Chapter 3

Materials and Methods

3.1 Materials

Hypertension has a vital role in producing cardiovascular diseases (CVDs) such myocardial infarction and stroke worldwide. Hypertension is the major risk factor for death and disability in India, according to research published in The Lancet on regional health (South-East Asia), 2022. The number of people living with hypertension (blood pressure of ≥140 mmHg systolic or ≥90 mmHg diastolic or on medication) doubled between 1990 and 2019, from 650 million to 1.3 billion. Antihypertensive are a group of medicines that are employed to treat hypertension (high blood pressure). Antihypertensive treatment is designed to prevent the consequences of high blood pressure, such as strokes and myocardial infarction [82].

Among the most important and most widely used drugs are thiazide diuretics, calcium channel blockers, Angiotensin-converting enzyme inhibitors (ACE), angiotensin II receptor antagonists (ARBs), and beta blockers.

Benidipine, a powerful and long-acting calcium channel blocker, functions by blocking three subtypes of calcium channels (L, N, and T) and showed a kidney protective effect. It also demonstrated a cardio-preventive effect due to enhanced nitric oxide generation with greater vascular selectivity [86]. Telmisartan, an azole class angiotensin II receptor antagonist, functions by decreasing the release of aldosterone by reversibly binding angiotensin II to the AT1 receptor found on vascular smooth muscle and adrenal glands. Thereby arterial blood pressure is lowered by decreasing the overall vascular resistance. Telmisartan also showed PPAR-γ agonistic function, which has positive effects on carbohydrate metabolism and antidiabetic property [87].

Different studies indicated that combination therapy with medicines having a different mechanism of action assists in the effective and speedy management of blood pressure. Combining different types of antihypertensive medicines together is one significant approach for obtaining blood pressure control in most hypertensive individuals. Benidipine and Telmisartan are the suggested combinations of calcium channel

blockers (CCBs) with angiotensin receptor blockers (ARBs) for the therapy of hypertension because of their anti-proteinuria properties [84].

3.1.1. Drug profile

a) Drug profile of Benidipine EP/JP [145,146]

Molecular formula: $C_{28}H_{31}N_3O_6$

Molecular weight: 505.571gm/mol

Chemical name Chemically Benidipine is 1,4-dihydro-2,6-dimethyl-4-(3-trophenyl)-3,5-pyridine-dicarboxylic acid methyl 1-(phenylmethyl)-3-piperidinyl ester hydrochloride.

Chemical structure

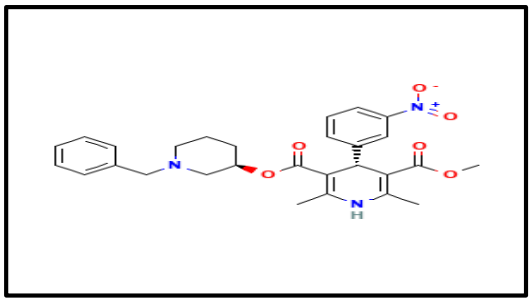

Figure 3.1: Chemical structure of Benidipine

Description

It has the appearance of a white to off white crystalline powder. This possesses a melting range of >193ºC.

Solubility

It is nearly undissolved in water 0.00243 mg/mL. Benidipine is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF). The solubility of benidipine in ethanol is approximately 10 mg/ml and approximately 30 mg/ml in DMSO and DMF.

Category

Calcium channel blocker

Indication

This is intended for hypertension.

Dose

For the treatment of hypertension and renal parenchymal hypertension, for adults, take 2 to 4mg of the active ingredient at a time, once daily, after breakfast. If the effect is insufficient, the dosage may be increased up to 1 tablet (8 mg) once daily, however, for patients with severe hypertension, take 4 to 8 mg at a time, once daily, after breakfast.

Pharmacodynamics

Benidipine lowers systolic and diastolic blood pressure as well as to provide drops in heart rate pulse following medication. It is observed also a reduction urine protein excretion and serum triglycerides. Multiple research studies have shown benidipine anti-oxidative activity, stimulation of NO production, suppression of adhesion molecules expression, activation of osteoblast differentiation, suppression of the proliferation of vascular smooth muscle cells and mesangial cells, and additionally myocardial protection. The increase of NO generation is connected with the cardio protective and anti-arteriosclerotic properties of benidipine.

Mechanism of action

Benidipine is a tripe calcium channel inhibitor by blocking L, N and T type calcium channel. It shows a highly long-lasting action that could be attributed to its extreme preference for cell membranes from the DHP binding site; this property suggested a long-lasting pharmacological activity of benidipine. The additional link of benidipine is the vascular choice towards peripheral blood vessels.

Absorption

Benidipine is easily absorbed upon oral administration reaching a maximal concentration within 2 hours.

Volume of distribution

Benidipine is extensively distributed to the tissues particularly in the liver and kidneys and plasma. It does not display a large accumulation with repeated oral treatments.

Protein binding

Benidipine is extensively coupled to plasma proteins and the bound form may contribute for even 98% of the injected dosage.

Metabolism

Benidipine is nearly fully metabolized in the liver. From numerous findings, it is considered that benidipine is largely metabolized by CYP3A and CYP3A5.

Route of elimination

The proportion of urine excretion following oral administration is of around 36% of the given dosage. Most of the remaining dosage is eliminated in feces, making bile excretion the principal elimination mechanism of benidipine.

Half-life

The elimination half-life of benidipine has been reported to be of around one hour.

Common side effects of this drug are:

- Vertigo
- Headache
- Dizziness
- Vomiting
- Orthostatic hypotension (a sudden decrease in systolic or diastolic blood pressure on standing after a prolonged sitting position)
- Palpitations
- Renal impairment
- Anemia
- Increased potassium in blood

Drug-Drug Interactions:

Benidipine may interact with high blood pressure lowering pills (benazepril, metoprolol, ramipril, hydrochlorothiazide etc.), anti-TB (rifampin), antifungal (itraconazole), cardiac-glycoside (digoxin) and H2 blockers (cimetidine).

Drug-Food Interactions:

Avoid foods with high fat or cholesterol. Avoid too much salt, like pickles, extra salt on salad, and grapefruit juice.

Drug-Disease Interactions:

Benidipine should not be given to people with low blood pressure (hypotension), liver disease, or kidney disease.

Marketed formulation

Drug Name	Brand Name	Dosage Form	Company Name	Dose of Drug
Benidipine	Biniplus 4	Tablet	Niksan Pharma	4 mg
	Z-Bene	Tablet	Corazone Pvt. Ltd.	4 mg
	Benidam 4	Tablet	Vridam Healthcare Pvt. Ltd.	4 mg
	Benepeck	Tablet	Koye	8 mg
	Bengreat-4	Tablet	Mankind	4 mg
	Benidep 8	Tablet	Ambica Pharma	8 mg

Table 3.1: Marketed formulations of Benidipine

b) Drug profile of Telmisartan BP/USP [147-149]

Molecular formula: $C_{33}H_{30}N_4O_2$

Molecular weight: 514.62 gm/mol

Chemical name

Chemically Telmisartan is 4'-[(1,4'-Dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'yl)methyl][1,1'-biphenyl]-2-carboxylic acid; 4'-[[4-methyl -6-(1-methyl-2-benzimidazoyl)-2-propyl-1-benzimidazoyl] methyl]-2- biphenyl carboxylic acid.

Chemical structure

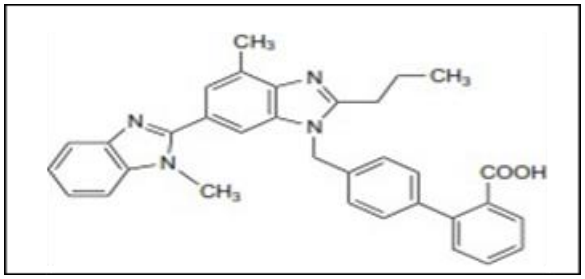

Figure 3.2: Chemical structure of Telmisartan

Description

This is a white or faintly yellowish, crystalline powder. It displays polymorphism. This has a melting temperature range of 261-263°C.

Solubility

It is basically insoluble in water (pH 3-9), sparingly soluble in strong acid (excluding insoluble hydrochloric acid), soluble in strong base. It displays modest solubility in methyl alcohol and it is sparingly soluble in dichloromethane.

Category

Angiotensin- II receptor antagonists

Indication

It is intended for hypertension.

Dose

40 mg once day (although 20 mg may be adequate), increasing if required after at least 4 weeks, to maximum 80 mg once daily.

Pharmacokinetics

Absorption: Telmisartan is readily absorbed from the gastrointestinal tract. **Bioavailability:** The absolute oral bioavailability generally dose-dependent and is around 42% after a 40-mg dosage and 58% after a 160-mg dose.

Tmax: Peak plasma concentration of telmisartan is attained around 0.5 to 1 hour after an oral dosage.

Plasma protein binding: Telmisartan is almost 99% bound to plasma proteins.

Excretion: It is eliminated nearly completely in the feces via bile, usually as unchanged drug.

Terminal elimination half-life: roughly 24 hours for telmisartan.

Adverse effects and precautions

Gastro-intestinal disturbances; abdomen pain; symptoms resembling influenza including pharyngitis and sinusitis; urinary tract infection; arthralgia, myalgia, back pain, leg cramps; eczema; less commonly dry mouth, flatulence, anxiety, vertigo, tendinitis-like symptoms, abnormal vision, elevated sweating; rarely bradycardia, tachycardia, dyspnoea, insomnia, depression, blood disorders, raised uric acid, eosinophilia, rash and pruritus; syncope and asthenia also reported.

Adverse effects of telmisartan have been found to be typically minor and temporary, and include dizziness, headache, and dose-related orthostatic hypotension. Hypotension may arise primarily in people with volume loss (for example those who have undergone high dosage diuretics).

Impaired renal function and infrequently, rash, urticaria, pruritus, angioedema and increased liver enzyme levels may occur. Hyperkalaemia, myalgia, and arthralgia have been documented. Telmisartan seems less likely than ACE inhibitors to produce a cough. Other side effects that have been documented with angiotensin-II receptor antagonists include respiratory-tract diseases, back discomfort, gastrointestinal abnormalities, tiredness, and neutropenia. Rhabdomyolysis has been recorded seldom. Telmisartan should be taken with care in individuals with hepatic impairment or biliary blockage.

Uses and administration

Telmisartan is an angiotensin-II receptor antagonist with anti-hypertensive effects largely owing to selective blockage of AT1 receptors and the resultant lowered pressure

impact of angiotensin-II. It is used in the control of hypertension, especially in individuals who develop a cough with ACE inhibitors and to minimize the risk of stroke in patients with left ventricular hypertrophy and in the treatment of diabetic nephropathy. It has also been tested in heart failure and in myocardial infarction. The maximal hypotensive impact is attained in around 3 to 6 weeks after commencing therapy.

Telmisartan is administered orally. After a dosage, the hypotensive effect peaks after 3 hours and sustains for at least 24 hours. The highest hypotensive impact occurs between around 4 to 8 weeks after commencing medication. In hypertension, telmisartan is administered at an initial dosage of 40 mg once day. This may be increased, if required, to a maximum dosage of 80 mg once day. Lower dosages should be explored in people with hepatic or renal impairment.

Marketed formulation

Drug Name	Brand Name	Dosage Form	Company Name	Dose of Drug
Telmisartan	Inditel	Tablet	Zydus Cadila	40 mg / 80 mg
	Anzitel	Tablet	Essweil	40 mg
	Arbitel	Tablet	Micro Cardicare	20 mg / 40 mg
	Angitel	Tablet	Molekule	40 mg / 80 mg
	Adcom	Tablet	Intel Pharma	40 mg
	Astel	Tablet	AS Pharma	20 mg / 40 mg

Table 3.2: Marketed formulations of Telmisartan

Procurement of Drugs

Benidipine received as a gift sample from Nikshan Pharmaceutical Ind. Ltd. Ankleshwar and Telmisartan was acquired as gift Sample from Alembic Research Centre, Vadodara, India.

3.1.2. Excipients profile

A list of excipients used for preformulation study, formulation of liquid SNEDDS and S- SNEDDS of Benidipine and Benidipine with Telmisartan.

Sr. No.	Name of excipients	Use	Source
1.	Oleic Acid	Oil	Loba Chemie Pvt Ltd, Mumbai, India
2.	Sunflower oil		Loba Chemie Pvt Ltd, Mumbai, India
3.	Olive oil		Loba Chemie Pvt Ltd, Mumbai, India
4.	Castor oil		Loba Chemie Pvt Ltd, Mumbai, India
5.	Sesame oil		Loba Chemie Pvt Ltd, Mumbai, India
6.	Peanut oil		Loba Chemie Pvt Ltd, Mumbai, India
7.	Eucalyptus oil		Loba Chemie Pvt Ltd, Mumbai, India
8.	Cottonseed oil		Loba Chemie Pvt Ltd, Mumbai, India
9.	Soyabean oil		Loba Chemie Pvt Ltd, Mumbai, India
10.	Labrafil M 1944		Gift Sample from Gattefosse, France
11.	Cremophore RH 40	Surfactant	Gift Sample from BASF, Germany
12.	Kolliphor EL		Gift Sample from BASF, Germany
13.	Tween 20		Merck chemical Pvt. Ltd. Germany
14.	Tween 80		Merck chemical Pvt. Ltd. Germany
15.	Span 20		Merck chemical Pvt. Ltd. Germany
16.	Span 80		Merck chemical Pvt. Ltd. Germany
17.	Solutol HS 15		Gift Sample from BASF, Germany
18.	Transcutol P	Cosurfactant	Gift Sample from Gattefosse, France
19.	Propylene glycol		BASF, Germany
20.	PEG 200		Merck chemical Pvt. Ltd. Germany
21.	PEG 400		Merck chemical Pvt. Ltd. Germany
22.	Aerosil® 200	Adsorbent	Gift Sample from Vardhman Healthcare Ahmedabad
23.	Aeroperl® 300		Gift Sample from Evonik Industries, India
24.	Neusilin® US2		Gift Sample from Fuji Chemical Industries USA

Table 3.3: List of chemicals used for experimental work

Oleic Acid [150]

Oleic Acid is a naturally occurring fatty acid with antibacterial properties added to a variety of drug products. Oleic acid, like other fatty acids, does not occur in the free state but is normally found as an ester of glycerol—i.e., as a glyceride or as an ester of a long-chain alcohol.

