

Development and In-Vitro Characterization of Self-nanoemulsifying Drug Delivery Systems of Valsartan

P. S. Rajinikanth, Yeoh Suyu, and Sanjay Garg

Abstract—The present study is aim to prepare and evaluate the selfnanoemulsifying drug delivery (SNEDDS) system of a poorly water soluble drug valsartan in order to achieve a better dissolution rate which would further help in enhancing oral bioavailability. The present research work describes a SNEDDS of valsartan using labrafil M 1944 CS, Tween 80 and Transcutol HP. The pseudo-ternary phase diagrams with presence and absence of drug were plotted to check for the emulsification range and also to evaluate the effect of valsartan on the emulsification behavior of the phases. The mixtures consisting of oil (labrafil M 1944 CS) with surfactant (tween 80), co-surfactant (Transcutol HP) were found to be optimum formulations. Prepared formulations were evaluated for its particle size distribution, nanoemulsifying properties, robustness to dilution, self emulsification time, turbidity measurement, drug content and in-vitro dissolution. The optimized formulations are further evaluated for heating cooling cycle, centrifugation studies, freeze thaw cycling, particle size distribution and zeta potential were carried out to confirm the stability of the formed SNEDDS formulations. The prepared formulation revealed t a significant improvement in terms of the drug solubility as compared with marketed tablet and pure drug.

Keywords—Self Emulsifying Drug Delivery System, Valsartan, Bioavailability, poorly soluble drug.

I. INTRODUCTION

Poor aqueous solubility is the predominant dilemma particularly associated with combinatorial chemistry and high throughput screening [1-3]. One of the pharmaceutical drugs that have such issue is valsartan ($\log P = 1.499$) [4,5]. Valsartan is an angiotensin II receptor blocker and it is indicated for hypertension, heart failure and post-myocardial infarction [4-6]. It is reported that marketed valsartan has an absolute bioavailability of 10 - 35% [5,6]. Furthermore, exposure (AUC) of valsartan decreases by 40% in the presence of food but increases by 70% in elderly [5,6]. Such variations may due to the pH-dependent solubility of valsartan, where the solubility are 16.8 g/L and 0.18 g/L in pH

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8.0 phosphate buffer (PBS) and water respectively [4,5]. Being a weakly acidic drug ($pK_a = 8.15$), valsartan is generally in the unionised form at the stomach, and thus poor solubility but great intestinal absorption. Thus, it is plausible that by addressing the solubility issue at low pH, enhanced absorption and subsequently greater bioavailability can be achieved.

There were several approaches taken in the past in order to improve the dissolution rate of valsartan and they included, but not limited to, solid dispersion, fast dissolving tablet and self-microemulsifying drug delivery system (SMEDDS) [7-10].

Among the various approaches in improving solubility, SNEDDS appears to surpass the rest as SNEDDS requires simple and cost-effective manufacturing facilities. This is because SNEDDS is a physically stable lipid solution and it omits the need of high energy emulsification process. Also, SNEDDS appears to reduce the effect of food on bioavailability [11] and improve the onset of action [12]. Besides, many lipophilic compounds have been used as model drugs in SNEDDS formulations and the results are remarkable [13-19].

SNEDDS is composed of an isotropic mixture of oil, surfactant, co-surfactant and drug [21]. Upon ingestion, the isotropic mixture will come in contact with the aqueous phase of gastrointestinal tracts and form an oil-in-water nanoemulsion with the aid of gastrointestinal motility. This nanoemulsion can provide a large interfacial area for partitioning of drug between oil and aqueous phase and subsequently offer better dissolution rate.

The aim of this research is to formulate SNEDDS of valsartan using optimised choice and ratio of oil, surfactant and co-surfactant.

II. MATERIALS AND METHODS

A. Materials

Valsartan API was purchased from Aurobindo Pharma Ltd (India). Excipients such as Capryol 90 (propylene glycol monocaprylate (type II) NF), Labrafac Lipophile WL 1349 (medium-chain triglycerides NF), Labrafil M 1944 CS (oleoyl polyoxyl-6 glycerides NF), Labrasol (caprylocaproyl polyoxyl-8 glycerides NF) and Transcutol HP (highly purified diethylene glycol monoethyl ether NF) were generously sponsored by Gattefosse (France). Surfactants such as tween

20 (polyoxyethylene (20) sorbitan monolaurate) and tween 60 (polyoxyethylene (20) sorbitan monostearate) were procured from R & M Chemical (UK) whereas tween 80 (polyoxyethylene (20) sorbitan monooleate) was obtained from Merck. As for PEG 300, PEG 400 and PEG 600, they were acquired from Aldrich (Germany). Olive oil was bought from Fluka. Other chemicals used were of analytical reagent grade.

