J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 48(2): 525-534, 2024
Doi: 10.33483/jfpau.1385707

ORIGINAL ARTICLE / ÖZGÜN MAKALE

LIQUID AND SOLID SELF-EMULSIFYING DRUG DELIVERY SYSTEMS (SEDDS) CONTAINING VALSARTAN: STABILITY ASSESSMENT AND PERMEABILITY STUDIES

VALSARTAN İÇEREN KATI VE SIVI KENDİLİĞİNDEN EMÜLSİFİYE OLAN SİSTEMLER (SEDDS): STABİLİTE DEĞERLENDİRMESİ VE PERMEABİLİTE ÇALIŞMALARI

Gülbeyaz YILDIZ TÜRKYILMAZ[1,2,3]*, Mine DİRİL[1], Eda GÜLMEZOĞLU[1], Hatice Yeşim KARASULU[1]

[1]Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 35100, İzmir, Türkiye
[2]Ege Üniversitesi, Faculty of Pharmacy, Department of Biopharmaceutic and Pharmacokinetic, 35100, İzmir, Türkiye
[3]Ege University, Research and Application Center of Drug Development and Pharmacokinetics (Argefar), 35100, İzmir, Türkiye

ABSTRACT

Objective: *Valsartan (VST) is a Biopharmaceutical classification system (BSC) class II active ingredient with a bioavailability of approximately 25% and is utilized to treat high blood pressure (hypertension). This study aimed was to showcase the stability and increase the permeability of VST by developing self-emulsifying drug delivery systems (SEDDS) and solidified SEDDS (S-SEDDS) formulations.*

Material and Method: *The ratios of the components were determined by the pseudo-ternary phase diagram, and the characterization studies were conducted in the previous study. Stability was performed in long-term (25±2˚C, 60±5% relative humidity) and accelerated (40±2˚C, 75±5% relative humidity) conditions. The intestinal permeability of SEDDS formulations was evaluated by Caco-2 cells.*

Result and Discussion: *Formulations for 12 month, droplet sizes were found to be 67.52 ± 5.26 nm and 176.93 ± 17.34 nm for SEDDS of VST (VST-SEDDS) and S-SEDDS of VST (VST-S-SEDDS), respectively. During this period, polydispersity indexes were: VST-SEDDS, 0.56±0.1; VST-S-SEDDS, 0.58±0.05. Both formulations increased VST permeability across Caco-2 cells: VST-SEDDS by 2.32x (powder) and 2.18x (commercial); VST-S-SEDDS by 1.38x (powder) and 1.30x (commercial). The formulation components did not have cytotoxic effects. These results demonstrated that newly developed VST-SEDDS and VST-S-SEDDS formulations with high permeability may be a desirable approach for antihypertensive therapy.*

Keywords: *Caco-2 cell line, permeability, self-emulsifying drug delivery system, solidified self-emulsifying drug delivery system, valsartan*

ÖZ

Amaç: *Valsartan (VST), biyoyararlanımı yaklaşık %25 olan Biyofarmasötik Sınıflandırma Sistemi (BSS) sınıf II aktif maddedir ve yüksek kan basıncını (hipertansiyon) tedavi etmek için kullanılır. Bu çalışmanın amacı, kendi kendine emülsifiye olan ilaç dağıtım sistemleri (SEDDS) ve katılaştırılmış*

* **Corresponding Author / Sorumlu Yazar:** Gülbeyaz Yıldız Türkyılmaz
e-mail / e-posta: gulbeyaz.yildiz@ege.edu.tr, **Phone / Tel.:** +902323113981

Submitted / Gönderilme : 03.11.2023
Accepted / Kabul : 07.03.2024
Published / Yayınlanma : 20.05.2024

SEDDS (S-SEDDS) formülasyonları geliştirerek VST'nin stabilitesini göstermek ve geçirgenliğini artırmaktır.

Gereç ve Yöntem: *Bir önceki çalışmada bileşenlerin oranları üçgen faz diyagramı ile belirlenmiş olup, karakterizasyon çalışmaları yapılmıştır. Bu çalışmada, uzun süreli (25±2˚C, 60±5% bağıl nem) ve hızlandırılmış (40±2˚C, 75±5% bağıl nem) koşullarda stabilite çalışmaları gerçekleştirildi. SEDDS formülasyonlarının bağırsak geçirgenliği Caco-2 hücreleri tarafından değerlendirildi.*

