250mm×4.6mm 5µ particle size). The data was acquired via Elite Solutions software. A Sartorius CPA225D balance was used for weighing standards. The initial mobile phase gradient condition was 70:30 of solvents A and B respectively, where A is a buffer of 0.02M KH_2PO_4 (pH 3.67±0.05) and B Acetonitrile. The accuracy of the pH meter used (Thermo Orion) was ±0.01 pH units. The elution was isocratic for the first 10 min and was altered gradually to Buffer–Acetonitrile (55:45) over 10 min. The initial eluent composition was restored in 2.6 min and maintained for an additional 7 min. The flow rate was set at 1.0 ml/min, the column temperature was set at 25±1 ◦C and the injection volume was 20µl .A mixture of Buffer and Acetonitrile at a ratio 55:45 was used as solvent for the preparation of standard and sample solutions. The buffer as well as Acetonitrile was filtered through a GHP (pore size 0.45µ)[9].

2.3. Preparation of standard and sample solutions

A standard solution consisting of Donepezil hydrochloride (100 ppm) was prepared with solvent. Also a sample solution was prepared with a placebo solution spiked with 100 ppm of Donepezil hydrochloride

Standard preparation:

Weigh accurately about 50 mg of Donepezil hydrochloride working standard to a 100 ml volumetric flask. Add about 30 ml Diluent to dissolve it completely (sonicate if necessary), Make up the volume with Diluent. Further dilute 5.0 ml of this solution to 25ml with Diluent.

Sample Preparation:

Weigh accurately 10 tablets of Donepezil hydrochloride 10mg equivalent to 100 mg of Donepezil hydrochloride to a 200 ml volumetric flask. Add about 100 ml Diluent to dissolve it completely and Sonicate for 10 minutes with intermediate shaking, Make up the volume with Diluent filter through 0.45µ GHP filter. Further dilute 5.0 ml this solution to 25 ml with Diluent.

Blank for UV-Spectroscopy: Water: Acetonitrile (80:20)

***Blank for HPLC*:** Buffer pH 3.6 KH_2PO_4 [10]: Acetonitrile (55:45)

2.4. Validation of assay-related method [11]

2.4.1. System suitability:

A sample solution was injected five times in order to obtain the retention times of the Donepezil and all the important parameters of system suitability testing were calculated (RSD of area of Donepezil peak and should be less than 2.

2.4.2. Selectivity/specificity

During specificity study a number of different solutions were prepared. Donepezil hydrochloride, standard solution, sample solution and placebo solution were injected. The spectra and purity plots were extracted through a diode array detector for each ingredient in the standard solution. Furthermore, forced degradation studies were conducted in order to prove selectivity of the method. The sample solution was subjected to acid and basic hydrolysis (using 3N HCl and 3N NaOH, respectively for 1 h), to oxidative hydrolysis (using 10% H_2O_2 for 1 h) to UV radiation (254 nm for 3 days).and to Heat degradation (using 105°C for 3 days).

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

Six standard solutions were prepared for the linearity test in the range of 25–150% of working concentration for Donepezil and in the range of LOQ—150% of working concentration. Each solution was injected in two replicates and linear regression analysis for each ingredient was performed.

2.4.4. System precision—repeatability

The system precision was examined by analyzing six determinations of the sample solution at working concentration versus a standard solution.

2.4.5. Intermediate precision (ruggedness)

A second analyst performed the same analysis as in repeatability, on a different day using new solutions and a different chromatographic system.

2.4.6. Accuracy

The recovery of three sample preparations at three concentration levels (50%, 100%, and 150%) of working concentration for Donepezil was examined.

2.4.7. Robustness

Several parameters of the method were purposely altered in order to determine the robustness of the method. The system suitability parameters as well as the recovery for the main ingredients in the sample solution were examined. The method parameters altered were the wavelength of detection, the column's temperature, the flow rate and the buffer's pH.

2.4.8. Stability of solutions

Both standard and sample solutions were prepared and analyzed for recovery of Donepezil at 0, 24, and 48 hr at room temperature.

2.4.9. Application of method

In order to evaluate the application of method, commercial preparations and the new formulation were analyzed. The samples were prepared as described above and the content of Donepezil was calculated.

3. RESULTS

3.1. Method development [12]

Several methods in literature describe the determination of Donepezil. The columns and detectors described vary between cases (C18, C30, enantiomers selective and UV, fluorescence, MS detection according to the article's method but the most common problem arising was the co-elution of impurities and Donepezil hydrochloride were seen.

