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Research paper

Development and bioavailability assessment of ramipril nanoemulsion formulation

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Abstract

The objective of our investigation was to design a thermodynamically stable and dilutable nanoemulsion formulation of Ramipril, with minimum surfactant concentration that could improve its solubility, stability and oral bioavailability. Formulations were taken from the o/w nanoemulsion region of phase diagrams, which were subjected to thermodynamic stability and dispersibility tests. The composition of optimized formulation was Sefsol 218 (20% w/w), Tween 80 (18% w/w), Carbitol (18% w/w) and standard buffer solution pH 5 (44% w/w) as oil, surfactant, cosurfactant and aqueous phase, respectively, containing 5 mg of ramipril showing drug release (95%), droplet size (80.9 nm), polydispersity (0.271), viscosity (10.68 cP), and infinite dilution capability. *In vitro* drug release of the nanoemulsion formulations was highly significant ($p < 0.01$) as compared to marketed capsule formulation and drug suspension. The relative bioavailability of ramipril nanoemulsion to that of conventional capsule form was found to be 229.62% whereas to that of drug suspension was 539.49%. The present study revealed that ramipril nanoemulsion could be used as a liquid formulation for pediatric and geriatric patients and can be formulated as self-nanoemulsifying drug delivery system (SNEDDS) as a unit dosage form.

Keywords: Nanoemulsion; Ramipril; Bioavailability; SNEDDS; Phase diagrams; Solubility; Sefsol 218

1. Introduction

Poor bioavailability can be due to poor solubility, degradation in GI lumen, poor membrane permeation and presystemic elimination [1,2]. By many estimates up to 40 percent of new chemical entities (NCEs) discovered by the pharmaceutical industry today and many existing drugs are poorly soluble or lipophilic compounds which leads to poor oral bioavailability, high intra- and inter-subject variability and lack of dose proportionality [3]. Thus, for such compounds, the absorption rate from the gastrointestinal (GI) lumen is controlled by dissolution [4]. The ability to deliver poorly soluble drugs will grow in significance in the coming years as innovator companies rely upon NCEs for a larger share of the revenue within the pharmaceutical market.

In recent years, much attention has focused on lipid-based formulations to improve the oral bioavailability of poorly water soluble drug compounds. In fact, the most popular approach is the incorporation of the active lipophilic component into inert lipid vehicles such as oils, surfactant dispersions, microemulsions, nanoemulsions, self-emulsifying formulations, self-microemulsifying formulations, emulsions and liposomes [5–19]. Most of them increase surface area of the drugs to improve solubilisation behaviour, as well as permeation. From the viewpoint of oral drug delivery, lipids are studied as components of various oily liquids and dispersions that are designed to increase solubility and bioavailability of

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drugs belonging to the class II and IV of the biopharmaceutical drug classification system [4].

One of the promising technologies is nanoemulsion drug delivery system, which is being applied to enhance the oral bioavailability of the poorly soluble drugs. Nanoemulsions are thermodynamically stable, transparent (or translucent); dispersions of oil and water stabilized by an interfacial film of surfactant molecules having the droplet size less than 100 nm. Nanoemulsion provides ultra low interfacial tensions and large o/w interfacial areas. Nanoemulsions have a higher solubilization capacity than simple micellar solutions and their thermodynamic stability offers advantages over unstable dispersions, such as emulsions and suspensions, because they can be manufactured with little energy input (heat or mixing) and have a long shelf life. The nanosized droplets leading to enormous interfacial areas associated with nanoemulsions would influence the transport properties of the drug, an important factor in sustained and targeted drug delivery [20,21]. The attraction of formulating o/w nanoemulsion systems lies in their ability to incorporate hydrophobic drugs into the oil phase thereby enhancing their solubility [21]. Nanoemulsions have been reported to make the plasma concentration profiles and bioavailability of drugs more reproducible [3,21–24].