Sonuç ve Tartışma: *Formülasyonların 12 ay boyunca partikül boyutları VST içeren SEDDS (VST-SEDDS) formülasyuonunda 67.52 ± 5.26 nm, VST içeren S-SEDDS (VST-S-SEDDS) formülasyonunda 176.93 ± 17.34 nm olarak bulundu. Bu süre boyunca VST-SEDDS ve VST-S-SEDDS formülasyonunun polidispersite indeksleri sırasıyla 0.56±0.1, 0.58±0.05 olarak bulundu. Her iki formülasyon da Caco-2 hücreleri boyunca VST geçirgenliğini artırdı: VST-SEDDS 2,32 kat (toz) ve 2,18 kat (ticari); VST-S-SEDDS 1,38x (toz) ve 1,30x (ticari). Formülasyonların bileşenlerinde sitotoksik etki görülmedi. Bu sonuçlar, yüksek geçirgenliğe sahip yeni geliştirilen VST-SEDDS ve VST-S-SEDDS formülasyonlarının antihipertansif tedavi için arzu edilen bir yaklaşım olabileceğini gösterdi.*

Anahtar Kelimeler: *Caco-2 hücre hattı, kendi kendine emülsifiye olan ilaç dağıtım sistemleri, katılaştırılmış kendi kendine emülsifiye olan ilaç dağıtım sistemleri, permeabilite, valsartan*

INTRODUCTION

Hypertension, a primary risk factor for cardiovascular diseases, impacts over 46% of the global adult population, totaling approximately 1.28 billion individuals worldwide, as The World Health Organization (WHO) reported in its 2023 report [1]. Valsartan (VST) is employed in managing high blood pressure and is an approved angiotensin II receptor blocker for treating hypertension in adults. It has been marketed in adults in Europe at doses of 80-160 mg since 1996; since 2006, it has been as the highest dose of 320 mg [2]. VST has an acidic structure, and its solubility is very low (<0.1 mg/ml), depending on pH, at the aqueous phase. VST belongs to the low solubility, high permeability (class II) category of the biopharmaceutical drug classification system (BCS) [3]. It has a bioavailability of approximately 25% because of low water solubility. Increasing the solubility, or reducing the first pass effect through the liver may be approached to increase bioavailability.

Self-emulsifying drug delivery systems (SEDDS) formulation approach used for years to increase solubility and bioavailability. SEDDS formulation contains oil as a solid or liquid form, surfactant, and cosurfactant. After the SEDDSs are taken orally, the drugs trapped in the triglycerides in their compositions are released from the enterocytes through the villus in the small intestine to the lymphatic pathway through exocytosis by chylomicrons and pass into the blood from the small intestine. Thus, the active substance enters the lymphatic system and passes into the blood circulation from there, and the drug is protected from the first-pass effect of the liver [4]. Developing self-emulsifying drug delivery systems (SEDDS) is crucial, particularly for drugs with low bioavailability. However, liquid SEDDS formulations present various drawbacks, including drug leakage, reduced stability, and limited drug loading capacity.

Different solidification techniques (such as adsorption on solid carriers, nanoparticulate systems, spray drying, melt extrusion, or melt granulation) are used for liquid SEDDS formulations that can be converted into solid SEDDS (S-SEDDS) formulations. Long-term storage and high stability can be achieved with S-SEDDS. The developed S-SEDDS can take various forms, such as dry emulsions, self-emulsifying capsules, micro/nano-particles, pellets/tablets, or suppositories [5,6].

In the previous study, SEDDS formulation was developed using VST active ingredient (VST-SEDDS), and characterization studies were carried out. In this study it was used isopropyl myristate as oil phase, Capyrol 90 and Tween 20 as surfactants and Transcutol HP as co-surfactant. After performing phase diagrams studies, 1:1 surfactant/co-surfactant ratio was determineted extensive microemulsion area. For the VST-SEDDS formulation, VST was dissolved in SEDDS formulation composition at 80 mg/0.5 ml ratio. The VST-S-SEDDS formulation from the VST-SEDDS formulation was carried out using a wet granulation for solid carrier adsorption technique using Avicel pH 101 and HPMC in previous study. The characterization results of the formulations obtained by using HPMC and Avicel pH 101 as solid carriers were compared, and studies were continued with Avicel 101 due to its higher flow properties.

[7]. The solid carrier adsorption technique was used in this study because it has many advantages, such as being simple and fast, and being able to be carried out without using organic solvents. This study aims is to complete the stability studies and show that the bioavailability of VST, which has low solubility and bioavailability from liquid and solid SEDDS formulations, increases by passing through Caco-2 cells. For this purpose, permeability studies on Caco-2 cell lines were divided into experimental groups: powder VST, VST-SEDDS, VST-S-SEDDS, and commercial product. Dispersions containing 40 µg/ml VST were applied to the well plates in all experimental groups, from apical to basolateral direction and from basolateral to apical direction. In addition, cytotoxicity studies were conducted using Caco-2 cell lines. According to the results, SEDDS formulations had increased permeability compared to the commercial product, and therefore, it would be predicted to increase bioavailability. It was also determined that the VST-S-SEDDS formulation had higher permeability values than the liquid VST-SEDDS formulation.

