2. Literature Review

2.1. Review on nose to brain delivery

The intranasal route is an attractive field of research for systemic and brain-targeted drug deliveries. Earlier, it has been restricted to local nasal treatment such as common cold, nasal allergies. rhinorrhea, sinusitis and nasal infections (Kozlovskaya L *et al.*, 2014). The recent studies shows that this route of administration has been used many small molecular weight drugs, peptides and proteins for systemic and targeted delivery (Cho HJ *et al.*, 2011; Graff CL *et al.*, 2005; Yang JP *et al.*, 2009; Zhang Q *et al.*, 2004). Nasal delivery offer several advantages like rapid absorption (fast onset of therapeutic effect), high bioavailability (rich, vascular submucosa and lymphatic system), circumventing of liver first-pass metabolism (resulting in higher and less variable bioavailability), no gastrointestinal drug degradation, non-invasive therefore reduced risk of infection, ease of convenience, self-medication, improved patient compliance and possible direct pathways to the CNS bypassing the blood–brain barrier over oral and parenteral delivery (Suter-Zimmermann K, 2008). However, it also shows some limitations such as active mucociliary clearance, enzymatic degradation by nasal peptidases and proteases, low permeability for hydrophilic drug, low pH for nasal epithelium and limited to potent drug/volume (25-200 µl) (Lochhead JJ and Thorne RG, 2012). In order to understand the complete aspects of nose-to-brain drug delivery it is necessary to have knowledge of the nasal anatomy and physiology.

2.1.1. Nasal anatomy and physiology

The primary functions of the nose are olfaction, regulation of humidity and temperature, particles filtration including microorganisms from the inhaled air. The nasal cavity extends from the nostrils (nares) to the nasopharynx and is divided longitudinally by the nasal septum. Each side comprises three regions; the nasal vestibule, the olfactory region and the respiratory region. The surface of the nasal cavity is lined by a mucous membrane that is well vascularized and covered by a mucous layer (Illum L, 2003). The nasal cavity of the human is about 5-8 cm large with total surface area of approximately 150 cm^2 (Wang X *et al.*, 2008). When it comes to intranasal delivery, olfactory and respiratory epithelia represent most important surface membrane for drug absorption.

Respiratory epithelium

The nasal respiratory epithelium constitutes about 50% of the nasal cavity in rats and 80–90% in humans. It is predominantly constitute of pseudostratified ciliated or nonciliated columnar secretory epithelium. The human respiratory epithelium is comprised of goblet cells, ciliated cells, intermediate cells, and basal cells. Serous glands, seromucous glands, and intraepithelial glands are also associated with the nasal respiratory epithelium (Figure 2.1). The seromucous glands are responsible for producing most nasal secretions while the goblet cells also secrete mucus. The production of mucus and the action of the ciliated epithelium are responsible for the main physical clearance mechanism of the nasal cavity, mucociliary clearance. Due

to its highly vascular nature, the respiratory epithelium is the surface that is responsible for the majority of absorption, which leads to systemic exposure following nasal dosing. Importantly, the nasal respiratory epithelium is innervated by branches of the trigeminal nerve, fibers from trigeminal ganglion cells (Illum L, 2015; Landis MS *et al.*, 2012; Lochhead JJ and Thorne RG, 2012; Mistry A *et al.*, 2009).

Olfactory epithelium

The nasal olfactory epithelium constitutes about 50% of the nasal cavity in rats and 10% in humans. In human, olfactory epithelium is located high in the nasal cavity. It partly overlies the cribriform plate, a bony structure that contains many pores that allow the passage of neuronal bundles from the olfactory epithelium to pass into the CNS. Olfactory epithelium may also lie partly on the nasal septum and on the superior turbinate. The human olfactory epithelium is composed of pseudostratified columnar epithelium that rests on a highly cellular lamina propria, which contains Bowman's glands and four major cell types: ciliated bipolar olfactory receptors, microvillar cells, sustetacular cells and basal cells (Figure 2.1). Commonly used preclinical species have similarly structured epithelium but the exact structure and olfactory receptor density may vary among species. It is also known that the size and function of the olfactory epithelium changes with age, disease state, trauma and medication. Located within the olfactory epithelium are the olfactory receptor cells or neurons. It is believed that human olfactory neurons function similarly to those of

other species and include standard second messenger systems for signaling. Human olfactory neurons have a nominal lifetime of only 6–10 weeks and have the ability to regenerate if damaged at the olfactory epithelium. This may be an important factor in both facilitated absorption of molecules and also mediating or reversing potential toxicity associated with drug delivery to this region. Olfactory receptor cells extend up through the cribriform plate as unmyleinated axons to the olfactory bulb, where they form the outer olfactory nerve layer of the olfactory bulb. Small populations of cells lined with numerous microvilli also exist in the olfactory epithelium. These cells are referred to as microvillus cells and have an unknown function. Blood vessels, inflammatory cells, and lymphatic vessels which drain into the deep cervical lymph nodes in the neck are also present in the submucosa (lamina propria) of the olfactory region (Illum L, 2015; Landis MS *et al.*, 2012; Lochhead JJ and Thorne RG, 2012; Mistry A *et al.*, 2009).

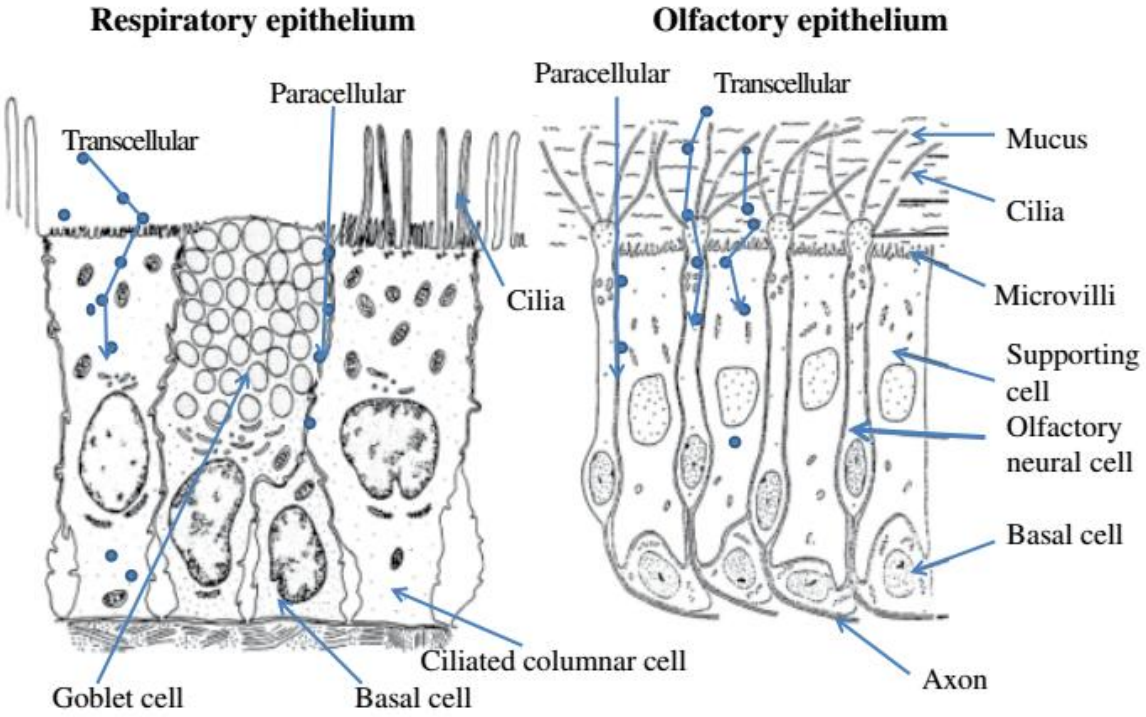

Figure 2.1: Schematic diagram of respiratory and olfactory epithelium.