Specification

Specification Limits	
Appearance	Oleic acid is a white to light yellow liquid
Empirical formula	$C_{18}H_{34}O_2$
Molecular weight	282.45
Density	0.895 g/mL at 25 °C (Lit.)
Solubility	Practically insoluble in water, Soluble in methanol, chloroform, ether or fixed and volatile oils, fixed & volatile oils, alcohol, benzene
Acid value	Less than Equal to 1
Water	Less than Equal to 2.0%
pH (10% in water)	6–7
HLB Value	Between14 to 17
Viscosity	25.6 cP at 30 °C
Melting point	13.4 °C
Refractive Index n20/D	1.459(Lit.)
Iodine value	89.9
Acid value	198.6

Storage

Oleic acid should be kept in a dry, cold and well-ventilated environment firmly closed containers concealed from light. Keep container closed when not in use.

Toxicity

Generally considered mostly nonirritant and harmless

Sunflower oil [151]

It is a viscous liquid obtained from the fruits and seeds (achenes) of the sunflower, Helianthus annus (Compositae), via mechanical means or by extraction. It is called as Huile de tournesol; oleum helianthi; sunflower seed oil. It is categorized as an oleic–linoleic acid oil. It is comprised linoleic acid (66%), oleic acid (21.3%), palmitic acid (6.4%), arachidic acid (4.0%), stearic acid (1.3%), and behenic acid (0.8%).

On Functional Category: Diluent; emollient; emulsifying agent; solvent; tablet binder Sunflower oil is commonly used as an edible oil, particularly in oleomargarine. It is additionally frequently employed in cosmetics and pharmaceutical formulations as a diluent, emollient, emulsifier, solvent, and tablet binder. Therapeutically, it is also employed to give energy and essential fatty acids for parenteral nutrition.

Specification

Specification Limits	
Appearance	Sunflower oil is a Pale Yellow-Colored Liquid
Empirical formula	$CH_3(CH_2)_{14}COOH$
Molecular weight	876.16
Density	0.915–0.919, g/mL at 25 °C(Lit.)
Solubility	Soluble with Benzene, Chloroform, Carbon Tetrachloride, Diethyl Ether, Light Petroleum; Extremely insoluble in Ethanol (95%)
HLB Value	7
Boiling point	40–60°C
Melting point	18°C
Refractive Index n20/D	1.472–1.474
Saponification number	188–192
Hydroxyl value	14–16
Iodine value	125–140
Acid value	not more than 0.9-1.1

Storage

Sunflower oil should be kept in an airtight, well-filled container, shielded from light. Stability may be increased by the incorporation of an antioxidant such as butylated hydroxytoluene.

Incompatibilities

Its stability gets disturbed in its incorporation of iron oxides and zinc oxide and it creates a 'skin' after having been exposed to air for 2–3 weeks.

Safety

It is typically recognized as a reasonably harmless and nonirritant substance.

Olive oil [152]

Olive oil is the fixed oil obtained by cold expression or other correct mechanical processes from the mature drupes of Olea europaea. Olive oil has been employed in enemas, liniments, ointments, plasters, and soap. It has also been exploited as oral capsules and solutions, and as a carrier for oily injections with specialized delivery methods. Olive oil has been used in combination with soybean oil to make a lipid emulsion for use in pre-term newborns.

Specification

Specification Limits	
Appearance	Clear, colorless or yellow, translucent greasy liquid.
Empirical formula	$CH_3(CH_2)nCOOH$ where n is a number from 12 to 22
Molecular weight	282.47
Specific gravity	0.908–0.914 at 25 °C(Lit.)
Solubility	Easily soluble in ethanol (95%); miscible with ether, chloroform, light petroleum (50–70°C), and carbon disulfide.
HLB Value	7
Smoke point	160–188°C
Flash point	225°C
Refractive Index n20/D	1.4657–1.4893

Saponification number	186-194
Iodine value	79-88
Acid value	less than 1

Storage

Olive oil should be kept in an airtight, well-filled container, away from light. Stability may be increased by the incorporation of an antioxidant such as butylated hydroxytoluene.

Incompatibilities

Olive oil could have been saponified by alkali hydroxides. As it consists of a high amount of unsaturated fatty acids, olive oil is prone to oxidation and is incompatible with oxidizing agents.

Safety

Olive oil is often considered as a largely nonirritant and harmless material when used as an excipient

Castor oil [153]

Castor oil is a triglyceride of fatty acids. The fatty acid content is roughly ricinoleic acid (87%); oleic acid (7%); linoleic acid (3%); palmitic acid (2%); stearic acid (1%) and tiny quantities of dihydroxystearic acid.

Castor oil is frequently utilized in cosmetics, culinary goods, and medicinal formulations. In pharmaceutical formulations, castor oil is most typically utilized in topical lotions and ointments at doses of 5–12.5%. However, it is also utilized in oral tablet and capsule formulations, ophthalmic emulsions, and as a solvent in intramuscular injections.

Specification

Specification Limits

Appearance	Viscous oil that is clear, light, or colorless yellow-colored oil.
Odor	Slight odor
Taste	Initially bland but afterwards slightly acrid
Empirical formula	$C_{57}H_{104}O_9$
Molecular weight	933.450 g/mol
Specific gravity	0.953–0.965 at 25 °C
Solubility	Miscible with chloroform, diethyl ether, ethanol, glacial acetic acid, and methanol; readily soluble in ethanol (95%) and petroleum ether; nearly insoluble in water
HLB Value	14
Boiling point	313°C
Flash point	229°C
Refractive Index n_D^{20}	1.473–1.477
Saponification number	176-187
Iodine value	80-90
Acid value	less than 1.5
Surface tension	39.0 mN/m at 20°C

Storage

Castor oil should be kept at a temperature not exceeding 25°C in well-filled airtight containers shielded from light.

Incompatibilities

Castor oil is incompatible to strong oxidizing agents.

Safety

Castor oil is typically considered as a reasonably nonirritant and harmless material when utilized as an excipient.

Sesame oil [154]

A typical study of refined sesame oil reveals the makeup of the acids, present as glycerides, to be: arachidic acid 0.8%; linoleic acid 40.4%; oleic acid 45.4%; palmitic acid 9.1%; and stearic acid 4.3%. Sesamin, a complex cyclic ether, and sesamolin, a glycoside, are also found in minor levels.

Sesame oil is frequently used as a solvent in the manufacturing of subcutaneous injections, oral capsules, rectal suppositories, and ophthalmic preparations; it may also be utilized in the formation of suspensions and emulsions. Multiple emulsion formulations, in which sesame oil was one of the oil phases added, have been explored as a prolonged-release approach for rifampicin.

Micro emulsions containing sesame oil have been produced for the transdermal administration of ketoprofen. Sesame oil has also been incorporated in self-microemulsifying drug delivery systems, and fast-disintegrating lyophilized dry emulsion tablets for oral administration. It has also been employed in the production of liniments, pastes, ointments, and soaps.

Specification

Specification Limits	
Appearance	Clear, colorless or pale yellow-colored viscous oil.
Odor	Slight odor
Taste	Bland
Empirical formula	$C_7H_{10}O_3$
Molecular weight	138.12 g/mol
Density	0.916–0.920 g/cm^{3}at 25 °C
Solubility	Insoluble in water; nearly insoluble in ethanol (95%); miscible with carbon disulfide, chloroform, ether, hexane, as well as light petroleum.
HLB Value	7
Freezing point	-5^{o}C
Flash point	338^{o}C
Specific rotation n_D^{25}	+1^o to +9^o
Saponification number	187-194

Iodine value	103-118
Acid value	less than 0.2
Viscosity (dynamic)	43 mPa s (43 cP)

Storage

Sesame oil should be kept in a well-filled, sealed, light resistant container, at a temperature not exceeding 40°C. Sesame oil used for use in the preparation of parenteral dosage forms should be kept under an inert gas in an airtight glass container.

Incompatibilities

Sesame oil could be saponified using alkali hydroxides.

Safety

Sesame oil is typically considered as a reasonably nonirritant and harmless material when utilized as an excipient.

Peanut oil [155]

BP: Arachis Oil

JP: Peanut Oil

USP-NF: Peanut Oi

A standard examination of refined peanut oil reveals the composition of the acids present as glycerides to be: arachidic acid 2.4%; behenicacid 3.1%; palmitic acid 8.3%; stearic acid 3.1%; lignoceric acid 1.1%; linoleic acid 26.0%, and oleic acid 56.0%. Peanut oil is utilized as an excipient in pharmaceutical formulations mainly as a solvent for sustained-release intramuscular injections. It is also utilized as a carrier for topical treatments along with a solvent for vitamins and hormones. Furthermore, it has been a component of sustained-release bead formulations, nasal drug delivery systems and controlled-release injectables. In terms of treatment, emulsions containing peanut oil have been used in consumption regimens, in enemas as a fecal softener, and in otic drops for softening ear wax.

Specification

Specification Limits

Appearance	Colorless or pale yellow-colored liquid
Odor	Faint
Taste	Bland, nutty
Empirical formula	$C_{22}H_{14}N_6Na_2O_9S_2$
Molecular weight	616.49 g/mol
Density	0.915g/cm^3at 25 °C
Solubility	Not significantly soluble in ethanol (95%); soluble in benzene, carbon tetrachloride, and oils; soluble with carbon disulfide, chloroform, ether, and hexane
HLB Value	7
Freezing point	-5°C
Flash point	283°C
Refractive index n_D^{25}	1.466–1.470
Saponification number	188-196
Iodine value	84-103
Acid value	less than 0.2
Viscosity (dynamic)	35.2 mPa s (35.2 cP) at 37°C

Storage

Substances designed for use in parenteral dosage forms should be kept in a glass container.

Peanut oil needs to be kept in a well-filled, sealed, light resistant container, at a temperature not higher than 40°C.

Incompatibilities

Peanut oil might be saponified through alkali hydroxides.

Safety

Peanut oil is slightly laxative at a dose of 15–60 mL orally or of 100–500 mL rectally as an enema. Adverse responses to peanut oil in meals and medicinal formulations have been described widely. These include severe allergic skin rashes and anaphylactic shock after eating of peanut butter.

Eucalyptus oil [156]

Eucalyptus oil is obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branchlets of various species of Eucalyptus rich in 1,8-cineole. The main components of essential oils of Eucalyptus oil are monoterpenes (1, 8-cineole, p-cymene, citronellal, citronellol, limonene, α-phellandrene, β-phellandrene, α -pinene, β -pinene, trans-pinocarveol, terpinolene, α-terpineol, α-thujene) and sesquiterpenes (β-caryophyllene, β -eudesmol, globulol, spathulenol and virdiflorol).

Eucalyptus oil has a wide application as a pharmaceutical, antiseptic, repellent, flavoring, fragrance and industrial uses. The main uses of the oil is for the pharmaceutical industry, perfumery and for industrial use.

Specification

Specification	Limits
Appearance	Colorless to pale yellow clear liquid
Odor	Aromatic, somewhat camphoraceous odor
Solubility	Soluble in alcohol & oils. Insoluble in water
Specific Gravity	0.909 to 0.919 @ 20°C
Optical Rotation	+2.0 to +9.0 @ 20°C
Refractive Index	1.459 to 1.465 @ 20°C
Taste	Pungent, spicy, cooling taste
Empirical formula	$C_{10}H_{18}O$
Molecular weight	154.25 g/mol
Density	0.906 to 0.927g/cm^3at 25 °C
Specific Gravity	Between 0.905 and 0.925
Boiling point	175°C
Melting point	15.4°C
HLB Value	9.8
Freezing point	-5°C

Flash point	53°C
Refractive index n_D^{25}	1.466–1.470

Use

Eucalyptus oil has been used for inhalation as a decongestant often in combination with other volatile substances. It has been used as a flavoring agent, rubefacient, cleaning solvent, as a fragrance, antiseptic, febrifuge and expectorant as well as orally for catarrh and coughs.

Storage

Goods containing eucalyptus oil should be kept at a temperature not exceeding 25°C in properly packed containers. Protect against light. Liquid goods containing eucalyptus oil are best kept in child protective containers.

Incompatibilities

Heat sources.

Safety

Proper grounding procedures to avoid static electricity should be followed. Ground/bond container and receiving equipment.

Use explosion-proof electrical, ventilating, lighting equipment.

Cottonseed oil [157]

USP-NF: Cottonseed Oil

A typical examination of refined cottonseed oil reveals the percentages of the acids present as glycerides which are outlined as follows: linoleic acid 39.3%; oleic acid 33.1%; palmitic acid 19.1%; stearic acid 1.9%; arachidic acid 0.6%, and myristic acid 0.3%. Also comprised are minor levels of phospholipid, phytosterols, and pigments.

