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Research Article

Design and development of bilastine-loaded self-nanoemulsifying drug delivery system (L-SNEDDS) for oral administration: Pharmacokinetic study (*In vivo* analysis)

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ABSTRACT

Oral administration of a drug is the most common, ideal and preferred route of administration. The main problem of oral drug formulations is their low bioavailability arising from poor aqueous solubility of drugs. The study developed bilastine-loaded lipid-based self-nanoemulsifying drug delivery systems (SNEDDS) in liquid formulations. The components used in the SNEDDS formulations included lipid (Capmul PG-NF), surfactant (Tween 40), and co-surfactant (Plurol Oleique). The optimized formulation was characterized through *in-vivo* analysis. The liquid-SNEDDS (L-SNEDDS) formulation [TP3PO1 (2:8)] demonstrated greater potential, showing a reduced particle size of 142.5nm, zeta potential −22.6mV, PDI 0.183, and a drug loading efficiency of 99.71 ± 0.67%, respectively. The optimized formulation of L-SNEDDS [TP3PO1 (2:8)] showed a stable nanoemulsion, and FT-IR studies indicated no interaction between the drug and excipients. The *in-vivo* pharmacokinetic study of the optimized bilastine-loaded L-SNEDDS formulation performed using albino rats (weight of single rat 250±25 g) showed that the area under the curve AUC_{0-24} for the optimized formulation [TP3PO1 (2:8)] was significantly higher 1129.45 ± 10.815 µg/mL/h compared to the drug suspension 179.96 ± 2.368 µg/mL/h. Cmax was also higher for TP3PO1 (2:8) 90.368 ± 2.596 µg/mL compared to the drug suspension 34.32 ± 2.31µg/mL. The plasma half-life ($t_{1/2}$) of the optimized formulation TP3PO1 (2:8) was increased significantly 22.45 ± 0.251 hrs compared to that of the drug suspension 18.57 ± 0.312 hrs. Overall, a range of lipid-based SNEDDS liquid formulations was successfully developed and appears to be a promising alternative for improving the solubility of poorly water-soluble drugs and evaluating pharmacokinetic parameters.

Keywords:
Self-nanoemulsifying drug delivery system (SNEDDS); Capmul PG-NF; Tween 40; Plurol Oleique; Bilastine; Solubility enhancement; Pharmacokinetic study.

1. INTRODUCTION

The development of nano-based delivery systems to get around these restrictions has been made possible by advances in nanotechnology. polymeric nanoparticles, lipid-based systems (liposomes, solid lipid nanoparticles, nanoemulsion), and niosome are examples of these systems. The most widely used nano-based approaches for lipophilic drugs involve the incorporation of drugs into lipid vehicles such as self-emulsifying drug delivery systems and oil/surfactant dispersion micro and nanoemulsions. Drug delivery systems that self-nanoemulsifying (SNEDDS) has become an essential tactic for effectively delivering medications with low water solubility[1, 3]. Due to their ability to increase the surface area and decrease the size of easily digestible oil droplets that are incorporated into mixed micelles that can pass through the intestinal lumen,

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SNEDDS are widely recognized for their potential to improve the solubility and absorption of lipophilic drugs. Furthermore, because SNEDDS can inhibit efflux pumps and increase the lipid fluidity of enterocyte membranes, they have also been shown to increase trans-cellular permeability and improve oral bioavailability[2, 3] Compared to traditional micro and nanoemulsion, SNEDDS are superior due to their unique features, which include long term stability, patient compliance, palatability, dose reduction, ease of formulation, and scale-up synthesis. Additional characteristics of SNEDDS that improve oral bioavailability include decreasing gut enterocyte cytochrome-P450 metabolism, promoting lymphatic transport through payer-patches, and guarding against first pass metabolism. There are types of SNEEDS that have been reported that's liquid SNEDDS (L-SNEDDS)[4]. Spray drying, melt granulation, and adsorption on inert solids such as lactose, microcrystalline cellulose, and aerosol are the methods used to achieve solidification. bilastine (log P = 5.02, aqueous solubility 0.00203 mg/mL) is an anti-histamine antagonist drugs from phenyl derivative group. It belongs to biopharmaceutical classification system (BCS) class II due to its low solubility high permeability. Chemically, it is 2-(4-(2-(4-(1-(2-ethoxyethyl)-1H-benzo[d]imidazol-2-yl) piperidin-1-yl) ethyl) phenyl)-2-methylpropanoic acid, as shown in Figure 1, with a molecular weight of 463.62 g/mol and

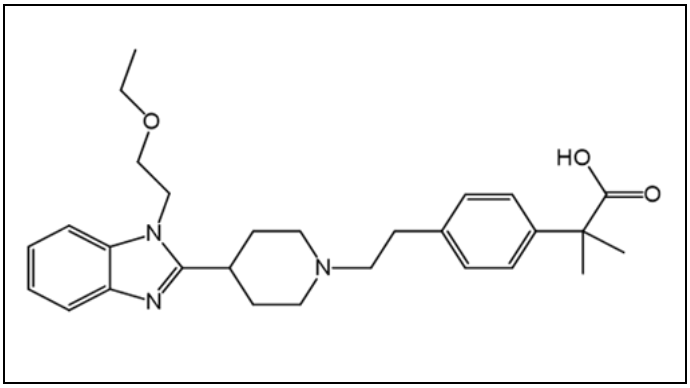

Figure 1. Structure of Bilastine

the chemical formula $C_{28}H_{37}N_3O_3$. When administered orally, it is incompletely absorbed with a bioavailability of approximately 61% (no accumulation pattern) and high protein binding of 84-90%. The time to reach maximum plasma concentration ranges between 1 and 13hrs following oral and intra-muscular administration respectively[6]. There is interaction with the cytochrome P_{450} system used to treat seasonal allergic rhinitis and chronic spontaneous urticarial. The extensive first-pass metabolisms are also thought to be responsible for such low oral bioavailability of bilastine. Moreover, there is a need to formulate bilastine in such a delivery system that can enhance its bioavailability by increasing solubility, permeability, and decrease first-pass metabolism. Therefore, incorporating bilastine into L-SNEDDS shall be a promising strategy to enhance its oral bioavailability[6].

This study aimed to improve the oral bioavailability of bilastine by designing and developing an optimized L-SNEDDS formulation using various oils, surfactants, and co-surfactants. After conducting a dispersibility test, the selected formulations were evaluated based on their percentage transmittance, drug content, zeta potential, particle size, and physical, chemical, and thermodynamic stabilities. Additionally, the formulations were assessed for the improvement of bilastine *in-vivo* oral bioavailability through the characterization of the plasma concentration-time profile in animal blood[7].