2.1.2. Transport of drug from nose to brain

The precise mechanism of drug transport from nose to brain is not completely elucidated. But the no of research paper discuss the different direct and indirect pathways for transportation (Illum L, 2000). It is suggested in the literature that a drug administered nasally is able to reach the CNS (i.e. CSF and brain tissue) by the various transport routes shown schematically in Figure 2.2. It is generally accepted that drugs administered nasally can reach the brain using three main pathways: (i) absorption across the nasal respiratory epithelium into the systemic circulation and, from there, across the BBB into the brain (systemic pathway), (ii) direct paracellular or transcellular transport via the olfactory neurons (olfactory neural pathway) or

the olfactory epithelial cells (olfactory epithelial pathway), or (iii) transport via the trigeminal nerves (trigeminal pathway) (Mistry A *et al.*, 2009). Apart for above pathways, different other factors also have a great impact in the rate and extent of nasal drug absorption. These factors can influence bioavailability and transport of drug from nose to brain. All these factors are presented in Figure 2.3.

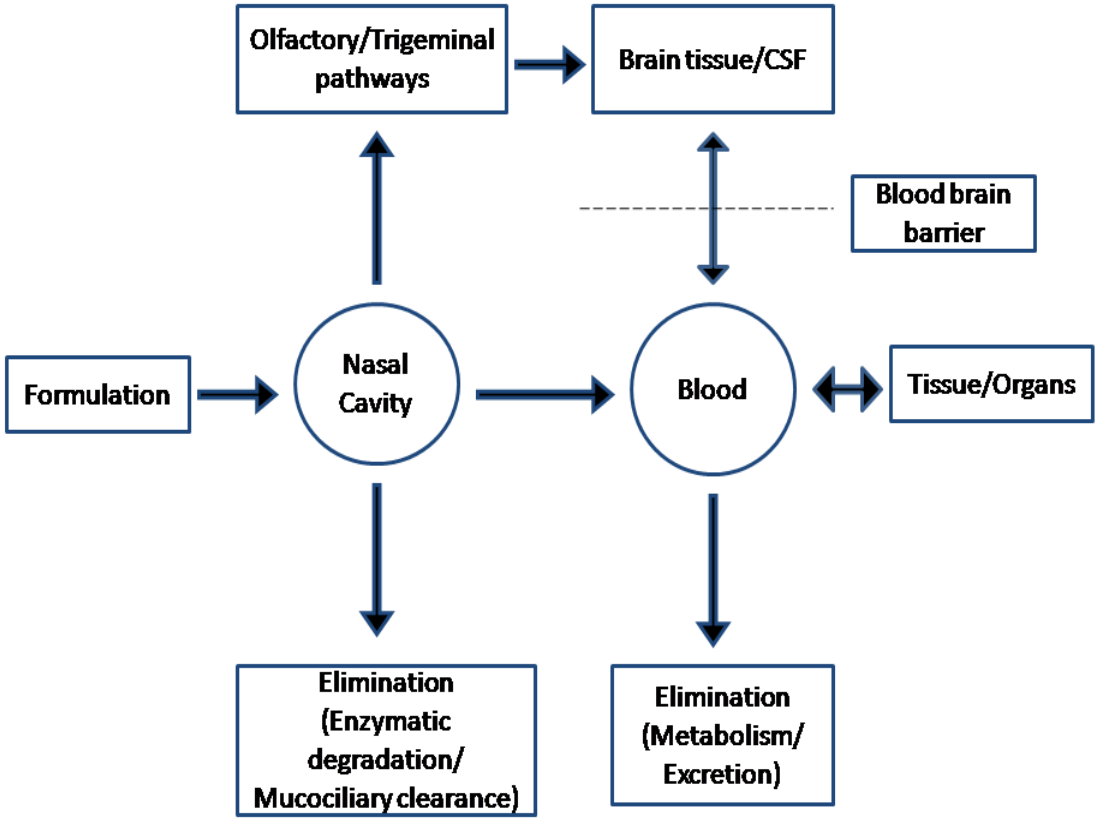

Figure 2.2: Fate of nanocariers from nasal cavity.

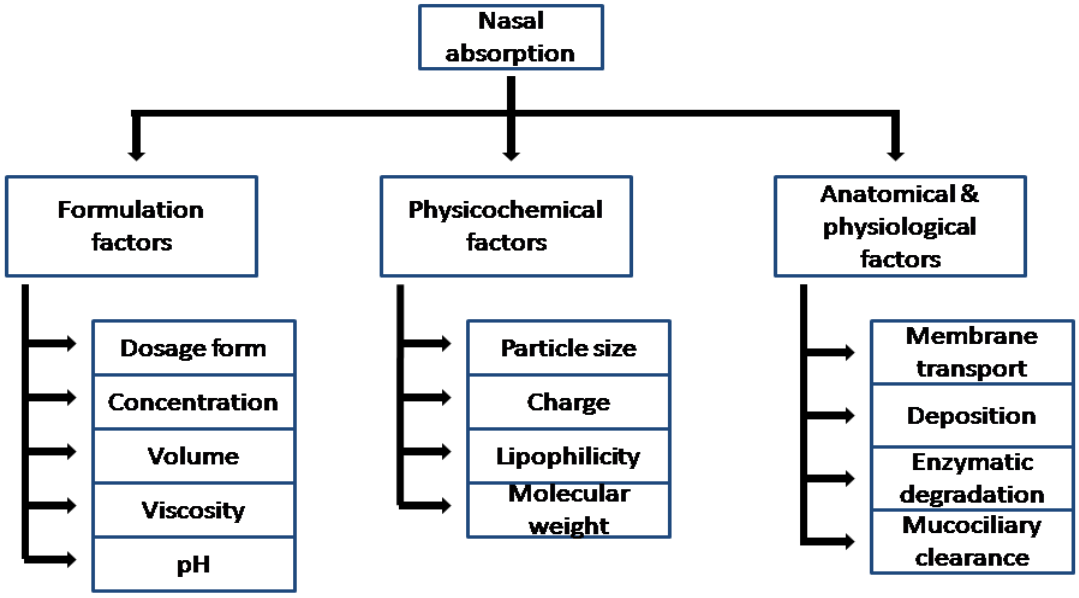

Figure 2.3: The physicochemical, anatomical, physiological and formulation factors affecting the nasal absorption of drugs.