Cottonseed oil is employed in pharmaceutical formulations mostly as a solvent for intramuscular injections. It is utilized as an emollient carrier for various medications

and also orally as a mild cathartic. It may also reduce stomach secretion and motility and boost calorie intake. It has been utilized in the creation of soaps, oleomargarine, lard substitutes, glycerin, lubricants, and cosmetics. Cottonseed oil has been utilized as a tablet binder for acetaminophen, for characterisation of the hot-melt fluid bed coating process, in the manufacturing of stable oral pharmaceutical powders, in encapsulation of enzymes, and as a component of an aqueous dispersion in pharmaceutical coating.

Specification

Specification Limits

Appearance	Pale yellow or bright golden yellow-colored
Odor	Odorless
Solubility	Relatively soluble in ethanol (95%); miscible in carbon disulfide, chloroform, ether, hexane, and petroleum ether
Specific Gravity	0.915–0.921@ 20°C
Refractive Index	1.4645–1.4655 @ 20°C
Taste	Bland, nutty taste.
Empirical formula	CH_4
Molecular weight	16.04246 g/mol
Density	0.916 g/cm^{3}at 25 °C
Specific Gravity	Between 0.905 and 0.925
HLB Value	7.5
Freezing point	-5 to 0^{o}C
Flash point	321^{o}C
Iodine value	109–120
Viscosity (dynamic)	Up to 70.4 mPa s (70.4 cP) at 20°C

Storage

Cottonseed oil must be kept in a well-filled, sealed, light resistant container in a cold, dry environment. Avoid exposure to severe heat.

Safety

Cottonseed oil is a flammable liquid when exposed to heat or flame. If it is permitted to saturate rags or greasy trash, there is a danger related to spontaneous heating. Dry chemicals such as carbon dioxide should be utilized to deal with any flames.

Soyabean oil [158]

BP: Refined Soya Oil

JP: Soybean Oil

PhEur: Soya-Bean Oil, Refined

USP: Soybean Oil

Soybean oil is the refined fixed oil derived from the seeds of the soya plant Glycine max Merr. Family: Fabaceae.

A common examination of refined soybean oil demonstrates the proportions of the acids, present as glycerides, to be: linoleic acid 50–57%; linolenic acid 5–10%; oleic acid 17–26%; palmitic acid 9–13%; and stearic acid 3–6%.

In medicinal products, soybean oil emulsions are largely employed as a fat source in total parenteral nutrition (TPN) programs. Additionally, soybean oil has been applied in the formulation of many different drug delivery systems, such as liposomes, microspheres, dry emulsions, self-emulsifying systems, microemulsions, nanoemulsions and nanocapsules, solid-in-oil suspensions, and multiple emulsions. Soybean oil may also be employed in cosmetics and is utilized as an edible oil. As soybean oil has emollient qualities, it is employed as a bath element in the treatment of skin that is dehydrated disorders.

Specification

Specification Limits

Appearance	Clear, pale-yellow coloured
Odor	Odorless
Taste	Bland

Solubility	Extremely insoluble in ethanol (95%) and water; miscible with carbon disulfide, chloroform, ether, and light petroleum.
Specific Gravity	0.916–0.922@ 20°C
Refractive Index	1.471–1.475 @ 20°C
Empirical formula	$C_{11}H_9N_3O_2$.Na
Molecular weight	920 kg/kmol
Density	0.916 -0.922 g/cm^{3}at 25 °C
Specific Gravity	Between 0.905 and 0.925
HLB Value	7
Freezing point	-10 to -16^{o}C
Flash point	282^{o}C
Saponification number	188-195
Iodine value	126-140
Acid value	less than 0.2
Viscosity (dynamic)	50.09 mPa s (50.09 cP) at 25°C

Storage

Soybean oil should be kept in a well-filled, sealed, light resistant container at a temperature not exceeding 25°C.

Incompatibilities

Soybean oil emulsions have been found to be incompatible at 25°C with a variety of compounds including calcium chloride, calcium gluconate, magnesium chloride, phenytoin sodium, and tetracycline hydrochloride. When plastic syringes are utilized for holding soybean oil emulsion, silicone oil could be extracted into the emulsion; expansion of the syringe pump also takes place which leads to the need for more strength to maintain the motion of the plunger.

Safety

Generally, soybean oil is recognized as a basically harmless and nonirritant substance. However, major adverse responses to soybean oil emulsions delivered parenterally

have been documented. These include instances of hypersensitivity, CNS responses, and fat embolism. Interference with the anticoagulant effect of warfarin has also been documented.

Labrafil® M 2125 CS [159-60]

Labrafil® M 2125 CS is derived from maize oil. It has an average of 6 ethoxy units. The substance is a blend of medium chain triglycerides, mostly derived from caprylic (C8) and capric (C10) acids. It may self-emulsify upon contact with water, creating a coarse dispersion (emulsion). When mixed with appropriate surfactants, it can create a fine dispersion (nanoemulsion). Therefore, it may serve as a lipid carrier for medications that are not soluble in water.

Labrafil M 2125 CS has been found to demonstrate bioavailability enhancing capabilities as well. Increase in oral bioavailability can be attributed to the long chain triglyceride composition absorption of highly lipophilic drugs by the lymphatic transport system reducing hepatic first-pass metabolism.

Specification

Specification Limits

Manufacturer

Gattefossé, Lyon, France

USP NF name

Linoleoyl polyoxyl-6 glycerides (or Linoleoyl macrogol-6 glycerides)

Chemical description

Consists of mono-, di- and triglycerides and PEG-6 (MW 300) mono- and diesters of linoleic (C18:2) acid

Physical appearance	Yellow liquid
Odor	Light
Empirical formula	$C_{43}H_{88}O_{10}$
Molecular weight	765.2 g/mol
Boiling point:	>150°C

Specific gravity	0.935-0.955 g/cm^3
HLB	9
Iodine value	89.9
Acid value	Less than 2
Saponification value	155 to 175
HLB value	3 to 4
Iodine value	90 to 110

Solubility

Dispersible in water, soluble in many organic solvents

Toxicity

Generally regarded as relatively nonirritant and nontoxic

Cremophor RH 40 [161,162]

Synonyms

Polyoxyl 40 hydrogenated castor oil.

Composition

Polyoxy1 35 hydrogenated castor oil acts as a non-ionic solubilizer and emulsifier produced by the reaction of hydrogenated castor oil with ethylene oxide in a 1:40 molar ratio. Cremophor RH 40 mostly is composed of glycerol polyethylene glycol hydroxystearate, which, together with fatty acid glycerol polyglycol esters, makes up the hydrophobic portion of the product. The hydrophilic portion comprises polyethylene glycols and glycerol ethoxylate.

Product type

Cremophor RH 40 creates transparent solutions in water. It is soluble in several organic solvents such as ethanol, n-propranol, isopropyl alcohol, ethyl acetate, chloroform, carbon tetrachloride, trichloroethylene, toluene, and xylene. In comparison with anionic emulsifying agents, Cremophor RH 40 becomes more difficult to dissolve in water at higher temperatures. The aqueous solutions become turbid at a given temperature. Cremophor RH 40 is miscible with all the other Cremophor grades and, on heating, also

with fatty acids, fatty alcohols and some animal and vegetable oils. It is therefore miscible with oleic and stearic acids, dodecyl and octa-decyl alcohols, castor oil, and a wide range of lipid-soluble compounds.

Specification

Specification Limits

Manufacturer	BASF – Germany
Appearance	White to pale yellow oily liquid
Empirical formula	$C_6H_{14}O_3$
Melting Range	160 – 260°C
Density	1.03 g/cm^3
Solubility	Soluble in water, ethanol, chloroform and oils
Acid value	Less than Equal to 1
Water	Less than Equal to 2.0%
pH (10% in water)	6–7
HLB Value	Between14 to 16
Acute oral toxicity (LD50/Rat)	20000 mg/kg

Storage

Cremophor RH 40 should be kept in firmly closed containers shielded from light. Prolonged storage is not suggested unless the containers are totally filled.

Incompatibilities

During aqueous solution, Cremophor EL is stable toward most electrolytes at the amounts commonly applied. However, it is incompatible with mercuric chloride when precipitation occurs. Some organic chemicals may generate precipitation at certain quantities, notably those containing phenolic hydroxyl groups, e.g. phenol, resorcinol, and tannins.

Regulatory Status

Specified in the FDA Inactive Ingredients Guide (IV injections and ophthalmic solutions). Mentioned in parenteral medications approved in the UK. Mentioned on the Canadian List of Acceptable Non-medicinal Ingredients.

Kolliphor® EL [163]

Ph.Eur.: Macrogolglycerol Ricinoleate 35

Kolliphor® EL is glycerol polyethylene glycol ricinoleate. The less hydrophylic components are composed of free polyethylene glycols and ethoxylated glycerol. Combined with the fatty acid esters of polyethylene glycol, this generates the hydrophobic component of the product. Kolliphor EL is employed as a non-ionic oil-in-water emulsifier and/or solubilizer. This tested product displays exceptionally good compatibility with other components and may be utilized with fat-soluble vitamins and essential oils or used as a pure solubilizer in formulations.

Specification

Specification Limits

Chemical Name	Polyoxyl 35 castor oil.
Form	Oily
Colour	Light yellow
Odour	Faint specific odour
pH value	6 - 8(100 g/l, 20 °C)
Melting point	Approx. 4 °C
Boiling point	> 100 °C
Flash point	257 °C
Ignition temperature	405 °C
Density:	1.05 – 1.06 g/cm^3 (25 °C)
Solubility	It form clear solution in water. It is also soluble in many organic solvent such as chloroform, carbon tetrachloride, ethyl alcohol, toluene, xylene
Solubility in water	100 g/l (20 °C)

Molecular weight 172.08 g/mol.

Molecular formulae: $C_4H_7Cl_2NO_2$

Viscosity, dynamic 650 - 800 mPa.s (25 °C)

General Applications:

1. Solubilizer.

2. Emulsifier.

Pharmaceutical Applications:

- Used to emulsify and solubilize oils and other water-insoluble substances.
- It was originally developed for use as solubilizers and emulsifier.
- Kolliphor EL has been used as a detergent to improve the solubility of dye and to increase the transmembrane permeation.
- It has been used as a vehicle for drug/stimulant delivery in cells.

Storage

Keep bottle well covered and dry; keep in a cool place. Extended storage is not suggested until the container is totally filled.

Tween® 20 (Polysorbate 20) [164]

Polysorbate 20 (Tween 20) is a hydrophilic non-ionic surfactant. Polysorbate 20 is produced by ethoxylation of sorbitan before the addition of lauric acid (Polyoxyethlene (20) sorbitan monolaurate).

The number 20 after the 'Polysorbate' component denotes the kind of the fatty acid (lauric acid) connected to the polyoxyethlene sorbitan part of the molecule.

They are also employed as solubilizing agents for poorly soluble medicines as well as wetting agents. These have been employed as emulsifying agents for the creation of stable oil-inwater emulsions. Tween 20 is used as an excipient in medicines as a stabilizer, specifically in tablet and ophthalmic solutions.

Specification

Specification Limits

Manufacturer	Swadesh life science, Ahmedabad
USP NF name	Polysorbate 20, PEG (20) sorbitan monolaurate,
Chemical description	A sequence of partial fatty acid esters of sorbitol and its anhydrides copolymerized with roughly 20 moles of ethylene oxide per each mole of sorbitol and its anhydrides.
Appearance	Yellow to yellow-green viscous liquid
Odor	Light
Empirical formula	$C_{58}H_{114}O_{26}$
Molecular weight	1,225 daltons
Density	1.1 g/mL
Solubility	Practically soluble in water, Anhydrous ethanol, Ethyl acetate, Methanol, Isopropanol, Propylene glycol, Ethylene glycol, Cottonseed oil.
Water	Less than Equal to 2.0%
pH (10% in water)	5–7
HLB Value	16.72
Viscosity	370-430 cps
Melting point	13.4 °C
Refractive Index n20/D	1.459(Lit.)
Iodine value	5
Acid value	2
Hydroxyl value	96-108
Saponification value	40-50

Storage

Aqueous solutions of polysorbates undergo autoxidation during storage, with changes being catalyzed by light, increased temperature, and copper sulfate. Tween 20 is heat sensitive and will darken when exposed to elevated temperatures.

Toxicity

Generally regarded as relatively nonirritant and nontoxic material.

Tween® 80 (Polysorbate 80) [165]

Polysorbate 80 (Tween 80) is a hydrophilic non-ionic surfactant. Polysorbate 80 is produced by ethoxylation of sorbitan before the addition of oleic acid (Polyoxyethlene (20) sorbitan monooleate).

The number 80 following the 'Polysorbate' part indicates the type of the fatty acid (oleic acid) linked to the polyoxyethlene sorbitan part of the molecule.

They are also utilized as solubilizing agents for poorly soluble drugs as well as wetting agents. These have been used as emulsifying agents for the preparation of stable oil-in water emulsions. Tween 80 is used as an excipient in pharmaceuticals as a stabilizer, particularly in tablet and ophthalmic solutions.

