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Development of validated RP-HPLC methods for quantification of donepezil and ranolzine from the in-house formulations

Dr.R.Srinivasan*, J.Kamal Chandra, D.Rajesh Kumar, D.Phanil Kumar
Siddhartha Institute of pharmaceutical sciences, Jonnalagadda, Narsaraopet, Guntur (DT), India.
***Corresponding author: Dr.R.Srinivasan**
E-mail id: rangusha75@gmail.com.

ABSTRACT

A simple, accurate, rapid, precise and novel Reverse phase High Pressure liquid chromatographic method (RP-HPLC) has been developed and validated for simultaneous determination of Donepezil & Ranolzine from the in-house formulations. Chromatographic separation for Donepezil was performed on Agilient Zorbax C18 (150x 4.6 mm,) 5µm at a wavelength of 220 nm using a isocratic program for 10 min, by using mobile phase of OPA buffer solution and Methanol in the ratio of 60:40v/v pH 3.0. Donepezil obeys linearity in the range of 2 to 20µg/ml. The retention time was found to be 6.184 min. Chromatographic separation for Ranolzine was performed on Phenomenex Zorbax C18 (250x 4.6 mm,) 5µm at a wavelength of 220 nm using a isocratic program for 15 min, by using mobile phase of Potassium Phosphate buffer solution and Methanol in the ratio of 35:65v/v pH 7.0. Ranolzine shows linearity in the range of 25 to175µg/ml. The retention time was found to be 10.454 min. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs. The method was validated for accuracy, precision, specificity, linearity, robustness, sensitivity, LOD and LOQ. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible.

Keywords: RP-HPLC, Donepezil , Ranolzine and retention time.

INTRODUCTION

CHROMATOGRAPHY

From Greek chroma "color" and graphein "to write"[1]
It is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase[2,3].

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY [4]

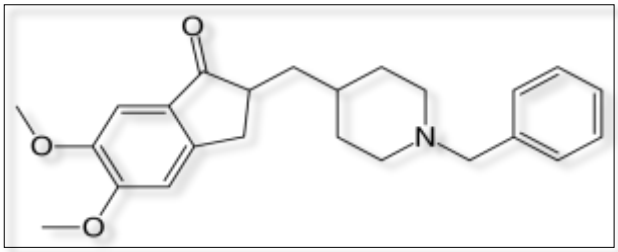
HPLC is a chromatographic technique used to separate the components in a mixture, to identify each component, and to quantify each component. HPLC is considered an instrumental technique of analytical chemistry. In general, the method involves a liquid sample being passed over a solid adsorbent material packed into a column using a flow of liquid solvent.

REVERSED-PHASE CHROMATOGRAPHY

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase

DRUG PROFILE OF DONEPEZIL

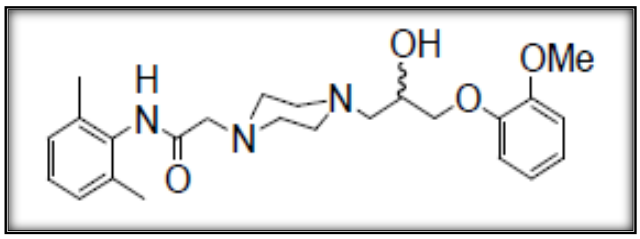
Structure of DONEPEZIL



Name	:	Donepezil Hydrochloride
IUPAC name	:	(RS)-2-[(1-benzyl-4-piperidyl) methyl]-5,6dimethoxy-1-Indanone hydrochloride
Molecular formula	:	$C_{24}H_{29}NO_3HCl$
Category	:	Anti-Alzheimer
Solubility	:	Donepezil hydrochloride is freely soluble in chloroform, soluble in water and in glacial acetic acid, slightly soluble in methanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane.
Pk_a	:	8.62
Absorbtion maxima	:	230nm
Melting point	:	206.72 °C

DRUG PROFILE OF RANOLZINE

Structure of Ranolzine



Name	Ranolzine
IUPAC name	1-piperazineacetamide,N-(2,6dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy) propyl]-, (±)-.
Molecular Formula	$C_{24}H_{33}N_3O_4$
Molecular weight	427.54 g/mole
Category	Anti angina
Description	Ranolazine is a white to off-white solid.
pKa	14.25
λ_{max}	220nm
Melting Point	119-1200°C

Storage	Store the medicine in a closed container at room temperature, away from heat, moisture, and direct light. Keep from freezing.
Solubility	Ranolazine is soluble in dichloromethane and methanol; sparingly soluble in ethanol acetonitrile tetrahydrofuran, and acetone; slightly soluble in ethyl acetate, isopropanol, toluene, and ethyl ether; and very slightly soluble in water.