MATERIAL AND METHOD

Materials

VST was generously provided by Bilim Pharmaceuticals (Beyoglu, Istanbul). Capyrol® 90 (Propylene glycol monocaprylate) and Transcutol® HP (Diethylene glycol monoethyl ether) were graciously supplied by Gattefossé (Saint-Priest, France). Isopropyl myristate, Tween® 20 (Polyoxyethylene sorbitan monolaurate), and Avicel pH 101 were acquired from Sigma Aldrich (Darmstadt, Germany). HBSS was also obtained from Sigma Aldrich (Darmstadt, Germany). All the solvents employed in the analytical studies were of high-performance liquid chromatography (HPLC) grade.

Methods

Preparation of VST-SEDDS and VST-S-SEDDS

A Pseudo-ternary phase diagram with oil, surfactant, and co-surfactant determined as a result of solubility studies was drawn using the water titration method. The emulsion area on the pseudo-ternary phase diagram was utilized to identify suitable phases and determine the proportion of each component [8]. Thermodynamic stability studies were carried out with the formulation giving the highest area in the ternary phase diagram. The thermodynamic stability of SEDDS formulations was evaluated by carrying out freeze-thaw, heating-cooling cycles, and centrifugation tests [9].
VST-SEDDS formulation was adsorbed onto Avicel pH 101, a type of inert carrier for preparing VST-S-SEDDS. VST-S-SEDDS formulation was prepared using a wet granulation technique. An oven at 45˚C for approximately 1 hour was used to dry the wet granulation. After that, characterization studies were performed for both formulations.

HPLC Studies

HPLC method was developed and validated for VST quantification in VST-SEDDS and VST-S-SEDDS formulations using HPLC with an Agilent (HP 1100, USA) Series. UV-DAD detector and Zorbax SB C18 (150 mm × 4.6 mm, 3.5 µm) column were used for VST analysis. As the mobile phase, a mixture of Acetonitrile: 0.1M phosphate buffer (55:45, v/v) was adjusted to pH 2.7 with trifluoroacetic acid. The injection volume was 10 µl, and the flow rate was set at 1 ml/min, with a wavelength of 250 nm [10]. The mobile phase was used as the solvent to determine the amounts of the developed VST-SEDDS and VST-S-SEDDS formulations of stability studies. The buffer solution (HBSS) was used as a dilution solution to evaluate permeability studies and observe the buffer solution effect [11]. Linearity, working range, limit of detection (LOD), and limit of quantification (LOQ) parameters were examined.

Chemical and Physical Stability

Chemical and physical stability were assessed over a 12-month storage period under two temperature/relative humidity (RH) conditions: 25 ± 2 °C/60 ± 5 RH% and 40 ± 2 °C/75 ± 5 RH%. Stability studies were carried out by filling the VTS-SEDDS into a vial and VST-S-SEDDS into the

bottle. Physical appearance, electrical conductivity, pH, density, refractive index, polydispersity index, droplet size, viscosity, and active ingredient quantification were monitored throughout stability.

In vitro Permeability Studies

Caco-2 human colon epithelial cancer cell lines are used to forecast the intestinal permeability of drugs because they mimic the small intestinal epithelium [12]. For permeability studies, Caco 2 cells were incubated (37°C/ 90% humidity and 5% CO_2) in flasks until the appropriate count and size. When the appropriate count was reached, permeability studies were conducted using Caco-2 cells prepared by seeding 5×10^5 cells in each of six wells with transwell polycarbonate membrane (pore size 0.4 µm, filtration area 4.67 cm^2, Corning, USA) and they were utilized 21 days after seeding [13]. Transepithelial electrical resistance (TEER) value was measured from the apical direction to the basolateral direction (A-B) and from the basolateral direction to the apical direction (B-A) to show that cell integrity was achieved at the beginning and end of the experiment. For *in vitro* permeability studies, 80 mg of VST, VST-SEDDS equivalent to 80 mg of VST, and commercial product were employed. The solutions obtained by diluting 80 mg VST, VST-SEDDS equivalent to 80 mg VST, and the commercial product in HBSS were applied (Valsartan concentration 40 µg/ml). A volume of 1.5 ml of solution was applied to A-B and, a volume of 2.6 ml of solution was applied to B-A direction for each well. Samples of 200 µl were collected at 0, 30, 60, 90, and 120 minutes (n=3). The apparent permeability coefficient (P_{app}, cm s^{-1}) was calculated from the slope (dQ/t) of the linear portion of the plots depicting the cumulative amounts of permeated VST (Q) over time (t), as per Equation 1 [14]. The quantity of drug remaining on the surface and inside of the polycarbonate membrane was also determined to assess the method's validity. The Efflux ratio was utilized to evaluate the P-gp inhibitory effect of the substances, and Equation 2 shows how the efflux value is calculated [15].