B. Solubility Studies

The solubility studies were conducted by excess known amount of valsartan was added to 1 ml of each vehicle and the mixtures were mixed using vortex mixer (Labnet International Inc). The mixtures were then stored in an incubator shaker at 150 rpm and 25°C for 24 hours. After that, the mixtures were spun using a centrifugator (Centrifuge Bench Top Refrigerated, Eppendorf) at 3000 rpm for 15 minutes. The supernatants were retrieved and quantified using UV-Vis spectrophotometer (Lambda 25, Perkin Elmer).

C. Pseudoternary Phase Diagram

The best oil, surfactant and co-surfactant were chosen based on solubility studies and consideration on the combined hydrophile-lipophile balance (HLB). Firstly, a mixture of surfactant and co-surfactant (Smix) was prepared in 1:1 ratio and combined with the selected oil in different volume ratio (e.g. from 1:9 to 9:1). When aqueous titration (addition of water in 5% increment from 5% to 95%) was performed on the lipid mixture of oil and Smix, the observation of clear to turbid mixture, which was the end point, was closely monitored. These end points were then marked on a pseudoternary phase diagram. The whole procedure was repeated for Smix 2:1 and 3:1. Finally, among the three pseudoternary phase diagrams, the one with the largest self-nanoemulsifying region was selected for the next phase of this research.

Aqueous titration was repeated for the selected Smix ratio in the presence of valsartan. This is because there were reports on the change in self-emulsifying property in the presence of drug [1]. Another pseudoternary phase diagram was constructed and six points were randomly picked from the self-nanoemulsifying region. The percentage of each component for all six points was calculated. Correspondingly, six formulations were prepared by mixing all specifically measured oil, surfactant, co-surfactant and valsartan.

D. Thermodynamic Stability Testing

The formulations were subjected to heating-cooling, centrifugation and freeze-thaw, where the physical appearances of the formulations were observed at the end of each testing. In heating cooling, all six formulations were heated at 45°C and then cooled at 4°C, with the duration of 24 hours at each temperature, for 2 cycles. Then, formulations which passed the heating-cooling cycles were subjected to centrifugation at 3500 rpm for 15 minutes. Finally, only formulations which passed the previous two steps were stored at alternating temperature of -21°C and 25°C, with the

duration of 24 hours at each temperature, for 2 cycles.

E. Robustness to Dilution

5µl of formulations were diluted infinitely (i.e. 900 times) with 4500 µl of water, pH 6.80 PBS and pH 1.20 acid buffer in three separate glass vials. The diluted formulations were shaken and then visually inspected after 24 hours for any form of instability.

F. Droplet Size and Zeta Potential Analyses

This analysis was carried out so as to determine the consistency in the size and stability of the emulsion at various dilutions (i.e. 100, 500 and 900 dilution factors) and dispersant media (miliQ water, pH 6.80 PBS and pH 1.20 acid buffer). Malvern Zeta Sizer Nano ZS with the conditions of backscatter detection at 173°; temperature of 25°C; refractive index of 1.330 were used. All were done in triplicates.

G. Transmission Electron Microscopy (TEM)

A drop of diluted formulation was placed on a carbon-coated copper grid, stained with 2% uranyl acetate aqueous solution, and examined using the TEM (Philips Tecnai 12).

H. In Vitro Dissolution Studies

Dissolution studies were carried out using USP Apparatus Type II (paddle type) with 900 ml of pH 6.80 ± 0.05 PBS, temperature at 37 ± 0.5°C and paddle rotation of 50 rpm. 5 ml of formulation, which contained 80 mg of valsartan, was instilled to the dissolution medium at time 0 minute. 5 ml of dissolution media was retrieved at timed intervals and the amount of valsartan was quantified using HPLC method. Dissolution studies were also done using pH 1.20 ± 0.05 acid buffer as dissolution medium. All were done in triplicates.

I. Emulsification Time

Under the same conditions as in vitro dissolution studies, time taken by the formulation to form homogenous mixture with the dissolution medium was noted in triplicates.

J. Dispersibility Test

Under the same conditions as in vitro dissolution studies, the type of emulsion formed was visually inspected and categorised as either clear, translucent with bluish tone or milky turbid emulsion.

K. Accelerated Stability Testing

All anhydrous formulations were stored in an incubator at 40°C and 75% relative humidity for four weeks. Visual assessment, droplet size and zeta potential analyses were conducted for selected formulations at the end of the study.