2. MATERIALS AND METHODS

Chromatographic analysis was performed using a chromatopak peerless column and an SPD M20A prominence auto sampler with a PDA detector, all operated with a Shimadzu Model (SHIMADZU USD-1100, Japan) HPLC system. The HPLC system was controlled using Lab Solution software. The method employed a room-temperature C18 column (150 mm × 4.60 mm, 5 µm particle size). The mobile phase was prepared by mixing acetonitrile and methanol in a 1:1 ratio[1, 4].

Bilastine was provided by Hetero Drugs Pvt. Ltd., Hyderabad. The material such as Olive oil, Capmul PG-NF, Crodamol PC-LQ, Crodamol P30, Labrasol, Tween 40, Labrasol ALF, Labrafac IF, Span 80, Transcutol P, Transcutol HP, Lauroglycol 90, Plurol Oleique, and Capryol were obtained from Gattefosse, Mumbai[9]. Acetonitrile, methanol, and hydrochloric acid of analytical reagent grade were purchased from Rankem Chemicals. NeusilinUS2 was a gift from Hetero Drugs Pvt. Ltd., Hyderabad. Purified water was used throughout the study. HPLC-grade acetonitrile and methanol were sourced from Merck Life Science Pvt. Ltd., Mumbai, India. All chemicals used in the research project were of analytical grade[8].

2.1 Method for analysis of bilastine using UV spectrophotometry

A UV spectrophotometric method was used for the quantitative analysis of bilastine. The working stock solution of bilastine aliquots 0.5, 1, 1.5, 2 and 2.5 mL were transferred into various volumetric flasks with a 10 mL capacity. Diluents were used to adjust the volume to the required concentration of 5, 10, 15, 20, and 25 µg/mL.

At 205nm, the solutions' absorbance was measured. Plotting absorbance against drug concentration allowed for the construction of the calibration curve, and regression equations were generated[11].

2.2 Method for analysis of bilastine using HPLC

A High-Performance Liquid Chromatography (HPLC) method was developed for the quantitative analysis of bilastine. A calibration curve was constructed over a concentration range of 5–100 µg/mL. To prepare the stock solution, 100 mg of pure bilastine was accurately weighed and transferred into a 100 mL volumetric flask. The volume was adjusted to the mark using HPLC-grade methanol to obtain a primary stock solution with a concentration of 1000 µg/mL. From this, 1 mL was pipetted into another 100 mL volumetric flask and diluted with HPLC-grade methanol to yield a 100 µg/mL working stock solution. Prior to analysis, the solution was filtered through a 0.45 µm membrane filter to remove any particulate matter[10].

Aliquots of 0.5, 1, 2, 2.5, 5, 7.5, and 10 mL of the working stock solution were transferred into separate 10 mL volumetric flasks, and the volume in each flask was brought up to the mark with the same diluent to prepare final concentrations of 5, 10, 20, 25, 50, 75, and 100 µg/mL, respectively. Each prepared solution was then injected into the HPLC system under optimized chromatographic conditions. The chromatograms obtained were used to record the mean peak area corresponding to each concentration. A calibration curve was plotted with bilastine concentration on the x-axis and the mean peak area on the y-axis. This curve was used to verify linearity and to quantify bilastine in subsequent analyses[6, 7].

2.3 The method for the FTIR study

Fourier Transform Infrared Spectroscopy (FTIR) was used to examine chemical bonding properties and analyse the functional groups in the samples. The (PerkinElmer Spectrum) spectrometer, which operates in the 4000–400 cm^{-1} range, was used to record the FTIR spectra. Using the attenuated total reflectance (ATR) technique and the potassium bromide (KBr) pellet method, samples were prepared based on compatibility and physical condition.

In the KBr technique, the sample was finely milled with dry KBr powder at an approximate ratio of 1:100 and then compacted into a clear pellet under vacuum conditions. The resolution of each spectrum was 4 cm^{-1}, and averages of 32 scans were made to minimize background noise and guarantee signal purity. Using the instrument's software, baseline correction and normalizing were carried out to ensure precise peak identification. In order to determine which distinctive absorption bands corresponded to different functional groups, such as -OH, -NH, -CH, C=O, C=C, and others relevant to the materials under the study, the resulting spectra were evaluated[11].

2.4 Solubility studies of bilastine

The solubility of bilastine was determined using different oils (Olive oil, Capmul PG-NF, Crodamol PC-LQ, Crodamol P30) as well as synthetic and semi-synthetic oils, such as Capmul MCM EP. Surfactants and co-surfactants, including Lauroglycol 90, Plurol Oleique, Labrasol, Tween 40, Labrasol ALF, Labrafac IF, Span 80, Transcutol P, Transcutol HP, and others, were also tested[10]. The drug was placed in a 5 mL stoppered glass vial and mixed with each component for 10 minutes using a vortex mixer. The vials were then placed in an isothermal shaker (GFL1092, Burgwedel, Germany) at 50 ± 1.0°C for 72 hours until homogeneity was achieved[11]. Centrifugation was performed at 3000 rpm for 10 minutes. After centrifugation, the supernatants were diluted in a mixed solvent and filtered through a 0.45 µm syringe filter. The drug concentration was determined using a UV spectrophotometer (Shimadzu UV-240 model, Japan) method[13].

2.5 Selection of excipients for formulations

The different ratios of oils, surfactants, and co-surfactants were used to assess bilastine solubility. Based on particle size and potential, four prepared formulations demonstrated optimal size in the nano range, along with favorable zeta potential and polydispersity index (PDI) characteristics. The optimized formulation used Capmul PG-NF (oil), Tween 40 (surfactant), and Plurol Oleique (co-surfactant)[4, 9].

2.6 Pseudo-ternary phase diagrams

To identify the nanoemulsion regions and determine the appropriate composition of oil, surfactant, and co-surfactant for the SNEDDS formulation, pseudo-ternary phase diagrams were created. These diagrams revealed that, despite the drug's higher solubility in the systems, the formulations containing Capmul PG-NF oil as the oily phase, Tween 40 as the surfactant, and Plurol Oleique as the co-surfactant demonstrated stable nanoemulsifying properties. The TP3PO1 formulation exhibited a blue-tinged emulsion (BTE) for oil: Smix ratios of 1:9 and 2:8, and a clear transparent emulsion for the 3.7, 4:6, 5:5, 6:4, and 9.1 ratios with Smix 3:1. No liquid crystal phase appeared during the titration process[14]. Furthermore, it was found that the ability to form nanoemulsions decreased in six systems as the co-

surfactant proportion increased, particularly when the systems contained Plurol Oleique, Tween 40 as the surfactant, and Capmul PG-NF oil as the oily phase[17]. This observation also highlights the role of the surfactant in forming the proper range of the nanoemulsion. A Figure 5 illustrates the nanoemulsion regions observed in the formulations. Table 2 provides the percentage composition of water: Smix and oil used during the titration[16].