MATERIALS & METHODS

HPLC METHOD DEVELOPMENT [5]

The process is influenced by the nature of the analytes and generally follows the following steps:

- step 1 - selection of the HPLC method and initial system
- step 2 - selection of initial conditions
- step 3 - selectivity optimization
- step 4 - system optimization
- step 5 - method validation.

ANALYTICAL METHOD DEVELOPMENT FOR DONEPEZIL

Optimized Conditions for Donepezil

Method Parameters	Optimized value
Column	C_{18} Agillent Zorbax
Analytical Wavelength	230 nm
Mobile phase	Methanol Water (pH 3) (40: 60 v/v)
Pump mode	Isocratic
Flow rate	1.0 ml/min
Volume of Injection	20 µl
Run Time	10 min

ANALYTICAL METHOD DEVELOPMENT FOR RANOLZINE

Optimized Conditions for Ranolzine

Mobile phase	Phosphate buffer :Methanol (65:35)
Stationary phase	Phenomenax C18 (4.6 x 250 m, 5 µ particle size)
Wave length	220nm
Run time	15 min
P.H of mobile phase	7.0
Flow rate	1.0 ml/min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution

PREPARATION OF PHYSICAL POWDER MIXTURES FOR HPLC

In order to evaluate Drug excipient interactions physical powder mixtures of drug and excipients commonly were selected for the study. The excipients and drug were taken in different ratios.

For Donepezil

Ingredients	Ratio
Mannitol	1:6.4
Micro crystalline Cellulose	1:0.2
Sodium starch Glycolate	1:0.8
Magnesium sterate	1:0.2
Talc	1:0.2

For Ranolzine

Ingredients	Ratio
Hypromellose phthalate grade HP-55	1:0.5
Ethocel standard 7FP Premium	1:0.5
Natrosol Type 250HHX	1:0.5
Klucel HF pharm	1:0.5
Avicel pH101	1:0.5
Magnesium Stearate	1:0.2

The powders are homogeneously mixed with a mortar and pestle for 10min, then powder mixture was placed in glass vials with a rubber stoppers. These vials were stored in at 40°C/75%RH for a period of 28 days. Samples were analyzed for related substances using previously described HPLC method and % of impurities was calculated.

SPECIFICITY

Blank solutions, Placebo, Standard solution sample solution were injected into the chromatographic system. Retention times obtained from standard and sample were compared for identification of analytes.

LINEARITY

A series of solutions of drug substance standard were prepared in the concentration range from 2 to 20µg/ml of test concentration to demonstrate linearity for assay by using single plot and injected in to the chromatographic system. A calibration graph is plotted between amount of drug (µg/mL) and chromatographic peak area (mV).

PREPARATION OF STANDARD STOCK SOLUTION FOR DONEPEZIL

Accurately 10 mg of Donepezil working reference standard was weighed and transferred into a 100 ml clean dry volumetric flask and 10 mL of diluent was added to it and sonicated for 10 min for complete dissolution of the drug. Finally the volume was made up to the mark with the diluent.

Preparation of 2µg/ml solution of Donapezil

0.2 mL of standard stock solution was pipetted into 10 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 4µg/mL of Donepezil

0.4 mL of standard stock solution was pipetted into 10 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 6 µg/mL of Donepezil

0.6 mL of standard stock solution was pipetted into 10 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 8 µg/mL of Donepezil

0.8 mL of standard stock solution was pipetted into 10 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 10 µg/mL of Donepezil

1.0 mL of standard stock solution was pipetted into 10 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 12 µg/mL of Donepezil

1.2 mL of standard stock solution was pipetted into 100 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 14 µg/mL of Donepezil

1 mL of standard stock solution was pipetted into 100 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 16 µg/mL of Donepezil

1.6 mL of standard stock solution was pipetted into 10 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 18 µg/mL of Donepezil

1.8 mL of standard stock solution was pipetted into 100 mL of volumetric flask and diluted up to the mark with diluent.

