



DEVELOPMENT AND EVALUATION OF NANOEMULSION OF RIVASTIGMINE FOR BRAIN DELIVERY

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Introduction

The development and evaluation of nanoemulsion of rivastigmine for brain delivery is the neurological condition affecting the aged population economically and socially .

- It is characterized by recent memory loss in the early stages accompanied by a profound cognitive decline in the later stage.
- Rivastigmine a reversible inhibitor of acetylcholinesterase enzymes.
- Literature review

- The various literatures have been reviewed in order to develop and characterize

(1:1) as a mixture of surfactant and co-surfactant and

NE for brain delivery.

- Formulation of rivastigmine

Brijesh and associates formulated Rivastigmine loaded ME and mucoadhesive ME (MME) and delivered intranasally to the brain for Alzheimer's disease. Titration method was used to prepare rivastigmine-loaded ME and MME and prepared formulations were characterized for drug content, globule size distribution, zeta potential, pH, viscosity and nasal ciliotoxicity study. ME was formulated with 8% w/w Capmul MCM EP as oil phase, 44% w/w Labrasol:Transcutol-P

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- Patent search of rivastigmine

Murata (WO2015156990A1) studied the effects of rivastigmine transdermal compositions with adhesive layer. Skin adhesive layer was prepared by Vitamin E and pressure-sensitive adhesive (e.g., Duro-tak 9301, Bio-PSA 4302). This step was followed by addition of ethyl acetate to reach the desired solid content weight. Transdermal flux test was performed on the epidermis of human cadaver skin using vertical type Frantz cell and transdermal patch was checked carefully for pinholes or air bubbles. Phosphate Buffer pH 7.5 with 0.01 % Gentamycin as antibiotic was used into the receptor compartment of Frantz cell. Samples were analyzed by HPLC using mobile phase 75 mM (NH₄)₂HPO₄, acetonitrile, and MeOH in a 2:1:1 ratio respectively. Results of

this study was clearly indicated a positive correlation between active agent layer, thickness and sustained release of rivastigmine

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• Nanoemulsions for intranasal delivery

Sumeet and associates prepared curcumin NE and delivered intranasally for Alzheimer's disease.

NE was prepared by spontaneous nanoemulsification method and optimized using Box–Behnken

design. In Box–Behnken design, oil, surfactant and co-surfactant concentration were used as independent variables and their affect on response y_1 (globule size) and y_2 (zeta potential) were as dependent variables.

Cont....

• Different formulations for intranasal delivery

Greco and associates (7989502) studied the effects of intranasal administration of modafinil in brain bioavailability. Modafinil was selectively delivered to the brain, minimized delivery to the blood of a person in need thereof by administering to the person a therapeutically effective dosage of modafinil, wherein the dosage was less than 1 mg, formulated in a lipid microemulsion and selectively delivered to the upper third of the nasal cavity. The method may be implemented with an intranasal pharmaceutical delivery device loaded with a modafinil composition and adapted to deliver the dosage to the upper third of the nasal cavity (Greco et al., 2010).

• Need of work

Mechanism of Action :-

Rivastigmine is a carbamate derivative of physostigmine which inhibits both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzyme. Thus, the concentration of acetylcholine due to inhibition of its hydrolysis into acetyl and choline by cholinesterase is increased.

• AIM AND OBJECTIVE

•The aim of the present study was to develop nanoemulsion of Rivastigmine that could deliver the drug into brain through nasal route to avoid first pass metabolism and to avoid the distribution to non-targeted site.

The following is the main objective of study:

To develop and evaluate the nanoemulsion of Rivastigmine for the treatment of Alzheimer's disease through intranasal route to bypass first pass effect in order to improve the bioavailability and reduces the dose and thereby side effects of the drug.

• PLAN OF WORK

The following plan of work was envisaged for accomplishing the objective

I. Physical characterization and identification of the Drug

- Organoleptic properties
- Melting point determination
- FTIR analysis
- UV spectral analysis
- DSC analysis

Cont....