Specification

Specification Limits

Manufacturer	Swadesh life science, Ahmedabad
USP NF name	Polysorbate 80, PEG (80)sorbitan monooleate
Chemical description	A sequence of partial fatty acid esters of sorbitol and its anhydrides copolymerized with roughly 20 moles of ethylene oxide per each mole of sorbitol and its anhydrides.
Appearance	Yellow to yellow-green viscous liquid
Odour	Light
Empirical formula	$C_{64}H_{124}O_{26}$
Molecular weight	1,225 daltons
Density	1.1 g/mL
Solubility	Practically soluble in water, Anhydrous ethanol, Ethyl acetate, Methanol, Isopropanol, Propylene glycol, Ethylene glycol, Cottonseed oil.
Water	Less than Equal to 3.0%

pH (10% in water)	5–7
HLB Value	15
Viscosity	425 (mPa s)
Specific gravity	1.065–1.095
Iodine value	19-24
Acid value	less than 2
Hydroxyl value	96-108
Saponification value	45-55
Surface tension	42.5

Storage

Polysorbates 80 are hygroscopic and have to be looked at for water content before to use and dried if required. Also, in accordance with other polyoxyethylene surfactants, extended storage might lead to the development of peroxides. Polysorbates 80 should be kept in a well-closed container, shielded from light, in a cold, dry location.

Toxicity

Generally considered as largely nonirritant and harmless substance.

Span 20 (Sorbitan monolaurate) [166]

Sorbitan monolaurate are frequently utilized in cosmetics, culinary items, and medicinal formulations as lipophilic nonionic surfactants. They are largely utilized in pharmaceutical formulations as emulsifying agents in the manufacture of creams, emulsions, and ointments for topical use. When used alone, sorbitan esters generate stable water-in-oil emulsions and microemulsions but are usually employed in conjunction with changing quantities of a polysorbate to make water-in-oil or oil-in water emulsions or creams of various consistencies.

Specification

Specification Limits

Manufacturer	Swadesh life science, Ahmedabad
USP NF name	Sorbitan monolaurate (sorbitan,esters monodecanoate)

Appearance	Cream- to amber-colored liquids or solid
Odour	Distinctive odor
Empirical formula	$C_{18}H_{34}O_6$
Molecular weight	346 Daltons
Density	1.01 g/mL
Solubility	Span 20 are normally soluble or dispersible in oils; they are additionally soluble in most organic solvents. In water, while insoluble, they are often dispersible.
Water	Less than Equal to 0.5%
pH (10% in water)	5–7
HLB Value	8.6
Viscosity	3900–4900 at 25°C (mPa s)
Specific gravity	0.98
Iodine value	Less than Equal to 7
Acid value	less than 7
Hydroxyl value	159-169
Saponification value	159-169
Surface tension	28

Storage

Span 20 should always be kept in a well-closed container in a cold, dry location.

Toxicity

Generally considered as largely nonirritant and harmless substance.

Handling Precautions Observe conventional precautions relevant to the conditions and amount of item handled. Eye protection and gloves are recommended

Span 80 (Sorbitan monooleate) [167]

Sorbitan monooleate are frequently utilized in cosmetics, culinary items, and medicinal formulations as lipophilic nonionic surfactants. They are largely utilized in pharmaceutical formulations as emulsifying agents in the manufacture of creams, emulsions, and ointments for topical use. When used alone, sorbitan esters generate stable water-in-oil emulsions and microemulsions but are usually employed in

conjunction with changing quantities of a polysorbate to make water-in-oil or oil-in water emulsions or creams of various consistencies.

Specification

Specification Limits

Manufacturer	Swadesh life science, Ahmedabad
USP NF name	Sorbitan monolaurate (sorbitan,esters monodecanoate)
Appearance	Cream- to amber-colored liquids or solid
Odour	Distinctive odor
Empirical formula	$C_{24}H_{44}O_6$
Molecular weight	429 daltons
Density	1.01 g/mL
Solubility	Span 80 are normally soluble or readily dispersed in oils; they are also soluble in most organic solvents. In water, while insoluble, they are often dispersible.
Water	Less than Equal to 1.5%
pH (10% in water)	5–7
HLB Value	4.3
Viscosity	3900–4900 at 25°C (mPa s)
Specific gravity	0.98
Iodine value	Less than Equal to 7
Acid value	less than 8
Hydroxyl value	193-209
Saponification value	149-160
Surface tension	30
Viscosity at 25°C	970–1080(mPa s)

Storage

Span 80 should be kept in a well-closed container in a cool, dry spot.

Toxicity

Generally viewed as largely nonirritant and harmless product.

Handling Precautions

Observe conventional precautions according to the conditions and amount of substance handled. Protection for the eyes and gloves are suggested.

Solutol HS®15 (Kolliphor® HS 15) [168]

Ph. Eur.: Macrogol 15 Hydroxystearate

USP/NF: Polyoxyl 15 Hydrostearate

Synonym: Kolliphor® HS 15

Kolliphor® HS 15 is a nonionic solubilizer and emulsifying agent obtained by reacting 15 moles of ethylene oxide with 1 mole of 12-hydroxy stearic acid.

Kolliphor® HS 15 is a potent, non-ionic solubilizer specifically designed for poorly water-soluble drugs in parenteral applications. Kolliphor® HS 15 is synthesized by reacting 12-hydroxystearic acid (I) with ethylene oxide (II) in presence of an alkaline catalyst to yield the major components, polyethoxylated derivatives III and V, which represent mono- and diesters. Physicochemical attributes of Kolliphor® HS 15 follow both the USP and Pharm. Eur. compendia.

Kolliphor® HS 15, one of the few efficient solubilizers, has frequently been employed in recent decades to increase the solubility of several poorly soluble substances. In compared with other known solubilizers such poloxamers, polysorbates, and castor oil based surfactants, it demonstrates an extraordinary solubilization capacity for several insoluble substances.

Specification

Specification Limits

Manufacturer	BASF, Germany
USP NF name	Sorbitan monolaurate (sorbitan, esters monodecanoate)
Appearance	Yellowish white paste at room temperature that becomes Liquid at approx. 30 °C.
Odour	Odourless
Composition	Consists of polyglycol mono- and di-esters of 12-hydroxystearic acid and of about 30% of free

	Polyethylene glycol
Solubility	Kolliphor® HS 15 is soluble in water, ethanol and 2-propanol to form clear solutions. Its solubility in water decreases with increasing temperature. It is insoluble in liquid paraffin.
Melting range	25-30°C
Flash point	272°C
pH (20% in water)	6.64
Density	0.03g/cm^3
HLB Value	14-16
Viscosity(Dynamic)	73 [mPas] at 60 °C
Peroxide Value	Less than 2
Iodine value	0.3
Acid value	0.1
Hydroxyl value	27

Storage

Kolliphor® HS should be kept in a well-closed container in a cold, dry area.

Toxicity

Generally considered as largely nonirritant and harmless substance.

Handling Precautions

Observe conventional precautions according to the conditions and amount of substance handled. Eye protection and gloves are suggested.

Transcutol® -P [169]

Synonyms

Diethylene glycol monoethyl ether (Highly purified)

Transcutol® P is a high purity grade of diethylene glycol monoethyl ether manufactured by the condensation of ethylene oxide and ethanol, followed by distillation. The

presence of an ether and an alcohol function explains the exceptional solubilizing capacity of this solvent

Specifications

Specification Limits

Source	Gattefosse, CAS # 111-90-0
Appearance	Colorless limpid liquid
Odour	Faint
Chemical name	Diethylene glycol monoethyl ether
Specific gravity at 20°C	0.985 to 0.991
Solubility	Soluble in ethanol and water. Partially soluble in vegetable oils and insoluble in mineral oils.
Chemical formula	$C_6H_{14}O_3$
Molecular weight	134.175 g/mol
Density	0.989 g/cm^3
Melting point	-76 °C
Boiling point	196-200 °C
Surface tension	31.3
Viscosity at 25°C	4.1 mPa.s at (25°C)
Flash point	96 °C
HLB Value	4.2
Specific gravity	0.985-0.991 g/cm^3 (20°C)
LD50 (Rat)	5500 µl/kg

Uses

Solubilizer of many active ingredients (i.e. indomethacin nifedipine, hormones, sterols). Absorption enhancer. Transcutol P can be used in topical, transdermal and oral pharmaceutical preparations. It can be associated to labrafil and vegetable oils.

Storage

Transcutol® P is susceptible to oxidation and hydrolysis; exposure to oxygen and humidity should be limited during handling and storage. Transcutol® P has a flash point

of 96°C, therefore exposure to external (or potential) sources of ignition at or above this temperature should be avoided. Gattefossé recommends avoiding heating above 85°C.

Propylene Glycol [170]

Synonyms

BP: Propylene glycol

JP: Propylene glycol

PhEur: Propylenglycolum

USP: Propylene glycol

Propylene glycol has been extensively employed as a solvent and preservative in a number of parenteral and nonparenteral medicinal formulations. It is a better generalized solvent than glycerin and dissolves a broad range of compounds, such as corticosteroids, phenols, sulfa medicines, barbiturates, vitamins (A and D), most alkaloids, and several local anesthetics.

Propylene glycol is often employed as a plasticizer in aqueous film-coating compositions. Propylene glycol is also employed in cosmetics and in the food sector as a carrier for emulsifiers and as a vehicle for tastes in preference to ethanol, because its lack of volatility offers a more homogeneous flavor.

Specifications

Specification Limits	
Appearance	Clear, colorless, viscous Liquid
Odor	Odorless
Taste	Sweet, slightly acrid taste
Chemical name	1,2-Propanediol
Specific gravity at 20°C	1.035–1.040
Solubility	Miscible with acetone, chloroform, ethanol (95%), glycerin, and water; soluble at 1 in 6 parts of ether; not miscible with light mineral oil or fixed oils,

Chemical formula	$C_3H_8O_2$
Molecular weight	76.09 g/mol
Density	1.038g/cm^3 at (20°C)
Boiling point	188°C
Melting point	-59 °C
Viscosity (dynamic)	58.1 mPa s (58.1 cP) at 20°C
Refractive Index	1.4324
Flash point	99 °C
HLB Value	4.5
LD50 (Rat, IV)	6.42 g/kg

Storage

At low temperatures, propylene glycol is stable in a well-closed container, but at high temperatures, in the open, it tends to oxidize, offering rise to compounds such as propionaldehyde, lactic acid, pyruvic acid, and acetic acid. Propylene glycol is hygroscopic and needs to be kept in a well-closed container, covered from light, in a cold, dry environment.

Safety

Propylene glycol is utilized in a broad range of pharmaceutical compositions and is usually considered as a reasonably harmless substance.

Polyethylene Glycol 200 [171]

Synonyms

BP: Macrogols

JP: Macrogol 200

Carbowax, PEG 200

The USPNF 23 identifies polyethylene glycol as having an addition polymer of ethylene oxide and water. Polyethylene glycol grades 200–600 are liquids; grades 1000 and higher are solids at ambient temperatures.

Polyethylene glycols (PEGs) are extensively utilized in several kinds of pharmaceutical formulations comprising parenteral, topical, ophthalmic, oral, and rectal treatments. It

has been employed experimentally in biodegradable polymeric matrices utilized in controlled-release systems.

Specifications

Specification Limits	
Appearance	Clear, colorless, viscous Liquid
Odor	Characteristic odor
Taste	Bitter, slightly burning taste
Chemical name	α-Hydro-ω-hydroxypoly(oxy-1,2-ethanediyl)
Specific gravity at 20°C	1.035–1.040
Solubility	Polyethylene glycols are soluble in acetone, alcohols, benzene, glycerin, and glycols.
Chemical formula	$H(OCH_2CH_2)_{5.2}OH$
Molecular weight	190- 210g/mol
Density	1.11-1.14 g/cm^3 at (20°C)
Flash point	182 °C
Boiling point	188°C
Viscosity (dynamic)	58.1 mPa s (58.1 cP) at 20°C
Refractive Index	1.459
Surface tension	44 dynes/cm
HLB Value	8
LD50 (Rat, oral)	28 g/kg

Storage

Polyethylene glycols should be kept in well-closed containers in an environment that is dry and cold. Stainless steel, aluminum, glass, or lined steel containers are more beneficial for the storage of liquid grades.

Safety

Polyethylene glycols are frequently employed in a range of medicinal compositions. Generally, they are regarded as nontoxic and nonirritant materials.

Polyethylene Glycol 400 [172]

Synonyms

BP: Macrogols

JP: Macrogol 400

Carbowax, PEG 400

The USPNF 23 identifies polyethylene glycol as being an addition polymer of ethylene oxide and water. Polyethylene glycol grades 200–600 are liquids; grades 1000 and higher are solids at ambient temperatures.

Polyethylene glycols (PEGs) are extensively utilized in a range of pharmaceutical formulations including parenteral, topical, ophthalmic, oral, and rectal treatments. It has been employed extensively in biodegradable polymeric matrices utilized in controlled-release systems.

Specifications

Specification Limits	
Appearance	Clear, colorless, viscous Liquid
Odour	Characteristic odor
Taste	Bitter, slightly burning taste
Chemical name	α-Hydro-ω-hydroxypoly(oxy-1,2-ethanediyl)
Specific gravity at 20°C	1.035–1.040
Solubility	Polyethylene glycols are soluble in acetone, alcohols, benzene, glycerin, and glycols.
Chemical formula	$H(OCH_2CH_2)_{9.7}OH$
Molecular weight	380- 420g/mol
Density	1.120 g/cm^3 at (20°C)
Flash point	238 °C
Boiling point	188°C
Viscosity (dynamic)	105-130 mPa s cP at 20°C
Refractive Index	1.465
Surface tension	44 dynes/cm
HLB Value	11.3

LD50 (Rat, IV) 7.3 g/kg

Storage

Polyethylene glycols should be kept in well-closed containers in a cold, dry environment. Stainless steel, aluminum, glass, or lined steel containers are more beneficial for the storage of liquid grades.