The replacement of methanol with acetonitrile results in separation of the two analytes an isocratic elution and a change in buffer concentration to 0.02M were chosen in order to shorten Retention times but also maintains specificity and selectivity of the method. Analysis of a sample solution revealed. A final adjustment of pH to 3.6 resulted in the separation of placebo and Donepezil along with blank and mobile phase.

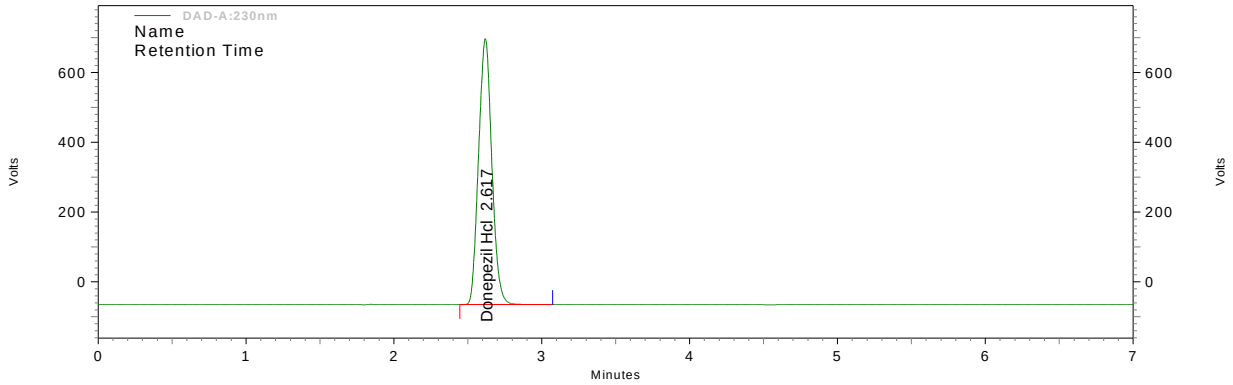

Fig II Standard solution of Donepezil hydrochloride (conditions: 0.02M KH_2PO_4/ACN 55:45, pH 3.6, flow 1 ml/min, 25 °C, 230 nm, Wakosil C18 , 250mm×4.6 mm, 5µ, run time 7 min).

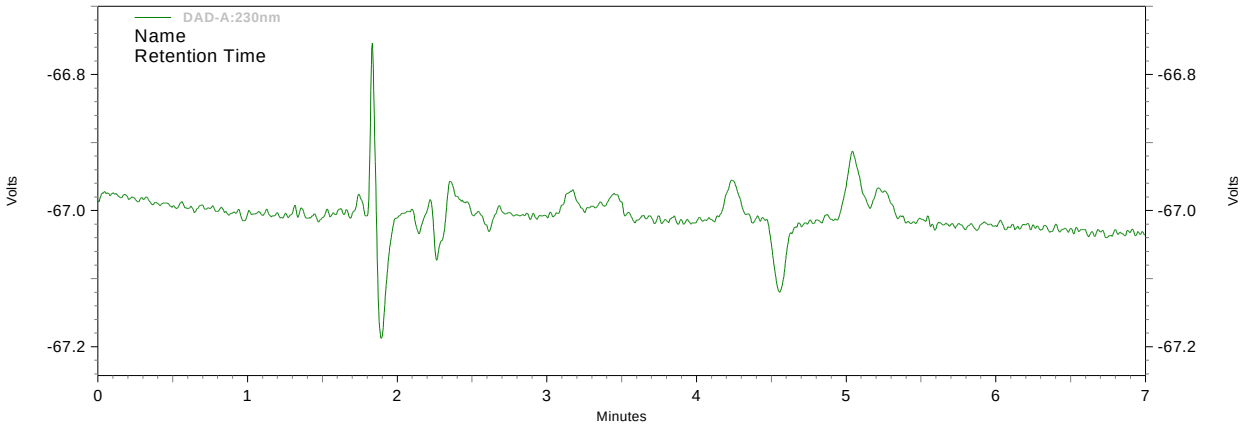

Fig III Placebo solution (conditions: 0.02M KH_2PO_4/ACN 55:45, pH 3.6, flow 1 ml/min, 25 °C, 230 nm, Wakosil C18 , 250mm×4.6 mm, 5µm, run time 7 min).

