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A Validated Method for Estimation of Rivastigmine Pure and Its Pharmaceutical Formulations by UV Spectroscopy

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ABSTRACT

A Simple, Rapid, Precise and Accurate UV Spectrophotometric method was developed and validated for the estimation of Rivastigmine in capsule dosage form. From the solubility profile 0.1M HCl was chosen as a solvent for the estimation of Rivastigmine. The sample solutions of 10 µg mL^{-1} of Rivastigmine prepared individually. 216 nm was selected as wavelength. The LOD and LOQ for estimation of Rivastigmine were 0.44 µg/ml, 1.44 µg/ml respectively. This method is simple, rapid and economically competitive with other published methods and also this method is very sensitive and free from interferences and can be used for routine analysis.

Keywords: Rivastigmine, UV, Ruggedness, Robustness.

INTRODUCTION

Qualitative analysis is performed to establish composition of a substance. The various qualitative tests are limit tests, reactions, determination of melting point, Determination of nuclear half-life. Quantitative analysis is mainly used to determine the amount or concentration of analyte in a sample and expressed as a numerical value in appropriate units. These techniques are based on suitable chemical reaction and either measuring the amount of reagent added to complete the reaction. [1].

Analytical chemistry is recognizing different substances and determining their constituents, Its supreme importance has caused it to be assiduously cultivated from a very early period in the history of chemistry, and its records comprise a large part of the quantitative work which is spread over the whole domain of science. The objective of analytical work is the achievement of reliable analytical results of a defined quality. Rivastigmine is a para sympathomimetic or cholinergic inhibitor used for the treatment of mild to moderate dementia of the Alzheimer's and Parkinson's disease. Rivastigmine is a cholinesterase inhibitor that inhibits both butyryl cholinesterase and acetylcholinesterase. [2,3].

MATERIALS AND METHODS

Rivastigmine pure drug was obtained as a gift sample from sun pharmaceuticals. All other chemicals and reagents used were analytical grade. The chemicals distilled water, ethanol, chloroform, benzene, 0.1M HCL, 0.1M H_2SO_4, 0.1 M HNO_3 and 0.1 M NaOH are purchased from Sigma Aldrich, Bangalore. JASCO V -730 UV-visible double beam spectrophotometer was used to perform the analysis.

Selection of solvents: The solubility of drugs was determined in a variety of solvents as per Indian pharmacopoeia

standards. Solubility was carried out in polar to non - polar solvents. Better absorption maximum was found to be 200 nm with 0.1M Hcl. So it was selected as optimized solvent in this method.

Preparation of standard stock solution: 100mg of rivastigmine pure drug was accurately weighed and transferred into a 100ml volumetric flask containing 0.1M Hcl. The volume is made up to the mark with 0.1M Hcl to get the stock solution (1mg/ml). This solution was further diluted with the same to get the working standard solutions.

Preparation of calibration curve: Aliquots of standard drug solutions in 0.1M Hcl transferred into a series of 10ml volumetric flask and the solution was made up to 10ml with 0.1M HCl. The absorbance of solutions was measured at 200 nm against reagent blank and the calibration curve of rivastigmine was constructed. Calibration curve was prepared by plotting concentration of rivastigmine on x-axis and their respective absorbance on y-axis.

Selection of detected wavelength: Appropriate dilutions of rivastigmine were prepared from stock solution. Using JASCO V-730 spectrophotometer was used to record absorption spectra the dilutions of rivastigmine were scanned over the range of 200-400 nm. It was observed that the drug showed maximum absorbance at 200 nm which was selected as the wavelength for detection.

Procedure for assay of pharmaceutical formulation: 10 tablets of rivastigmine were weighed and powdered in a glass mortar. A quantity of tablet powder equivalent to 100 mg of rivastigmine was transferred into a 100ml volumetric flask and to this 25ml of 0.1M HCl was added and the solution was sonicated for 25 minutes and solution was then filtered through a Whatman filter paper No.41 and the final volume was made up to 100ml to obtain concentration of 1mg/ml rivastigmine. This solution was further diluted to obtain concentration 10µg/ml and was used for analysis.

