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Research Article

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RP-HPLC and UV spectrophotometric method development and validation of donepezil hydrochloride tablets

Jagadeesh Kumar Ega and Kavitha Siddoju*

Department of Chemistry, Chaitanya Postgraduate College,(Autonomous), Kakatiya University, Warangal, Telangana State., India

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ABSTRACT

Donepezil hydrochloride is used in the Alzheimer's disease used in neurodegenerative disorder characterized by progressive attribute some of them to a deficiency cholinergic neurotransmission. Its therapeutic effect by enhancing cholinergic function it was accomplished by increasing the concentration of acetyl cholinesterase through reversible inhibition of its hydrolysis. High-performance liquid chromatography is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. The Donepezil hydrochloride is used as a active ingredient in the pharmaceutical dosage form. Pharmacopoeias have not yet provided an official method for its quantization. Hence present aim of the work is to develop a specific. precise, accurate, linear, simple, rapid and cost effective analytical method for Donepezil hydrochloride in tablet dosage form by RP-HPLC and UV spectrophotometric method. The work extends to validate for the developed methods as per ICH guidelines.

Keywords: Donepezil, Alzheimer's disease, RP-HPLC and UV spectrophotometric method

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INTRODUCTION

Donepezil Hydrogen chloride **(** (±)-2, 3-dihydro-5, 6-dimethoxy2-[[1-(phenyl methyl 4- piperidinyl] methyl]-1H-inden-1-one hydrochloride**)** **(**MF $C_{24}H_{29}NO_3 \cdot HCl$**)** is white crystalline power and freely soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane. This is however no reported HPLC method for the analysis of Donepezil in its technical grade and formulation. The HPLC method described here is simple, sensitive and reproducible for Donepezil determination in formulations with low background interference.

HPLC is the term used to describe liquid chromatography in which the liquid mobile phase is forced through the column at high speed as a result, the analysis time is reduced by 1-2 orders of the magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possibly increasing the column efficiency substantially. The importance of chromatography is increasing rapidly in pharmaceutical analysis for the exact differentiation, selective identification and quantitative determination of structurally closely related compounds and purity testing of final products and the intermediates. Its suitability for separating non-volatile species or thermally fragile ones and its wide spread applicability to substances that are of prime interest to the industry. Sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation.

Adsorption chromatography employs high-surface area particles that adsorb the solute molecules. Usually a polar solid such as a silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptane, octane or chloroform are used in adsorption chromatography. In adsorption chromatography, adsorption process is described by competition model and solvent interaction model. Competition model assumes that entire surface of the stationary phase is covered by mobile phase molecules as a result of competition for adsorption site. In solvent interaction model the retention results from the interaction of solute molecule with the second layer of adsorbed mobile phase molecules. The differences in affinity of solutes for the surface of the stationary phase account for the separation achieved.

EXPERMENTAL SECTION

Table-1

Goal	Comment
Resolution	Precise and rugged quantitative analysis requires that R_s be greater than 1.5
Separation time	<5-10 min is desirable for routine procedures.
Quantization	2% for assays; 5% for less-demanding analyses15% for trace analyses.
Peak height	Narrow peaks are desirable for large signal/noise ratios.
Solvent consumption	Minimum mobile-phase use per run is desirable.

Table-2 Chromatographic Mode

Sample type	Analytes type	Common mode
Macromolecules	Organic polymers	GPC
(MW > 2,000)	Biomolecules	SEC, RPC, IEC, HILIC, HIC
Organics	Polar	RPC, NP, HILIC
(MW < 2,000)	Medium polarity	RPC
	Nonpolar	RPC, NARP, NP
	Ions, ionizable compounds	RPC (ion suppression), RPC-IP, IEC, HILIC
Preparative	All	NP, RPC, GPC, IEC

Reversed-phase (RPC), ion-pair (IP), ion-exchange (IEC), hydrophilic interaction (HILIC),hydrophobic interaction (HIC), normal-phase (NP), gel permeation (GPC), size-exclusion (SEC), nonaqueous RP (NARP).

Table- 3 Commonly Used Buffers For RP-HPLC

Buffer	PKa	Buffer range	UV cutoff(nm)
Phosphate	2.1 7.2 12.3	1.1-3.1 6.2-8.2 11.3-13.3	200
Formic acid	3.8	2.8-4.8	210
Citrate	3.1 4.7 5.4	2.1-4.1 3.7-5.7 4.4-6.4	230
Acetic acid	4.8	3.8-5.8	210
Tris	8.3	7.3-9.3	205
Triethylamine	11.0	10.0-12.0	200
Pyrrolidine	11.3	10.3-12.3	200

Table-4 List of specifications for typical spectrophotometer

SPECIFICATION	λmax VALUE
Wavelength range	190 – 900 nm
Wavelength accuracy	±0.3 nm at slit width of 0.2 nm
Wavelength repeatability	±0.1 nm
Wavelength scanning speed	Fast, medium ,slow and super slow
Band width	0.1 – 7.5 nm (variable and selectable)
Resolution	±0.1 nm
Stray light	<0.015 % at 220 and 340 nm <0.0003 % at 220 and 340 nm (drift instrument)
Photometric accuracy	±0.002 in 0 – 5 AU ±0.004 in 0.5– 1.0 AU
Photometric repeatability	±0.001 in 0 – 0.5 AU ±0.00 in 0.5– 1.0 AU ±0.3 %T (0 – 100 %T)
Photometric mode	Abs, %T, %Reflectance, Energy (E)
Drift (AU / h)	<0.0004 after warm up
Response time	~ 0.1 – 5
Photometric range (AU)	4 – 5
Base line flat ness (AU)	Within ±0.001 Abs (scan speed dependent)
Scan speed	50 nm/min to ~ 2100 nm/min