Equation 1.

$$\text{Papp(Permeability)} = \frac{\text{dQ}}{\text{dt}} \text{x} \frac{1}{\text{Co x A x 60}}$$

In Equation 1, P_{app} is the apparent permeability (cm/s), dQ/dt is the steady state flux, A is the diffusion area of the monolayer (in cm^2), Co is the initial concentration of the drug in the donor compartment (µM), and 60 is a conversion factor for time.

Equation 2.

$$\text{The efflux ratio} = \frac{\text{Papp(B} - \text{A)}}{\text{Papp(A} - \text{B)}}$$

In Equation 2, P_{app} (A-B): Apparent permeability of apical direction to basolateral direction (cm/s), P_{app} (B-A): Apparent permeability of basolateral direction to apical direction (cm/s).

Cytotoxicity Test

A cytotoxicity study was conducted to observe the potential cytotoxic effect of formulation components on Caco-2 cells [16]. Caco-2 cells were seeded in a 96-well plate (pore size 0.4 µm, Corning, USA), each well at a density of 1×10^{-4} cells and incubated for 24 h at 37 ± 0.5 °C in a CO_2 incubator. To examine cytotoxicity powder VST, VST-SEDDS formulation, VST-S-SEDDS formulation, and commercial tablet product, were dissolved in Hanks' Balanced Salt Solution (HBSS) and incubated with Caco-2 cells for 24, 48, and 72 hours. At the end of these periods, 100 µl of (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) (5 mg/ml of stock MTT / phosphate buffer saline (PBS) solution) was added to each of the plate and incubated for 4 hours. The solutions were removed, and 100 µl dimethyl sulfoxide (DMSO) was added to each 96-well. To test cell viability, absorbance values at 540 nm were used an ELISA microplate reader UV spectrophotometer (Thermo vario scan-FHA multi-plate reader) [17].

Statistical Evaluation

Statistical significance was assessed using the Student t-test and one-way ANOVA test. Differences were deemed significant at the 95% confidence level ($p < 0.05$). The results were presented as the mean of the obtained values, and the corresponding standard deviation (±).

RESULT AND DISCUSSION

Preparation of VST-SEDDS and VST-S-SEDDS

Details of the method development, ternary phase diagram and characterization studies of the optimum formulation were described in a previous [7]. As per the study, isopropyl myristate was employed as the oil phase, Capyrol 90 and Tween 20 as surfactants, and Transcutol HP as a co-surfactant. 0.5 ml of the developed SEDDS formulation contains 80 mg VST. Thermodynamic stability studies evaluate the ability of the SEDDS formulation to withstand stress conditions [18]. In the optimal SEDDS formulation, the heating-cooling, freeze-thaw cycles, and centrifugation test were carried out within the scope of the thermodynamic study. At the end of these studies, no phase separation was observed in the VST-SEDDS formulation, and it was shown that the developed formulation was not affected by stress conditions. The droplet size of the VST-SEDDS formulation was obtained at 72.63 ± 0.45 nm, and the zeta potential value was found to be 0.069 ±0.005 mV. In addition, the VST-SEDDS formulation polydispersity index (PDI) was found to be 0.449. Self-emulsifying systems, in particular, utilize a classification system known as the Lipid Formulation Classification System (LFCS). This system, introduced by Pouton, facilitates a systematic and rational approach to formulation [19]. According to LFCS, SEDDS formulations with a 50-100 nm droplet size are in the Type IIIB class. Due to the droplet size of the developed VST-SEDDS formulation, it was observed to have a Type IIIB classification system that shows greater dispersion rates then other types [20]. When the SEDDS formulation was diluted 1:1000 with water, the droplet size (<100nm) did not change over 24 hours.

VST-S-SEDDS formulation was developed based on VST-SEDDS formulation using the wet granulation technique with Avicel pH 101. 500 mg of the developed VST-S-SEDDS formulation contains 80 mg VST. After diluting with the amount of water that creates the optimum formulation, droplet size and PDI analyzes were performed and these results were found to be 186±5.23 nm and 0.51±0.30, respectively. Due to the droplet size of the developed VST-S-SEDDS formulation, it was observed to have a Type IIIA classification system. According to the Lipid Formulation Classification System (LFCS) proposed by Pouton, while Type III A systems need to be digested more than Type IIIB systems for absorption in the in vivo environment. Since the solvent capacity of the Type IIIB systems is lower, the possibility of precipitation in the in vivo environment may be higher than the Type IIIA systems [19]. While VST-SEDDS is in the Type IIIB class according to droplet size, the VST-S-SEDDS formulation developed by the solid phase absorption method is in the Type IIIA class according to the droplet size. The reason for this change can be interpreted as the change in dispersibility behavior and droplet size after the absorption of VST-S-SEDDS into Avicel. In solidification drug delivery systems, predicting the flow characteristics of powders for manufacture is especially important. Powder characterization evaluations of VST-S-SEDDS formulation were carried out using European Pharmacopoeia guidance [21]. Therefore, the Hausner ratio and Carr index, derived from bulk and tapped density were found to predict powder flowability. Bulk density, tapped density, Hausner ratio and Carr index results calculated for the developed VST-S-SEDDS formulation were 0.288, 0.4301, 1.492, and 32.98%, respectively. Granules with a Hausner ratio below 1.25 and 16-20% compressibility percentage are known to have good fluidity [21]. For this reason, it may be recommended to add glidants and/or lubricants as formulation components to increase the flow properties of the developed formulation [22]. However, since using a lubricant to increase flowability would cause problems fitting into capsule No. 00, the dosage form was planned to be a sachet. Accordingly, in the characterization studies carried out, the dimensional analysis of VST-S-SEDDS was found to be 98.4% in the 2000-1400 µm mesh size range. The emulsification time of the solidified self-emulsions and the droplet size after redispersibility were measured and found to be 30 seconds and 186.3±1.362 nm, respectively. It has been observed that this formulation belongs to Type IIIA from the LFCS with its particle size. Its rapid