Safety

Polyethylene glycols are frequently employed in a range of medicinal compositions. Generally, they are considered as harmless and nonirritant materials.

Aerosil® 200 [173]

Aerosil® 200 Pharma is a high purity amorphous anhydrous colloidal silicon dioxide for use in pharmaceutical products which fulfils the analytical requirements of the currently valid versions of the European Pharmacopeia (Ph. Eur.), United States Pharmacopeia (USP/NF), Japanese Pharmacopeia (JP) and Indian Pharmacopoeia (IP). Aerosil® 200 is a hydrophilic fumed silica with a specific surface area of 200 m^2/g. It's used as an anti-caking, free-flowing, gelling, suspending, dispersing, and thickening agent. It's also used in cosmetics for its light-diffusing properties.

Features:

- In toothpaste formulations, whether in all-silica formulations, in carbonate pastes, or in those using phosphate abrasives, it gives the rheological body.
- It can help increase the volume of the hair, counteracting the flattening effect of the conditioning agents.
- It prevents a premature reaction and caking of powdered colorant component (hair dyes).
- It helps improve the homogeneous distribution of the pigments in color cosmetics.
- It prevents the agglomeration of active ingredients—generally aluminum and zirconium salts (AP salts)-helping to ensure that they are evenly distributed and have an intense and long-lasting effect.
- It also avoids white residue.

- Aerosil® 200 is suggested for use in skin care, oral care, hair care (beaches, dyes, conditioners), make-up (powder, nail polish, lipstick/lip gloss) and antiperspirants & deodorants (sticks) applications.

Specifications

Specification Limits

Manufacturer	Evonik Operations GmbH
BET Surface Area	175-225[m^2/g]
pH	3.7 - 4.5
Loss on drying (%wt)	less than or equal to 1.5
Carbon content (%wt)	4 to 6.5

Storage

Aerosil® 200 must be kept in a closed container under dry condition protected from volatile compounds and utilized within 1 to 2 years after production.

Aeroperl® 300 [174]

Aeroperl® 300 Pharma is a high purity colloidal silicon dioxide is suited for use in pharmaceutical products (tested according to USP/NF and Ph. Eur. Monograph 0434). It is an unaltered, unprocessed and micro-granulated hydrophilic fumed silica. It is a very absorptive colloidal silicon dioxide.

Specifications

Specification Limits

Manufacturer	Evonik Operations GmbH
Physical state	Solid
Colour	White
Form	Powder
Odor	Odorless
pH	3.5 - 5.5 (40 g / l) (20 °C)(suspension)
Melting point	1700 °C
Density	2.2 g/cm^3 (20 °C)

Water solubility > 1 mg/l

Features:

- It has minimal dust formation, high adsorption capacity, quick absorption and good free flow behavior.
- Hydrophilic fumed silica, Aeroperl® 300/30 from Evonik functions as a carrier and filler.
- It may be employed as a carrier to facilitate the dissolving of poorly soluble active medicinal components.
- Improving the dissolution of poorly soluble APIs The respond to to the solubility problem may be employed as an inorganic carrier for solid dispersions
- Due to the tiny pore size, it is able to stabilize APIs in the amorphous form It demonstrates minimal thickening of solvents and relatively easy separations from liquids & gases

Storage

Keep containers tightly closed in a dry, cool place.

Neusilin® US2 [175]

Neusilin® is a synthetic, amorphous form of Magnesium Alumino metasilicate. It is a multifunctional excipient that can be used in both direct compression and wet granulation of solid dosage forms. Neusilin® is widely used for improvement of the quality of tablets, powder, granules and capsules.

Neusilin® is an excellent adsorbent carrier for solid dispersion.

Solid dispersions can be prepared via hot melt granulation and Hot Melt Extrusion (HME) to improve dissolution profile of poorly water soluble drugs.

Neusilin® solves common problems associated with tableting by facilitating improved and consistent flow of powder mix, providing optimum tablet hardness at low compression forces, protecting the active ingredient from moisture related issues and converting oily or sticky APIs into free flowing powder.

For drugs with high melting points, HME can be prepared simply by mixing of crystalline drug and Neusilin® before passing it through the extruder. The extruded sample can be recovered as amorphous powder and then converted to tablets through direct compression.

Specifications

Specification Limits

Manufacturer	Fuji Chemical Industries Co., Ltd.
Appearance	White powder or granule
Chemical formula	$Al_2O_3 \cdot MgO \cdot 1.7SiO_2 \cdot xH2O$
Form	Amorphous
True specific gravity	2.0-2.2
Solubility	Practically insoluble in water and ethanol
Compositions	
(Dried at 110˚) (%)	Al_2O_3 29.1-35.5
	MgO 11.4-14.0
	SiO_2 29.2-35.6

Features:

- Neusilin® US2 show higher oil adsorption capacity when compared to MCC or colloidal silica.
- Neusilin® keeps the drug amorphous and stable under accelerated stability as well as long term storage conditions. Neusilin® is well accepted by formulators world-wide as an aid for formulations containing antibiotics, oily actives, poorly water soluble APIs, herbal mixtures, vitamins, etc.
- Neusilin® is also used as carrier for solid dispersions and self-micro emulsifying drug systems.
- Improves tablet and powder capsule quality
- Compact tablets with relevant hardness
- Improves powder flowability
- Excellent carrier for solid dispersion
- Anticaking agent for hygroscopic powders
- Stabilization of deliquescent drugs

Sr. No.	Application	Qty. required
1)	Tablet - binder, disintegrator, increase hardness (%)	1-10
2)	Stabilization of deliquescent drugs (%)	5-15
3)	Excipient/diluent (%)	30-90
4)	Solidification of liquid pharmaceutical preparations (%)	30-50
5)	Carrier for Solid dispersion, SMEDDS	20-50

Table 3.4: Typical Application and Quantity Required for Neusilin® US2

3.1.3. Equipment & Software

A list of equipment used for the preformulation study, formulation, and characterization of the development of benidipine and benidipine with telmisartan SNEDDS is listed in Table 3.4. Similarly, Table 3.5 presents the equipment used for the development of benidipine and benidipine with telmisartan SNEDDS. Torrent Research Centre (Ahmedabad, India) voluntarily provided empty hard gelatin capsules.

Sr. No.	Name of Instruments	Company Name
1)	UV visible spectrophotometer	UV 1800, Shimadzu, Japan
2)	FTIR spectrophotometer	Bruker Alpha, USA
3)	Differential scanning calorimetry (TA 60AW)	Jade PerkinElmer, USA
4)	Rotary Shaker	Remi Equipments PVT LTD, Mumbai
5)	Digital weighing balance	Aczet Balance, Mumbai
6)	Sonicator	Equitron, Mumbai, India
7)	USP dissolution apparatus	TDT-081, Electrolab, India
8)	Cyclomixer mixer	Remi Equipments PVT LTD, Mumbai
9)	Laboratory Centrifuge	R-9C Centrifuge, Remi Equipments PVT LTD, Mumbai
10)	Abbe's Refractometer	Krishna scientific, Haryana, India
11)	pH meter	Lab India, Mumbai, India
12)	Micropipettes	HiMedia, Mumbai, India
13)	Brookfield viscometer	Brookfield Engineering Labs .Inc, USA

14)	Malvern particle size analyzer	Zetatrac, Microtac Inc, USA
15)	Stability chamber	Thermolab, India
16)	Transmission Electron Microscope(TEM)	FEI Company, USA

Table 3.5: List of Equipment's used

Techniques	Software used
Experimental design	Design expert ® software (version 13.0.4.1, StateEase Inc, Minneapolis, MN, UA)
Standard result ±SD	Micro soft Excel 2007 software (Microsoft, USA)
Statistics	Microsoft Excel 2007 software (Microsoft, USA)
Shelf- life	Minitab 19 software.

Table 3.6: List of software

3.2 Methods

3.2 Methodology for development of Benidipine and Benidipine with Telmisartan SNEDDS

Preliminary investigations before formulation

The objectives of the pre-formulation studies are:

- To measure the solubility of the drug substance in different excipients and determine the excipients with high solubility for the drug substance.
- To conduct emulsification research with oils, surfactants, and co-surfactants (with high drug solubility) for the selection of the best combination of oil, surfactant, and co-surfactant where the maximum amount of oil emulsified and greater nanoemulsion region are identified.
- To carry out physical and chemical compatibility of drug substances with selected ingredients.
- Plotting of a ternary phase diagram to determine the range of components necessary for nanoemulsion production.
- Assessing the solubility of the drug material in various dissolving medium for determine necessary dissolution media for SNEDDS formulation [125].

3.2.1 Characterization of Benidipine (BD) and Telmisartan (TEL)

A) Physicochemical characterization

I. Organoleptic characteristics

The BD and TEL samples were analyzed to determine their physicochemical characteristics, including their nature, color, odor, and solubility.

II. Measurement of solubility

The solubility of BD and BD with TEL was assessed in both water and methanol. Excess amounts of benidipine and benidipine with telmisartan were dissolved separately in 10 mL of water and methanol to create saturated solutions. A limited quantity of these samples was collected, filtered, and appropriately diluted. Subsequently, the solubility characteristics of the benidipine drugs were assessed using UV spectrophotometry, specifically at a wavelength of 237 nm. The solubility profile of benidipine (BD) and telmisartan (TEL) was determined by analyzing certain wavelengths. This investigation used the dual-wavelength approach.

B) Spectral analysis is used to identify BD and TEL.

The authenticity of the acquired benidipine and telmisartan samples was verified using UV spectroscopy, Fourier transform infrared (FTIR) spectroscopy (Bruker Alpha, USA), and DSC (Jade PerkinElmer, USA) prior to conducting further study [126].

I. UV spectroscopy

UV spectroscopy has been carried out to find the maximum wavelength (λmax) of BD and TEL in methanol. For the experiment, two mg of each medication were individually put in five mL of methanol and sonicated for five minutes for full dissolution. After that, the volume was made up to 10 mL with identical solvent in a volumetric flask. Further, the acquired samples were scanned by a UV spectrophotometer in the range of 200–400 nm and recorded as the λmax of both drug and compared with the standard λmax of the same.

II. FTIR spectroscopy

The Fourier transform infrared (FTIR) spectra of BD and TEL were acquired using a FTIR spectrometer (Bruker Alpha, USA) linked to a crystal diamond universal ATR (UATR) sampling accessory. Accurately weighed, two mg of solid samples of both medications were individually combined and triturated with dried potassium bromide

(KBr) (5% weight of the samples) using a mortar and pestle. The homogeneous mixtures were crushed under a pressure of 1000 psig to make pellets of each medicine individually. Then, the pellets were positioned at the top of the ATR sampler, and an average spectrum of 32 scans was collected in the region of 4000-500 cm^{-1} wave number. The acquired spectra of both medications were compared with defined standard spectra of the same.

III. DSC

The DSC (Jade PerkinElmer, USA) was utilized to determine the physical state of BD and TEL. It is based on principle of measurement of heat flow in and out of sample and reference for the period of controlled temperature cycle. For the analysis, accurately weighed 2 mg each of BD and TEL were put in an aluminum pan separately and sealed by a sealing machine. Then, these pans were heated at a heating rate of 10°C/min from 50 to 450° C in the presence of nitrogen gas at a flow rate of 25 mL/min. The thermal behaviors of pure drug substance BD and TEL were compared with the physical mixture of BD and TEL.

3.2.2 Analytical method development and validation for estimation of BD and BD with TEL

For the determination of BD and BD with TEL in different samples during preformulation investigations, formulation development, and in-vitro studies, analytical procedures were created using techniques like UV spectroscopy.

A) Analytical method development and validation for the determination of BD

The standard stock solution of BD was made by transferring, correctly weighed, 100 mg of BD to a 100-ml volumetric flask containing 10 ml of methanol. Dissolving drugs correctly. Then volume was raised to the mark by using methanol to provide a concentration of 1000µg/ml. From this, 10 ml of the solution was transferred to a 100 ml volumetric flask and the volume was filled with methanol and 0.1 N HCL to reach a concentration of 100 µg/ml. It is a standard stock solution that is further diluted with methanol and 0.1 N HCL to achieve a concentration of 3–15 µg/ml. The calibration

curve of the benidipine was produced by taking the absorbance obtained on the y-axis and the concentration of the solution on the x-axis [105].

B) Analytical technique development and validation for estimation of BD with TEL

A comprehensive number of HPLC techniques are illustrated in the literature for the measurement of benidipine and telmisartan in formulations and plasma [117, 119]. The suggested dual-wavelength spectrophotometric approach employed in the present investigation consists of scanning a standard solution comprising 1–5 µg/ml of benidipine (BD) and 10–50 µg/ml of telmisartan (TEL) in the UV range of 200–400 nm using a 1cm cell. Methanol was utilized as a blank for reference. The wavelength at which each medication displayed maximum absorbance was determined (λ max) from the overlain spectra. Specific wavelengths for BD and TEL were determined based on this study, allowing the quantification of both medications utilizing the dual wavelength approach as stated in previous research work [118]. The accuracy and precision of the method were established and tested statistically. Accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100%, 120%) taking into consideration percentage recovery of added bulk drug samples.

3.2.3. Compatibility studies

Compatibility of BD and TEL in a solid state

Solid-state compatibility examinations were done to identify any concerns of interaction utilizing physical observation, such as color, texture, and spectrum analysis. The dried samples of BD, TEL, and their combination in a ratio of 1:1 were kept for 12 hours in glass vials separately at room temperature for the below-specified tests.