INSTRUMENTATION

S. No.	Name of Instrument	Model	Make
1	Semi micro balance	CPA225D	Sartorius
2	Micro balance	CPA2P	Sartorius
3	Precision balance	GPA5202	Sartorius
4	pH Meter	3 Star	Thermo Orion
5	HPLC	1200 series	Agilent
6	HPLC	LC-2010 CHT	Shimadzu
7	Column	C18(250X4.6),5µ	Wakosil
8	Column	C18(250X4.6),5µ	Waters
9	UV-Spectrophotometer	UV-1800	Shimadzu
10	Sonicator	UCB 70	Spectralab

Reagents and Chemicals

Acetonitrile, Potassium dihydrogen orthophosphate, Sodium Hydroxide, Triethylamine, Hydrochloric acid, Hydrogen peroxide all are Merck (GR-Grade) and TKA water.

Working/reference standards

Donepezil Hydrochloride Working Standard.

Filter

0.45µm GHP membrane filter (Manufactured by PALL).

Test sample Donepezil Hydrochloride tablets 10 mg.

S.No.	Ingredients	10mg tablet (mg)
1	Lactose Monohydrate powder grade	154.20
2	Micro crystalline celluose 102	22.0
3	Hydroxyl propylmethyl celluose	7.0
4	Maize Starch B	92.40
6	Magnesium stearate	2.80
7	Opedray yellow	8.0
8	Opedray White	-
9	Donepezil hydrochloride API	10.0
Total		288.0

System suitability Parameters	Donepezil hydrochloride
RSD	0.63 %
Tailing factor	1.39
No. of theoretical plates	4458

LINEARITY

Linearity Level	Volume of Linearity Stock solution	Final dilution (ml)	Conc. of Donepezil hydrochloride (In ppm)
Level 1 (25%)	1	25	25.2
Level 2 (50%)	2	25	50.4
Level 3(75%)	3	25	75.6
Level 4 (100%)	4	25	100.8
Level 5 (125%)	5	25	126.0
Level 6 (150%)	6	25	151.2

Linearity response

Levels	Conc. (ppm)	Response (mean area)
Level 1(25%)	23.529	121416
Level 2(50%)	47.058	2359538
Level 3(75%)	70.586	3621026
Level 4(100%)	94.115	4888025
Level 5(125%)	117.644	5842717
Level 6(150%)	141.173	7046995
Y – intercept		**73999.5**
Slope		**49642**
Correlation Coefficient		**0.99934**

Range Dilutions

Sample Name	Amount of Placebo to be weighed (mg)	Amount of Donepezil hydrochloride API to be weighed (mg)	Conc. of Donepezil hydrochloride (ppm)
Recovery 50% -1	2780	50	50
Recovery 50% -2	2780	50	
Recovery 50% -3	2780	50	
Recovery 100% -1	2780	100	100
Recovery 100% -2	2780	100	
Recovery 100% -3	2780	100	
Recovery 150% -1	2780	150	150
Recovery 150% -2	2780	150	
Recovery 150% -3	2780	150	

Recovery data

Recovery level I (50%)			
Analysis no.	**Quantity added in ppm**	**Quantity recovered in ppm**	**% Recovery**
1	47.24	47.22	100.0
2	48.22	47.81	99.2
3	47.42	47.39	99.9
		Mean	**99.7**
		SD	**0.44**
		% RSD	**0.44**

Recovery level II (100%)			
Analysis no.	**Quantity added in ppm**	**Quantity recovered in ppm**	**% Recovery**
1	94.56	93.23	98.6
2	94.58	94.48	99.9
3	94.52	94.15	99.6
		Mean	**99.4**
		SD	**0.68**
		% RSD	**0.69**

Recovery level III (150%)			
Analysis no.	**Quantity added in ppm**	**Quantity recovered in ppm**	**% Recovery**
1	142.16	140.30	98.7
2	141.84	139.66	98.5
3	141.53	142.92	101.0
		Mean	**99.4**
		SD	**1.40**
		% RSD	**1.40**

Recovery Level	**% Mean Recovery**
Recovery level -50%	99.7
Recovery level -100%	99.4
Recovery level-150%	99.4
Mean Recovery	**99.5**
SD	**0.17**
% RSD	**0.17**

Repeatability

Analyst I

Sample. No	**% Assay**
Sample Preparation-1	98.9
Sample Preparation-2	99.6
Sample Preparation-3	100.3
Sample Preparation-4	100.4
Sample Preparation-5	99.3
Sample Preparation-6	100.4
Avg	**99.8**
SD	**0.643**
%RSD	**0.64**

Precision

Sample No.	% Assay	Intermediate Precision data
1	98.9	Analyst-I(Repeatability data)
2	99.6	
3	100.3	
4	100.4	
5	99.3	
6	100.4	
7	99.7	Analyst-II(Intermediate Precision data)
8	101.7	
9	101.7	
10	101.4	
11	101.4	
12	101.0	
Cum. %RSD	**0.96**	

Analyst II

Sample. No	% Assay
Sample Preparation-1	99.7
Sample Preparation-2	101.7
Sample Preparation-3	101.7
Sample Preparation-4	101.4
Sample Preparation-5	101.4
Sample Preparation-6	101.0
Avg	**101.2**
SD	**0.756**
%RSD	**0.75**