2.1.3. Reported research work based on nose to brain delivery

Westin UE et al., 2006 studied the olfactory transfer of morphine to the brain hemispheres by comparing brain tissue and plasma morphine levels after nasal administration with those after intravenous administration. In this study, morphine (1.0 mg/kg body weight) was administered via the right nostril or intravenously as a 15-min constant-rate infusion to male rats. The content of morphine and its metabolite morphine-3-glucuronide in samples of the olfactory bulbs, brain hemispheres, and plasma was assessed using high performance liquid chromatography, and the areas under the concentration-time curves (AUC) were calculated. Finally, at both 5 and 15 min after administration, brain hemisphere

morphine concentrations after nasal administration were similar to those after i.v. administration of the same dose, despite lower plasma concentrations after nasal administration. The brain hemispheres/plasma morphine AUC ratios for the 0-5 min period were thus approximately 3 and 0.1 after nasal and i.v. administration, respectively, demonstrating a statistically significant early distribution advantage of morphine to the brain hemispheres via the nasal route. It indicated that morphine is transferred via olfactory pathways to the brain hemispheres, and drug transfer via this route significantly contributes to the early high brain concentrations after nasal administration to rats (Westin UE *et al.*, 2006).

Czapp M et. al. 2008 reported brain penetration of phenobarbital following its intranasal administration in rats by microdialysis and brain homogenates analysis. The anticonvulsant efficacy of intranasal phenobarbital was determined in the amygdala kindling model. The results of this study indicated that phenobarbital was efficiently delivered to the rat brain following intranasal administration. Phenobarbital concentrations in the olfactory bulb exceeded more in caudal parts of the brain at ten minutes, thus, indicating that phenobarbital has been partially targeted to the brain via local pathways. The amygdala kindling model also demonstrated that effective brain concentrations were reached with intranasal phenobarbital delivery. In conclusion, intranasal administration of phenobarbital in rats is associated with efficient brain penetration rates allowing to achieve therapeutic concentrations (Czapp M *et al.*, 2008).

Piao HM et al., 2010 developed fexofenadine microemulsion system composed of oil, surfactant and co-surfactant for intranasal delivery. The microemulsions were characterized by phase behavior, particle size, viscosity and solubilization capacity. Histopathology and in vivo nasal absorption of the optimized microemulsion formulations were also investigated in rats. A single isotropic region was found in the pseudo-ternary phase diagrams developed at various ratios with Lauroglycol 90 as oil, Labrasol as surfactant and Plurol Oleique CC49 or its mixture with PEG-400 (1:1) as cosurfactant. The optimized microemulsion formulations showed higher solubulization of fexofenadine, i.e., (22.64 mg/mL) and (22.98 mg/mL), compared to its intrinsic water solubility (1.51 mg/mL). Nasal absorption of fexofenadine from these microemulsions was found to be fairly rapid. Tmax was observed within 5 min after intranasal administration at 1.0 mg/kg dose, and the absolute bioavailability (0–4 h) was about 68% compared to the intravenous administration in rats. This results suggested that these microemulsion formulations could be used as an effective intranasal dosage form for the rapid-onset delivery of fexofenadine (Piao HM *et al.*, 2010).

Md S et al., 2014 have prepared bromocriptine (BRC) chitosan NPs (CS NPs) by ionic gelation method. BRC-loaded CS NPs showed greater retention into the nostrils (42 ± 8.5% radioactivity) for about 4 h, whereas the 44 ± 7.5% could be retained up to 1 h for BRC solution. The brain:blood ratios of 0.96 ± 0.05 > 0.73 ± 0.15 > 0.25 ± 0.05 of BRC-loaded CS NPs (i.n.) > BRC solution (i.n.) > BRC-loaded CS NPs (intravenous), respectively, at 0.5 h indicated direct nose-to-brain transport

bypassing blood--brain barrier. BRC-loaded CS NPs administered intranasally showed significantly high dopamine concentration (20.65 ± 1.08 ng/ml) as compared to haloperidol-treated mice (10.94 ± 2.16 ng/ml) ($p < 0.05$). Histopathology of brain sections showed selective degeneration of the dopaminergic neurons in haloperidol-treated mice which was markedly reverted by BRC-loaded CS NPs. This study showed potential of nose-to-brain drug delivery carrier for the treatment of Parkinson's disease (Md S *et al.*, 2014).

Gartziandia O et al., 2015 reported a suitable chitosan coated nanostructured lipid carrier (CS-NLC) formulation with the capacity to reach the brain. The optimal formulation displayed a particle size of 114 nm with a positive surface charge of +28 mV. The in vitro assays demonstrated the biocompatibility of the nanocarrier and its cellular uptake by 16HBE14o-cells. Furthermore, no haemagglutination or haemolysis processes were observed when the particles were incubated with erythrocytes, and no toxicity signals appeared in the nasal mucosa of mice after the administration of CS-NLCs. Finally, the biodistribution study of CS-NLC-DiR demonstrated an efficient brain delivery of the particles after intranasal administration (Gartziandia O *et al.*, 2015).

2.2. Review on Nanostructured lipid carriers

In recent decay, the deliveries of drug in the form of polymeric or lipid carriers have been discussed enormously. Among them lipid based delivery systems emerged as promising vehicles for targeted delivery and to overcome the problem of poorly solubility and low bioavailability (Beloqui A et al., 2014). Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are new generations of lipid nanoparticles produced from solid lipids. SLN includes only solid lipids as matrix, while NLC is consisting of solid lipid matrix entrapped liquid lipid inside (Muller RH et al., 2002). These carriers are natural products with good biocompatibility, low toxicity and low cost. The NLC has been considered as an alternative to SLN, liposomes and emulsions due to improved properties such as ease of manufacture, high drug loading, and more flexibility in modulating the drug release profile (Jia L et al., 2010). Additionally, the lipids around the drugs might protect the drugs from oxidation, hydrolysis and enzymatic degradation. Generally, NLC gives higher drug stability and provides a sustained release, which might prolong the drug circulation time and improve the therapeutic efficacy (Li M *et al.*, 2013). NLC is successfully applied for delivery of different drugs via various routs of administration, such as oral, intravenous, intranasal, pulmonary and transdermal (Beloqui A *et al.*, 2014; Han F *et al.*, 2008; Patil-Gadhe A *et al.*, 2014; Singh SK *et al.*, 2016; Zhang P *et al.*, 2012).

2.2.1. Ingredient of Nanostructured lipid carriers (NLC)

The essential ingredients for NLC preparation involve solid lipid, liquid lipids and surfactant. Both solid and liquid lipids are included in NLCs for constructing the inner cores while surfactant mostly present on the surface for stabilization of nanoparticles. The selection of specific solid lipid, liquid lipid and surfactant is based on the solubility of drug in lipid, compatibility, stability of formulation and route of administration. The different ingredients for NLC preparation are mention in Table 2.1

Table 2.1: List of ingredients used in the preparation of NLC (Fang CL *et al.*, 2013).