Recovery studies: The recovery experiment was done by adding known concentrations of rivastigmine raw material to the 50% pre analyzed formulation. Standard rivastigmine in the range of 80%, 100% and 120% to the 50% pre analyzed formulation into a series of 10 mL volumetric flasks and dissolved with 0.1M HCl and the contents were sonicated for 15 minutes then the solution was made up to mark with 0.1M HCl. After sonication the solutions were filtered through Whatman filter paper No.41.

The absorbance of the resulting solutions was measured at their selected wavelength for the determination of rivastigmine. The amount of each drug recovered from the formulation was calculated for the drugs by simultaneous equation method. The procedure was repeated for three times. The linearity was established across the range and the absorbance of standard stock solution in the range of 2-10µg/ml was measured at 200nm. The calibration curves were prepared by plotting graph between absorbance and concentration. Linearity was determined by least square regression method.

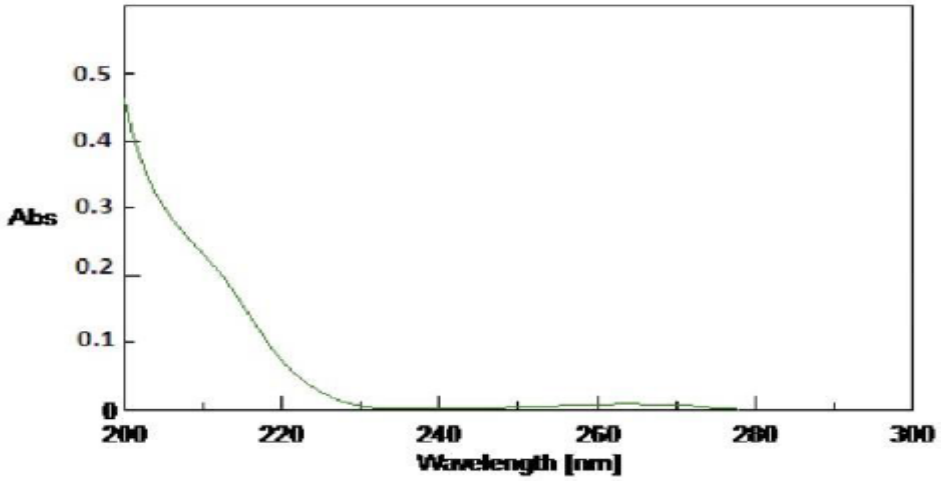
RESULTS AND DISCUSSION

The solubility test for Rivastigmine was performed with the following solvents based on the solubility profile of the drug:

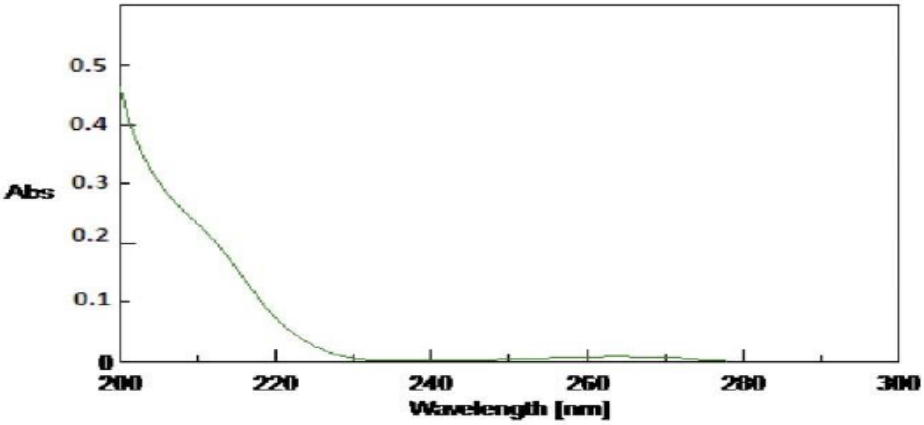
SOLVENTS	SOLUBILITY TESTING
Distilled water	Freely soluble
Chloroform	Soluble
Benzene	Soluble
Ethanol	Freely soluble
0.1M HCl	Freely soluble
0.1M H_2SO_4	Freely soluble
0.1M HNO_3	Freely soluble
0.1M NaOH	Freely soluble

SELECTION OF SOLVENT: Many trails were carried out to find out the ideal solvent system for dissolving the drug Rivastigmine. The following spectrum shows the absorbance of Rivastigmine with the freely soluble solvents. Hence, 0.1M HCL satisfies

the above-mentioned criteria it is chosen as an ideal solvent.