II. Analytical methodology

Development and validation of analytical methods for routine analysis like solubility study, in-vitro release study and ex-vivo diffusion study.

Development and validation of bioanalytical method for plasma and brain homogenate.

III. Formulation design and development

Cont...

Screening of oil, surfactant and cosurfactant: Preliminary screening of various oils, surfactants and co-surfactants was done to study their accommodation ability for the drug.

Construction of Pseudo-Ternary phase diagram: On the basis of solubility studies

selection of appropriate oil, surfactant and co-surfactant was done. Aqueous phase titration was used for construction of Pseudo-Ternary phase diagrams.

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IV. Preparation of formulation

V. Optimization of formulation using design expert

VI. Physical stress stability screening

Centrifugation

Heating cooling cycle

Freeze-thaw cycle

VII. Characterization of optimized formulation for their globule size, polydispersity index (PDI), surface charge (zeta potential) and surface morphology by using appropriate techniques.

Cont....

VIII. In-vitro release study of optimized formulation.

IX. Ex-vivo diffusion study of optimized formulation.

X. In-vivo study of the optimized formulation.

Animal studies were performed as per the accorded approval from CPCSEA.

Wistar albino rats weighing between 200-250g were selected for study

. Biodistribution studies

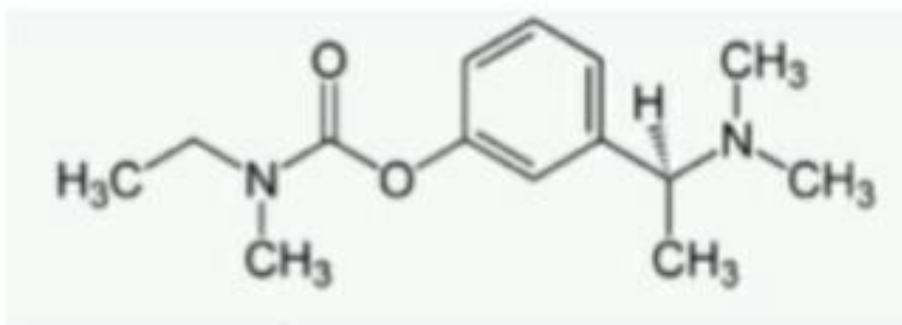
. Stability study

DRUG AND EXCIPIENTS PROFILE

Drug profile: rivastigmine hydrochloride

Category: Parasympathomimetic or Cholinergic agonist

Molecular formula : C₁₄H₂₂N₂O₂.HCL Molecular weight : 250.3367 gm/mol Structure formula:.



IUPAC name : 3-[(1S)-1-(dimethylamino)ethyl]phenyl N-ethyl-N-methylcarbamate

Characters

Appearance : White crystalline powder

Solubility : very soluble in water, soluble in methanol, slightly soluble in n-octanol and very slightly soluble in ethyl acetate.

Melting point : 124-125°C Half life : 1.5 h

λ_{max} : 224.5 nm in methanol, 264 nm in buffer pH 7.4 and 261 in pH 6.4 pKa : 8.85

Excipients profile

Surfactant (Tween 80)

i. Chemical name: Polyoxyethylene sorbitan monooleate ii. Molecular formula: C₆₄H₁₂₄O₂₆ iii. Molar mass: 1310 g/mol iv. Physical state and appearance: Liquid (oily liquid) v. Odor: Fatty (slight) vi. Color: Clear amber, yellow vii. HLB value: 15.0 viii. pH (1% aqueous soln): 7 (Neutral) ix. Dispersion properties: High solubility in water, methanol.

x. Solubility: Easily soluble in cold water, hot water. Soluble in methanol, toluene, cotton seed oil corn oil, ethyl acetate. Insoluble in mineral oil. xi. Toxicity to animals: Acute oral toxicity (LD₅₀): 25000 mg/kg by oral in mouse.