Solution stability data

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

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 (232)	0.103	1.36	2940

System suitability

Sample Name	%RSD
Blank (diluent)	NA
Standard solution (System Suitability)	0.283

Specificity	Interferences
Diluent	No interference
Mobile phase	No interference
Blank solution	No interference

LINEARITY

Linearity Level	Volume of Linearity Stock solution	Final dilution (ml)	Conc. of Donepezil hydrochloride (In ppm)
Level 1 (25%)	1	25	25.2
Level 2 (50%)	2	25	50.4
Level 3(75%)	3	25	75.6
Level 4 (100%)	4	25	100.8
Level 5 (125%)	5	25	126.0
Level 6 (150%)	6	25	151.2

Calculations

Level	Conc. (ppm)	Response (mean area)
Level 1(25%)	23.529	0.2365
Level 2(50%)	47.058	0.4995
Level 3(75%)	70.586	0.7120
Level 4(100%)	94.115	1.0135
Level 5(125%)	117.644	1.2270
Level 6(150%)	141.173	1.4950
Y – intercept		**-0.0137**
Slope		**0.0107**
Correlation Coefficient		**0.99927**

Range Dilutions

Sample Name	Amount of Placebo to be weighed (mg)	Amount of Donepezil hydrochloride API to be weighed (mg)	Conc. of Donepezil hydrochloride (ppm)
Recovery 50% -1	2780	50	50
Recovery 50% -2	2780	50	
Recovery 50% -3	2780	50	
Recovery 100% -1	2780	100	100
Recovery 100% -2	2780	100	
Recovery 100% -3	2780	100	
Recovery 150% -1	2780	150	150
Recovery 150% -2	2780	150	

Recovery level I (50%)			
Analysis no.	Quantity added in ppm	Quantity recovered in ppm	% Recovery
1	47.24	47.22	100.5
2	48.22	47.81	98.3
3	47.42	47.39	100.1
		Mean	**99.7**
		SD	**1.20**
		% RSD	**1.20**

Recovery level II (100%)			
Analysis no.	Quantity added in ppm	Quantity recovered in ppm	% Recovery
1	94.56	93.23	101.8
2	94.58	94.48	101.9
3	94.52	94.15	102.1
		Mean	**101.9**
		SD	**0.12**
		% RSD	**0.12**

Recovery level III (150%)			
Analysis no.	Quantity added in ppm	Quantity recovered in ppm	% Recovery
1	142.16	140.30	99.6
2	141.84	139.66	99.8
3	141.53	142.92	100.2
		Mean	**99.9**
		SD	**0.29**
		% RSD	**0.29**

Recovery Level	% Mean Recovery
Recovery level -50%	99.7
Recovery level -100%	101.9
Recovery level-150%	99.9
Mean Recovery	**100.5**
SD	**1.25**
% RSD	**1.24**

PRECISION

Sample. No	% Assay
Sample Preparation-1	98.2
Sample Preparation-2	99.2
Sample Preparation-3	98.8
Sample Preparation-4	98.7
Sample Preparation-5	99.4
Sample Preparation-6	99.2
Avg	**98.9**
SD	**0.44**
%RSD	**0.44**

Linearity response

Level	Conc. (ppm)	Response (mean area)
Level 1(0.2ppm)	0.19800	0.0261
Level 2(0.4ppm)	0.39600	0.0512
Level 3(0.6ppm)	0.59400	0.0762
Level 4(0.8ppm)	0.79200	0.1044
Y – intercept		**-0.000500**
Slope		**0.131263**
Correlation Coefficient		**0.9996**

LIMIT OF QUANTITATION (LOQ)

Level	Conc. (ppm)	Response (mean area)
Level 1(0.2ppm)	0.19800	0.0261
Level 2(0.4ppm)	0.39600	0.0528
Level 3(0.6ppm)	0.59400	0.0781
Level 4(0.8ppm)	0.79200	0.1060
Level 5(1.0ppm	0.99000	0.1321
Level 6(1.2ppm)	1.18800	0.1591
Y – intercept		**-0.0007**
Slope		**0.1343**
Correlation Coefficient		**0.9999**

VALIDATION REPORT FOR DONEPEZIL HYDROCHLORIDE TABLETS BY RP-HPLC

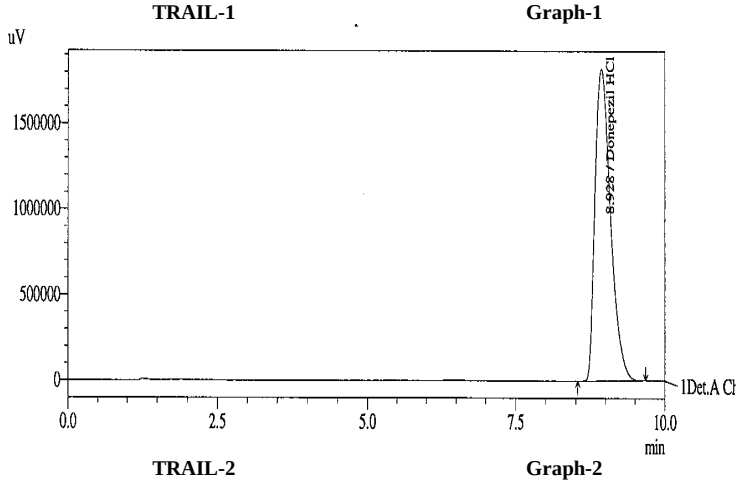
S.No	PARA-METERS	LIMIT	OBSERVATIONS	PASSES/ FAILS
1	Specificity	No Interferences at retention time of the analyte peak.	Interference at retention time of the analyte peak	Passes
2	Precision System Precision Method Precision	 RSD NMT 2.0% RSD NMD 2.0%	 Donepezil -0.63% Donepezil -0.64%	 Passes
3	Linearity of detector response	Correlation co-efficient NLT 0.999	Donepezil -0.99934	Passes
4	Accuracy	% Recovery range 98-102%	Donepezil -99.6-100.0%	Passes
5	Ruggedness	RSD NMT 2.0%	Donepezil -0.75%	Passes
6	Robustness	RSD NMT 2.0%	Within limits	Passes