I. DSC

DSC thermograms of BD, TEL, and their combination in the ratio of 1:1 were produced using Jade PerkinElmer, USA. 2–3 mg of the samples were enclosed in a sealed

aluminum pan, and analysis was done from 40 to 300° C at a scanning rate of 10°C/min utilizing a stream of nitrogen gas.

II. FTIR analysis

The Fourier transform infrared (FTIR) spectra of BD, TEL, and the combination thereof were acquired using a FTIR spectrometer (Bruker Alpha, USA) linked with a crystal diamond universal ATR (UATR) sampling accessory. Solid samples of pharmaceuticals were put at the top of the ATR sampler, and an average spectra of 32 scans were acquired in the region of 4000-500 cm^{-1} wave number.

3.2.4. Application of Quality by Design (QbD)

Quality by Design (QbD) is the foundational element of the systematic process of Formulation by Design (FbD). Quality by Design was designed by quality expert Joseph Moses Juran for application in the planning of production. To assure a specified degree of product quality, all industries employ the QbD method while building pharmaceutical processes [132–133]. The ICH Q8 (R1) standard defines QbD as "a systematic approach to development that begins with" the development of a quality target product profile (QTPP), assessments of critical quality attributes (CQAs), critical material attributes (CMAs), and critical process parameters (CPPs), as well as the integration of screening and risk assessment, optimization, and validation [134].Quality by design gives value and quality to the final pharmaceutical product. It is vital to identify and regulate the key parameters connected to the process and the product throughout formulation development. The main objective of employing QBD in the pharmaceutical sector is to build a strong pharmaceutical product that can achieve the greatest therapeutic effectiveness, quality features, and extended shelf life over storage.

Setting the quality target product profile (QTPP) and significant characteristics of quality (CQAs)

The QTPP was first set up in order to include an expected overview of the drug's outstanding attributes that may boost BD and BD with TEL oral bioavailability for the greatest appropriate pharmaceutical benefits. This was done by utilizing FbD-based technology for the production of solid self-nano emulsifying devices. The QTPP is one of the requirements mentioned by the product development team using the QbD

technique for creating therapeutic effects in conformity with the label claim. To be compatible with the QTPP, drugs must display observable qualities, such as globule size and flocculation, burst timings (which signal more quickly solubilization of the drug in the gastrointestinal fluid), and other patient-focused quality characteristics (QAs). The QTPP-established CQAs were responsible for providing the product with significant characteristics, safety, and effectiveness, indicating major alterations when QTPP is adjusted [135].

Risk assessment

The study of components and process characteristics that have a more pronounced influence on the efficacy of the medicine is taken into consideration when producing dosage forms within the framework of quality by design. To study the probable interactions between drugs integrating excipients using various unit operations and a risk assessment approach were applied to forecast the probable risks or failures, if any. Using Minitab 16, the Ishikawa fish-bone diagram (Minitab 17 software, M/s Minitab Inc., Philadelphia, USA) was designed to speed the risk assessment process and identify the main likely causes and minor details that have an influence on the CQAs of drugs [136].

3.2.5A Formulation and development of benidipine SNEDDS

3.2.5A1. Screening of Oil, Surfactants, and Co-Surfactants

Screening of Oils

In the shaking flask technique, numerous modified oils, surfactants, and co-surfactants have been proven to exhibit transparency as well as quick emulsification, and such characteristics were applied in selecting the oils. Excess BD was transferred to screw-capped vials and mixed (Vortex mixer, Remi, Mumbai, India) for 30 seconds with 2 mL of each excipient. The container was shaken for 72 hours at 120 rpm in a water bath shaker at 37 ± 0.5°C (Rivotek, Mumbai, India). Following 72 hours, each container was rotated in a lab centrifuge (Remi Equipment, Mumbai, India) for 15 minutes at 3000 rpm. The supernatant had been separated using membrane filtration and filter paper with a 0.45 µm particle size. Methanol had been utilized to dilute a particular component. The total amount of the solubilized drugs has been estimated using a

predetermined equation. The study was performed three times, and the average results have been reported [36-38]. The excipients utilized were all generally regarded as safe (GRAS) and human-safe.

Surfactant and Co-Surfactant Screening

In order to find out the most suitable surfactant system for the given oily phase, the emulsification capacities of several surfactants have been evaluated. In summary, 300 mg of the defined oil phase was combined with 300 mg of the approved surfactant. To facilitate the mixing of the oil and surfactant, the liquid was then vortexed for 60 seconds. This isotropic system was measured appropriately at 100 mg and mixed with 25 mL of distilled water to produce a fine emulsion. The number of volumetric flasks required for producing a homogeneous emulsion has been calculated as a measure of the emulsification method's performance. After settling for two hours, the emulsions' % transmittance was assessed at 638.2 nm using a UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan). Many surfactants have been studied for their ability to emulsify, utilizing double-distilled water as a control in order to find the best surfactant for the purpose it serves.

Co-surfactants may improve the capacity of nano-emulsification [123]. By adding 200 mg of surfactant to 100 mg of co-surfactant, a total of 300 mg of Labrafil M2125 CS was added, and the mixture was vortexed to generate homogeneity in order to analyze the relative efficiency of surfactants. To generate a fine emulsion, the isotropic system was weighed appropriately and diluted with double-distilled water. For testing emulsification ease, the number of volumetric flask inversions necessary to generate a homogeneous emulsion was calculated. The emulsions were then tested for transparency at 638.2 nm using double-distilled water as a blank on a UV spectrophotometer. As an outcome of examinations of solubility, surfactants and co-surfactants have been used to emulsify the oil [124].

3.2.5A2. Building of a Ternary Phase Diagram

Several lipid-to-emulgent ratios ranging from 1:9 to 9:1 were employed to define the boundaries of the nanoemulsion area. Ternary phase diagrams have been created at 37

°C, utilizing chosen surfactants and co-surfactants in different weight ratios in the oil phase and surfactant to co-surfactant mixer (Smix) (1:1, 2:1, and 3:1). The oil phase and each Smix ratio were properly incorporated utilizing a vortexing method. The mixtures were vortexed for two to three minutes before being first incubated at 37 °C with continuous shaking to generate equilibrium. The transparency of the mixture was evaluated visually. The nanoemulsion was developed using the sample, which was clear or slightly blue. The transparent and isotropic characteristics of the ternary diagrams were identified employing oil, surfactant, and co-surfactant [125]. Each trial was done three times.

3.2.5A3. Preparation of BD-Loaded SNEDDS

A predefined amount of oil was placed into a screw-capped glass vial bearing 4 mg of correctly weighed BD, which was then heated in a water bath at 37°C. This oily mixture was combined with the suitable surfactant and co-surfactant before being homogenized in a rotating motion. The formulations were then held at room temperature for one further 15 minutes of continuous sonication [126].

3.2.5A4. Factor Screening Studies

A few factors among the innumerable are shown in screening tests to explain a majority of the experimental variance, resulting in a phenomenon known as the "sparsity effect." These factor screening tests were carried out as a resource to develop the test for identifying the core few CMAs and/or key process parameters (CPPs) that have a large influence on the response variable, or CQA [127]. To analyze independent variables' effects on essential features, the influences of the oil (Labrafil M2125 CS), the surfactant (Kolliphor EL), and the cosurfactant (Transcutol P) were investigated as shown in table 3.8.

The optimization process of the SNEDDS has been carried out using response surface methodology (RSM) with the assistance of design expert® software (version 13.0.4.1, State Ease Inc., Minneapolis, MN, USA). This is a frequently utilized statistical model that is used to examine the impact of certain factors on experimental results. It is additionally utilized to optimize many different parameters in the multivariable system.

Sr. No.	Independent variables	Dependent variables	Goals for dependent variables
1	Quantity of oil (X_1)	Emulsification time	Minimize
2	Quantity of surfactant (X_2)	Droplet size	Minimize
3	Quantity of co-surfactant (X_3)	%Drug release at 15 min	Maximize
4		% Transmittance	Maximize

Table 3.7. Three-factor CCD experiment design grid with variable for SNEDDS of BD

Composition and limits of the experimental domain						
Factor	**Role**	**Values**				
		-α	**-1**	**0**	**1**	**+ α**
Labrafil M2125 CS	Oil	13.18	20	30	40	46.82
Kolliphor EL	Surfactant	36.59	40	45	50	53.41
Transcutol P	Cosurfactant	16.59	20	25	30	33.41

Table 3.8. Variables in CCD for SNEDDS of BD

3.2.5A5. Optimization of Benidipine SNEDDS

The relationship between the topography of the response surface, which includes the maximum, local, minimum, and ridge lines, and the regions where the most optimized response arises is the basis of this study. The Box-Behnken design (BBD) and the Central Composite Rotatable Design (CCRD) are two well-known experimental designs used in Response Surface Methodology (RSM). The CCRD was selected to evaluate the influence of process parameters on the expected outcomes. The CCRD uses mathematical and statistical tools to define and analyze issues connected to the response of interest, which includes variable matrices. Additionally, it is helpful to obtain significant data on the desired reaction while decreasing the number of experimental tries. Therefore, it accelerates up the optimization process by simplifying the identification of variable components and determining the optimal range for the intended outcome [128].

CCRD is often favored over BBD due to the inclusion of two additional extreme values, namely + α and − α, in addition to the low, mid, and high values of independent variables. The two extreme values give essential rotatability to the design. In order to improve the formulation, a central composite screening technique was adopted to analyze the main impacts of the interaction terms of different elements on the various SNEDDS characteristics. An SYSTAT version 13 experimental design component is currently in development for a five-level, three-factor rotating CCD (α = 1.68). Eight factorial points and six axial points composed the design [129].

According to Table 3.7, the extremely important features for investigation in the highly influential formulation have included globule size (D_{nm}), percentage transmittance (%T), self-emulsification time (T_{emul}), and percentage of drug release in 15 minutes (Rel_{15}). 15 experimental runs were carried out based on the finalized CCD, with the component factors such as oil ((X_1), surfactant (X_2), and co-surfactant (X_3) as shown in Table 3.9.

The optimization of drug release within a 15-minute time period in the context of S-SNEDDS of Benidipine is determined by many variables. First, it assures that Benidipine will have the expected therapeutic effects quickly since it conforms to its pharmacokinetic profile and therapeutic window. Second, it assists in achieving the goals of improving drug solubility, dissolution, and absorption that were set out during formulation design. The third factor might be the laws and instructions for determining bioequivalence and drug release characteristics, as well as the criteria for in vitro dissolution testing. Overall, receiving fast drug release within 15 minutes increases therapeutic effectiveness, achieves formulation goals, and fulfills regulatory expectations.

These SNEDDS formulations were further examined for the previously mentioned critical features. The coefficients for every single component of the key quality parameters have been obtained as follows after the elimination of the interaction effects between the variables using the design's non-linear quadratic model.

Batch	Coded Level			X1	X2	X3
	X_1	X_2	X3	Labrafil M2125 CS (%)	Kolliphor EL (%)	Transcutol P (%)
BD1	-1	-1	-1	20	40	20
BD2	1	-1	-1	40	40	20
BD3	-1	1	-1	20	50	20
BD4	1	1	-1	40	50	20
BD5	-1	-1	1	20	40	30
BD6	1	-1	1	40	40	30
BD7	-1	1	1	20	50	30
BD8	1	1	1	40	50	30
BD9	-1.682	0	0	13.18	45	25
BD10	1.682	0	0	46.82	45	25
BD11	0	-1.682	0	30	36.59	25
BD12	0	1.682	0	30	53.41	25
BD13	0	0	-1.682	30	45	16.59
BD14	0	0	1.682	30	45	33.4
BD15	0	0	0	30	45	25

Table 3.9: Coded value and converted value for the proposed matrix according to the CCD design batches

These SNEDDS formulations were further examined for the previously mentioned significant characteristics. The coefficients for every single component of the key quality parameters have been obtained as follows after elimination of the interaction effects between the variables using the design's generated non-linear quadratic model.

$$Yi = b_0 + b_1X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{23} X_2 X_3 + b_{13} X_1 X_3 + b_{11} X_{12} + b_{22}X_{22} + b_{33} X_{32} \quad \text{……. (3.1)}$$

Yi is the dependent variable, b_0 is the average answer over 15 runs, and bi is the predicted coefficient for factor Xi. The primary affects (X_1, X_2 and X_3) represent the average outcome of collectively modifying each element from its minimal to its maximum values.

When two or more components undergo modifications at the same time, the composition changes, as shown by the interaction terms (X_1, X_2, X_3, and X_1 X_3). The introduction of quadratic elements (X_{12}, X_{22}, and X_{32}) is designed to assess the model's non-linearity.

Also, by producing an overlay plot, the components of the optimal (checkpoint) batch were examined. The following formula was applied in obtaining the percentage of relative accuracy of each response to measure the effectiveness of the model:

$$\%\text{Relative Error} = \frac{\text{Predicted value} - \text{Experimental value} \times 100}{\text{Predicted value}} \quad \ldots\ldots (3.2)$$

3.2.5A6. Assessment of SNEDDS for Selected Responses

Responses that have been taken into consideration are Dnm, %T, Temul, and Rel15. Each formulation was diluted with water at a ratio of 1:100 while being gently stirred to attain equal dispersion of the formulation in the aqueous medium. Using the L-SNEDDS Malvern Zeta sizer, the average droplet size in nanometers of all 15 developed formulations was measured. Using a UV-visible spectrophotometer, the transmittance of these solutions was evaluated at 638.2 nm [177].