Solid lipid	Liquid lipid	Surfactant
▪ Tristearin, ▪ Stearic acid, ▪ Cetyl palmitate, ▪ Behenic acid, ▪ Palitic acid, ▪ Glyceryl monostearate ▪ Precirol ATO 5 (Glyceryl distearate), ▪ Compritol 888 ATO (Glyceryl dibehenate), ▪ Dynasan116, 118	▪ Medium chain triglycerides, ▪ Paraffin oil, ▪ 2-octyl dodecanol, ▪ Oleic acid, ▪ Squalene, ▪ Isopropyl myristate, ▪ Vitamin E, ▪ Polyvinyl alcohol ▪ Sodium oleate, ▪ Miglyol 812	▪ Pluronic F68 (poloxamer 188), ▪ Pluronic F127 (poloxamer 407), ▪ Polyvinyl alcohol, ▪ Solutol HS15, ▪ Sodium deoxycholate, ▪ Sodium glycocholate, ▪ Polysorbate 20 (polyoxyethylene (20) sorbitan

(Glyceryl tripalmitate), ▪ Softisan 154 (Hydrogenated palm oil), ▪ Cutina CP (Cetyl Palmitate), ▪ Imwitor 900 P (Glycerol mono and distearate), ▪ Emulcire 61 (Cetyl Alcohol) ▪ Geleol (Glycerol monoand distearate)	(caprilyc/capric medium chain triglycerides), ▪ Transcutol HP (diethylene glycol monoethyl ether), ▪ Labrafil Lipofile WL 1349 (Caprylic/Capric triglyceride), ▪ Capryol 90 (Propylene glycol monocaprylate) ▪ Solutol HS15 (Polyethylene glycol (15)-hydroxystearate)	monolaurate), ▪ Polysorbate 40 (polyoxyethylene (20) sorbitan monopalmitate), ▪ Polysorbate 60 (polyoxyethylene (20) sorbitan monostearate), ▪ Polysorbate 80 (polyoxyethylene (20) sorbitan monooleate)

Glyceryl monostearate

Nonproprietary Names

BP: Glyceryl Monostearate 40–55

JP: Glyceryl Monostearate

PhEur: Glycerol Monostearate 40–55

USP-NF: Glyceryl Monostearate

Synonyms

Capmul GMS-50; Cutina GMS; 2,3-dihydroxypropyl octadecanoate; Geleol; glycerine monostearate; glycerin monostearate; glycerol monostearate; glyceroli monostearas; glycerol stearate; glyceryl stearate; GMS; HallStar GMS; Imwitor 191; Imwitor 900; Kessco GMS; Lipo GMS; monoester with 1,2,3-propanetriol; monostearin; Myvaplex 600P; Myvatex; 1,2,3-propanetriol octadecanoate; Protachem GMS-450; Rita GMS; stearic acid, monoester with glycerol; stearic monoglyceride; Stepan GMS; Tegin; Tegin 503; Tegin 515; Tegin 4100; Tegin M; Unimate GMS.

Chemical Name and CAS Registry Number

Octadecanoic acid, monoester with 1,2,3-propanetriol [31566-31-1]

Empirical Formula and Molecular Weight

$C_{21}H_{42}O_4$ (358.6)

Description

Glyceryl monostearate is a white to cream-colored, wax-like solid in the form of beads, flakes, or powder. It is waxy to the touch and has a slight fatty odor and taste.

Typical Properties

Acid value: 44.0

Iodine value: 90.0–110.0%

Hydroxyl value: 90.0–110.0%

Saponification value: 90.0–110.0%

Melting point: ≈55°C

Solubility: Soluble in hot ethanol, ether, chloroform, hot acetone, mineral oil, and fixed oils. Practically insoluble in water, but may be dispersed in water with the aid of a small amount of soap or other surfactant.

Stability and Storage Conditions

If stored at warm temperatures, glyceryl monostearate increases in acid value upon aging owing to the saponification of the ester with trace amounts of water. Effective antioxidants may be added, such as butylated hydroxytoluene and propyl gallate. Glyceryl monostearate should be stored in a tightly closed container in a cool, dry place, and protected from light.

Safety

Glyceryl monostearate is widely used in cosmetics, foods, and oral and topical pharmaceutical formulations, and is generally regarded as a nontoxic and nonirritant material. Included in the FDA Inactive Ingredients Database (oral capsules and tablets; ophthalmic, otic, rectal, topical, transdermal, and vaginal preparations). Included in nonparenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients (Excipients HoP, 2013) .

Oleic acid

Nonproprietary Names

BP: Oleic acid

PhEur: Oleic acid

USPNF: Oleic acid

Synonyms

Acidum oleicum; Crodolene; Crossential 094; elaic acid; Emersol; Glycon; Groco; Hy-Phi; Industrene; Metaupon; Neo-Fat; cis-9-octadecenoic acid; 9,10-octadecenoic acid; oleinic acid; Priolene.

Chemical Name and CAS Registry Number

(Z)-octadec-9-enoic acid [112-80-1]

Empirical Formula and Molecular Weight

C18H34 O2 (282.47)

Description

A yellowish to pale brown, oily liquid with a characteristic lard-like odor and taste. Oleic acid consists chiefly of (Z)-9-octadecenoic acid together with varying amounts of saturated and other unsaturated acids. It may contain a suitable antioxidant.

Typical Properties

Density: 0.895 g/mL

Melting point: 13 to 14 °C

Boiling point: 360 °C

Solubility: Miscible with benzene, chloroform, ethanol (95%), ether, hexane, and fixed and volatile oils; practically insoluble in water.

Stability and Storage Conditions

On exposure to air, oleic acid gradually absorbs oxygen, darkens in color, and develops a more pronounced odor. At atmospheric pressure, it decomposes when heated at 80–100°C. Oleic acid should be stored in a well-filled, well-closed container, protected from light, in a cool, dry place.

Safety

No specific health effects are reported for oleic acid. It is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol. However, its ability to raise HDL is still debated. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil (Excipients HoP, 2013).

Tween 80

Nonproprietary Names

BP: Polysorbate 80

JP: Polysorbate 80

PhEur: Polysorbatum 80

USPNF: Polysorbate 80

Synonyms

Atlas E; Armotan PMO 20; Capmul POE-O; Cremophor PS 80; Crillet 4; Crillet 50; Drewmulse POE-SMO; Drewpone 80K; Durfax 80; Durfax 80K; E433; Emrite 6120; Eumulgin SMO; Glycosperse O-20; Hodag PSMO-20; Liposorb O-20; Liposorb O-20K; Montanox 80; polyoxyethylene 20 oleate; Protasorb O-20; Ritabate 80; (Z)-sorbitan mono-9-octadecenoate poly(oxy1,2- ethanediyl) derivatives; Tego SMO 80; Tego SMO 80V; Tween 80.

Chemical Name and CAS Registry Number

Polyoxyethylene 20 sorbitan monooleate [9005-65-6]

Empirical Formula and Molecular Weight

C64H124O26 - 1310

Description

Polysorbates have a characteristic odor and a warm, somewhat bitter taste.

Typical Properties

Acidity/alkalinity: pH= 6.0-8.0 for a 5% w/v aqueous solution.

Critical micelle concentration (CMC): 0.012 mM

Aggregation number: 60

Solubility: Soluble in ethanol and water; insoluble in mineral oil and vegetable oil.

Stability and Storage Conditions

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive

to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides. Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

Safety

Polysorbates are widely used in cosmetics, food products for oral, parenteral, and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials. There have been occasional reports of hypersensitivity to polysorbates following their topical and intramuscular use. Polysorbates have also been associated with serious adverse effects, including some deaths, in low-birth weight infants intravenously administered a vitamin E preparation containing a mixture of polysorbates 20 and 80 (Excipients HoP, 2013).