Preparation of 20 µg/mL of Donepezil

2.0 mL of standard stock solution was pipetted into 10 mL of volumetric flask and diluted up to the mark with diluent.

PREPARATION OF STANDARD STOCK SOLUTION FOR RANOLZINE

Preparation of standard stock solution

Accurately 100 mg of Ranolzine working reference standard was weighed and transferred into a 100 ml clean dry volumetric flask ant the volume was made up to the mark get a concentration of 1000µg/ml.

Preparation of 25µg/ml solution of Donapezil

0.25 mL of standard stock solution was diluted to 10ml to get a concentration of 25 µg/ml.

Preparation of 50µg/mL of Donepezil

0.5 mL of standard stock solution was diluted to 10ml to get a concentration of 50 µg/ml.

Preparation of 75 µg/mL of Donepezil

0.75 mL of standard stock solution was diluted to 10ml to get a concentration of 75 µg/ml.

Preparation of 100 µg/mL of Donepezil

1.0 mL of standard stock solution was diluted to 10ml to get a concentration of 100 µg/ml.

Preparation of 125 µg/mL of Donepezil

1.25 mL of standard stock solution was diluted to 10ml to get a concentration of 125 µg/ml.

Preparation of 150 µg/mL of Donepezil

1.5 mL of standard stock solution was diluted to 10ml to get a concentration of 150 µg/ml.

ACCURACY

Standard stock solution for Donepezil

Accurately 100 mg of Donepezil working reference standard was weighed and transferred into a 100 mL clean dry volumetric flask. 10 mL of diluent was added and sonicated for 10 min for complete dissolution of the drug then volume was made up to the mark with the diluent.0.4ml of above solution was taken and transferred to a 10ml volumetric flask and made up to the mark with methanol.

Sample Stock Solution for Donepezil

An accurately weighed sample powder equalent to 100mg of Donepezil was transferred to 100ml volumetric flask, to this 40ml of methanol was added and sonicated for a period of 15min and then volume was made up to mark with methanol. 1ml of above solution was transferred to 100ml volumetric flask and the volume was made up to the mark using mobile phase. 0.2 ml of above solution was taken and transferred to a 10ml volumetric flask and made up to the mark with methanol.

Preparation of 50% standard addition solution

To 1.0 mL of supernatant sample stock solution in a 10 mL volumetric flask, 1 mL of standard stock solution was added and diluted up to the mark with diluent.

Preparation of 100% standard addition solution

To 1.0 mL of supernatant sample stock solution in a 10 mL volumetric flask, 2.0 mL of standard stock solution was added and diluted up to the mark with diluent.

Preparation of 150% standard addition sample solution

To 1.0 mL of supernatant sample stock solution in a 10 mL volumetric flask, 3 mL of standard stock solution was added and diluted up to the mark with diluent.

Preparation of Solutions for Ranolzine

Preparation of Standard solution for Ranolzine

100mg of Ranolzine was accurately weighed and transferred to 100 volumetric flask, to this 40ml of diluent was added and sonicated for a period of 15min and then volume was made up to mark with diluent.

Preparation of sample Solution for Ranolzine

An accurately weighed sample powder equivalent to 100mg was weighed and transferred into a 100 mL clean dry volumetric flask. 10 mL of diluent was added and sonicated for 10 min then volume was made up to the mark with the diluent. 5mL of the above solution was diluted to 10 mL to get a concentration of 500 µg/ml.

Preparation of 50% standard addition solution

To 1.0 mL of supernatant sample stock solution in a 10 mL volumetric flask, 0.25 mL of standard stock solution was added and diluted up to the mark with diluent.

Preparation of 100% standard addition solution

To 1.0 mL of supernatant sample stock solution in a 10 mL volumetric flask, 0.5 mL of standard stock solution was added and diluted up to the mark with diluent.

Preparation of 150% standard addition sample solution

To 1.0 mL of supernatant sample stock solution in a 10 mL volumetric flask, 0.75 mL of standard stock solution was added and diluted up to the mark with diluent.

Procedure

Sample solutions prepared separately by addition of standard stock at 50%, 100% and 150% of working sample concentration were injected in triplictate into the chromatographic system.

Precision

System Precision

The system precision was established by injecting six replicate injections of standard solution of Donepezil, Ranolzine in to the chromatographic system by maintaining the optimized chromatographic conditions.