emulsification in water compared to the emulsification time supported the idea of using it in the sachet dosage form.

This formulation has greater dispersion rates and requirements of enzymatic digestion are not necessary [19].

HPLC Studies

HPLC method was developed and validated to determine drug content [7]. The calibration curve was drawn for HBSS medium, and LOD and LOQ values were calculated (Table 1).

Table 1. Linearity, LOD and LOQ results of permeability medium

Media	Concentration range (µg/ml)	Equation	R^2	LOD (µg/ml)	LOQ (µg/ml)
HBSS	0.1-50	y= 17.516x+3.3711	0.9992	0.700	0.210

Chemical and Physical Stability

It was observed that there were no significant physical and chemical changes in the stability results of the developed formulation carried out at 25±2˚C, 60±5% relative humidity (long term) and 40±2˚C, 75±5% relative humidity (accelerated) for 12 months (Table 2 and Table 3).

Table 2. The stability results of VST-SEDDS formulation at 25 ± 2˚C/60 ± 5 RH% and at 40 ± 2˚C/75 ± 5 RH% (n=3)

Conditions* / Parameters	0. Month		1. Month		3. Month		6. Month		9. Month		12. Month	
	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.
pH*	3.74	3.74	3.75	3.76	3.75	3.75	3.76	3.75	3.75	3.75	3.74	3.76
Refractive index **	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46
Electrical conductivity (µs) **	86.0	86.0	86.0	87.0	86.0	87.0	87.0	87.0	86.0	87.0	86.0	86.0
Density (g/ml)	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Droplet size (nm)**	72.6 ±1.56	72.6 ±1.56	70.2 ±0.96	73.7 ±1.94	73.3 ±1.56	79.0 ±2.95	62.1 ±1.25	63.8 ±0.96	65.6 ±1.4	67.5 ±1.76	61.3 ±1.62	63.9 ±1.85
Polydispersity index	0.45 ±0.21	0.45 ±0.35	0.77 ±0.25	0.42 ±0.21	0.48 ±0.30	0.67 ±0.31	0.64 ±0.29	0.58 ±0.32	0.49 ±0.34	0.59 ±0.33	0.64 ±0.30	0.56 ±0.28
Viscosity (cP)	47.0	47.0	47.1	47.0	47.1	47.0	47.0	47.1	47.0	47.0	47.1	47.1
Assay (%)	95.2 ±0.50	95.2 ±0.50	95.8 ±0.60	95.0 ±0.50	95.5 ±0.30	96.3 ±0.60	95.5 ±2.00	96.8 ±0.50	95.5 ±0.91	96.8 ±1.01	96.5 ±1.56	97.3 ±1.49
Physical appearance	Homogeneous, Transparent, No Phase Separation											

* 1. condition: 25 ± 2 ◦C/60 ± 5 RH%; 2. Condition: 40 ± 2 ◦C/75 ± 5 RH%; ** Diluted with the amount of water that creates the optimum formulation.

Droplet sizes for VST-SEDDS and VST-S-SEDDS during the long-term stability condition were 67.52 ± 6.07 nm and 176.93 ± 17.34 nm, respectively. The polydispersity index (PDI) gives a measure of particle size distribution. A PDI <0.5 indicates a homogeneous system and a narrow particle size distribution [23]. VST-SEDDS formulation polydispersity index (PDI) was found to be 0.449. After diluting with the amount of water that creates the optimum VST-S-SEDDS formulation, PDI analyzes were performed and it was found to be 0.51±0.30. During stability period, Polydispersity indexes (PDI) of VST-SEDDS and VST-S-SEDDS formulation were found to be 0.56±0.1, 0.58±0.05, respectively. The PDI values of the formulations did not change for 12 months and were approximately 0.5, indicating

that the formulations maintained their monodisperse structure throughout this period. The accelerated stability test, it was found to be 70.11 ± 6.07 nm for VST-SEDDS and 192.53 ± 22.16 nm for SEDDS. During all stability studies, when VST-SEDDS and VST-S-SEDDS are evaluated on their own, there were no statistically significant changes in droplet size and active substance assay ($p<0.05$). Solidified SEDDS increases the solubility of lipophilic drugs, reduces their biodegradation, and provides a better stability profile.