2.2.2. Application of nanostructured lipid carriers in targeted delivery

Taratula O et al. 2013 have developed, synthesized, and tested a multifunctional nanostructured lipid nanocarrier-based system (NLCS) for efficient delivery of an anticancer drug and siRNA directly into the lungs by inhalation. The NLCS was tested in vitro using human lung cancer cells and in vivo utilizing mouse orthotopic model of human lung cancer. After inhalation, the proposed NLCS effectively delivered its payload into lung cancer cells leaving healthy lung tissues intact and also significantly decreasing the exposure of healthy organs when compared with

intravenous injection. The NLCS showed enhanced antitumor activity when compared with intravenous treatment. The data obtained demonstrated high efficiency of proposed NLCS for tumor-targeted local delivery by inhalation of anticancer drugs and mixture of siRNAs specifically to lung cancer cells and, as a result, efficient suppression of tumor growth and prevention of adverse side effects on healthy organs (Taratula O *et al.*, 2013)

Beloqui A et al., 2013 evaluated the potential of nanostructured lipid carriers (NLCs) as a tool to enhance the oral bioavailability of poorly soluble compounds using saquinavir (SQV), a BCS class IV drug and P-gp substrate as a model drug. SQV transport across Caco-2 monolayers was enhanced up to 3.5-fold by NLCs compared to SQV suspension. M cells did not enhance the transport of NLCs loaded with SQV. The size and amount of surfactant in the NLCs influenced SQV's permeability, the transcytosis pathway and the efflux of SQV by P-gp. An NLC of size 247 nm and 1.5% (w/v) surfactant content circumvented P-gp efflux and used both caveolae- and clathrin-mediated transcytosis, in contrast to the other NLC formulations, which used only caveolae-mediated transcytosis. By modifying critical physicochemical parameters of the NLC formulation, researchers were able to overcome the P-gp drug efflux and alter the transcytosis mechanism of the nanoparticles. These findings support the use of NLCs approaches for oral delivery of poorly water-soluble P-gp substrates (Beloqui A *et al.*, 2013).

Tian B-C et al., 2013 evaluated the application of nanostructured lipid carriers (NLC) in ocular delivery system. NLC was labeled with fluorescent marker rhodamine B or coumarin-6 were produced by a melt emulsification method. By confocal laser scanning microscopy (CLSM), the interaction of NLC with corneal epithelia was traced and evaluated in rabbits in vivo. The labeled NLC were characterized to be solid spherical in shape with an average diameter of 70 nm and zeta potential of −8 mV by transmission electron microscopy and dynamic light scattering, respectively. CLSM results demonstrated NLC were not directly internalized by corneal epithelia, whereas the markers themselves transferred from NLC to corneal epithelia with subsequent staining of intracellular lipophilic compartments. Furthermore, the in vitro release study using liposome dispersions as mimic biomembranes demonstrated an efficient transfer of fluorescence marker into the liposomes. This implied the deceptive particle uptake was due to a collision-induced process, during which the rapid transfer of fluorescence marker occurred by forming a complex between the nanoparticles and the biomembranes. Thus, these evidences provide further insights into NLC as an ocular delivery system (Tian B-C *et al.*, 2013).

2.3. Review on Asenapine Maleate

2.3.1. Physico-chemical properties

Asenapine maleate is chemically (3aR*,12bR*)-5-Chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz [2,3:6,7] oxepino[4,5-c] pyrrole maleate. The molecular formula of asenapine maleate is $C_{17}H_{16}ClNO.C_4H_4O_4$ having molecular weight 401.84 (285.8 as the free base) (Figure 2.4). It is a white to off-white non hygroscopic crystalline powder in bitter test (Figure 2.5). It was developed by Organon laboratories (Org 5222) having CAS registry number 85650-56-2. Asenapine is slightly soluble in water and sparingly soluble in 0.1 M HCl. It is stable in crystalline form, although excessive light can induce degradation (Minassian A and Young JW, 2010). The pKa of the protonated free base is 8.51. Log P for neutral species and cationic species is 4.9, 1.4, respectively (Bartlett JA and van der Voort Maarschalk K, 2012; Carlotta M *et al.*, 2015).

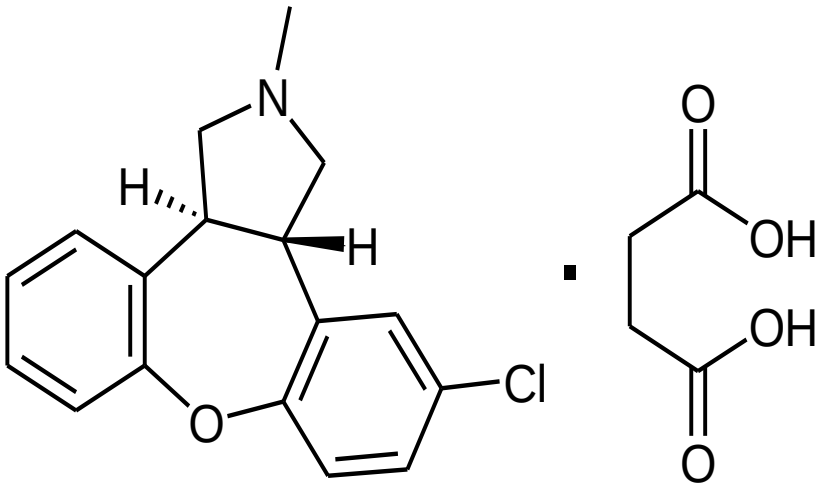

Figure 2.4: Chemical structure of asenapine maleate

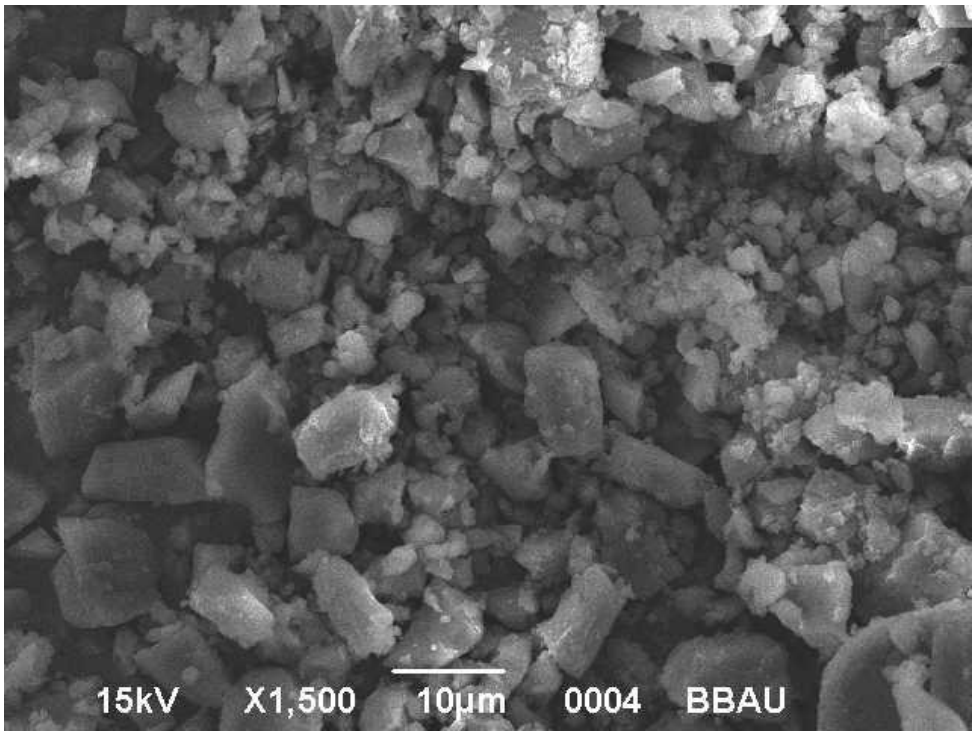

Figure 2.5: Scanning electron micrograph of asenapine maleate (API)