III. RESULTS

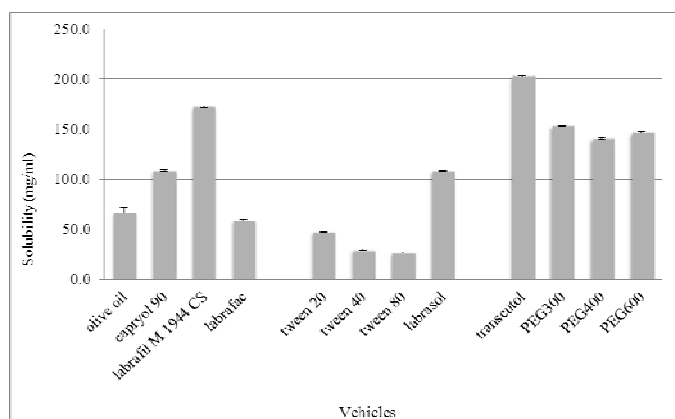


Fig. 1 Graph of solubility of valsartan in various vehicles

Oil with the greatest solubilizing capacity for the drug under study was desired in order to obtain maximum drug loading [21, 22]. The solubility of drug in different oils, surfactant and co-surfactants are shown in Fig. 1.

TABLE I
 THE PERCENTAGE OF EACH COMPONENT IN THE SELECTED SIX FORMULATIONS

Formulation	Percentage of Labrafil M 1944 CS (% v/v)	Percentage of Tween 80 (% v/v)	Percentage of Transcutol HP (% v/v)
A	68.5	23.5	8.0
B	60.0	30.0	10.0
C	50.0	37.5	12.5
D	35.5	48.5	16.0
E	23.0	58.0	19.0
F	43.0	42.5	14.5

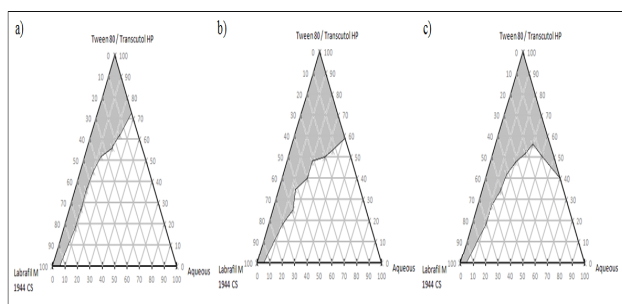


Fig.2. Pseudoternary phase diagrams of Labrafil M 1944 CS (oil), Tween 80 and Transcutol HP (Smix) and water, in the absence of valsartan, with Smix ratios: a) Smix 1:1; b) Smix 2:1 and c) Smix 3:1

The self-nanoemulsifying regions were represented by the shaded region.

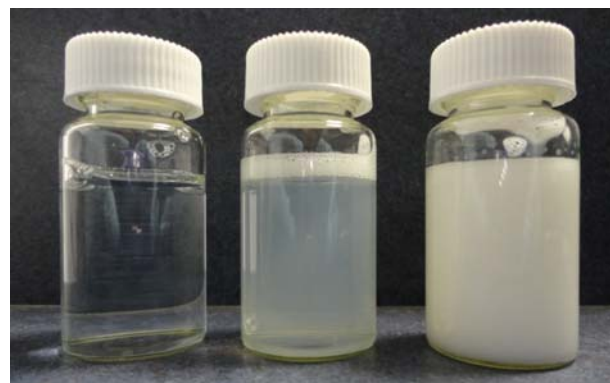


Fig. 3 Observation of (from left to right) transparent; translucent with bluish tone; and milky turbid emulsions

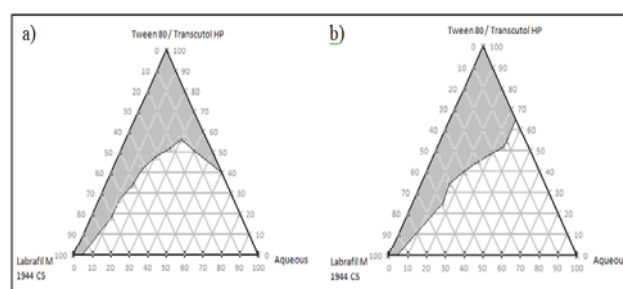


Fig. 4 Pseudoternary phase diagrams of Labrafil M 1944 CS (oil), Tween 80 and Transcutol HP (Smix) and water a) in the absence of valsartan; and b) in the presence of 80 mg of valsartan

IV. DISCUSSION

A. Solubility of Drug

Oils with medium or short hydrocarbon chains were known to nanoemulsify better than long chain triglycerides such as olive oil [21]. Based on these two aspects, Labrafil M 1944 CS, with the solubility of 172.6 mg/ml, was selected as the oil. As for the selection of Smix, these components must be of GRAS status, which suggested safe for oral consumption. Although Labrasol appeared to be the surfactant with maximum solubility (Fig. 1), tween 80 was chosen due to material availability. Transcutol HP was regarded as the best co-surfactant and the solubility was found to be 202.8 mg/ml. Addition of co-surfactant into the SNEDDS formulation was advantageous as it can improve drug loading and self-emulsification time [21].