ESTIMATION OF λ max:

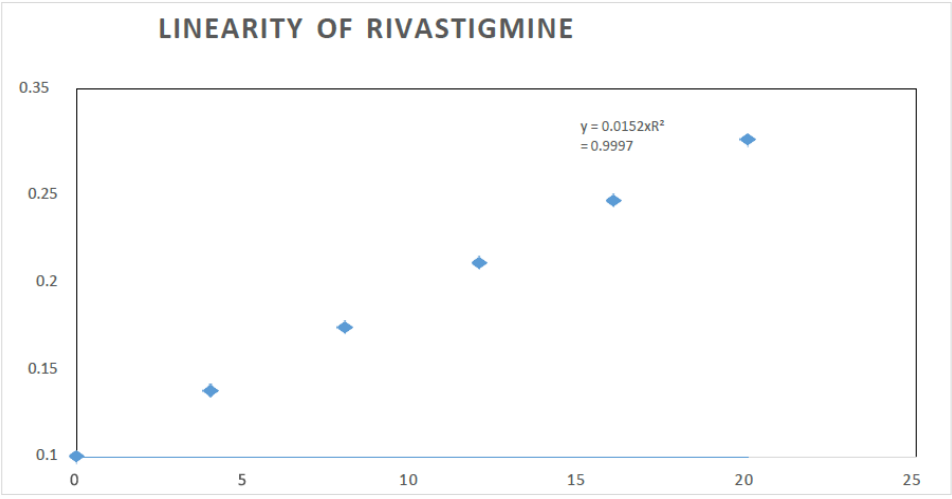


VALIDATION OF DEVELOPED METHOD:

The proposed method obeys Beer's law in the concentration range of 2-20 µg/ml with good correlation coefficient of $r^2 = 0.9997$.

LINEARITY DATA FOR RIVASTIGMINE

Concentration (µg/ml)	Absorbance
0	0
4	0.0632
8	0.1232
12	0.1846
16	0.2438
20	0.3012



Accuracy:

Recovery studies were carried out for the developed method by addition of known amount of standard drug solution of rivastigmine to pre-analyzed capsule sample solution at three different concentration levels. The resulting solutions were analyzed by proposed methods. The recovery was in the range of 98.61 to 100.89 percentages.

DETERMINATION OF ACCURACY RESULTS FOR RIVASTIGMINE

Brand Name	Amount of sample (mcg / ml)	Amount ofdrug added(mcg / ml)	Amount Recovered	% Recovery ± SD**	% RSD
RIVAMER	15	7.5	22.46	100.89 ±0.46	0.45
	15	15	29.95	99.09 ± 0.48	0.48
	15	22.5	37.492	100.32 ± 17	0.17

Precision:

Conc. mcg / ml	Intra-day Absorbance Mean± SD**	% RSD	Inter-day Absorbance Mean ± SD**	% RSD
10	0.0295 ± 0.00055	1.85	0.029 ± 0.00055	1.85
20	0.06185 ± 0.00075	1.21	0.061 ± 0.0089	1.46
30	0.0901 ± 0.00075	0.83	0.09 ± 0.0089	0.99
40	0.1203 ± 0.00082	0.67	0.12 ± 0.00089	0.74
50	0.152 ± 0.00126	0.83	0.152 ± 0.0011	0.76

RUGGEDNESS: Ruggedness results for at 216nm

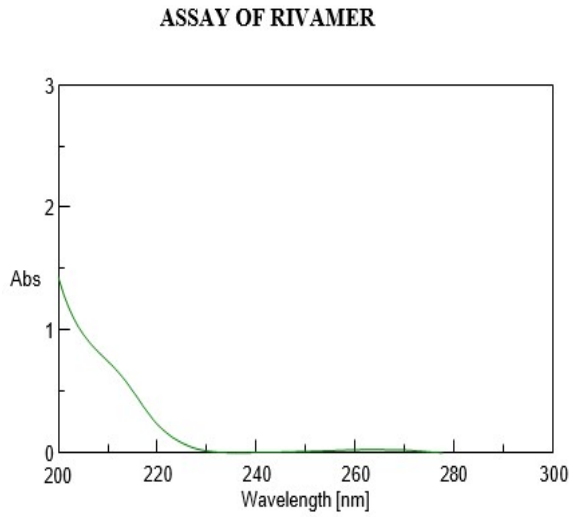
Tablet	Labelclaim(mg)	Analyst I		Analyst II	
		Amountfound (mg)	Recovery ±SD** (%)	Amountfound (mg)	Recovery ±SD** (%)
RIVAMER	1.5	1.486	98.087±0.48	1.492	99.52 ± 0.34