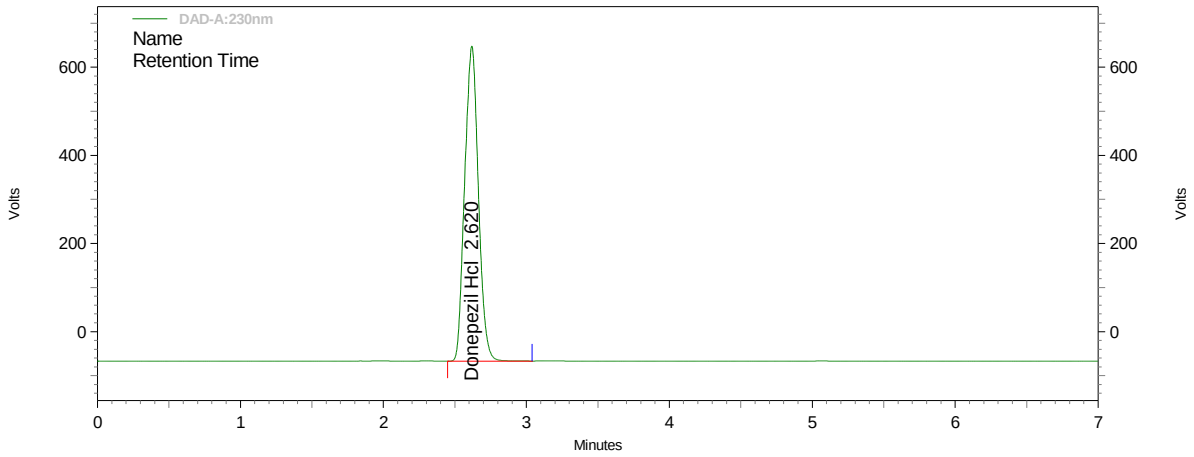

Fig IV Sample solution (conditions: 0.02M KH_2PO_4/ACN 55:45), pH 3.6, flow 1 ml/min, 25 °C, 230 nm, Wakosil C18, 250mm×4.6 mm, 5µm, run time 7 min).

3.2. Validation of assay method

3.2.1. System suitability

The observed retention time was presented in Table I (Calculated by the system software) was set as the system suitability parameter (<1.5). Also the RSD of the peak area of Donepezil (<2.0%) was calculated.

Table-I: System suitability results.

System suitability Parameters	Donepezil hydrochloride by HPLC	Donepezil hydrochloride by UV
RSD	0.63 %	0.28%
Tailing factor	1.39	NA
No. of theoretical plates	4458	NA

3.2.2. Selectivity/specificity

As shown in Fig-4 Donepezil hydrochloride was well separated in a sample solution injection. Also, the placebo solution shows no interfering peak with the rest of the analytes as shown in Fig III. The purity values of peaks derived from the purity plots were acceptable (Table I). Furthermore, the specificity of the method was confirmed through forced degradation studies. Donepezil showed degradation products after acid hydrolysis, alkaline hydrolysis (3N NaOH) and oxidation (10% H_2O_2), light degradation at 254 nm and heat degradation at 105°C.The purity angle of the main peak maintained an acceptable value and mass balance was maintained during all stress conditions (Table II).

Table-II: Specificity results.

Sample	%Assay by HPLC	Peak Purity	% Assay by UV
Control Sample	99.6	1.0000	99.1
Acid degradation sample	98.5	1.0000	99.4
Alkali degradation sample	98.9	0.9998	98.6

Peroxide degradation sample	98.1	0.9998	98.6
Heat degradation sample	100.0	1.0000	100.6
Light degradation sample	99.6	1.0000	101.4

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

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

Table-III: linearity results.

Levels	**Conc. (ppm)**	**Response (mean area) by HPLC**	**Response (mean area) by UV**
Level 1(25%)	23.529	121416	0.2365
Level 2(50%)	47.058	2359538	0.4995
Level 3(75%)	70.586	3621026	0.7120
Level 4(100%)	94.115	4888025	1.0135
Level 5(125%)	117.644	5842717	1.2270
Level 6(150%)	141.173	7046995	1.4950
Y – intercept		**73999.5**	**-0.0137**
Slope		**49642**	**0.0107**
Correlation Coefficient		**0.99934**	**0.99927**

3.2.4. System precision—repeatability

The RSD of sample solutions recovery was calculated and found to be below acceptable values (Table IV).

3.2.5. Intermediate precision (ruggedness)

The RSD of sample solutions recovery, as well as the overall RSD was calculated and found less than 2.0 (Table IV), which demonstrates good precision of the method.

Table-IV: Precision and intermediate precision results.