2.7 Formulation of L-SNEDDS of bilastine

The formulation of L-SNEDDS, various ratios of oil, surfactant, and co-surfactant were selected. An accurately weighed 100 mg of bilastine was dissolved in the appropriate amounts of oil/surfactant mixture. The formulations were then heated in a water bath at 40 °C to aid in drug solubilization, after being cyclomixed for one minute to ensure uniform mixing. Following this, all formulations were cycle-mixed until transparent preparations were achieved[18]. The finalized L-SNEDDS of bilastine were left aside for 48 hours at room temperature. The formulations were characterized for various parameters and checked for any signs of turbidity or phase separation[7, 10].

2.8 Evaluations of L-SNEDD

2.8.1 Self-nanoemulsification and visual assessment

The nanoemulsion was added to 100 milliliters of water. An opaque, milky white appearance indicated the formation of a macroemulsion, while the presence of a clear, isotropic, transparent solution following the dilution of L-SNEDDS with water confirmed the formation of the nanoemulsion. The stability of the formulation was demonstrated by the absence of phase separation and/or precipitation[19].

2.8.2 Thermal stability testing

The effect of temperature and centrifugation on the stability of nanoemulsion was investigated through thermal stability testing conducted on the prepared L-SNEDDS formulations[15]. According to ICH guidelines, the SNEDDS formulation was put through thermal stability tests under different conditions such as accelerated (40 ± 2 °C/75 ± 5% RH) and long-term (25 ± 2 °C/60 ± 5% RH). To evaluate stability, samples were routinely examined for droplet size, PDI, drug content, and physical appearance.

2.8.3 In-vitro drug dissolution testing

An *in-vitro* drug dissolution study was performed on a standard drug using a USP Apparatus II (paddle method) in 900 mL of 1N HCl, used as the dissolution medium. The temperature of the dissolution medium was maintained at 37 ± 0.5 °C and the paddle rotation speed was set at 50 rpm. Aliquots of 5 mL were withdrawn at predetermined time intervals and immediately filtered and analysed using UV spectrophotometer system (Shimadzu UV-240 model). The standard curve of bilastine was plotted between absorbance and concentrations. From these values, the cumulative percentage drug release and the percentage drug release of L-SNEDDS of bilastine were calculated. The *in vitro* drug release study was carried out using 1N hydrochloric acid to replicate extremely acidic environment of the human stomach (pH.1.2), which is relevant for evaluating the release behaviour of orally administered drugs, especially those that are expected to dissolve in the gastric fluid before absorption[16, 19].

2.8.4 Determination of drug loading efficiency (DLE)

The drug loading efficiency was evaluated by quantifying the amount of drug successfully incorporated into the SNEDDS pre-concentrate.

$$\text{Drug Loading Efficiency (\%)} = \frac{\text{Amount of drug solubilized in SNEDDS}}{\text{total amount of drug added}} \times 100\%$$

2.8.5 Drug content studies

The methanol was used to dilute the required amount of L-SNEDDS formulation. The volume was adjusted to 25 mL using 1 mg/mL of methanol. The resulting solution was withdrawn, and 0.1mL (100 µg) was further diluted with 10 mL of methanol to obtain a concentration of 10 µg/mL. Aliquots of 40 ppm diluted sample were put into cuvettes then kept into the UV spectrophotometer system for analysis at the specified λmax[9, 10].

2.8.6 Centrifugation observation

After adding the formulations in a 1:20 ratio to deionized water, the mixture was centrifuged for 30 minutes at 3500 rpm to check for phase separation or precipitation[3, 5].

2.8.7 Freeze thaw cycle

The freeze-thaw cycle was applied to the formulations that remained stable after centrifugation. In this study, SNEDDS formulations were diluted in a 1:20 ratio with deionized water and then stored at two different temperatures for 48 hours each, resulting in two freeze-thaw cycles between -20 °C and +25 °C. After 48 hours, the samples were checked for phase separation or precipitation[7, 9].

2.8.8 Determination of droplet size and zeta potential

By using a cyclomixer, 1mL of nanoemulsion from the prepared SNEDDS formulation was mixed with 10 mL of distilled water for one minute. It was then set aside for 30 minutes. The droplet size, PDI, and zeta potential of the emulsions were determined using a zetasizer (Malvern)[20].

2.9 In vivo pharmacokinetic study

In vivo pharmacokinetic studies were conducted using albino rats (with an average body weight of 250±25 g), through oral administration as shown in Figure 8. The animals free from infections were kept at a temperature of 25 to 30°C and a humidity of 65% along with food and water. The study was conducted according to protocol #IAEC-III-PRIP-FEB-2023-Protocol 04, which was approved by the Animal Ethical Committee for the use of animals in *in-vivo* studies[1, 4]. The animals were divided into 4 groups (n = 2) and housed one day prior to the experiment, with free access to food and water. The optimized nano formulation TP3PO1 (2:8) and drug suspension were administered orally through gavage at a concentration of 25 mg per 250±25g body weight of albino rats[7, 9]. Blood samples (0.5 mL) were collected from the tail vein at predetermined time points (0, 1, 2, 4, 6, 8, 12, and 24 hours) into anti-clotting tubes and centrifuged at 5,000 rpm for 10 minutes at 4 °C temperature (Thermo Fisher Scientific, Germany, ST-8R model). The plasma was separated, transferred into separate containers, and stored in a freezer at -20°C until further analysis. The drug was extracted from plasma samples using liquid-liquid extraction by adding 200 µL of chilled acetonitrile and 150 µL of methanol, followed by vortexing for 5 minutes and centrifugation at 1,500 rpm for 10 minutes. The supernatant of each sample was then transferred to labeled HPLC vials and analyzed sequentially on HPLC using the method described above[1, 9]. Peak areas corresponding to concentrations were recorded, and a graph of areas versus time was plotted to calculate the plasma drug concentration for the formulations. Pharmacokinetic parameters of orally administered bilastine were obtained using non-compartmental pharmacokinetic analysis of plasma concentration-time data[5, 3]. PK Solver (a free Microsoft Excel add-in) was used to calculate the area under the curve from the concentration versus the time curve to the last measured time ($AUC_{0–24}$) and other pharmacokinetic parameters. All values were expressed as mean ± SD[21].