2.3.2. Bio-pharmaceutical properties

2.3.2.1. Absorption

Asenapine is absorbed rapidly from oral or gastric mucosa. After sublingual administration, C_{max} was obtained within 0.5 to 1.5 hours. Moreover, increasing the dose two fold from 5 to 10 mg twice daily results in 1.7 times increase in both the Cmax and AUC indicates that absorption of asenapine is non linear with dose. The sublingual and oral absolute bioavailability of Asenapine at 5 mg is 35% and < 2%, respectively (Citrome L, 2014). The low oral bioavailability was due to high hepatogastrointestinal first-pass metabolism in human. However, in preclinical

studies, oral bioavailability was found to be in between 20-65% in rats and up to 10% in dogs. The presence of water and food markedly affect the sublingual and oral bioavailability of asenapine (Djupesland PG *et al.*, 2014) .

2.3.2.2. Distribution

Asenapine is rapidly distributed with large volume of distribution (~20-25 l/kg), indicating extensive extra vascular distribution. It is highly bound to plasma proteins (95%), especially with albumin and α1-acid glycoprotein. Asenapine and its metabolite N-desmethyl asenapine are weak substrates of the human P-gp, resulting very less impact on in-vivo disposition of asenapine and N-desmethyl asenapine. (Citrome L, 2014; Djupesland PG *et al.*, 2014) .

2.3.2.3. Metabolism and excretion

Asenapine is primarily metabolized by direct glucuronidation (UGT1A4) and oxidative metabolism (predominantly CYP1A2) into two inactive compounds: N-glucuronide-asenapine and N-desmethyl-asenapine (George M *et al.*, 2013) . In asenapine treatment, caution should be taken when using CYP1A2 inducers (carbamazepine or rifampin) or CYP1A2 inhibitors (fluvoxamine, ciprofloxacin, ketoconazole). In radiolabel isotope study of asenapine, approximately 50% and 40% drug was recovered in urine and feces respectively. Asenapine is an active moiety, however other metabolites have negligible effects due to their lower affinity towards dopamininergic and serotonergic receptors (Djupesland PG *et al.*, 2014; Nutt DJ and Attridge J, 2014).

2.3.2.4. Population Pharmacokinetic

In population pharmacokinetic studies, the higher C_{max} and AUC were observed in elderly as compared to adult patient might be credited to slow drug clearance. In older population the asenapine C_{max} values were 12% higher with 5 mg BID and 21% higher with 10 mg BID. AUC (0-12 h) values were 21% higher with 5 mg BID and 30% higher with 10 mg BID. However, no dose adjustment is required in elderly patient as per USFDA drug label information (Nutt DJ and Attridge J, 2014). Moreover, safety and efficacy of asenapine (Dose: 2.5, 5 & 10 mg bid) have been established in child patient (10 to 17 years) for bipolar mania in recent clinical trials. (NCT01244815; NCT01349907). However, no safety and efficacy studies have been reported for pediatric patients below 10 years of age.

Potential gender differences in asenapine pharmacokinetics have not been studied in a dedicated trial; however in a population analysis, no significant differences between men and women on asenapine pharmacokinetics were observed. There was 14% decrease in clearance in black subjects compared to other ethnic origins but these differences are smaller compared to overall variability in pharmacokinetics observed for asenapine. In one of the study, pharmacokinetics of asenapine was similar in Caucasian and Japanese subjects (Citrome L, 2014; Nutt DJ and Attridge J, 2014).

2.3.3. Pharmacodynamic

The precise mechanism of action of asenapine is not clearly understood in the treatment of schizophrenia. Asenapine has similar receptor binding profile as of

other atypical antipsychotic drugs; exhibiting potent antagonism at serotonin, dopamine, noradrenaline and histamine receptors. It has relatively higher potency at serotonin receptors compared to dopamine receptors. The efficacy of asenapine is mediated through a combined effect of antagonist activity at dopamine D_2 and serotonin 5-HT_{2A} receptors. This unique receptor signature and functional activity of asenapine supports its distinct psychopharmacological profile for the treatment of schizophrenia and bipolar mania (Nutt DJ and Attridge J, 2014; Tarazi FI and Neill JC, 2013).

Further, study of the receptor activity of asenapine suggested that it has an upregulating effect on D_1-like receptors, most likely secondary to direct blockade of these receptors. This finding is important as up-regulation of D_1 and D_2 receptors is associated with a decreased extrapyramidal symptom related adverse events. This finding is different from those of other antipsychotics, such as fluphenazine, olanzapine, and risperidone, which do not significantly cause a variation in D_1-receptor levels. The dopaminergic and glutamatergic activity of asenapine in medial prefrontal cortex of rat revealed not only advantages in maintenance of positive symptoms but also in negative and cognitive symptoms in patients with schizophrenia (Franberg O *et al.*, 2008; Marston HM *et al.*, 2009). These pharmacological profiles suggested asenapine can be used for effective treatment in schizophrenia and bipolar disorder with minimum metabolic disorder and EPS.

2.3.4. Safety and tolerability

The several efficacy studies demonstrated asenapine is well tolerated in schizophrenia and bipolar I disorder. Most common adverse effects associated with asenapine are somnolence, dizziness, and weight gain. Occurrence of EPS with asenapine was similar to risperidone while it was lower than haloperidol (Auclair AL *et al.*, 2006). The lesser affinity for D_2 receptors compared to atypical antipsychotic offers a benefit by decreasing the risk of EPS and hyper prolactinemia. The risk of EPS was higher in asenapine in comparison to olanzapine, with a 35.4% increased incidence with asenapine versus 19% with olanzapine (Ahlenius S and Hillegaart V, 1986). Although there is a risk of weight gain with asenapine but it is lesser than that associated with olanzapine. This has been attributed to the lower binding affinity of asenapine for the histamine receptor (H_1) as compared to olanzapine and quetiapine which have strong H_1 binding affinity and thereby carry a greater risk of weight gain (Djupesland PG *et al.*, 2014). Asenapine is classified as a pregnancy category 'C' drug demonstrated maternal and embryo-fetal toxic effects in animal studies at dose dependent manner. However, safety and tolerability of asenapine was not studies in pregnant women yet but neonates exposed to antipsychotic drugs during the third trimester of pregnancy are at risk for extrapyramidal and/or withdrawal symptoms. In preclinical reproduction studies, it was not teratogenic at intravenous doses up to 1.5 mg/kg in rats and 0.44 mg/kg in rabbits administered during organogenesis. These doses are 0.7 and 0.4 times,

respectively, of the maximum recommended human dose (MRHD) of 10 mg twice daily given sublingually on a mg/m^2 basis (Nutt DJ and Attridge J, 2014).