Method Precision

Six replicate samples of drug product at 100% of concentration were prepared and injected into the chromatographic system.

Intermediate Precision

Six assay samples of drug product at 100% of working sample concentration were prepared and injected into the chromatographic system on different day.

Robustness

As part of evaluation of robustness, change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

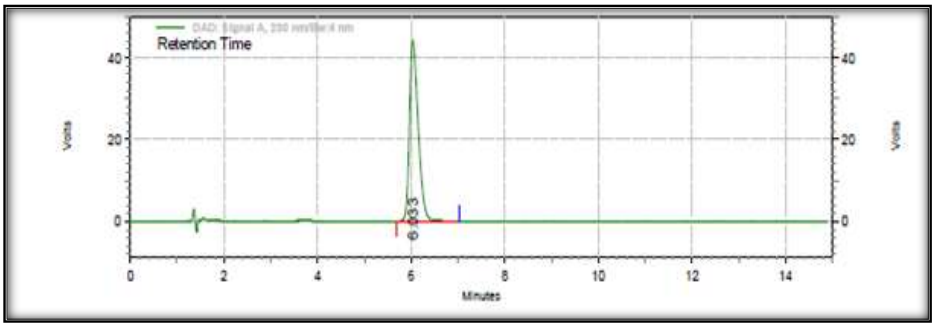
Effect of variation of Flow Rate

Sample solutions were prepared and analysed by injecting into the chromatographic system maintaining flow rates i.e. less flow (0.8 mL/min), more flow (1.2 mL/min) and actual flow (1.0 mL/min).

RESULTS & DISCUSSION

Chromatographic Conditions of Donepezil

Mobile phase	Buffer: Methanol in (60:40)
Stationary phase	Agilient Zorbax C18 (4.6 x 250 m, 5 µ particle size)
Wave length	230nm
Run time	10 min
P.H of mobile phase	3.0
Flow rate	1.0 ml/min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution



Standard Chromatogram of Donepezil

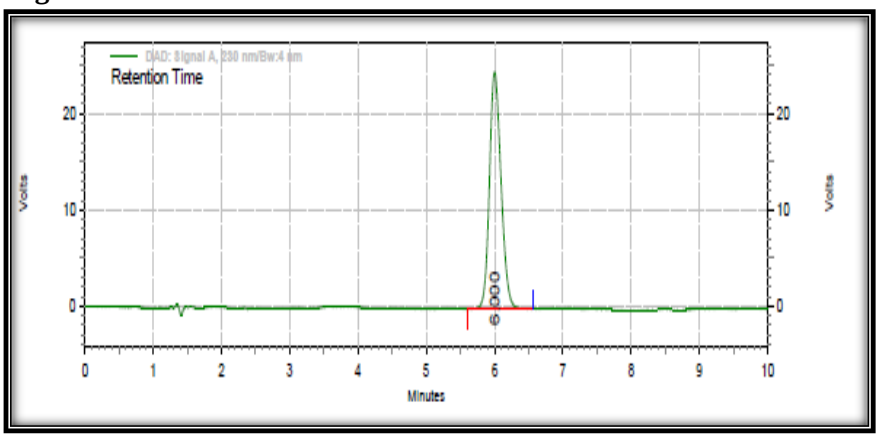
SYSTEM SUITABILITY PARAMETERS OF ASSAY

System suitability parameters	Results
Retention time (min)	6.184
Area (V)	1056396
Theoretical plates	4637
Tailing factor	1.57