3. RESULTS AND DISCUSSION

3.1 Calibration curve by using UV spectrophotometry

A calibration curve for bilastine was draw based on the absorbance values obtained using UV spectrophotometry. Standard solutions with concentrations of 5, 10, 15, 20, and 25 µg/mL were analyzed at 205 nm, and their absorbance was recorded. The resulting data was used to plot a calibration curve of absorbance versus concentration, as shown in Figure 2. The plot demonstrated a strong linear relationship between concentration and absorbance, indicating adherence to Beer-Lambert's law over the studied range. The regression equation derived from the calibration curve exhibited a correlation coefficient (R^2) of 0.994, confirming the linearity and reliability of the method for quantitative analysis of bilastine.

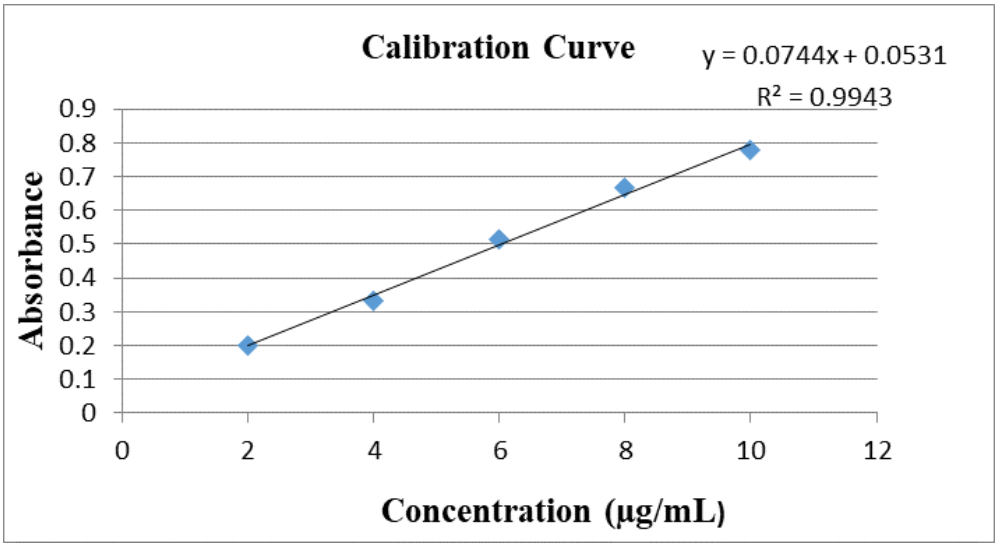

Figure 2. Standard curve of Bilastine

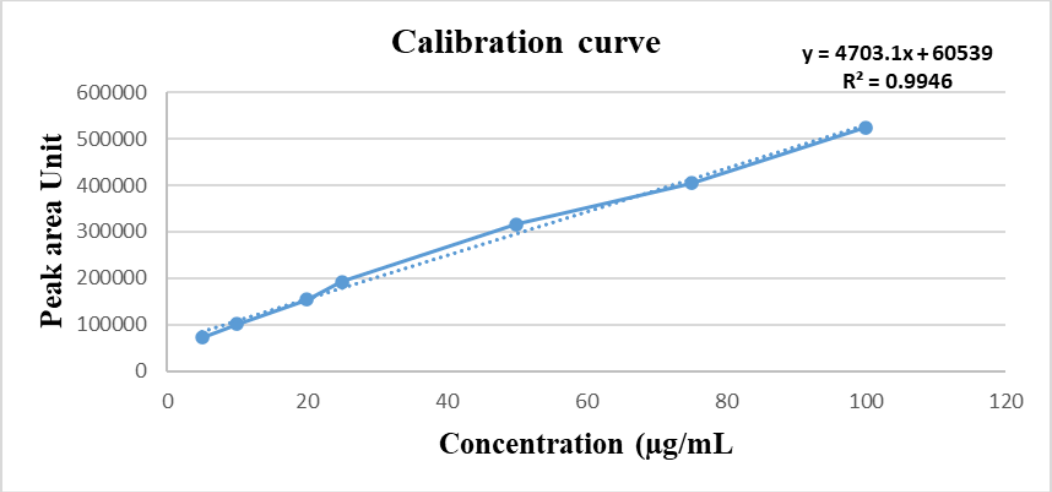

Figure 3. Standard curve of bilastine using HPLC

3.2 Calibration curve by using HPLC

The calibration curve for bilastine was draw using High-Performance Liquid Chromatography (HPLC) over a concentration range of 5–100 µg/mL. The analysis demonstrated good linearity within this range. The linear regression equation obtained was: y = 4703.1x + 60539, with a correlation coefficient (R^2) of 0.9946, indicating a strong linear relationship between concentration and peak area. The calibration curve is presented in Figure 3[2, 3].

3.3 Solubility study

The solubility of the drug in the ingredients plays a crucial role, as formulations often cause precipitation before experiencing *in situ* solubilization, which can affect the stability of the formulation. High drug solubilization is essential for increasing the efficiency of drug loading into carriers, with a concomitant improvement in pharmacokinetic parameters[9]. Additionally, the recommended amount of bilastine SNEDDS must be miscible in the chosen excipients with the least amount of mixture to be developed into an effective product. The results of bilastine solubility in various ingredients are shown in Figure 4. The self-emulsification feature depends on the selection of a suitable combination of ingredients. This study demonstrated that Tween 40, combined with the highest quantity of Capmul PG-NF, produced the best emulsification, as shown in Table 1. The surfactant forms a layer around the oil droplets, reducing the surface tension between the aqueous and oil phases. Additionally, increasing the surfactant concentration enhances the spontaneity of self-emulsification. An increase in co-surfactant concentration reduces

Table 1. Solubility study of bilastine

Components	Excipients	Solubility (mg/mL)
Oils	Olive oil	0.41±0.02
	Capmul MCM	0.206±0.02
	Capmul PG-NF	0.475±0.02
	Crodamol PC-LQ	0.089±0.02
	Crodamol P30	-0.038±0.02
Surfactants	Labrasol	0.009±0.02
	Tween 40	0.119±0.02
	Labrasol ALF	-0.005±0.02
	Labraf IF	0.146±0.02
	Span 80	0.443±0.02
Co-surfactants	Transcutol P	0.419±0.02
	Transcutol HP	0.015±0.02
	Lauroglycol 90	-0.037±0.02
	Plurol Oleique	0.168±0.02
	Capryol	0.235±0.02