Co-surfactant Transcutol-P

i. Chemical name: Diethylene glycol monoethyl ether ii. Molecular formula: C₆H₁₄O₃ iii. Molar mass: 134.17 g/mol iv. Appearance: Liquid

V. Odor: Characteristics odor

Vi. Color: Clearvii. HLB value: 4.2

viii. Solubility: Soluble in methanol and water ix. Toxicity to animals: Acute oral toxicity (LD₅₀): 10502 mg/kg by oral and 4000 mg/kg by intravenous route in rats.

Cont....

Oil phase (Capmul MCM)

i. Chemical name: Glyceryl caprylate/caprate ii. Molecular formula: C₁₃H₂₆O₄ iii. Molar mass: 246.34 g/mol iv. Appearance: Oily liquid

v. Odor: Characteristics odor vi. Color: Colorless or slightly yellow vii. HLB value: 5.5-6.0 viii. Solubility: Soluble in water

Pharmacokinetics

1. Protein binding– 40% bound to plasma proteins, mainly to albumin.
2. Absorption –Pharmacokinetics are linear over a dose range of 1.5-3.0 mg given every 12 h. Rivastigmine is well-absorbed (approximately 100%) orally within 1 h.
3. Metabolism – Extensively via cholinesterase-mediated hydrolysis in the brain; metabolite undergoes N-methylation and/or sulfate conjugation hepatically; CYP minimally involved; linear kinetics at 3 mg twice daily, but nonlinear at higher doses.
4. Distribution–The volume of distribution (Vd) of rivastigmine is about 1.8-2.7 L/kg; penetrates blood brain barrier (CSF levels are ~40% of plasma levels following oral administration)
5. Elimination – Rivastigmine is eliminated mainly in the urine (97% as metabolites); faeces (0.4%).
6. Bioavailability – Oral 36% to 40%
7. Half life – 1.5 h (oral), 3 h (transdermal after removal)
8. Peak time – 1 h (oral), 10-16 h (transdermal following first dose)

Indications and usage

For Alzheimer's disease, cognitive (thinking and memory), functional (activities of daily living) and behavioural problems associated with Alzheimer's and Parkinson's disease dementias.

Contraindications:-

Patients who have a history of hypersensitivity reactions to rivastigmine or any other carbamate derivatives (e.g. Neostigmine, pyridostigmine, physostigmine), or any other component of the formulation.

Drug interactions:-

Antipsychotics: acetylcholinesterase inhibitors (central) may enhance the neurotoxic (central) effect of antipsychotics. Severe extrapyramidal symptoms have occurred in some patients.

Beta blockers: acetylcholinesterase inhibitors may enhance the bradycardic effect of beta blockers.

Cholinergic agonists: acetylcholinesterase inhibitors may enhance the adverse/toxic effect of cholinergic agonists.

Corticosteroids (systemic): may enhance the adverse/toxic effect of acetylcholinesterase inhibitors. Increased muscular weakness may occur.

Ginkgo biloba: may enhance the adverse/toxic effect of acetylcholinesterase inhibitors.

Neuromuscular blocking agents (nondepolarizing): acetylcholinesterase inhibitors may diminish the neuromuscular blocking effect of neuromuscular blocking agents.

Succinylcholine: acetylcholinesterase inhibitors may enhance the neuromuscular blocking effect of succinylcholine.

Adverse effects:-

Many concentration-related effects are reported at a lower frequency by transdermal route

Central nervous system: dizziness, headache, fatigue, insomnia, confusion, depression, anxiety, malaise, hallucinations, aggressiveness, parkinsonism symptoms worsening, vertigo

Gastrointestinal: nausea, vomiting, diarrhoea, anorexia, abdominal pain, dyspepsia, constipation, flatulence, weight loss, eructation, dehydration

Cardiovascular: syncope, hypertension

Genitourinary: urinary tract infection

Neuromuscular and skeletal: weakness, tremor

Respiratory: rhinitis

Miscellaneous: diaphoresis, flu-like syndrome

Ethanol/Nutrition/Herb interactions:-

Smoking: nicotine increases the clearance of rivastigmine by 23% Ethanol: avoid ethanol (due to risk of sedation; may increase GI irritation).