2.3.5. Clinical efficacy in schizophrenia and bipolar disorder

Asenapine provides an alternative treatment option in the management of schizophrenia and bipolar I disorder under second generation antipsychotics (SGAs). Asenapine is well tolerated in adults, shows low anti-muscarinic adverse effects owing to less affinity towards these receptors. Its 'atypical' pharmacological profile (high $5HT_{2A}$:D_2 affinity ratio) also suggests a low risk of EPS-related adverse effects. Asenapine effects on prolactin and metabolic parameters are modest and it has a more favorable weight gain profile than olanzapine. It may be a suitable treatment option in patients who are at increased risk of metabolic disturbances or diabetes or obese. Moreover, it may also be a safer alternative in patients with impaired renal function because no dosage adjustments are required in these individuals (as in the case with risperidone and paliperidone). Asenapine's sublingual formulation may be an advantage in people with swallowing difficulties (Bishara D *et al.*, 2011; Citrome L, 2009; McIntyre RS and Wong R, 2012; Minassian A and Young JW, 2010; Stoner SC and Pace HA, 2012). The summary of recent clinical trial based on safety/efficacy studies of asenapine were discussed in Table 2.2-2.4. These clinical trials would be helpful in safety/efficacy studies in different age group and post-marketing surveillance studies for approved indications.

Table 2.2: Completed asenapine clinical trials (with results) registered at http://www.clinicaltrials.gov, first received after August 14, 2009 (data assess on July 10, 2015).

Clinical trial No.	Title	Phase, Study type	Duration	Asenapine/Control	Comments
NCT01190254	An 8-week, placebo-controlled, double-blind, randomized, fixed-dose efficacy and safety trial of asenapine in adolescent subjects with schizophrenia	Phase-3, Interventional (Parallel assignment)	8 weeks	Asenapine (2.5, 5 mg, bid)/ Placebo	The least square mean difference between the asenapine and placebo on the PANSS total score at the day 56 were not significant (-4.8 for 2.5 mg bid, p=0.070; -5.6 for 5.0 mg bid, p=0.064). However, the significant improvement in the CGI-score was observed in the 5.0 mg bid group versus placebo.
NCT01190267 (Extension study of NCT01190254)	A 26-week, multi-center, open-label, flexible dose, long-term safety trial of asenapine in adolescent subjects with schizophrenia	Phase- 3, Interventional (Single group assignment)	26 week	Asenapine (2.5, 5.0 mg, bid)	The primary outcome measured number of participants with a treatment-emergent adverse event (AE) during extension study [time frame: up to 30 weeks] and number of participants who discontinued study drug during extension study due to an AE [time frame: up to 26 weeks]. No statistical data was available.
NCT01549041	A randomized comparison of twice-daily versus once-daily asenapine for schizophrenia	Phase- 4, Interventional (Parallel assignment)	2 weeks	Asenapine (10 mg at evening)/ Asenapine (5.0 mg, bid)	The final result was not available. Here, the investigators hypothesize that patient and staff acceptance will be better in once daily dosing and that improvements in psychopathology will be similar across once daily and twice daily dosing. Primary outcome measure was patient acceptance at day 14. The secondary end point was the change in BPRS total Score from baseline to day 14.
NCT01244815	Efficacy and safety of 3-week fixed-dose asenapine treatment in pediatric acute manic or mixed episodes associated with bipolar I disorder (Protocol no. P06107)	Phase- 3, Interventional (Parallel assignment)	3 weeks	Asenapine (2.5, 5.0, 10.0 mg, bid)/ Placebo	In primary outcome, the least square mean difference between the asenapine and placebo on Young Mania Rating Scale (Y-MRS) total score at Day 21 from baseline were significant (-3.2 for 2.5 mg bid, p=0.008; -5.3 for 5.0 mg bid, p<0.001; -6.2 for 10 mg bid, p<0.001). However, the significant difference in the secondary

					end point CGI-score was observed in all asenapine groups compared to placebo.
NCT01349907 (This was an extension study of NCT01244815)	A 50-week open-label, flexible-dose trial of asenapine extension treatment to P06107 in pediatric subjects with acute manic or mixed episodes associated with bipolar I disorder.	Phase- 3, Interventional (Single group assignment)	50 week	Asenapine/Asenapine (2.5, 5.0, 10.0 mg, bid). Placebo/ Asenapine (2.5, 5.0, 10.0 mg, bid).	The primary outcomes, which measure the number of participants who experienced clinical or laboratory adverse events [Time frame: Baseline (Day 1) to 30 days after the last dose of study drug (up to approximately 54 weeks)] was found to be 197, 74 in reporting group Asenapine/Asenapine (No of participants analyzed: 241) and Placebo/Asenapine (No of participants analyzed: 80) respectively.
NCT01460290	Asenapine in the treatment of older adults with bipolar disorder	Phase- 4, Interventional (Single group assignment)	12 weeks	Asenapine (at 5 mg and increased as tolerated to a maximum of 20 mg/day). No control group	The primary outcome measures were found to be change in depressive symptoms as measured by the Hamilton Depression Rating Scale (HAM-D), 17.5 (4.8) and 10.3 (6.3) and change in manic symptoms as measured by the YMRS, 27.9 (4.4) and 18.1 (14.2) for baseline and 12 weeks respectively. The results were expressed in mean (SD), no statistical data was available.
NCT01098110	A multicenter, randomized, double-blind, fixed-dose, 6-week trial of the efficacy and safety of asenapine compared with placebo in subjects with an acute exacerbation of schizophrenia (phase 3)	Phase- 3, Interventional (Parallel assignment)	6 weeks	Asenapine (5.0, 10.0 mg, bid)/ Placebo	In primary outcomes, least square mean difference between asenapine and placebo on PANSS total score at day 42 were found to be significant (-11.29 for 10 mg bid, p<0.0001; -13.22 for 5 mg bid, p<0.0001). However, significant improvement in mean difference in the percentage of participants who were clinical global impressions- Improvement (CGI-I) responders were observed in 5 mg & 10 mg bid group versus placebo (22.2, 28.8 for 5, 10 mg respectively, p<0.0001).

PANSS: Positive and Negative Syndrome Scale; CGI: Clinical Global Impressions; BPRS: Brief Psychiatric Rating Scale; Y-MRS: Young Mania Rating Scale; CGI-I: Clinical Global Impressions- Improvement

Table 2.3: Completed asenapine clinical trials (without results) registered at http://www.clinicaltrials.gov, first received after August 14, 2009 (data assess on July 10, 2015).