Table 3. The stability results of VST-S-SEDDS formulation (n=3)

Conditions* / Parameters	0. Month		1. Month		3. Month		6. Month		9. Month		12. Month	
	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.
Droplet size (nm)**	186 ±5.23	186 ±5.23	184 ±4.96	179 ±4.78	142 ±3.56	169 ±4.26	181 ±9.47	233 ±6.73	178 ±4.82	195 ±3.42	189 ±8.62	190 ±5.41
Polydispersity index**	0.51 ±0.30	0.53 ±0.28	0.49 ±0.22	0.58 ±0.40	0.51 ±0.33	0.61 ±0.28	0.58 ±0.18	0.63 ±0.26	0.60 ±0.25	0.62 ±0.35	0.61 ±0.28	0.63 ±0.32
Assay (%)	100.0 ±0.51	100.0 ±0.51	95.7 ± 1.45	104.3 ±0.96	103.0 ± 1.03	104.0 ± 1.53	102.0 ± 1.02	104.0 ± 0.95	106.1 ± 0.62	103.0 ± 1.52	104.0 ± 1.32	104.0 ± 0.85
Physical appearance**	Homogeneous, Transparent, No Phase Separation											

* 1. condition: 25 ± 2 ◦C/60 ± 5 RH%; 2. Condition: 40 ± 2 ◦C/75 ± 5 RH%; ** Dilute with the amount of water that creates the optimum formulation.

In vitro Permeability Studies

Caco-2 cells resemble intestinal epithelium due to their tight junctions and microvilli-like structure [24]. For this purpose, permeability studies of powder VST, VST-SEDDS, VST-S-SEDDS, and commercial product were performed with Caco-2 cell lines. They calculated P_{app} for apical to basolateral (A-B) and basolateral to apical (B-A) direction (Table 4). If $P_{app} > 10^{-6}$ cm/s, it can be said to have high permeability [25]. When the efflux value is >2, there is a possibility that the formulation will be exposed to efflux flow, which is a factor that reduces permeability (Table 4).

Table 4. Apparent permeability coefficient and Efflux value (n=3)

	Powder Valsartan	Commercial product	VST-SEDDS	VST-S-SEDDS
$P_{app\ A-B}$±SD (cm/s)	$37.3x10^{-5}$ ±0.01	$39.7x10^{-5}$ ±0.006	$86.5x10^{-5}$ ±0.007	$51.5x10^{-5}$± 0.002
$P_{app\ B-A}$±SD (cm/s)	$72.9.x10^{-5}$±0.01	$63.4x10^{-5}$± 0.008	$75.6x10^{-5}$ ± 0.016	$52.1x10^{-5}$± 0.009
Efflux	1.95±0.698	1.59±0.69	0.87±0.047	1.01±1.66

According to the results, it was observed that VST-SEDDS and VST-S-SEDDS formulations increased the permeability of VST by 2.32 and 1.38 times, respectively, compared to powder VST and by 2.18 and 1.30 times, compared to the commercial product. It was observed that the permeability was increased similarly with the S-SEDDS formulation developed in the study of Timur et al. [26]. The surfactants and co-surfactants used in SEDDS formulations increase permeability by opening tight junctions and reducing efflux membrane transport activity [27]. In addition, when the amount of VST drug remaining on the surface and inside of the polycarbonate membrane was analyzed, no amount of VST was found in the samples. This result indicates that VST is not retained in the membrane and that all added represents a permeability study.

Transepithelial electrical resistance (TEER) is measured across the cellular monolayer and is a convenient and relatively sensitive measure of the integrity and permeability of the monolayer on cell lines. The TEER value range of cells with cell integrity s hould be 500-1200 Ω.cm^2 [28]. Throughout

this study, TEER values in the Caco-2 cell line for A-B and B-A were in the range of 1100-1200 Ω .cm^2 in all pre- and post-experiment measurements and were found to comply with the acceptance criteria.

In vitro permeability studies showed that SEDDS formulations increased the permeability of Caco-2 cell lines. Finally, developing valsartan-containing VST-SEDDS and VST-S-SEDDS may be a desirable for improved bioavailable antihypertensive therapy.

Cytotoxicity Test

As Caco-2 cells were used as a permeability model, biocompatibility and tolerability of developed VST-SEDDS and VST-S-SEDDS formulations on Caco-2 cells were important [29]. For this reason, formulations were assessed in Caco-2 cells by MTT assay to check their safety. In addition to the developed formulations, the effect of powder VST and commercial product on cell viability was investigated. In order to predict any cytotoxic effects that may arise from the formulation components, MTT studies were also conducted for the blank formulations and no change in cell viability was observed in blank formulations. In the cytotoxicity study, cell viability greater than 95% was obtained for powder VST, VST-SEDDS, VST-S-SEDDS, and the commercial product, 98.5%, 96%, and 96.5%, 98%, respectively. Therefore, there is no cytotoxic effect from formulations on Caco-2 cells.