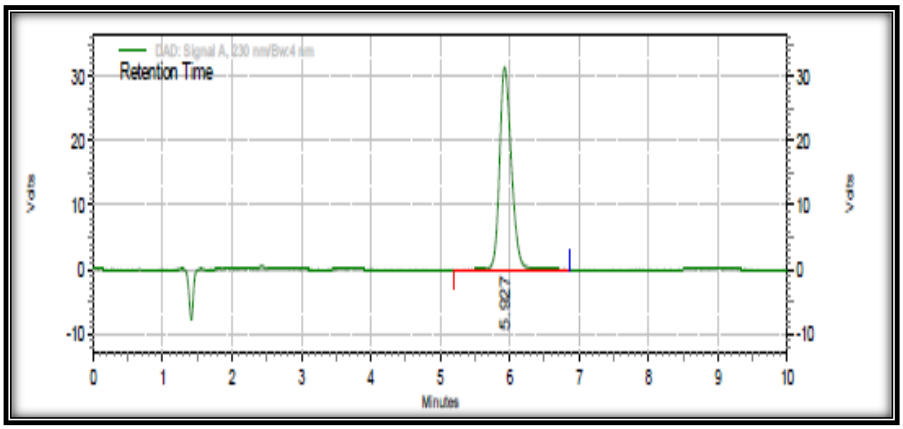
Validation results of Donepezil

Parameter	**Result**		
Linearity	2-20 µg/mL Correlation coefficient = 0.998		
System precision	%RSD = 0.03		
Method precision	%RSD = 0.22		
Accuracy	Mean recovery = 99.958		
	ROBUSTNESS		
	Change in flow rate		
Flow rate (mL/min)	**Rt (min)**	**Efficiency**	**Asymmetry**
0.8	5.82	4899	1.66
1.2	6.3	4622	1.54
Change in wave length			
Wavelength	**Rt (min)**	**Efficiency**	**Asymmetry**
225	6.067	4550	1.65
235	6.033	4625	1.69
Change in % of organic phase			
Mobile phase composition	**Rt (min)**	**Efficiency**	**Asymmetry**
10% low	6.4	4652	1.66
10% high	5.91	4422	1.54

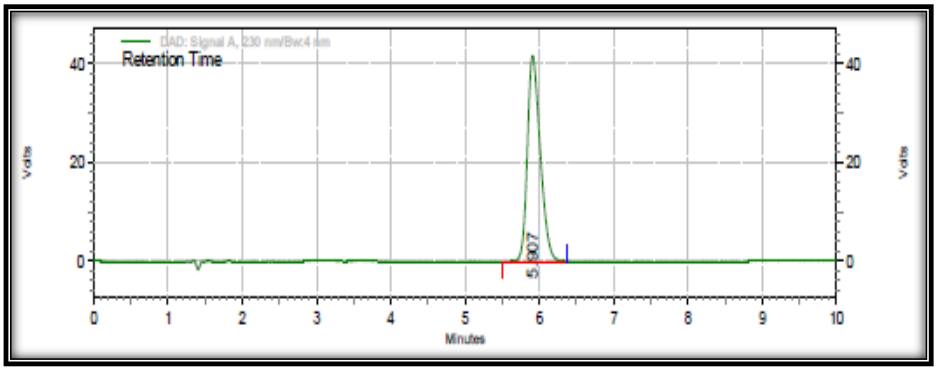
Linearity Chromatograms



Chromatogram recorded at a concentration of 6µg/ml i.e., 50% of Standard solution



Chromatogram recorded at a concentration of 8µg/ml i.e., 100% of Standard solution



Chromatogram recorded at a concentration of 10µg/ml i.e., 150% of Standard solution

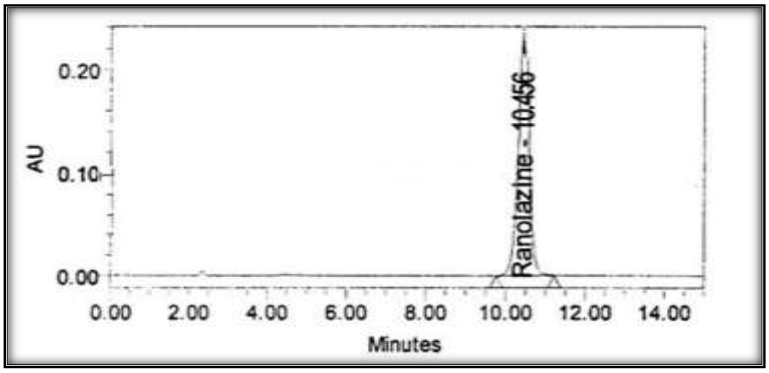
Chromatographic Conditions of Ranolzine

Mobile phase	Phosphate buffer :Methanol (65:35)
Stationary phase	Phenomenax C18 (4.6 x 250 m, 5 µ particle size)
Wave length	220nm
Run time	15 min
P.H of mobile phase	7.0
Flow rate	1.0 ml/min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution

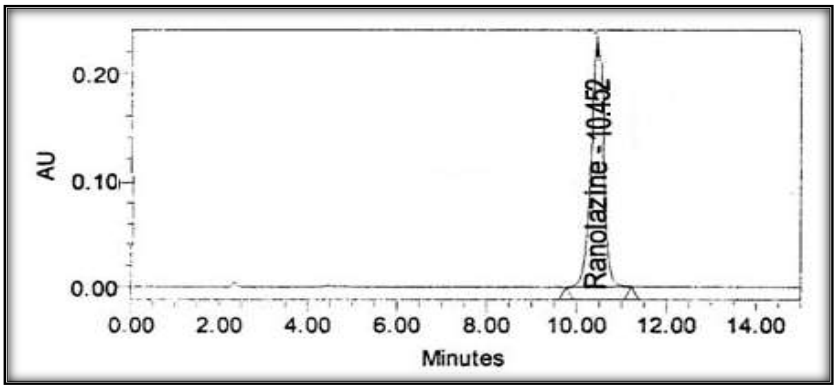
System Suitability Parameters of Assay

System suitability parameters	Results
Retention time (min)	10.454
Area (V)	17865132
Theoretical plates	3897
Tailing factor	1.05

Chromatograms indicating Specificity of Ranolzine



Chromatogram of Standard



Chromatogram of Sample

Validation results of Ranolzine

Parameter	**Result**		
Linearity	25-150 µg/mL Correlation coefficient = 0.999		
System precision	%RSD= 0.0240		
Method precision	%RSD = 0.161		
Accuracy	Mean recovery = 100.97		
Change in flow rate			
Flow rate (mL/min)	**Rt (min)**	**Efficiency**	**Asymmetry**
0.8	10.539	3320	1.1
1.2	10.410	3292	0.99
Change in wave length			
Wavelength	**Rt (min)**	**Efficiency**	**Asymmetry**
215	10.460	3225	1.1
225	10.459	3321	1.1
Change in % of organic phase			
Composition of Organic phase	**Rt (min)**	**Efficiency**	**Asymmetry**
10% low	10.556	3297	1.0
10% high	10.351	3235	1.0

Comparison of Donepezil observed values with I.P limits

S.NO	**PARAMETERS**	**LIMIT**	**OBSERVATION**
1	System suitability (%RSD of tailing factor)	Suitable	1.05
2	Specificity	No interference	Specific
3	Precision		
	a)System precision	RSD NMT 2.0%	0.03
	b)Method precision	RSD NMT 2.0%	0.22
4	Linearity	Correlation coefficient NLT 0.999	0.998
5	accuracy	%Recovery range 98-102%	99.958
6	Robustness	RSD NMT 2.0%	Robust (<2%)
7	LOD	0.40 µg/ml	0.0031 µg/ml
8	LOQ	0.978 µg/ml	0.0096 µg/ml

Comparison of Ranolzine observed values with I.P limits

S.NO	**PARAMETERS**	**LIMIT**	**OBSERVATION**
1	System suitability (%RSD of tailing factor)	Suitable	1.05%
2	Specificity	No interference	Specific
3	Precision		
	a)System precision	RSD NMT 2.0%	0.0240
	b)Method precision	RSD NMT 2.0%	0.161
4	Linearity	Correlation coefficient NLT 0.999	0.999
5	accuracy	%Recovery range 98-102%	100.97
6	Robustness	RSD NMT 2.0%	Robust (<2%)
7	LOD		0.018 µg/ml
8	LOQ		0.054 µg/ml

CONCLUSION

The method was validated by evaluating Specificity, linearity, accuracy, precision, robustness, limit of quantification, limit of detection. The results conclude that the method was suitable for the estimation of Donepezil in the prepared Oro dispersible formulation and also for the estimation of Ranolzine in the extended release formulation.

REFERENCES

[1] Pharmaceutical Analysis. http://www.pharmatutor.org/pharma-analysis

[2] "Chromatography". Online Etymology Dictionary.

[3] Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 μm particle columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice". Retrieved 10/23/2012.

[4] H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle, "Instrumental Methods of Analysis", 7th ed, CBS publishers and Distributors, New Delhi, 1986, pp.518-521, 580-610.

[5] Third edition, lloyd r. Snyder, joseph j. Kirkland, john w. Dolan. Introduction to modern liquid chromatographya: New Jersey; john wiley & sons, inc., publication;2010.

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