All value are expressed as mean ± SD (n = 0.5)

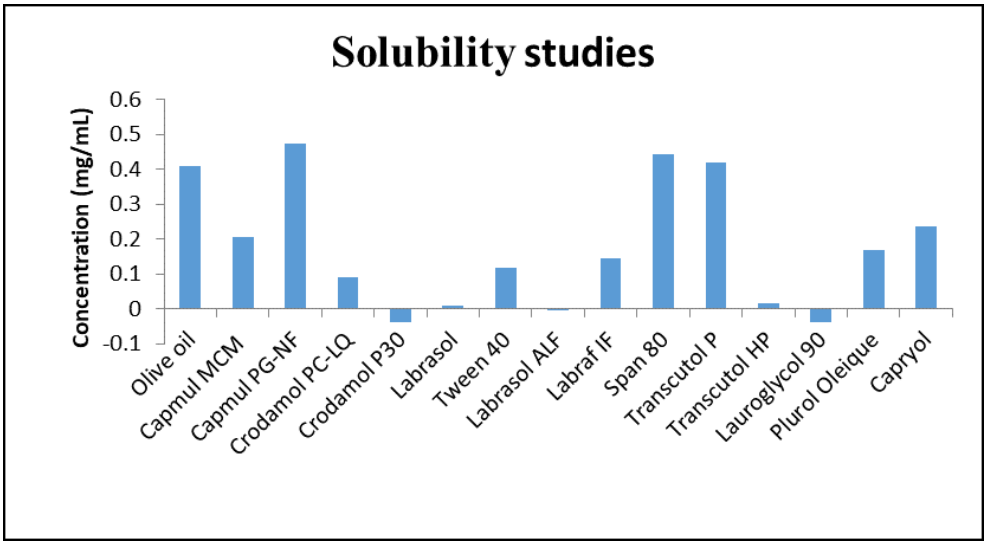

Figure 4. Solubility study of bilastine (mean value ± SD of three distinct experiments is displayed in the results)

the emulsion formation area but has little effect on the reduction of interfacial tension. A higher HLB value is required to produce an o/w type emulsion. The primary purpose of the co-surfactant in the preparation of beta-alanine is to reduce the surfactant ratio in the formulation. To improve the solubilization of model lipophilic drug compounds, Plurol Oleique was added to the formulation[6, 15].

3.4 Pseudo-ternary phase diagrams

To determine the self-nanoemulsifying region, as well as the optimal concentration and combination of oil, surfactant, and co-surfactant, a ternary phase diagram was developed. Ternary mixtures were prepared using excipients with exceptional ability to solubilize bilastine. Percentage transmittance was used to assess whether the ternary mixtures formed good or poor emulsions. Various formulations with different oil, surfactant, and co-surfactant concentrations were created, and their SNEDDS physicochemical characteristics were evaluated. A nanoemulsion with a percentage transmittance of ≥ 85% was considered a good emulsion. Different concentrations of surfactants and co-surfactants were used in the development of SNEDDS, and the ternary diagrams with varying concentrations of excipients in a 2:1 ratio are shown in Figure 5. With varying ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, and 9:1, the concentration of Capryol 90 oil ranged from 4–29%, while the surfactant and co-surfactant concentrations ranged from 65–67% and 4–32%, respectively. This data was used to construct a ternary phase diagram, where each vertex represents 100% of a specific ingredient. In Figure 5, the bluish-white area indicates the transparent and low-viscosity region of the nanoemulsion[7, 9].

3.5 Formulations of L-SNEDDS

The ratio of oil, surfactant, and co-surfactant used to prepare the L-SNEDDS formulations varied. A 100 mg dose of bilastine was used for the formulation. The drug was dissolved in 200 mg of oil, and 1600 mg of Smix was added. The mixture was then mixed using a cyclomixer until a transparent preparation was achieved. The formulations were stored at room temperature and allowed to equilibrate prior to evaluation[1, 3].

3.6 Evaluation of bilastine L- SNEDDS

The self-nanoemulsification times for the prepared L-SNEDDS were evaluated. It took only 46 ± 1.5 seconds to emulsify the prepared L-SNEDDS of bilastine. The results showed that the prepared emulsions exhibited stable efficiency under the given conditions. All values are expressed as mean ± SD (n = 3). A dispersibility test was conducted in distilled water and phosphate buffer (pH 6.8), The formulations produced grade 1 emulsions, which indicate emulsions free impurities, with a long shelf life, resistant to separation, uniform and in small droplets for a smooth and maintained emulsion under stress[4, 9].

The prepared L-SNEDDS formulations were monitored for stability and phase separation at time intervals of 2, 4, 6, 8, 12, and 24 hours. Over time, it was observed that none of the formulations exhibited phase separation or precipitation. The optimized L-SNEDDS formulation was diluted with 10 mL of methanol in 1 mL increments. After suitable dilution, the resultant solution was examined using UV spectrophotometry (Shimadzu UV-240 model, Japan). The absorbance of each solution was measured at 205 nm, and the drug loading efficiency was found to be

99.71 ± 0.67%. All values are expressed as mean ± SD (n = 3). The L-SNEDDS [TP3PO1 (2:8)] formulation was subjected to particle size analysis. The droplet size, PDI, and zeta potential of the emulsions were found to be 142.5 nm, 0.183, and -22.6 mV, respectively in the nano region. A value of -22.6 mV indicates moderate electrostatic repulsion between particles and similarly**,** and PDI (< 0.2) indicates a uniform particle population, contributing to predictable release, absorption, and stability.

Table 2. Percentage composition of oil (Capmul PG-NF), surfactant (Tween 40) and co-surfactant (Plurol Oleique) upon titration with water.