VALIDATION REPORT FOR **DONEPEZIL HYDROCHLORIDE** *TABLETS BY UV SPECTROSCOPY*

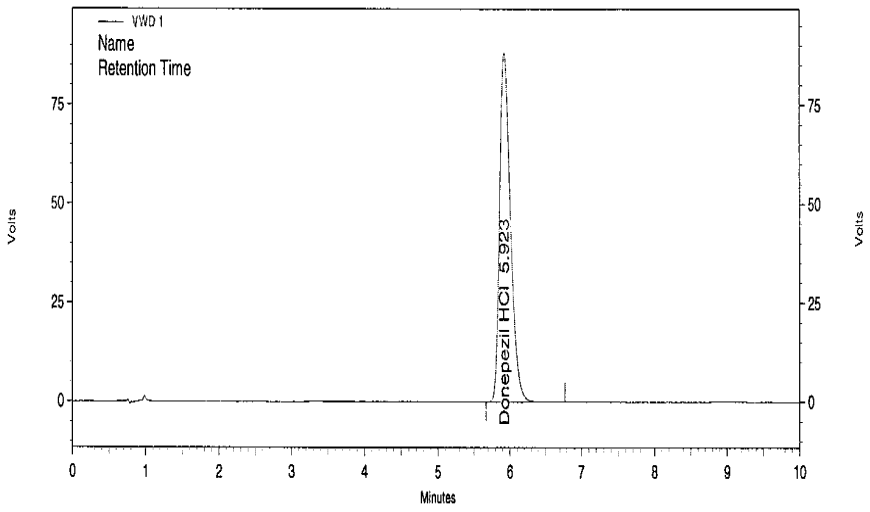
S.No	PARA-METERS	LIMIT	OBSERVATIONS	PASSES/ FAILS
1	Specificity	No Interferences at retention time of the analyte peak.	Interference at retention time of the analyte peak	Passes
2	Precision System Precision Method Precision	RSD NMT 2.0% RSD NMD 2.0%	Donepezil -0.283% Donepezil -0.44%	Passes
3	Linearity of detector response	Correlation co-efficient NLT 0.999	Donepezil -0.99927	Passes
4	Accuracy	% Recovery range 98-102%	Donepezil -99.7-101.9%	Passes
5	Limit of detection (LOD)	Correlation co-efficient NLT 0.995	Donepezil -0.8 ppm	Passes
6	Limit quantitation (LOQ)	Correlation co-efficient NLT 0.998	Donepezil -1.2 ppm	Passes