The study was carried out on a total of three distinct times. The L-SNEDDS self-emulsification efficacy was assessed by adding 0.1 mL of each formulation to 100 mL of distilled water maintained at 37±0.5°C. The formulations were tested while the solution was being stirred with a stainless steel paddle at an average speed of 50 rpm. Using USP Equipment II (paddle type) at 50 rpm with 0.1 N HCl as the dissolving fluid, the in vitro solubility of L-SNEDDS and pure drugs was evaluated. After appropriate dilution and examination on a UV spectrophotometer at 237 nm, an aliquot of the 5 mL sample was taken at different intervals. After 15 minutes, the drug release by different formulations was measured [130].

3.2.5B Formulation and development of benidipine with telmisartan SNEDDS

3.2.5B1. Screening of oils, Surfactants, and Co-Surfactants

Screening of oils

The shake flask process consisted of the evaluation of several kinds of modified oils, surfactants, and co-surfactants. Their transparency as well as their ability to quickly create an emulsion were among the criteria for assessment. The scores played an important role in selecting the appropriate oils for the study. Excessive amounts of benidipine (BD) combined with TEL are introduced into screw-capped vials and blended with 2 mL of each excipient for a duration of 30 seconds using a vortex mixer (Remi, Mumbai, India). Afterward, the vials were securely closed and agitated in a water bath shaker at a speed of 120 strokes per minute, maintaining a constant temperature of 37 ± 0.5°C (Rivotek, Mumbai, India) for a period of 72 hours. After this particular time period, a laboratory centrifuge (Remi Equipment, Mumbai, India) was used to rotate each vial at a speed of 3000 revolutions per minute for a duration of 15 minutes. The liquid portion was then isolated by passing it through a membrane filter and filter paper with a pore size of 0.45 μm. The obtained substance was mixed with methanol, and the quantity of dissolved medicine was measured using a standard formula. The experiment was repeated three times in its entirety, and the average results were reported. Furthermore, all the additives used for the research were universally acknowledged as safe (GRAS) and officially declared safe for human consumption [120-122].

3.2.5B2. Surfactant and co-surfactant screening

To establish the best-suited surfactant system for the necessary oily phase, several surfactants' emulsification abilities were tested. 300 milligrams of the specified oil phase were mixed with 300 milligrams of the required surfactant, followed by vortexing for 60 seconds. The resulting isotropic system was carefully measured at 100 mg and diluted with 25 mL of distilled water to form a fine emulsion. The minimal number of volumetric flask inversions required for homogeneity was assessed as an indicator of emulsification efficiency.

After a two-hour rest, the % transmittance of the emulsions at 638.2 nm was evaluated employing a UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan). To select the most effective surfactant, emulsification experiments were done using double-distilled water as a control. Co-surfactants were applied to increase nano-emulsification

capabilities [123]. After mixing 200 mg of surfactant with 100 mg of co-surfactant and 300 mg of eucalyptus oil, the liquid was vortexed for homogeneity. The generated emulsions were evaluated by a UV spectrophotometer for transparency at 638.2 nm, using double-distilled water as the blank [124]. The emulsifying procedure for the oil was carried out by adding surfactants and co-surfactants in accordance with the findings of solubility tests.

3.2.5B3. Construction of the Ternary Phase Diagram

In order to determine the limit of the nanoemulsion zone, a series of lipid-to-emulgent ratios ranging from 1:9 to 9:1 were employed. Ternary phase diagrams were constructed at a temperature of 37°C. The size of the nanoemulsion zones in the phase diagrams has been examined using an interchangeable combination of surfactant and co-surfactant at ratios of 1:1, 2:1, 3:1, 1:2, and 1:3. The oil phase and each Smix ratio have been thoroughly mixed using a vortexing process. After vortexing for two to three minutes, the mixtures were incubated at 37°C with vigorous shaking until equilibrium was established. The transparency of each combination was visually tested. Samples that were clear or slightly blue in color were selected for the purpose of producing nanoemulsion. A mixture of oil, surfactant, and co-surfactant has been employed to construct the ternary diagrams, focusing on increasing the transparency and isotropic qualities. Each experimental setup was done in triplicate to assure accuracy and reproducibility [125].

3.2.5B4. Preparation of Benidipine with Telmisartan-loaded SNEDDS as per the experimental design

The BD with TEL-SNEDDS was systematically optimized using the Box-Behnken design (BBD) in Design Expert® 13 software. BBD has been applied to optimize and analyze the main effects, interaction effects, and quadratic effects of the formulation ingredients of BD with TEL-SNEDDS. Fifteen formulations were produced through Design Expert® 13 software for the response surface approach based on the BBD at three variables and three-level designs. This methodology is useful for exploring quadratic response surfaces and creating second-order polynomial models. The design

includes repeating center points and a group of points sitting at the midpoint of each edge of the multidimensional cube.

Sr. No.	**Independent variables**	**Dependent variables**	**Goals for dependent variables**
1	Quantity of oil (X_1)	Emulsification time (Y_1)	Minimize
2	Quantity of surfactant (X_2)	Droplet size (Y_2)	Minimize
3	Quantity of co-surfactant (X_3)	%Benidipine release at 15 min (Y_3)	Maximize
4		%Telmisaratan release at 15 min (Y_4)	Maximize
5		% Transmittance (Y_5)	Maximize

Table 3.10: Three-factor BBD experiment design grid with variable for SNEDDS of BD with TEL

In screening tests, a select number of variables have been identified among the countless, contributing to the bulk of the experimental variance and resulting in the "sparsity effect. These factor screening tests were done as a strategy to identify the important critical material attributes (CMAs) and/or critical process parameters (CPPs) that considerably impact the response variable or critical quality attributes (CQAs) [126]. Critical quality attributes (CQAs) for L-SNEDDS of Benidipine with Telmisartan and their justifications were developed.

Formulations were made by heating accurately determined quantities of 4 mg BD and 40 mg Telmisartan in a screw-capped glass vial with a predetermined oil at 37°C. The heated oily mixture was then blended with appropriate surfactant and co-surfactant, homogenized utilizing a vortex motion, and sonicated for fifteen minutes at room temperature.

3.2.5B5. Factor screening studies

In the present work, extensive research was done utilizing a Box-Behnken design (BBD) to examine the effect of various components and their relationships on different elements of SNEDDS. The experiment involves 15 runs based on a Box-Behnken Design (BBD), with the component elements being oil (X1), surfactant (X2), and co-surfactant (X3). The influence of independent variables, such as Eucalyptus oil (oil), Kolliphor EL (surfactant), and Transcutol P (cosurfactant), on significant aspects was evaluated as shown in Table 3.11.

Composition and limits of experimental domain				
Factor	**Role**	**Values**		
		Low	**Medium**	**High**
X_1 % Eucalyptus Oil	Oil	50	55	60
X_2 % Conc. of Kolliphor EL	Surfactant	30	35	40
X_3% Conc. of Trancutol P	Cosurfactant	10	12.5	15

Table 3.11: Three-factor BBD experiment design grid with variable for SNEDDS of BD with TEL

According to Table 3.10, the extremely important features for investigation in the highly influential formulation have included. The response factors tested were self-emulsification time (Y1), droplet size Dnm (Y2), percentage of BD release in 15 minutes (Y3), percentage of TEL release in 15 minutes (Y4), and transmittance percentage (Y5). To analyze the generated SNEDDS, multiple regression analyses were carried out and a second-order polynomial model was created to match the BBD model. This enabled us to explore the relationships between the different factors and their influence on SNEDDS characteristics [127].

3.2.5B6. Assessment of SNEDDS for Selected Responses

The study measured numerous responses, which include globule size (Dnm), percentage transmittance (%T), self-emulsification time (Temul), and the amount of pure drugs released in 15 minutes. Every combination was diluted with water (1:100 ratio) and rotating quickly to produce uniform dispersion. The average droplet size of 15 formulations was assessed using the Malvern zeta sizer of the L-SNEDDS. Furthermore, the transmittance of these solutions has been determined at 638.2 nm using a UV-visible spectrophotometer (UV1800, Shimadzu, Japan). The self-

emulsification ability of L-SNEDDS has been assessed by adding each formulation to water at 37±0.5°C and rotating at 50 rpm. Furthermore, L-SNEDDS and pure drugs were studied for in vitro dissolution using USP Equipment II with 0.1 N HCl as the dissolving media and measured on a UV spectrophotometer at certain wavelengths for Benidipine and Telmisartan. A drug release study was done at 15 minutes across various formulation batches [128].

Batch	**Coded Level**			**X1**	**X2**	**X3**
	X1	**X2**	**X3**	**%Eucalyptus Oil**	**%Koliphor El**	**%Transcutol P**
BT1	1	1	0	60	40	12.5
BT2	0	0	0	55	35	12.5
BT3	0	1	-1	55	40	10
BT4	-1	0	1	50	35	15
BT5	-1	0	-1	50	35	10
BT6	-1	1	0	50	40	12.5
BT7	0	0	0	55	35	12.5
BT8	1	0	1	60	35	15
BT9	1	-1	0	60	30	12.5
BT10	0	1	1	55	40	15
BT11	1	0	-1	60	35	10
BT12	0	-1	-1	55	30	10
BT13	0	0	0	55	35	12.5
BT14	0	-1	1	55	30	15
BT15	-1	-1	0	50	30	12.5

Table 3.12: Design plan of Box-Behnken Design batches for the prepared SNEDDS of BD with TEL

3.2.6. Characterization of L-SNEDDS Formulations of BD and BD with TEL

3.2.6.1. Visual Characterization

The L-SNEDDS formulation, consisting of BD, and BD with TEL has been diluted with 100 ml of distilled water and properly mixed. The spontaneously formed nanoemulsion was evaluated for homogeneity, clarity, and drug precipitation [130].

3.2.6.2. Emulsification Time Determination

After diluting the BD-loaded L-SNEDDS as well as BD with TEL–loaded L-SNEDDS formulations with distilled water, the amount of time required for nanoemulsion creation was observed [131].

3.2.6.3. Calculating the Liquefaction Time

Each BD-containing L-SNEDDS and BD with TEL loaded L-SNEDDS mixture had been placed in an opaque polythene bag, which was subsequently attached to the thermometer's bulb. The melting time was obtained using a thermo-regulated heating mantle that maintained the thermometer with the accompanying formula at 37°C. For the purpose of this study, a round-bottomed flask containing 250 mL of 2.0% SLS has been utilized [132].

3.2.6.4. Cloud Point Temperature Measurement

The temperature at which a clear nanoemulsion turns cloudy is referred to as the cloud point temperature. This was done by periodically raising the temperature of BD-loaded SNEDDS and BD with TEL–loaded L-SNEDDS (1 mL) while shaking them on a hot plate magnetic stirrer until they created a cloudy emulsion [132].

3.2.6.5. *Invitro* Dissolution

The current study applied the USP dissolution test equipment-II paddle method (Electrolab, test apparatus, USA) to analyze in vitro drug dissolution for BD-loaded SNEDDS as well as BD with TEL–loaded L-SNEDDS formulations. The following conditions were applied in the dissolution study: 900 ml of the dissolving media, a 50 rpm paddle speed, and a 37±0.5°C mean temperature. The experiment was done employing three distinct dissolving media, namely simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and distilled water.

At predetermined time intervals of 5, 10, 15, 20, 25, 30, 45, and 60 minutes, the test solution of 5 ml was calculated and replenished using the same quantity of the dissolving medium. After that, the mixtures were passed through a 0.45 µm Whatman filter paper (Whatman, NJ, USA). Before being spectrophotometrically analyzed at 237 nm using a UV-visible spectrophotometer for BD SNEDDS and analyzed at specified

wavelengths for the estimation of benidipine and telmisartan for BD with TEL SNEDDS using a UV-visible spectrophotometer, the collected samples were suitably diluted. Every sample has been taken three times [133].

3.2.7. Evaluation of Optimized Formulation of L-SNEDDS of BD and BD with TEL

3.2.7.1 Droplet Size and Zeta Potential Measurement

With the use of a Zetasizer Nano, dynamic light scattering (DLS) was performed to analyze the mean droplet diameter (Z-average) of the improved SNEDDS. For this technique, 1 mL of BD-loaded SNEDDS and BD with TEL -loaded SNEDDS was extracted from the stock formulation. It was centrifuged for 15 minutes at 10,000 rpm. This centrifuged SNEDDS formulation's supernatant solution was extracted and mixed with 5 mL of distilled water. To achieve maximum dispersion, mild agitation was also utilized. The polydispersity index, particle size, and zeta potential were all examined [134].

3.2.7.2 pH

A digital pH meter (Systronics, India Ltd., Ahmedabad, India) had been used for determining the nanoemulsion pH [134].

3.2.7.3 Drug Encapsulation Efficiency

To measure drug entrapment efficacy, one mL of the BD-loaded SNEDDS and BD with TEL -loaded SNEDDS mixture was generated and dispersed into 10 mL of methanol. This was rotating at 3000 rpm for 10 minutes. The resultant solution was vortexed, filtered, and diluted with methanol. Using a UV-visible spectrophotometer (UV 1800 Shimadzu, Mumbai, India), the drug's content of BD was determined at 237 nm for BD SNEDDS and the drug concentration for Benidipine and Telmisartan at particular wavelengths for BD with TEL SNEDDS [135].

3.2.7.4 Rheological Study

The viscosity of the developed nanoemulsion was determined using a Brookfield viscometer (Brookfield Engineering Labs, St. Louis, MO, USA) with spindle no. 61

getting started at 20 rpm at 25°C after the BD-loaded and BD with TEL loaded optimized L-SNEDDS formulation had been blended with water in a ratio of 1:250 [135].