S.N	Formulation Name	Smix (mg)	Oil (mg)	Water (mg)	Total (mg)	% Smix	% water	% oil	Remarks
1	TP3PO1(1:9)	450	50	950	1450	31.03	65.51	3.44	Bluish white
2	TP3PO1(2:8)	400	100	916	1416	28.24	64.68	7.06	Bluish white
3	TP3PO1(3:7)	350	150	1030	1530	22.87	67.32	9.80	Bluish white
4	TP3PO1(4:6)	300	200	967	1467	20.44	65.91	13.63	Milk white
5	TP3PO1(5:5)	250	250	1012	1512	16.53	66.93	16.53	Milk white
6	TP3PO1(6:4)	200	300	1018	1518	13.17	67.06	19.86	Milk white
7	TP3PO1(7:3)	150	350	1014	1514	9.90	66.97	23.11	Milk white
8	TP3PO1(8:2)	100	400	914	1414	7.07	64.63	28.28	Milk white
9	TP3PO1(9:1)	50	450	1016	1516	3.29	67.01	29.68	Milk white

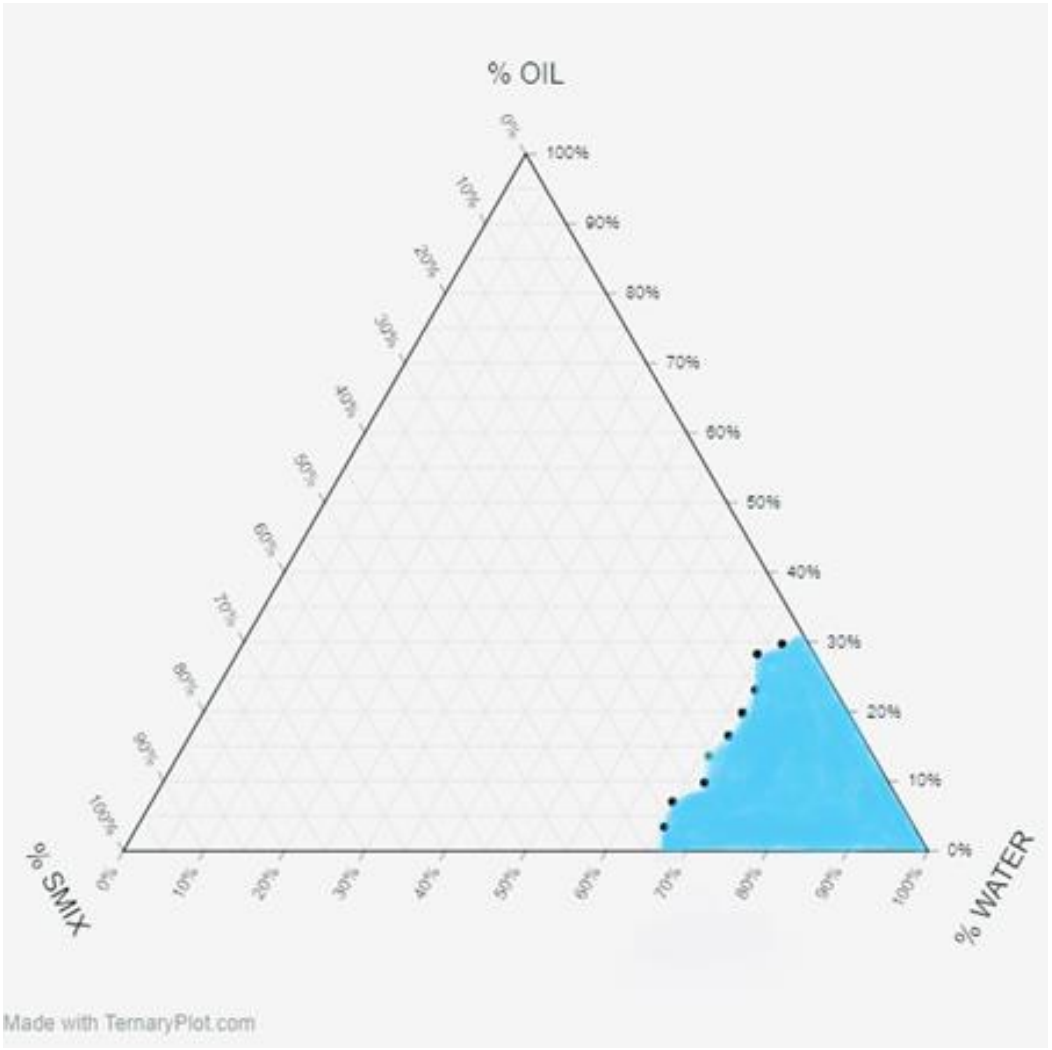

Figure 5. Pseudo- ternary phase diagrams (TP3PO1)**.**

System

Temperature (°C):	25.0	Zeta Runs:	12
Count Rate (kcps):	113.1	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	7

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-22.1	Peak 1: -20.6	100.0	7.62
Zeta Deviation (mV):	19.9	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm):	0.0242	Peak 3: 0.00	0.0	0.00
Result quality :	Good			

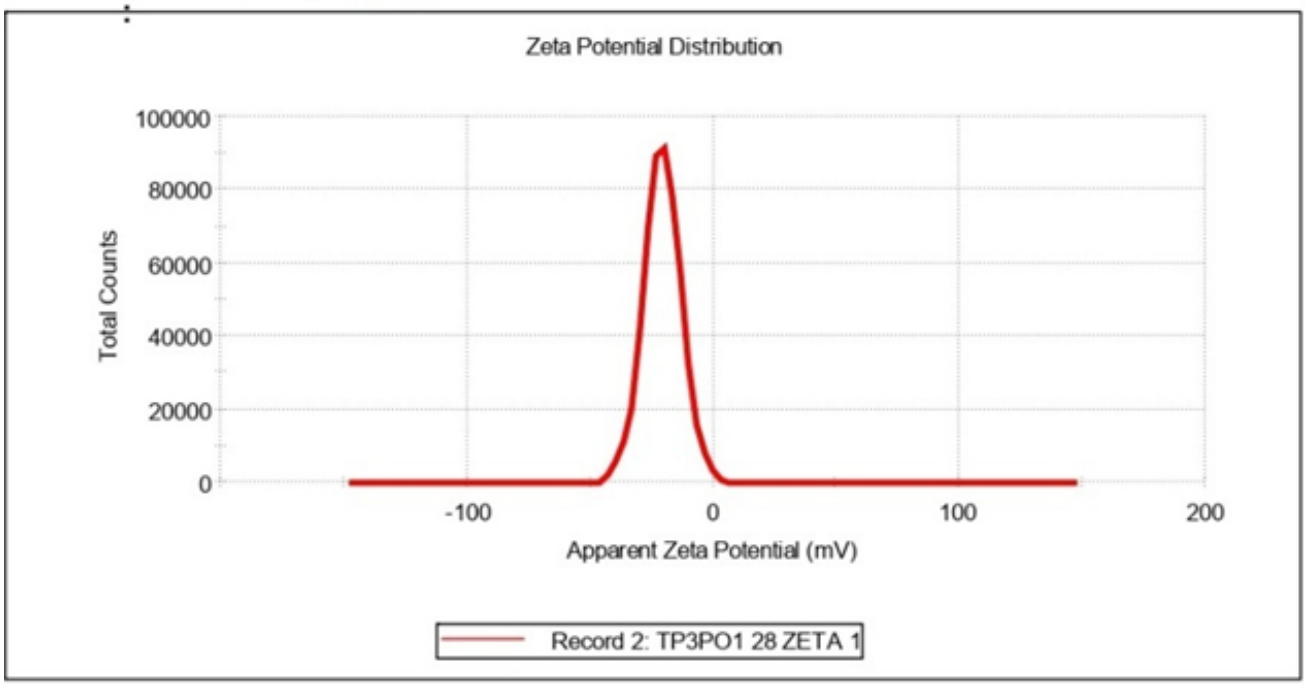

Figure 6. Droplet size and zeta potential (TP3PO1 (2:8)

The compatibility study of the pure drug and formulation was conducted using FT-IR spectroscopy. The results were almost identical, as the functional group regions were within ±10 values. This indicates that there was no interaction between the drug and the excipients used in the formulation[13].