In this study, stability evaluations and permeability studies of the developed SEDDS and S-SEDDS formulations containing the active ingredient VST, which shows low solubility depending on pH, were successfully carried out. No significant change was observed in the characterization results performed during the stability follow-ups of both SEDDS and S-SEDDS formulations. Moreover, in the permeability study conducted on the Caco-2 cell line, it was observed that the permeability increased in SEDDS and S-SEDDS formulations depending on the active ingredient. These results show that SEDDS and S-SEDDS formulations containing VST may be an alternative approach in antihypertensive treatment.

ACKNOWLEDGEMENTS

This study was supported by The Scientific and Technological Council of Turkey (TUBİTAK Project No: 117S821).

AUTHOR CONTRIBUTIONS

Concept: G.Y.T., E.G., H.Y.K.; Design: G.Y.T., H.Y.K.; Control: H.Y.K, M.D.; Sources: G.Y.T., M.D., E.G., H.Y.K.; Materials: E.G., H.Y.K.; Data Collection and/or Processing: G.Y.T., E.G., M.D.; Analysis and/or Interpretation: G.Y.T., M.D., H.Y.K.; Literature Review: G.Y.T., M.D., H.Y.K.; Manuscript Writing: G.Y.T., M.D., H.Y.K.; Critical Review: G.Y.T., M.D., H.Y.K.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. World Health Organization Web site. (2023). Hypertension. Retrieved November 17, 2023, from https://www.who.int/news-room/fact-sheets/detail/hypertension. Accessed date: 16.03.2023.
2. European Medicines Agency (2010). Assessment report for DIOVAN and associated names, from https://www.ema.europa.eu/en/documents/referral/assessment-report-diovan-emeaha-29-pad1220_en.pdf. Accessed date: 19.03.2023.
3. Himawan, A., Djide, N.J.N., Mardikasari, S.A., Utami, R.N., Arjuna, A., Donnelly, R.F., Permana, A.D. (2022). A novel in vitro approach to investigate the effect of food intake on release profile of valsartan in solid dispersion-floating gel *in-situ* delivery system. European Journal of Pharmaceutical Sciences, 168, 106057. [CrossRef]