Clinical trial No.	Title	Phase, Study type	Duration	Asenapine/Control	Comments
NCT01395992	A multicenter, double-blind, fixed-dose, long-term extension trial of the safety of asenapine in subjects diagnosed with bipolar I disorder who completed protocol P05691 (formerly 041044) (Phase 3b, protocol P05692 [formerly 041045])	Phase- 3, Interventional (Parallel assignment)	26 weeks	Asenapine (5.0, 10.0 mg, bid)	The primary purpose of this trial was to evaluate the long-term safety of asenapine. Participants who have completed the 3-week trial P05691 (NCT00764478) can be screened for eligibility for this 26-week extension study in which they will continue treatment. Primary outcome will measure number of participants experiencing clinical and laboratory adverse events (AEs) [Time frame: Baseline up to 212 days].
NCT01142596	Long-term extension trial of asenapine in subjects with schizophrenia (phases 3; protocol no. P06125)	Phase- 3, Interventional (Parallel assignment)	52 weeks	Asenapine (5.0, 10.0 mg, bid)	This is a safety study of asenapine. The assigned primary outcome measure was to change in weight gain/loss, body mass index, extrapyramidal symptoms, fasting glucose, insulin, prolactin level, serious and non-serious adverse events from baseline [Time frame: 52 weeks].
NCT01617200	A multicenter, double-blind, fixed-dose, long-term extension trial of the safety of asenapine using olanzapine as an active control in subjects diagnosed with schizophrenia who completed protocol P05688	Phase- 3, Interventional (Parallel assignment)	26 weeks	Asenapine (2.5, 5.0 mg, bid)/ Olanzapine	The purpose of this trial was to evaluate the long-term safety of 2.5 and 5 mg asenapine administered sublingually twice daily in schizophrenia participants. Olanzapine administered 15 mg orally once daily is used as an active control. The assigned primary outcome measure was to change in weight from baseline to day 182 [Time frame: baseline (day-1of short term trial) day

					182 (long-term extension)].
NCT01400113	Treating acutely agitated patients with asenapine sublingual tablets: a single-dose, randomized, double-blind placebo controlled trial	Phase- 4, Interventional (Single group assignment)	2 hours	Asenapine (10 mg)/ Placebo	Mean (SE) baseline PANSS-EC scores for the asenapine-treated and placebo-treated subjects were 19.40.66 and 20.10.61, respectively. Mean PANSS-EC scores at endpoint (LOCF) was 7.40.65 for the asenapine-treated subjects and 14.70.98 for the placebo-treated subjects. Change in PANSS-EC score at 2 h was statistically significantly greater for the asenapine-treated subjects compared with the placebo-treated subjects. NNT for response vs. placebo was 3 (95% CI).
NCT01617187	A multicenter, randomized, double-blind, fixed-dose, 6-week trial of the efficacy and safety of asenapine compared with placebo using olanzapine as an active control in subjects with an acute exacerbation of schizophrenia (P05688)	Phase- 3, Interventional (Parallel assignment)	6 weeks	Asenapine (2.5, 5.0 mg bid)/ Active comparator: Olanzapine (15 mg qd)/ Placebo	The purpose of this trial was to assess the effect of asenapine 2.5 and 5 mg sublingually twice daily (BID) compared with placebo in the treatment of schizophrenia (overall symptoms) as measured by PANSS total score [Time frame: baseline, day 42]. Olanzapine administered 15 mg orally once daily (QD) was used as an active control.
NCT01244828	Long-term study of asenapine in subjects with residual subtype, receiving multiple or/and high dose drugs, or treatment refractory schizophrenia (Protocol P06238)	Phase- 3, Interventional (Single group assignment)	52 weeks	Asenapine (5.0, 10.0 mg, bid)	This was a safety long term study of asenapine. The primary outcome measure was to determine baseline changes in body weight, BMI, EPS, HbA1c, fasting glucose, insulin and prolactin to time frame 52 weeks.

PANSS-EC: Positive and Negative Syndrome Scale- Excited Component; NNT: Number Needed to Treat

Table 2.4: Asenapine clinical trials (open studies) registered at http://www.clinicaltrials.gov, first received after August 14, 2009 (data assess on July 10, 2015).

Clinical trial No.	Title	Phase, Study type	Duration	Asenapine/Control	Comments
NCT01587118	An open label pilot study of adjunctive asenapine for the treatment of posttraumatic stress disorder	Phase- 4, Interventional (Single group assignment)	12 weeks	Antidepressant plus asenapine	This is a safety/efficacy study in PTSD patient, who have not fully remitted to an adequate trial of standard antidepressant treatment. Primary outcome will be measure the clinical administered PTSD scale (CAPS) [Time frame: baseline, week 4, 8, and 12].
NCT01968161	An open-label switch study to asenapine in the early stage of psychosis	Phase- 4, Interventional (Single group assignment)	3 months	Asenapine (5 mg, bid)	This study is safety/efficacy study, providing data on a particularly important population, i.e., subjects who are at the inception of treatment for a psychotic disorder and who are likely to remain on a given drug on a long-term basis. Primary outcome will measure the impact of switching to asenapine.
NCT01807741	Asenapine for bipolar depression	Phase- 2, Interventional (Parallel assignment)	8 weeks	Asenapine (5-10 mg, bid)/ Placebo	This is an efficacy study, compare asenapine with placebo in the treatment of depression associated with bipolar I disorder. Primary outcome will be measure the change in MADRS total scores from baseline to endpoint over 8 weeks.

2.3.6. Regulatory status

USA

Asenapine is available in the name of "Saphris" in USA. It was approved by US-FDA on August 13, 2009 for the acute treatment of schizophrenia as well as for the acute treatment of manic or mixed episodes associated with bipolar 1 disorder with or without psychotic features. The orange book of US FDA indicated that three patents (US 5763476, US 7741358B2 and US 8022228B2) listed against asenapine with new patient population and pediatric exclusivity upto March 17, 2018 and September 17, 2018 respectively. Moreover, the exclusivity of new chemical entity was expired on August 13, 2014.

European Union

It was approved by European Medicines Agency on September 01, 2010 and prescribed in the brand name "Sycrest". In contrary to USFDA indication, it was only approved for treatment of moderate to severe manic episodes associated with bipolar I disorder in adults.

India

It was approved in India in April 7, 2011 by Central Drugs Standard Control Organization (CDSCO), India. The approved indication was acute treatment of schizophrenia in adults only. There is no information available for marketing of asenapine by Innovator Company. Moreover generic version of formulation by Sun Pharmaceutical Limited was available in the name of "Asenapt" in 5.0 mg dose strength.

Australia

It was approved in March 07, 2011 by Therapeutic Goods Administration (TGA-Australia). As per Australian Public assessment record (AusPAR), the therapeutic indication was same as approved in USA.

2.3.7. Future market aspects

Asenapine is one of the eleven SGAs approved in USA. It was authorized for sale just after the approval of iloperidone (Approved on May 2009) and before the approval of lurasidone (Approved on Oct 28, 2010) and brexpiprazole (Approved on Jul 10, 2015) (Brexpiprazole)). In coming year, results of ongoing and completed clinical trial will provide further in-depth safety and efficacy data of asenapine in large patient population. However, the presence of low cost generic, availability of different dosage from (orodispersible, immediate release tablet and long acting depot) and other relatively metabolic safe (aripiprazole, iloperidone, and lurasidone) SGAs will provide a strong competition to asenapine in widespread acceptability by clinicians and patients. Further, next five year in USA, there is very less possibility for availability of inexpensive generic version owing to Orange book (USFDA) listed patent (US5763476, US7741358 and US8022228) against this drug, which are going to expired on December 9, 2020, October 6, 2026 and October 6, 2026, respectively with it's pediatric exclusivity.