Ramipril {(2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl] amino]-1-oxopropyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid}, a potent antihypertensive drug, is almost completely converted to its active metabolite ramiprilat (a dicarboxylic acid) by hydrolytic cleavage of the ester group in the liver which has about six times angiotensin converting enzyme (ACE) inhibitor activity of ramipril. Ramipril is a highly lipophilic (log *P* (octanol/water), 3.32), poorly water soluble drug with absolute bioavailability of 28–35%, when 5 mg of oral ramipril is compared with the same dose given intravenously [25–27]. Ramipril and ramiprilat inhibit ACE which catalyses the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Inhibition of ACE receptor decreases tissue and circulating ACE activity, which leads to decreased vasopressor activity and decreased aldosterone secretion and therefore, causes general vasodilatation and lowers blood pressure effectively [28,29]. The HOPE (heart outcomes prevention evaluation) trial showed that ramipril therapy significantly reduced the incidence of myocardial infarction, stroke or cardiovascular death (relative risk 0.78) and reduced total mortality (relative risk 0.84) versus placebo in patients (mean age of patients was 65.9 years) with atherosclerotic diseases or diabetes mellitus [30]. According to study conducted by ESCAPE trial group, ramipril is a very effective antihypertensive and antiproteinuric agent in children with chronic renal failure associated with hypertension [31].

The dose of ramipril varies between 2.5 mg and 20 mg and frequently prescribed dose is 5 mg for the adult. Therefore, for the present study, 5 mg dose was selected for the development of nanoemulsion formulation. Thus, the objectives of the present study were to develop and characterize an optimal nanoemulsion formulation of ramipril using minimum surfactant concentration, so that nano-sized droplets could be maintained on dilution by the gastrointestinal (GI) fluids with an aim to increase its bioavailability and compare it with a marketed capsule formulation as well as the drug suspension. The other objective was to develop a liquid formulation that can be used for pediatric patients as well as to develop a self-nanoemulsifying drug delivery (SNEDDS) unit dose formulation.

2. Materials and methods

2.1. Materials for component selection

Ramipril base was a gift sample from Ranbaxy Research Laboratories (Haryana, India). Medium chain triglyceride (Labrafac®), Caprylo caproyl macrogol-8-glyceride (Labrasol®), Polyglyceryl-6-dioleate (Plurol oleique®) were gift samples from Gattefossé (Saint Priest, Cedex France), Propylene glycol mono caprylic ester (Safsol 218®) was gift sample from Nikko Chemicals (Tokyo, Japan). Isopropyl myristate (IPM), Glycerol triacetate (Triacetin), Castor oil, Polyoxyethylene (20) sorbitan mono oleic acid (Tween 80®), Diethylene glycol monoethyl ether (Carbitol®), Sodium perchlorate AG, and Acetonitrile (HPLC grade) were purchased from Merck (Schuchardh, Hokenbrunn, Germany). Water was obtained from Milli-Q-water purification system (Millipore, MA). For LC/MS/MS study, all the chemicals and C-18 solid phase extraction cartridges (Oasis HLB, 30 ng/cc) were a gift from Ranbaxy Research Lab. Ltd. (Haryana, India). All other chemicals were of analytical grade.

2.2. Screening of components

The most important criterion for the screening of components for nanoemulsion is the solubility of poorly soluble drug in oils, surfactants and cosurfactants. Since the aim of this study is to develop an oral formulation, therefore, solubility of drug in oils is more important as the ability of nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in oil phase. The solubility of ramipril in various oils was determined by adding an excess amount of drug in 2 mL of selected oils (Sefsol 218, Triacetin, IPM, Labrafac, Castor oil), distilled water separately in 5 mL capacity stopper vials, and mixed using a vortex mixer. The mixture vials were then kept at 25 ± 1.0 °C in an isothermal shaker (Nirmal International, Delhi, India) for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 μm membrane filter. The concentration of ramipril was determined in oils and water using HPLC at 210 nm.