TRAILS FOR THE METHOD FINALIZATION

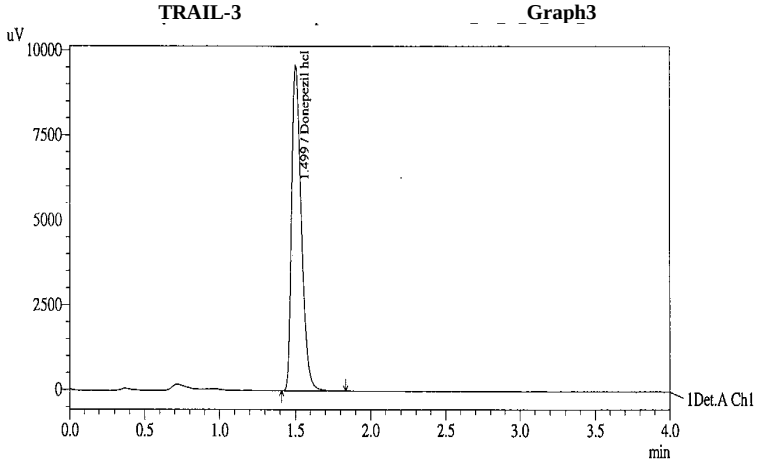
TRAIL-1 **Graph-1**



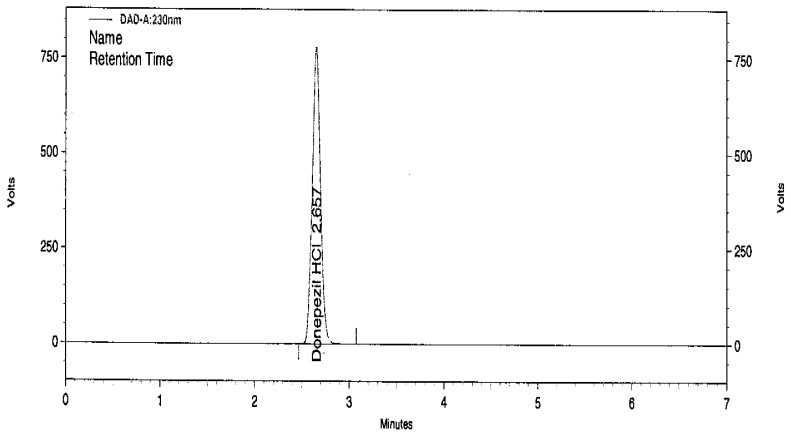
TRAIL-2 **Graph-2**



TRAIL-3 **Graph3**



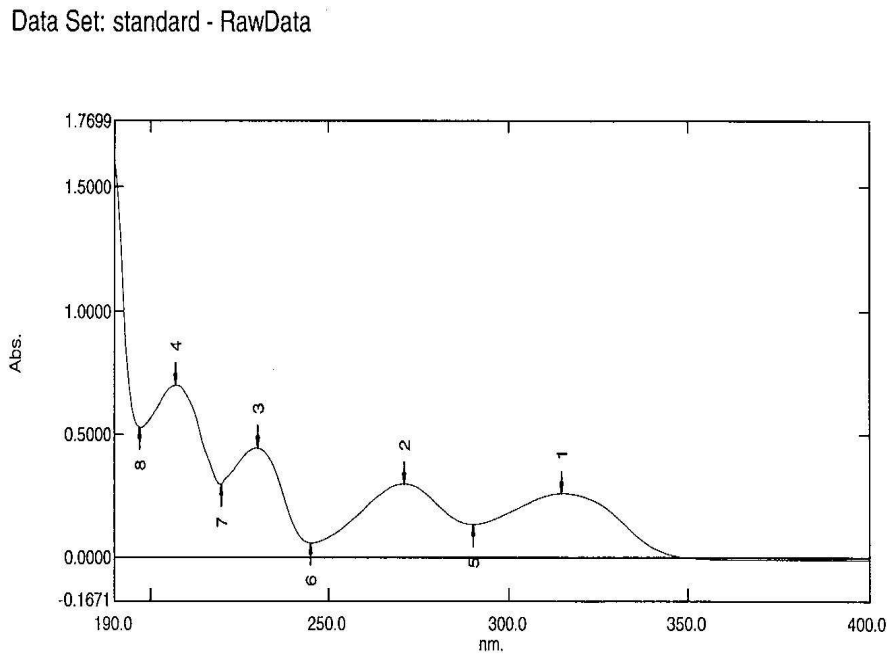
TRAIL-4 **Graph-4**



DAD-A:230n

DONEPEZIL HYDROCHLORIDE STANDARD BY UV

GRAPH-5



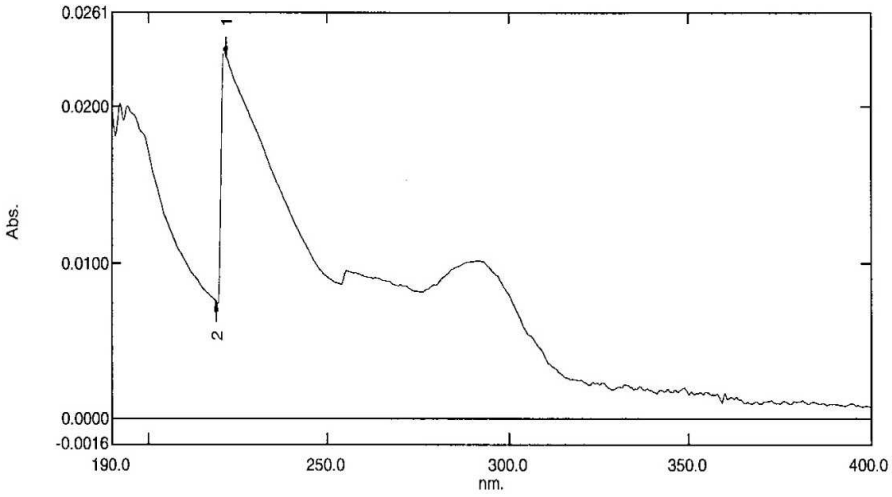
Measurement Properties	
Wavelength Range (nm.):	190.0 to 400.0
Scan Speed:	Medium
Sampling Interval:	0.2
Auto Sampling Interval:	Enabled
Scan Mode:	Auto
Instrument Properties	
Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	360.0 nm
S/R Exchange:	Normal
Attachment Properties	
Attachment:	None
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	10 mm
Additional Information:	

No.	P/V	Wavelength	Abs.
1	P	315.0	0.2626
2	P	271.0	0.3006
3	P	230.0	0.4452
4	P	207.0	0.6987
5	V	290.0	0.1371
6	V	245.0	0.0615
7	V	220.0	0.2988
8	V	197.0	0.5297

DONEPEZIL HYDROCHLORIDE BLANK BY UV

Graph-6

Data Set: Blank - RawData



No.	P/V	Wavelength	Abs.
1	▲	222.0	0.0233
2	▼	219.0	0.0075

Measurement Properties
Wavelength Range (nm.): 190.0 to 400.0
Scan Speed: Medium
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Auto

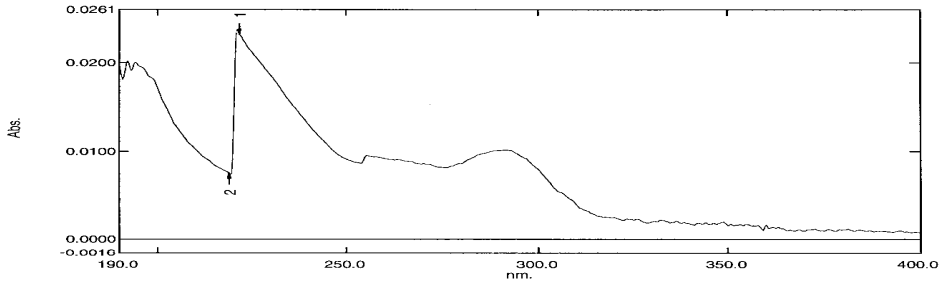
Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 360.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length: 10 mm
Additional Information:

RECOVERY STUDIES BY UV

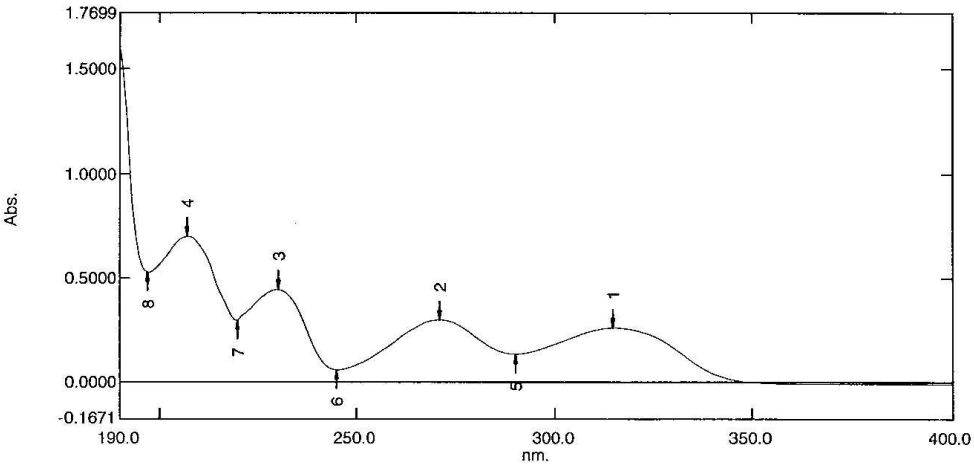
Data Set: Recovery Blank - RawData



No.	P/V	Wavelength	Abs.	Description
1		222.0	0.0233	
2		219.0	0.0075	

Measurement Properties	
Wavelength Range (nm.):	190.0 to 400.0
Scan Speed:	Medium
Sampling Interval:	0.2
Auto Sampling Interval:	Enabled
Scan Mode:	Auto
Instrument Properties	
Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	360.0 nm
S/R Exchange:	Normal
Attachment Properties	
Attachment:	None
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	10 mm
Additional Information:	

Data Set: Recovery standard - RawData

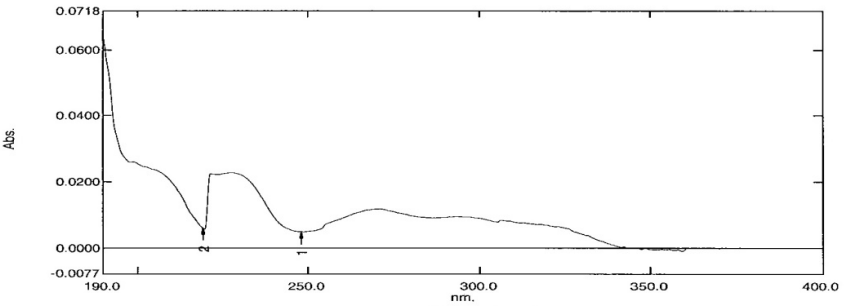


No.	P/V	Wavelength	Abs.
1		315.0	0.2626
2		271.0	0.3006
3		230.0	0.4452
4		207.0	0.6987
5		290.0	0.1371
6		245.0	0.0615
7		220.0	0.2988
8		197.0	0.5297

Measurement Properties	
Wavelength Range (nm.):	190.0 to 400.0
Scan Speed:	Medium
Sampling Interval:	0.2
Auto Sampling Interval:	Enabled
Scan Mode:	Auto
Instrument Properties	
Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	360.0 nm
S/R Exchange:	Normal
Attachment Properties	
Attachment:	None
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	10 mm
Additional Information:	

LOQ & LOD SAMPLES:

Data Set: LEVEL-1 - RawData



No.	P/V	Wavelength	Abs.	Description
1		248.0	0.0049	
2		219.0	0.0060	

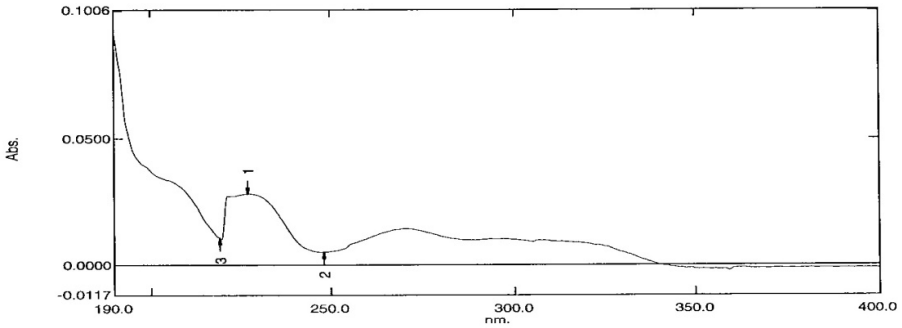
Measurement Properties
Wavelength Range (nm.): 190.0 to 400.0
Scan Speed: Medium
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 360.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length: 10 mm
Additional Information:

Data Set: LEVEL-2 - RawData



No.	P/V	Wavelength	Abs.	Description
1		227.0	0.0280	
2		248.0	0.0049	
3		219.0	0.0106	