3.2.7.5 Conductivity Measurement

A deluxe conductivity meter from MS Electronics (Haryana, India), operating at 50 Hz, was the instrument utilized to measure the conductivity. Thermostatic control was applied to maintain the temperature at 30°C with a limit of 0.5°C [135]. After dilution with water, SNEDDS were examined for conductivity after dilution with water (1:50).

3.2.7.6 Thermodynamic Stability Investigations

Numerous thermodynamic stability studies have been conducted utilizing BD-loaded L-SNEDDS formulations and BD with TEL loaded L-SNEDDS, including centrifugation stress testing and heating-cooling cycles [136]. For the cooling and heating cycles, the SNEDDS formulation was maintained at ambient temperature for no more than 48 hours, and it was additionally stored in a refrigerator between 4 and 8°C. Each of these configurations, all of which are constant at this temperature, was further studied using centrifugation. After completing the heating-cooling cycle, the formulations undergo a 30-minute centrifugation at 3500 rpm. Three freeze-thaw cycles at -21°C and +25°C were successfully carried out for the BD-loaded L-SNEDDS and BD with TEL loaded L-SNEDDS formulations, with storage at each setting lasting at least 48 hours.

3.2.7.7 Robustness to Dilution and pH Change

To study the ways the composition behaved when exposed to increasing amounts of fluids in the stomach, dilution testing of BD-loaded SNEDDS of BD14 and BD with TEL loaded L-SNEDDS of BT11 in 0.1N HCL, a phosphate buffer pH 6.8, and water has been carried out [136]. So each sample was periodically examined for purity or precipitation instantly, after 1, 2, and 3 hours.

3.2.8. Preparation of S- SNEDDS

Issues with L-SNEDDS, such as using solid carriers with high surface areas, may help with poor stability, drug leakage, SNEDDS interactions with capsule shells, etc. This

S-SNEDDS combines the benefits of a solid dosage form with L-SNEDDS. L-SNEDDS is absorbable and may be transformed into combining carriers such as Aerosil 200, Aeroperl 300, and Neusilin US2 to generate a free-flowing powder in different weight ratios of 1:1, 1:1.5, and 1:2.

Carriers are porous in nature and provide a high surface area for the adsorption of a significant quantity of oils. L-SNEDDS were incorporated progressively into an adsorbent-filled mortar and pestle, mixed for adsorption onto a solid carrier, and then passed through a # BSS 30 sieve to generate a consistent, fluid powder [137].The selection of the most effective solid carrier and its concentration required for formulation solidification was determined by adding increment quantities (100mg) of solid carrier to the optimized liquid SNEDDS until the liquid was entirely adsorbed and solidified effectively. The powder has been produced and then put inside hard hydroxy propyl methyl cellulose (HPMC) capsules for further investigation.

A micromeritic analysis of the powders' bulk density, tapped density, Hausner's ratio (HR), Carr's index (CI), and angle of repose (AR) were also undertaken. As per the scientific literature, bulk density has been utilized to calculate HR and CI. AR was measured using the static funnel method. The flow ability of the porous carriers was tested employing the flow property analysis of the micromeritic data. Finally, based on these responses, the most effective solid carrier-L-SNEDDS ratio was determined [138].

3.2.9. Characterization of S-SNEDDS of BD and BD with TEL

3.2.9.1. Fourier Transform Infrared Spectroscopy (FTIR) Study

Compatibility studies between BD, TEL, oil, Smix, and Neusilin US2 were identified by evaluating the peaks from an FTIR study. The FTIR for BD, Neusilin US2, and S-SNEDDS of BD14 were generated as a physical combination using the FTIR-6100 (JASCO, Tokyo, Japan) for BD. The FTIR for BD, TEL, Neusilin US2, and S-SNEDDS of BT11 were generated as a physical combination for BD with TEL. From 4000 to 400/cm, the compounds were scanned using an FTIR spectrophotometer [134].

3.2.9.2. Thermo-Scanning Differential (DSC)

The metal containers with crimped tops were used to store the samples. Using a nitrogen gas stream, the sample and reference pans were continuously heated in the heating chamber from 100 to 400°C at a scanning rate of 10°C/min [135].

3.2.9.3. Morphology Analysis of S-SNEDDS

An analysis of the S-SNEDDS of BD's and S-SNEDDS of BD with TEL's exterior characteristics was carried out using scanning electron microscopy (SEM). On aluminum stubs were attached the S-SNEDDS of BD-14 powder and pure drug samples using one-sided tape. Similarly for S-SNEDDS of BT11 powder and pure drug samples BD with TEL using one-sided tape. The sample coated with gold was tested using an activation voltage of 15 kV and a working distance of 8 mm [136].

I. Surface morphology using Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) (Zeiss, Germany) was used to determine the surface morphology of BD, TEL, the pure solid carrier neusilin US2 and S- SNEDDS of BD 14 and S- SNEDDS of BT11. All samples were spread over the double sided bi-adhesive carbon tape, which was attached with a metallic stud. The sample coated with gold was tested using an activation voltage of 15 kV and a working distance of 8 mm.

II. Surface morphology using Transmission Electron Microscopy (TEM)

The shape and size of droplets produced following the reconstitution of L-SNEDDS were determined using TEM. A single drop of reconstituted sample was placed on a 200 mesh carbon coated grid and then stained with 2% uranyl acetate. After drying, the grid with stained samples was visualized under TEM (FEI Company, USA) at an accelerating voltage of 100 KV using the FEI Xplore3D software TEM (FEI Company, Hillsboro, USA).

III. Powder X-ray Diffraction (PXRD)

Making use of particle diffraction with X-ray methods, the crystalline phase of the pure drug and the S-SNEDDS of BD 14 and S-SNEDDS of BT 11 were examined [137]. Using a powder X-ray diffractometer with Ni-filtered CuK radiation at a voltage of 40 kV and an electrical current of 25 mA, the patterns of X-ray diffraction of pure drug,

powder S-SNEDDS of BD 14 and powder S-SNEDDS of BT11 have been collected. Using a scanning rate of 1°/min, the pattern of diffraction was analyzed across a 20-degree range from 10-80°.

3.2.9.4. In vitro dissolution of S-SNEDDS

For the purpose of investigating in vitro drug dissolving characteristics, S-SNEDDS of BD14 and S-SNEDDS of BT11 were inserted in hard HPMC capsules. The filled capsules were placed in a beaker that dissolves in 900 mL of dissolving medium (0.1 N HCl, pH 1.2). Capsule sinkers were initially used to avoid the capsule floating in the medium. For this approach, the controlling strategies were 50 rpm and 37 ± 0.5 °C. Five mL samples were collected, filtered, and, at different times, an equal amount of the same medium was added. With the support of a UV-visible spectrophotometer, each specimen was analyzed. Experiments were done three times in order to produce a systematic and consistent mean result [135]. The S-SNEDDS of BD14 formulation's drug release profile was constructed utilizing a pure drug, the L-SNEDDS of BD14, and the marketed brand Z-Bene, Corazon (A Division of Arlak Biotech Private Limited), Punjab, India. Similarly drug release studies of the pure drug (BD with TEL), liquid SNEDDS of BT11, S-SNEDDS of BT11 compositions, and commercially available tablet Benidip T 4 mg/40 mg Tab (Precia Pharma Pvt. Ltd.) in Thane, India, have been carried out [136].

3.2.10. Optimization of S-SNEDDS of BD and BD with TEL

The quadratic model, encompassing the linear mixture and interactions between two components, was created for all the responses using an MLRA approach. Statistical analysis was performed using one-way analysis of variance with the level of statistical significance set at $p < 0.05$ [136]. Each model was graded based on various statistical criteria, including R^2, Adj. R^2, Pred. R^2 and AP. The significant effect ANOVA's F test, which was done using the development tool and had a 95% confidence level of $P < 0.05$, was used to assess the response. Depending on the models for each response, a contour map was constructed. The contour plots of each model were blended to generate an overlay contour plot that was used to find the most appropriate location based on product efficacy [137].

3.2.11. Pharmacodynamics Research of Benidipine and Benidipine with Telmisartan

All the experiments on animal were conducted as per the Institutional Animal Ethical Committee (IAEC) guidelines, New Delhi, with approved protocol number, ARI/PT/712/2022. The procedure had been authorized by Ahmedabad's 20th Institutional Animal Ethics Committee (IAEC) of Accuprec Research Labs Pvt. Ltd. The committee is duly approved by Government of India for the control and management of experimentations on animals. The animal center of Accuprec Research Labs Pvt. Ltd. (Ahmedabad, India) supplied the animals. Animals were acclimatized in normal laboratory atmosphere of temperature 25 ± 2 ° C with relative humidity of 65± 5%. Animals were kept overnight fasting with free access to water before the commencement of experiment. [139-140].

Ethical clearance for the experiments: The study has been approved by Institutional Animal Ethics Committee in 20th IAEC Meeting at Accuprec Research Labs Pvt Ltd, Ahmedabad (ARL/PT/712/2022).

The antihypertensive effectiveness of pure BD drug, BD with TEL drugs, optimized S-SNEDDS of BD14 and S-SNEDDS of BT11 was investigated in adult Wistar albino rats of either sex with weights ranging from 220 to 240 g.

Husbandry conditions:

a. Housing: Animals were housed in a polypropylene cage with a stainless steel grill on top. The bedding material was dry wheat husk (post hulled) that was changed every morning hours during acclimatization periods.

b. Acclimatization: The animals were acclimatized for one week prior to the start of the experiment.

c. Environment: Animals were exposed to 12 hours day and night cycles with a standard temperature of 23±3°C and the relative humidity was 50–60%.

d. Diet: The animals were fed with standard rat pellet feed provided by Keval sales Corporation, Vadodara throughout study period. The drinking water was provided in polypropylene bottles with stainless steel sipper tube at all times.

Six rats were allocated randomly to each of the six groups: hypertension control, normal control, hypertensive treated with the S-SNEDDS formulation, and hypertensive treated with standard drug BD and BD and TEL.

The study utilized 10% fructose dissolved in the water and delivered to Wistar rats to produce hypertension (the equivalent of taking in a meal containing 48–57% fructose) for two weeks, and it was associated with increased plasma levels of insulin, glucose, and triglycerides. All of the animals had unrestricted access to food and 1 percent NaCl.

Dose fixation: The drug dose was derived from the previously mentioned dose of Benidipine for animal studies.

Route of drug administration: The drugs were administered through oral route to respective groups and were prepared in distilled water (vehicle) as per dose level and same was administered in a uniform volume of 5 ml/kg body weight of albino rats.

Drug schedule: The test drug and vehicle were administered orally between 09:00 am to 09:30 am.

A tail cuff sensor technology was employed to non-invasively monitor blood pressure after two weeks utilizing a Biopack MP36 collection machine, the NIBP200A—small animal tail non-invasive blood pressure system (BIOPAC System Inc., USA). Following 2 weeks, systolic and diastolic blood pressure were measured by applying the Biopack data collection equipment, which represented the hypertension stage in rats except for the control group. All rats with a systolic blood pressure of 150 mm Hg were chosen. The species were separated into six groups:

Groups	Treatments	No. of animals
G1	Normal control group	1-6
G2	Hypertensive control (Disease Control)	7-12
G3	Hypertensive treated with formulation (SNEDDS of Benidipine)	13-18
G4	Hypertensive treated with standard Benidipine	19-24
GS	Hypertensive treated with formulation (SNEDDS of Benidipine with Telmisartan)	25-30
G6	Hypertensive treated with standard Benidipine with Telmisartan	31-36

Table 3.13: Study design for Pharmacodynamics study

After administration of test drugs to respective groups the fall of blood pressure in albino rats at different time intervals. Animals in groups 3 and 4 were administered a dosage of 2 mg/kg of S-SNEDDS of BD and standard BD. After administering the test medicines to each group, we measured the decrease in blood pressure at different time intervals (0, 2, 4, 6, and 8 hours). Group 5 and 6 animals were administered a dosage of 4 mg/40 mg BD with TEL/kg/day of S-SNEDDS of BD with TEL, along with regular BD with TEL. After administering the test drugs to their respective groups, blood pressure (BP) was recorded at 2, 6, 12, and 24 hours using the BioPack data acquisition devices. Each measurement has been carried out three times, and the mean result has been recorded [141, 142].

All experimental data's were expressed as mean values (measurement of BP) ± S.E.M and were subjected to bio statistical interpretation by SPSS windows version 20 statistical packages all the way through a one-way ANOVA followed by post-hoc test (Dunnett's 't' test) for multiple comparisons of the mean differences and responses of drugs and SNEDDS. Statistical significance of $P < 0.05$ were considered as level of significance [176,178].

3.12. Accelerated Stability Research

The optimized S-SNEDDS of BD-14 and S-SNEDDS of BT11 formulation was submitted to a six-month accelerated stability test in a stability chamber (Nova Instruments Private Limited, Mumbai, India) at 40 ± 2°C temperature and 75± 5% RH. S-SNEDDS BD-14 and S-SNEDDS BT 11 was packed in HPMC capsules, sealed, and preserved in the stability chamber in a glass container with a cotton stopper. After 0, 1, 2, 3, and 6 months, the specimens were removed from the stability chamber and examined in 15 minutes for emulsification efficiency, globule size, percent transmittance, and release of drugs [135].

Statistical analysis

All the experiments were performed in triplicates, and all data are presented as mean ± SD. Statistical analysis was performed using one-way analysis of variance with the level of statistical significance set at $p < 0.05$ [136].