Food: food delays absorption by 1.5 h, lowers Cmax by 30% and increases AUC by 30%

Herb/Nutraceutical: avoid ginkgo biloba (may increase cholinergic effects)

Warning/precautions

Anorexia/weight loss: significant anorexia and weight loss are associated with use; occurs more frequently in women and during the titration phase. Monitor weight during therapy.

Nausea/vomiting: significant nausea and vomiting have been associated with use; occurs more frequently in women and during the titration phase. May be severe, particularly at doses higher than recommended.

Vagotonic effects: cholinesterase inhibitors may have Vagotonic effects which may cause bradycardia and/or heart block with or without a history of cardiac disease.

Disease related concerns:-

Disease related concerns

Cardiac conduction abnormalities: use with caution in patients with sick-sinus syndrome, bradycardia or conduction abnormalities. AD treatment guidelines consider bradycardia to be a relative contradiction for use of centrally active cholinesterase inhibitors.

Peptic ulcer disease: use with caution in patients at risk of ulcer disease (e.g. previous history or NSAID use); may increase gastric acid secretion. Monitor for symptoms of bleeding.

Respiratory disease: use with caution in patients with COPD and/or asthma.

Seizure disorder: use with caution in patients with a history of seizure disorder.

Urinary tract obstruction: use with caution in patients with bladder outlet obstruction or prostatic hyperplasia; cholinomimetics may cause or worsen outflow obstructions, including possible exacerbation of BPH symptoms.

Equipments

Sr. No	Equipment
1	U.V-Visible spectrophotometer
2	Differential scanning calorimeter
3	Fourier Transform Infrared Spectroscopy
4	Magnetic stirrer
5	Oven
6	Melting point apparatus
7	pH meter
8	Weighing balance
9	Sonicator
10	High performance liquid Chromatography
11	Freeze dryer

Materials

Sr. No	Materials
1	Rivastigmine hydrochloride
2	Capmul MCM
3	Transcutol-P, tween 80
4	Acetonitrile HPLC grade
5	Methanol, Methanol HPLC grade
6	HPLC grade water
7	Disodium hydrogen phosphate
8	Sodium chloride
9	Potassium dihydrogen phosphate
10	Water(unltrapurified), diethyl ether
11	Coptex 200-P, Cremophor EL
12	Cremophor RH-40,

METHODOLOGY

Physical characterization and identification of rivastigmine hydrochloride (RHC):

The procured sample of rivastigmine hydrochloride (RHC) was characterized on the basis of its physiochemical properties such as color, odor, solubility in water and other solvents. Melting point determination, DSC, UV spectroscopy and FTIR were carried out on the obtained sample and matched with those reported in the earlier literature.

1 Organoleptic properties:

RHC was characterized for its organoleptic properties viz. Nature, colour and odour.

2.Melting point determination:

Melting point of RHC was determined using melting point determination apparatus. The drug was introduced into a capillary glass tube. A sufficient quantity of drug powder formed a compact column of 4-6 mm height. This capillary tube was then inserted into HICON melting point apparatus along with the calibrated thermometer. The temperature at which the drug melted was recorded.

3.Differential scanning calorimeter (DSC):

The sample of RHC (about 5 mg) was loaded and sealed into DSC pan with a DSC loading puncher.

4.Fourier Transform Infrared (FTIR) spectroscopy:

FTIR spectroscopic analysis of the RHC was carried out using Potassium Bromide (KBr) pellet technique. An accurately weighed quantity of RHC (5 mg) was mixed with KBr (1:1) and later converted into a pellet using

hydraulic press. The pellet was scanned between 4000 to 400 wavelength (cm⁻¹) to get the characteristic spectra.