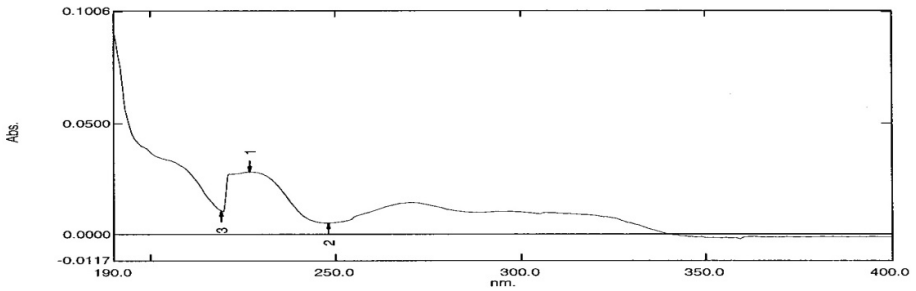
Measurement Properties
Wavelength Range (nm.): 190.0 to 400.0
Scan Speed: Medium
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 360.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length: 10 mm
Additional Information:

Data Set: LEVEL-3 - RawData



No.	P/V	Wavelength	Abs.	Description
1		227.0	0.0280	
2		248.0	0.0049	
3		219.0	0.0106	

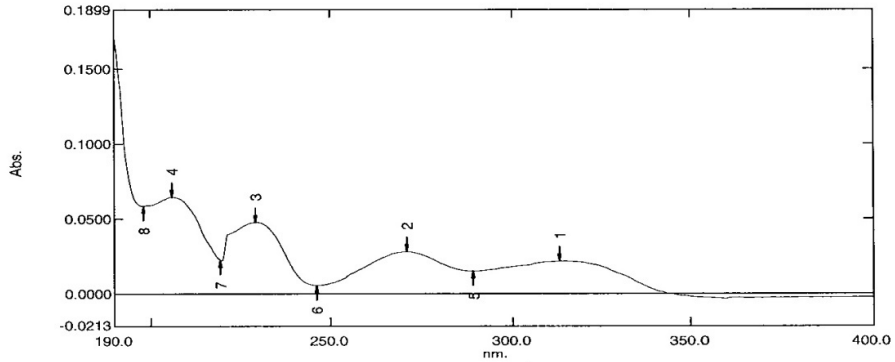
Measurement Properties
Wavelength Range (nm.): 190.0 to 400.0
Scan Speed: Medium
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 360.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length: 10 mm
Additional Information:

Data Set: LEVEL-4 - RawData



No.	P/V	Wavelength	Abs.	Description
1		313.0	0.0215	
2		271.0	0.0278	
3		229.0	0.0476	
4		206.0	0.0644	
5		289.0	0.0148	
6		246.0	0.0053	
7		219.0	0.0226	
8		198.0	0.0585	

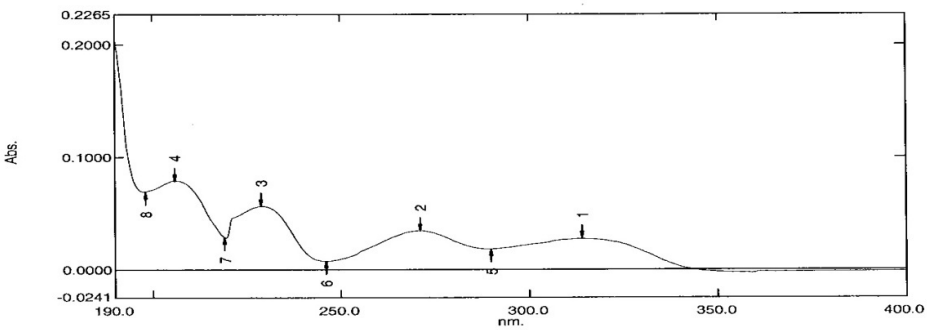
Measurement Properties
Wavelength Range (nm.): 190.0 to 400.0
Scan Speed: Medium
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 360.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length: 10 mm
Additional Information:

Data Set: LEVEL-5 - RawData



No.	P/V	Wavelength	Abs.	Description
1		314.0	0.0273	
2		271.0	0.0343	
3		229.0	0.0563	
4		206.0	0.0788	
5		290.0	0.0182	
6		246.0	0.0074	
7		219.0	0.0291	
8		198.0	0.0692	

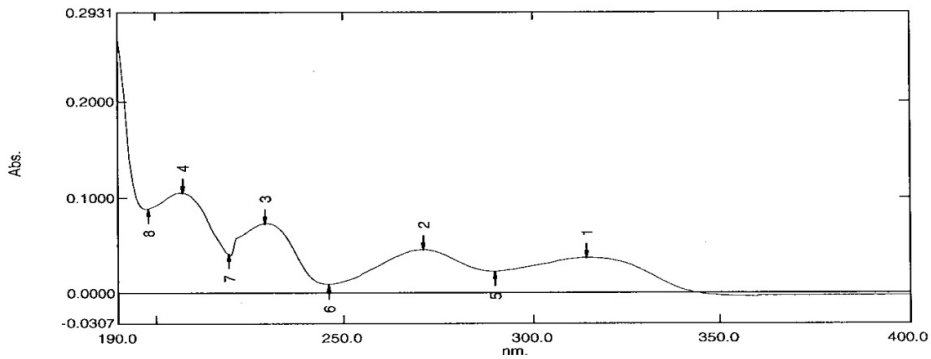
Measurement Properties
Wavelength Range (nm.): 190.0 to 400.0
Scan Speed: Medium
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 360.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length: 10 mm
Additional Information:

Data Set: LEVEL-6 - RawData



No.	P/V	Wavelength	Abs.	Description
1		314.0	0.0368	
2		271.0	0.0453	
3		229.0	0.0729	
4		207.0	0.1048	
5		290.0	0.0228	
6		246.0	0.0095	
7		219.0	0.0408	
8		198.0	0.0882	

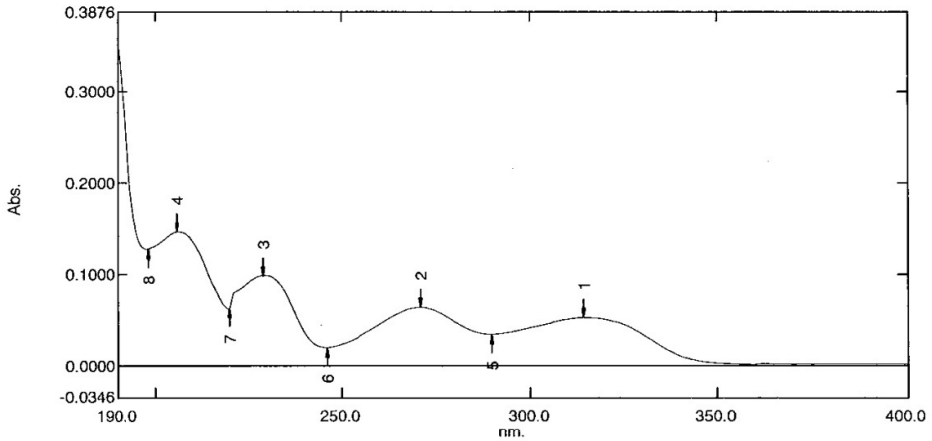
Measurement Properties
Wavelength Range (nm.): 190.0 to 400.0
Scan Speed: Medium
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 360.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length: 10 mm
Additional Information:

Data Set: LEVEL-7 - RawData



Measurement Properties

Wavelength Range (nm.):	190.0 to 400.0
Scan Speed:	Medium
Sampling Interval:	0.2
Auto Sampling Interval:	Enabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	360.0 nm
S/R Exchange:	Normal

Attachment Properties

Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	10 mm
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	⊕	314.0	0.0522	
2	⊕	271.0	0.0635	
3	⊕	229.0	0.0988	
4	⊕	206.0	0.1464	
5	⊖	290.0	0.0340	
6	⊖	246.0	0.0196	
7	⊖	220.0	0.0630	
8	⊖	198.0	0.1276	

RESULT AND DISCUSSION

The working condition for the HPLC & UV method was established for Donepezil hydrochloride and then was applied on pharmaceutical dosage forms. A simple reverse phase High Performance Liquid Chromatographic & UV method has been developed and subsequently validated.

The separation method was carried out by using a mobile phase consisting of acetonitrile and (0.02M) potassium dihydrogen orthophosphate pH 3.6±0.05 in the ratio 55:45. The detection was carried out by using VWD detector at 230nm. The column was Wakosil C18 250 X 4.6mm 5 µ. The flow rate was selected as 1ml/min.

The retention time of Donepezil hydrochloride was found to be 2.6. The asymmetry factor or tailing factor of Donepezil hydrochloride was found to be 1.39, which indicates symmetrical nature of the peak. The number of theoretical plates of Donepezil hydrochloride was found to be 4458, which indicates the efficient performance of the column. These parameters represent the specificity of the method.

From the linearity studies, specified concentration levels were determined. It was observed that Donepezil hydrochloride were linear in the range of 25% to 150% for the target concentration by RP-HPLC. The linearity range of Donepezil hydrochloride 25-150µg/ml was found to obey linearity with a correlation coefficient of 0.99934.

The validation of the proposed method was verified by system precision and method precision by RP-HPLC. The %RSD of system suitability for Donepezil hydrochloride was found to be 0.63.

The validation of the proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The robustness studies were performed by changing the pH, flow rate, mobile phase and wavelength. The ruggedness study was also performed.

From the linearity studies, specified concentration levels were determined. It was observed that Donepezil hydrochloride was linear in the range of 25% to 150% for the target concentration by UV. The linearity range of Donepezil hydrochloride 25-150µg/ml for Donepezil hydrochloride was found to obey linearity with a correlation coefficient of 0.99927.

The validation of the proposed method was verified by system precision and method precision by UV method. The %RSD of system suitability for Donepezil hydrochloride was found to be 0.283.

The validation of the proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The limits of LOQ were found to be 1.2 ppm and LOD was found to be 0.8 ppm. These are all comes under the specified limits and passes.

The analytical method validation was carried out by UV and RP-HPLC as per ICH guidelines and given below are the tables are the summary of the results.

CONCLUSION

Agilent 1200 series with VWD Detector and Wakosil C18 (250x4.6mm, 5µ) column, injection of 20 µl is injected and eluted with the mobile phase of potassium dihydrogen orthophosphate buffer with 0.02M of 3.6 pH and acetonitrile in the ratio 55:45, which was pumped at a flow rate of 1.0 ml at 230 nm. The peak of Donepezil Hydrochloride was found well separated at 2.6 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, ruggedness, robustness and solution stability.

Shimadzu 1800 series of UV-Spectrophotometry was used with water and acetonitrile in the ratio 55:45, which was detected at single point 230 nm. The absorbance of Donepezil Hydrochloride was found. The developed method was validated for various parameters as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, ruggedness, LOQ and LOD.

The analytical method validation of Donepezil Hydrochloride by RP HPLC & UV method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Donepezil Hydrochloride.

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