Table 3. Functional groups stretching studies by FT-IR

Formulation name	O-H stretch	C=N Stretch	C=O stretch	Alkyl C-H stretch
Pure drug	3440.66	1457.27	1726.32	2932.13
TP3PO1 (2:8)	3439.61	1462.88	1735.70	2923.77

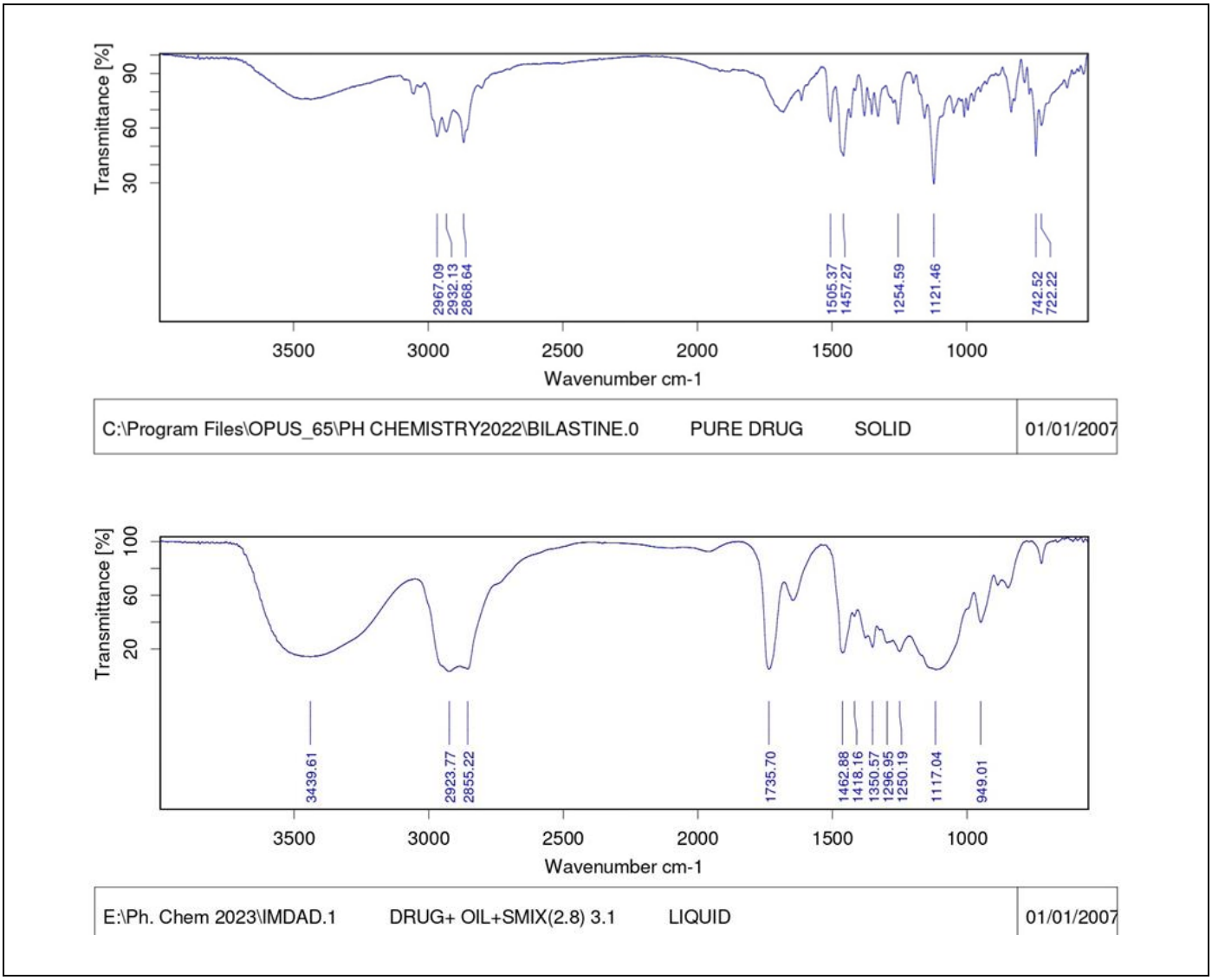

Figure 7. FT-IR studies for liquid formulation

Thermodynamic stability studies of the L-SNEDDS formulation were conducted using centrifugation and a freeze-thaw cycle. It was observed that the emulsion remained stable during centrifugation at 3500 rpm, and the alternating temperature cycle of -20°C and +25°C.

The optimized formulation of TP3PO1 (2:8) underwent an accelerated stability study at 25 ± 0.5°C/60 ± 5% RH and 40 ± 0.5°C/75 ± 5% RH for 1-3 months to assess parameters such as the effect of dilution, droplet size, PDI, zeta potential, and *in-vitro* drug release. The L-SNEDDS formulation was successful in the effect of dilution test. The droplet size was found to be 138 nm with a PDI of 0.225 and a zeta potential of -21.5 mV indicating no significant effect on droplet size after 1 and 3 months of the stability study. The percentage of bilastine released was found to be 105.24 ± 0.678% at the end of 1 and 3 months, indicating no change in drug release over this period. Based on the results, the formulation was stable during the 1-3 month accelerated stability study[2, 3].

3.7 *In vivo* animal study

3.7.1 Pharmacokinetic studies

Plasma samples obtained from the test and control groups of rats (n = 2) at predefined intervals were injected into an HPLC system to analyze the drug concentration using a validated HPLC method, as previously described. The retention time of bilastine was observed to be 2.926 minutes. Plasma drug concentration was plotted against time to obtain the plasma concentration-time curve (Figure 9), which was used to study the pharmacokinetics of bilastine loaded in L-SNEDDS. The pharmacokinetics of bilastine in L-SNEDDS were significantly higher compared to the control (drug suspension), with the maximum observed in the TP3PO1 (2:8) formulation[1, 3].