Robustness:

S.NO	$^{1}\lambda$ max	$^{2}\lambda$ max
Mean	0.9883	0.9872
SD	0.001118	0.00129
%RSD*	0.001131	0.00139

Assay of Rivastigmine

Formulation	Labeled amount	Amount obtained µg/ml	%Rivastigmine ±S.D
RIVAMER	1.5mg	1.486	98.087±0.48



DISCUSSION

Pradeep Vavia et al., concluded that the Rivastigmine-loaded nanoparticles were prepared successfully using the single emulsion solvent evaporation method in the presence of PVA as a stabilizer. The particle size and drug entrapment were optimized based on the study of the effect of different formulation variables. The synthesized L-lactide-depsipeptide copolymer produced RT-loaded nanoparticles with low particle size and high entrapment efficiency of rivastigmine. A change in the concentration of the stabilizer, polymer and the amount of RT was found to vary the size, poly dispersity and entrapment efficiency of the prepared nanoparticles. The *in vitro* release study revealed that sustained release of RT for 72 h. The effect of different formulation variables used for the optimization of the formulation in achieving higher efficacy with an improved safety profile of polymer-based nanoparticle formulation[4].

Hossein Aminia B et al., reported the HPLC-UV method for quantification of

rivastigmine in human plasma and the simple extraction procedure on liquid–liquid extraction followed by back- extraction into diluted acid. The sensitivity of the assay is sufficient to follow the pharmacokinetics of rivastigmine after administration of a low dose of rivastigmine to human subjects[5]. Kapil et al., developed that a spectrofluorimetric method for the estimation of rivastigmine in bulk drug and pharmaceutical formulations. The relative fluorescence intensity of rivastigmine was measured in triple distilled water at an excitation wavelength of 220 nm and an emission wavelength of 289 nm. Linearity range was found to be 100 to 4000 ng/ml. The detection and quantitation limits were found to be 20.5 and 62.1 ng/ml, respectively[6].

Rishi Kapil et al., concluded that Buccoadhesive drug delivery has been considered as an effective formulation strategy to enhance bioavailability of drugs undergoing significant hepatic first-pass metabolism. This study embarked upon developing controlled release buccoadhesive films of rivastigmine not only to improve its bioavailable fraction but also to attain a smooth plasma drug level profile in order to decrease the incidences of severe GI adverse effects[7]. Murat Emre concluded that patients who manage to stay on Cholinesterase inhibitors therapy for longer have a greater chance of slowing the progression of their symptoms and also decreased risk of institutionalization. The pharmacokinetic profile of the rivastigmine transdermal patch reduces side effects, allowing patients to more easily reach target doses. Cholinesterase inhibitors and memantine are currently used for the treatment of AD. The Rivastigmine patch provides an efficient option and considered a first-line therapy for mild to moderate AD patients[8].

Seyed Javad Torabi et al., reported that different MIPs were synthesized by precipitation polymerization with different cross-linkers for RVS delivery as a novel drug carrier. MIP2 was selected as the best among the prepared samples due to monodisperse morphology and the highest amount of RVS absorption and characterized by FTIR, TGA, FESEM, BET and also binding capacity via HPLC. The morphology of the synthesized MIPs was spherical with a dimension of about 66 nm while the synthesized NIP was layered, and its dimensions were about 427 nm[9].

The BET results confirmed that the porosity of the MIP particles was more than the NIP. The prepared polymeric nanoparticles can selectively re-bind the RVS in aqueous media. The obtained results showed that PETA network-builder agent is a good factor for making MIP for the controlled release of RVS due to the capacity of its polymer and its low drug release rate. *in vitro* release of RVS in a similar fluid to the unconsolidated cerebrospinal fluid showed that hydrogen interactions resulted in controlled release and the slowness of the drug at long hours, aimed at increasing the half-life of the RVS. The toxicity of the synthesized polymer showed that the synthesized material is biocompatible for biocompatible and bioconvenient biological cells. The selective absorption capacity and extraction ratio and controlled release behaviour of optimized prepared MIP (MIP2) and these nanoparticles can be considered as a suitable carrier for RVS.