5. UV spectroscopy:

UV spectrum of RHC in methanol, phosphate buffer pH 6.4 and pH 7.4 was obtained using UV spectrophotometer (Shimadzu Corp, Kyoto, Japan). Scanning was carried out over a wavelength region of 200-400 nm and the λ_{max} was determined.

Analytical Methodology:

Towards the objective of quantification of RHC at various stages and different samples of the studies, efforts have been made towards the development and validation of analytical method by UV spectrophotometry and high pressure liquid chromatography (HPLC) as follows.

- 1 UV Spectrophotometry
- 2 Method validation of UV spectrophotometer in methanol
 - (i) Linearity and range
 - (ii) Precision
 - (a) Repeatability
 - (b) Intermediate precision (iii) Accuracy as recovery studies
 - iv) Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following formulae as per ICH guidelines.

$$\text{LOD} = 3.3 \times \sigma/S \dots \dots \dots (4.1)$$

$$\text{LOQ} = 10 \times \sigma/S \dots \dots \dots (4.2)$$

Where σ = the standard deviation of the intercept of the calibration plot
 S = the slope of the regression line

High performance liquid chromatography (HPLC)

2.1 HPLC method validation for determination of RHC in rat blood plasma and brain homogenate

- (a) Preparation of mobile phase
- (b) sample preparation
- (c) Preparation of calibration curve
- (d) Validation parameters

Formulation of nanoemulsion

- 1 Selection of oil, surfactant and cosurfactant
- 2 Construction of pseudo-ternary phase diagrams
- 3 Preparation of RHC loaded NE
- 4 Optimization of nanoemulsion formulation by Experimental design using Box–Behnken

Physical stability testing of nanoemulsions

- 1 Heating-cooling cycle
- 2 Centrifugation study
- 3 Freeze-thaw cycle

Characterization of optimized nanoemulsion

- 1 Percentage transmittance (%T)
- 2 Determination of globule size and polydispersity index
- 3 Zeta potential measurement
- 4 Transmission electron microscopy

RESULTS AND DISCUSSION

Summary and Conclusion

- From the physical properties and identification tests of the drug sample, it was concluded that the drug sample RHC was authentic, pure and confirming to the standards.
- The highest solubility of RHC was achieved with Capmul MCM. The solubility in Capmul MCM was 80 ± 2.64 mg/ml, so it was selected as an oil phase for making nanoemulsion. Nanoemulsions were formulated by titration method. The phase behavior of different surfactant, co-surfactant and their combinations was determined by constructing ternary phase diagrams. Percentage nanoemulsion region obtained for different groups were determined. The 4 : 1 ratio of Tween 80 : Transcutol-P yielded broad regions of nanoemulsion, so this combination was selected for further studies

Conclusion

In the present study, optimized RHC-loaded NEs was developed using Box-Behnken statistical experiment design which gives the optimum concentration of oil, Smix and water at which the globule size of 35.75 ± 0.21 nm, PDI of 0.247 ± 0.04 and zeta potential of -24.4 ± 0.67 mV could be successfully achieved. The release of RHC from NEs was significantly higher than RHC solution and the release mechanism was found to be diffusion controlled (Higuchi model). The results of permeation study demonstrated great potential of developed NEs formulation for enhancing the permeation of RHC across nasal mucosa in contrast to RHC solution. Significantly high level of RHC was achieved in brain via NEs as evident in result of pharmacokinetic studies in comparison of RHC solution. Further optimized formulation was non-toxic and safe as demonstrated by nasal ciliotoxicity studies. The present study clearly demonstrated that i.n. administration of optimized NEs is highly efficient for attaining the higher brain concentration of RHC which could be of great benefit for enhancing its therapeutic prospect.

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