4. Caliph, S.M., Charman, W.N., Porter, C.J.H. (2000). Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. Journal of Pharmaceutical Sciences, 89, 1073-1084. [CrossRef]
5. Czajkowska-Košnik, A., Szekalska, M., Amelian, A., Szymańska, E., Winnicka, K. (2015). Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin. Molecules, 20, 21010-21022. [CrossRef]
6. Parmar, B., Patel, U., Bhimani, B., Sanghavi, K. (2012). SMEDDS: A dominant dosage form which improve bioavailability. American Journal of Pharmtech Research, 2, 54-72.
7. Diril, M., Türkyılmaz, G.Y., Gülmezoğlu, E., Karasulu, H.Y. (2023). Development, characterization and *in vitro* evaluation of solid self-emulsifying drug delivery systems (S-SEDDS) containing valsartan. Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 16(3), 672-686. [CrossRef]
8. Yang, X., Gao, P., Jiang, Z., Luo, Q., Mu, C., Cui, M. (2021). Preparation and evaluation of self-emulsifying drug delivery system (SEDDS) of cepharanthine. AAPS PharmSciTech, 22, 1-12. [CrossRef]
9. Ali, M.S., Alam, M.S., Alam, N., Siddiqui, M.R. (2014). Preparation, characterization and stability study of dutasteride loaded nanoemulsion for treatment of benign prostatic hypertrophy. Iranian Journal of Pharmaceutical Research. 13, 1125-1140.
10. Şatana, E., Altinay, Ş., Göger, N.G., Özkan, S.A., Şentürk, Z. (2001). Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC. Journal of Pharmaceutical and Biomedical Analysis, 25, 1009-1013. [CrossRef]
11. Scheeren, L.E., Nogueira-Librelotto, D.R., Fernandes, J.R., Macedo, L.B., Marcolino, A.I.P., Vinardell, M.P., Rolim, C.M.B. (2018). Comparative study of reversed-phase high-performance liquid chromatography and ultraviolet-visible spectrophotometry to determine doxorubicin in ph-sensitive nanoparticles. Analytical Letters, 51, 1445-1463. [CrossRef]
12. Karaküçük, A., Taşhan, E., Öztürk, N., Çelebi, N. (2021). *In vitro* caco-2 cell permeability studies of ziprasidone hydrochloride monohydrate nanocrystals. Turkish Journal of Pharmaceutical Sciences, 18, 223-227. [CrossRef]
13. Brück, S., Strohmeier, J., Busch, D., Drozdzik, M., Oswald, S. (2017). Caco-2 cells-expression, regulation and function of drug transporters compared with human jejunal tissue. Biopharmaceutics and Drug Disposition, 38, 115-126. [CrossRef]
14. Diril, M., Karasulu, Y., Toskas, M., Nikolakakis, I. (2019). Development and permeability testing of self-emulsifying atorvastatin calcium pellets and tablets of compressed pellets. Processes 7(6), 365. [CrossRef]
15. Matsumoto, T., Kaifuchi, N., Mizuhara, Y., Warabi, E., Watanabe, J. (2018). Use of a Caco-2 permeability assay to evaluate the effects of several Kampo medicines on the drug transporter P-glycoprotein. Journal of Natural Medicines, 72, 897-904. [CrossRef]
16. Sawadogo, W.R., Luo, Y., Elkington, B., He T.C., Wang, C.Z., Yuan, C.S. (2020). Assessment of the anti-proliferative effect and preliminary analysis of cell cycle arrest and pro-apoptotic effects of balanites aegyptiaca (L.) Delile on colorectal cancer cells HCT-116 and HT-29. Journal of Pharmaceutical Research International, 11, 9-21. [CrossRef]
17. Zein, R., Alghoraibi, I., Soukkarieh, C., Salman, A., Alahmad, A. (2020). *In-vitro* anticancer activity against Caco-2 cell line of colloidal nano silver synthesized using aqueous extract of Eucalyptus Camaldulensis leaves. Heliyon 6 (8), e04594. [CrossRef]
18. Suvarna, V., Pagdhar, U., Kadu, A., Oza, M. (2017). Development and characterization of solid self-emulsifying drug delivery system containing nateglinide. Asian Journal of Pharmaceutics, 11, 27-36 [CrossRef]
19. Pouton, C.W. (2000). Lipid formulations for oral administration of drugs: Non-emulsifying, self-emulsifying and "self-microemulsifying" drug delivery systems. European Journal of Pharmaceutical Sciences, 11, 93-98. [CrossRef]
20. Tengsh, S.D., Karande, K.M. (2020). A review on self micro-emulsifying drug del,very system: a tool for solubility enhancement. International Journal of Research and Analytical Reviews (IJRAR), 7, 101-114.
21. Council of Europe. European Pharmacopoeia, 6th ed. (2007). 20936 (01/2008). Strasbourg, France, p. 320-324.
22. Giri, T.K., Kumar, K., Alexande,r A., Ajazuddin, Badwaik, H., Tripathi, D.K. (2012). A novel and alternative approach to controlled release drug delivery system based on solid dispersion technique. Bulletin of Faculty of Pharmacy, 50, 147-159. [CrossRef]
23. Bernkop-Schnurch A, Malkawi A, Jalil A, Nazir I, Matuszczak B, Kennedy R. (2020). Self-emulsifying drug delivery systems: Hydrophobic drug polymer complexes provide a sustained release *in vitro*. Molecular Pharmaceutics,17(10), 3709-3719.

24. Lea, T. (2015). Caco-2 Cell Line. In: Verhoeckx, K (Eds of Chief), The Impact of Food Bioactives on Health. Springer, Cham. [CrossRef]
25. Cárdenas, P.A., Kratz, J.M., Hernández, A., Costa, G.M., Ospina, F., Baena, Y., Maria, C., Simõe,s O., Jimenez-kairuz, Á., Aragon, M. (2017). *In vitro* intestinal permeability studies, pharmacokinetics and tissue distribution of 6-methylcoumarin after oral and intraperitoneal administration in Wistar rats. Brazilian Journal of Pharmaceutical Sciences, 53, 1-9. [CrossRef]
26. Timur, S.S., Gürsoy, R.N. (2020). Design and *in vitro* evaluation of solid SEDDS for breast cancer therapy. Journal of Drug Delivery Science and Technology, 60, 102023. [CrossRef]
27. Sha, X., Yan, G., Wu, Y., Li, J., Fang, X. (2005). Effect of self-microemulsifying drug delivery systems containing Labrasol on tight junctions in Caco-2 cells. European Journal of Pharmaceutical Sciences, 24, 477-486. [CrossRef]
28. Chen, S., Einspanier, R., Schoen, J. (2015). Transepithelial electrical resistance (TEER): A functional parameter to monitor the quality of oviduct epithelial cells cultured on filter supports. Histochemistry and Cell Biology, 144, 509-515. [CrossRef]
29. Silva, A.C., González-Mira, E., García, M.L., Egea, M.A., Fonseca, J., Silva, R., Santos, D., Souto, E.B., Ferreira, D. (2011). Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): High pressure homogenization versus ultrasound. Colloids and Surfaces B: Biointerfaces, 86, 158-165. [CrossRef]