Ganesa Sundararajan Subramanian et al., indicated the method for rivastigmine in the bulk drug and in pharmaceutical formulations, in this method is suitable for the quantitative determination of rivastigmine in the capsule formulation. The method uses a two-component mobile phase. The method is advantageous in that a large number of samples can be analyzed simultaneously[10]. Buchi N. Nalluri et al., concluded that the efficient RP-HPLC-PDA method was developed for the analysis of RVS in bulk and transdermal patch and in transdermal permeation and/or release studies. The method provides selective quantification of RVS without interference from diluent. Therefore, this method can be

employed in quality control to estimate the amount of RVS[11].

Avijit Choudhury et al., indicated the RP- HPLC method for the estimation of Rivastigmine effect by enhancing cholinergic function in bulk and pharmaceutical dosage form. The mobile phase used was 2.02 g of 1-octane sodium sulfonate in 1000 ml Milli – Q water and the pH was adjusted to 3.0 with ortho phosphoric acid and filter through 0.45mm nylon 66 membrane filter. The mobile phase was prepared by mixing buffer and acetonitrile in the ratio of (70:30 % v/v). The specification of the chromatographic system, Column 4.6 mm ´ 250 mm, ODS, Xterra RP18, 5 mm, flow rate 1.0 ml/min, detection 217 nm, injection volume 40 ml and run time 15 min[12].

S. Alexandar et al., proposed RP-HPLC method for the simultaneous estimation of Rivastigmine in pharmaceutical dosage form, this RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies. An isocratic RP-HPLC Method for analysis of Rivastigmine in pharmaceutical dosage forms has been developed and validated. Best separation was achieved on a Thermo Hypersil C4 column (25 cm X 4.6 mm, 5 µm) using a mobile phase of 0.01 M ammonium acetate buffer adjusted to pH 4.0 with orthophosphoric acid and Acetonitrile (60:40, v/v) at a flow rate of 1.0 mL min-1. UV detection was performed at 220 nm. Atorvastatin was used as an internal standard. The retention time of Rivastigmine and Atorvastatin was 4.75 and 8.83 min, respectively. This method is applied for the quantitative determination of Rivastigmine in commercial formulations[13].

Naz Hasan Huda et al., concluded that to assist the development of dual-ligand NP formulations for brain drug delivery, in this HPLC method has been validated for analysis of RHT loading in dual-ligand NPs preparations, in vitro drug release, and cellular transport studies. chromatographic separation was achieved using a C18 column maintained at 50°C and an isocratic mobile phase consisting of TFA containing ACN and water with a flow rate of 1.5 mL/min. exhibited good linearity over the assayed concentration range and good intra- and inter day precision[14].

CONCLUSION

A Simple, Rapid, Precise and Accurate UV Spectrophotometric method was developed and validated for the estimation of Rivastigmine in capsule dosage form. From the solubility profile 0.1M HCl was chosen as a solvent for the estimation of Rivastigmine and it results linear and consistent. The sample solutions of 10 µg mL^{-1} of Rivastigmine prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using 0.1M HCl as blank. The overlaid spectra of Rivastigmine were recorded. From the spectra, 216 nm was selected as wavelength. The optical characteristics and the data concerning to the proposed method is represented. The percentage label claim present in capsule formulation was found to be 98.087±0.48 Rivastigmine respectively.

The percentage recovery was found to be in the range of 99.09-100.89% for Rivastigmine. The LOD and LOQ for estimation of Rivastigmine were 0.44 µg/ml, 1.44 µg/ml respectively. Ruggedness was performed by two different analysts in the same experimental conditions. The %RSD was calculated. Therefore, the developed method results the linearity, accuracy and precision which indicates that the method is exclusive. So, this method is simple, rapid and economically competitive with other published methods and also this method is very sensitive and free from interferences and can be used for routine analysis.

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Conflict of Interest: None
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Ethical Approval: Approved

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