Sample. No	% Assay by HPLC	% assay by UV
Sample Preparation-1	98.9	99.7
Sample Preparation-2	99.6	101.7
Sample Preparation-3	100.3	101.7
Sample Preparation-4	100.4	101.4
Sample Preparation-5	99.3	101.4
Sample Preparation-6	100.4	101.0
Avg	**99.8**	**101.2**
SD	**0.643**	**0.756**
%RSD	**0.64**	**0.75**

3.2.6. Accuracy

The recovery of three sample preparations was examined and ranged from 97.9% to 100% as shown in Table V.

Table-V: Accuracy results.

Accuracy Level	% Mean Recovery by HPLC	% Mean Recovery by UV
Accuracy level -50%	99.7	99.7
Accuracy level -100%	99.4	101.9
Accuracy level-150%	99.4	99.9
Mean Recovery	**99.5**	**100.5**
SD	**0.17**	**1.25**
% RSD	**0.17**	**1.24**

3.2.7. Robustness

The results obtained from the robustness study were well within the limits, apart from the case of changing flow rate and pH (Table VI).

Table-VI: Robustness results.

Robustness Criteria	% RSD	TF	TP
Change in mobile phase flow rate by - 0.2ml / minutes (Flow rate =0.8 ml/minutes)	0.159	1.39	3236
Change in mobile phase flow rate by +0.2ml / minutes (Flow rate =1.2 ml/minutes)	0.084	1.33	2642
Change in wavelength by-2nm (228nm)	0.045	1.35	2917
Change in wavelength by +2nm (232nm)	0.103	1.36	2940

3.2.8. Stability of solutions

The recovery of 48 h solutions versus 0 h solutions was calculated. Both standard and sample solutions were found to be stable for 48 h, since the recovery was within specifications (Table VII).

Table VII: Solution stability results.

Preparation	Standard		Sample	
	%Assay	% Difference	%Assay	% Difference
0 hours	100.0	N/A	98.9	N/A
After 24 hours	101.3	1.30	100.7	1.8
After 48 hours	101.2	1.20	100.0	1.0

3.2.9. Application of method

The results of the Aricept 10 mg analysis and new formulation analysis are compared. The assay value was determined and it was within the accepted criteria.

4. Results and Discussion

The method described in this paper is a reverse phase HPLC and UV-spectrometric method. The peaks elute from the C18 column in order of decreasing polarity, that is more polar compounds elute first and less polar and bigger compounds are retained strongly and elute later. Compounds with very similar structure elute close to each other. The interactions between mobile phase, stationary phase and analytes result in the observed order of elution. Parameters that affect retention and separation are % of organic solvent and type of organic solvent, because they define the polarity of the mobile phase and the kind of interactions between molecules and stationary phase. This is the reason for altering these parameters during method development and optimization. pH of the mobile phase is also a very significant parameter, influencing retention and separation of peaks. Different pH values were tried in order to achieve the best separation, and it was concluded that it should be controlled within strict limits throughout the analysis.

Table-VIII: Method validation report for donepezil hydrochloride by RP- HPLC &uv spectroscopy.

PARA-METERS	LIMIT	OBSERVATIONS FOR HPLC	OBSERVATIONS FOR UV	PASSES/ FAILS
Specificity	No Interferences at retention time of the analyte peak.	Interference at retention time of the analyte peak	Interference at retention time of the analyte peak	Passes
Precision • System Precision • Method Precision	RSD NMT 2.0% RSD NMD 2.0%	Donepezil -0.63% Donepezil -0.64%	Donepezil -0.283% Donepezil -0.44%	Passes
Linearity of detector response	Correlation co-efficient NLT 0.999	Donepezil -0.99934	Donepezil -0.99927	Passes
Accuracy	% Recovery range 98-102%	Donepezil -99.6-100.0%	Donepezil -99.7-101.9%	Passes

Limit of detection (LOD)	Correlation co-efficient NLT 0.995	Donepezil -0.8 ppm	Donepezil -0.8 ppm	Passes
Limit quantitation (LOQ)	Correlation co-efficient NLT 0.998	Donepezil -1.2 ppm	Donepezil -1.2 ppm	Passes
Ruggedness	RSD NMT 2.0%	Donepezil -0.75%	Donepezil -0.75%	Passes

5. Conclusion

Based on the validation results the proposed HPLC and UV methods are proven to be suitable for the determination of Donepezil hydrochloride assay content in Donepezil tablets.

The method is robust with respect to variations in method parameters. Care should be taken regarding the flow rate and the adjustment of buffer's pH, since deviations from the proposed pH (3.6±0.05) result in inadequate peaks separation.

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