Figure 8. *In vivo* animal studies (experiments), (Animal dose for oral administration of = 25mg (based on body weight)

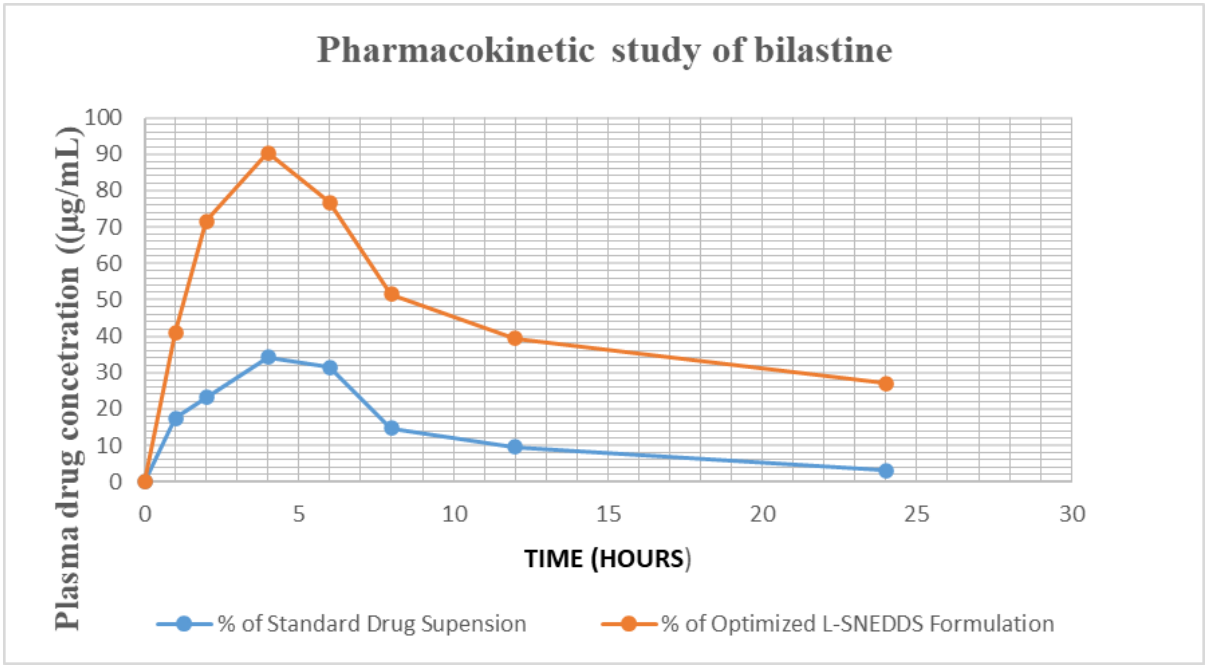

Figure 9. Plasma concentration-time curve of bilastine (25 mg) after oral administration.

The pharmacokinetic study of bilastine using the non-compartmental pharmacokinetic model was conducted with PK Solver. The observed values for different pharmacokinetic (PK) parameters for the test and control formulations are presented in Table 4. The area under the curve (AUC_{0-24}) for the optimized formulation [TP3PO1 (2:8)] was found to be significantly higher 1129.45 ± 10.815 µg/mL/h compared to the drug suspension 179.96 ± 2.368 µg/mL/h. Cmax was also higher for TP3PO1 (2:8) 90.368 ± 2.596 µg/mL compared to the drug suspension 34.32 ± 2.31 µg/mL. The plasma half-life ($t_{1/2}$) of the optimized formulation TP3PO1 (2:8) of bilastine was significantly increased to 22.45 ± 0.251 hrs., compared to the bilastine suspension 18.57 ± 0.312 hrs.

4. CONCLUSION

The formulations of bilastine-loaded self-nanoemulsifying drug delivery systems (SNEDDS) were developed in liquid form. The aim of the present study was to improve the solubility, dissolution, and pharmacokinetic parameters of the poorly water-soluble drug bilastine. Different components, including oil (capmul PG-NF), surfactant (Tween 40), and co-surfactant (plurol oleique), were used as excipients. In this study, three different bilastine-loaded SNEDDS formulations [TP3PO1 (1:9), TP3PO1 (2:8), and TP3PO1 (3:7)] were prepared. Among these, TP3PO1 (2:8) was selected as the stable and optimized formulation. The optimized formulation

Table 4. Pharmacokinetic value of bilastine

Parameters	Results	
	Standard drug Formulation	Optimised SNEDDS formulation
C_{max} (µg/mL)	34.32	90.368
T_{max} (h)	4	4
$t_{1/2}$ (h)	18.57	22.45
K_e (h^{-1})	0.03730	0.03086
AUC_{0-24} (µg/mL/h)	179.96	1129.45

was characterized by several *in vitro* and *in vivo* analyses. Among these, L-SNEDDS [TP3PO1 (2:8)] demonstrated greater potential in terms of reduced particle size 142.5nm, zeta potential −22.6mV, PDI 0.183, and drug loading efficiency, which was found to be 99.71 ± 0.67%. The optimized formulation of L-SNEDDS [TP3PO1 (2:8)] exhibited stable nanoemulsion characteristics, and FT-IR studies showed no interaction between the drug and excipients.

The *in vivo* pharmacokinetic study of the optimized formulation of bilastine-loaded SNEDDS showed that the area under the curve ($AUC_{0–24}$) for the optimized formulation [TP3PO1 (2:8)] was significantly higher, 1129.45 ± 10.815µg/mL/h, compared to the drug suspension, 179.96 ± 2.368µg/mL/h. Cmax was also higher for TP3PO1 (2:8), 90.368 ± 2.596 µg/mL, compared to the drug suspension, 34.32 ± 2.31µg/mL. The plasma half-life ($t_{1/2}$) of the optimized formulation TP3PO1 (2:8) was significantly increased to 22.45 ± 0.251 hrs as a compared to the drug suspension 18.57 ± 0.312 hrs. Hence, the current approach demonstrated that bilastine's solubility and dissolution rate were significantly improved after oral administration, leading to enhanced oral absorption and improved pharmacokinetic parameters for the optimized formulation.

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Author contributions

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Conflict of interest (If any)

The authors declare no conflict of interest. All authors have read and agreed with the final manuscript.

Ethics approval

The animal study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Pulla Reddy Institute of Pharmacy, approval number IAEC-III-PRIP-FEB-2023-Protocol 04, in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

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