Besides, the combinatory HLB_a value for this SNEDDS combination (i.e. Labrafil M 1944 CS + Tween 80 + Transcutol HP) was found to be in the range of 8 - 18 and formation of oil-in-water nanoemulsion can be ensured.

B. Construction of Pseudoternary Phase Diagram

Based on Fig. 2, the self-nanoemulsifying regions appeared to increase with increasing Smix ratio where Smix 3:1 resulted in the largest self-nanoemulsifying region. It was also noticeable that at higher ratio of Smix, the mixture can take up greater amount of water and still remain as translucent mixture with bluish tone (Fig. 3). This could be explained by the fact

that higher amount of surfactants can be adsorbed at the interface and hence, stabilised the formation of nanoemulsions.

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C. Formulations of SNEDDS

The self-nanoemulsifying region with the incorporation of 80 mg of valsartan appeared to be significantly larger ($p < 0.05$) than that of in the absence of valsartan (Fig. 4). Next, six points were randomly picked from the self-nanoemulsifying region and the formulations were summarised in Table I.

D. Thermodynamic Stability Testing

All six formulations passed the thermodynamic stability testing as there was no sign of phase separation or drug precipitation at the end of all cycles. This suggested that the formulations were robust against storage at extreme conditions.

E. Robustness to Dilution/Precipitation

Formulation A and B showed some white precipitation on the surface but the precipitation resolved upon gentle shaking. As for formulation C, D, E and F, all appeared to remain homogenous with no sign of phase separation or drug precipitation. This implied that these formulations were stable at infinite water dilution.

F. Droplet Size Analysis

Droplet size is of great concern in the formulations of SNEDDS as small globule size of emulsion contributes to greater interfacial area which can then provide better drug partitioning and absorption. However, there was no consensus on the exact size range of nanoemulsion [15, 17, 21, 23]. In the present study, average droplet size of less than 200 nm

with low polydispersity index (PDI), ideally < 0.5 was desirable. Referring to Table II, formulation A, B and C showed an average droplet size of more than 200 nm and so, these formulations were dropped from this study. As for formulation F, it showed inconsistency in the droplet size at various dilution factors. Only formulation D and E were included for further testing as both formulations showed small droplet sizes, especially formulation E which gave rise to less than 100 nm.

TABLE II
THE AVERAGE DROPLET SIZE OF ALL SIX FORMULATIONS AT VARIOUS DILUTION FACTORS WITH WATER

Formulation	Dilution factor	Droplet size (nm)	PDI
A	100	335.9 ± 12.91	0.177 ± 0.059
A	500	426.2 ± 6186	0.218 ± 0.056
A	900	412.1 ± 129.1	0.222 ± 0.124
B	100	285.7 ± 3.15	0.012 ± 0.032
B	500	334.5 ± 9.57	0.197 ± 0.022
B	900	257.0 ± 15.89	0.237 ± 0.031
C	100	272.7 ± 6.43	0.248 ± 0.108
C	500	213.9 ± 8.05	0.347 ± 0.006
C	900	210.6 ± 4.43	0.214 ± 0.041
D	100	163.7 ± 1.98	0.178 ± 0.013
D	500	185.8 ± 2.95	0.201 ± 0.013
D	900	173.2 ± 2.69	0.350 ± 0.087
E	100	93.6 ± 0.97	0.204 ± 0.004
E	500	84.5 ± 0.62	0.232 ± 0.006
E	900	87.2 ± 1.19	0.168 ± 0.031
F	100	178.1 ± 2.50	0.144 ± 0.005
F	500	256.5 ± 9.07	0.396 ± 0.073
F	900	191.2 ± 20.81	0.316 ± 0.025

G. Effect of Different Media on Droplet Size and Zeta Potential

Due to considerable pH variations along gastrointestinal tracts, it is rational to observe the consequence of different media on the SNEDDS. Although the droplet sizes of both formulations were of less than 200nm, the droplet sizes changed significantly ($p < 0.05$) at different dispersant media (Table III). This may be due to the weakly acidic valsartan, which was mainly unionised at lower pH, which remained in the oil droplets and hence resulting in bigger droplet size at acidic condition. As opposed, the weakly acidic valsartan was mainly ionised at higher pH, had the tendency to diffuse to the continuous phase of PBS, resulting in smaller droplet size at basic condition. Zeta potential was also measured in order to determine the interaction between colloidal particles, where large positive or negative values suggesting high inclination of emulsion repulsion and hence, stability [24]. Formulation E was considered to be less stable at PBS as the obtained zeta

potential was very near to the isoelectric point (i.e. 0 mV), where there would be high chance of droplet coalescence.

TABLE III
 THE AVERAGE DROPLET SIZE, PDI AND ZETA POTENTIAL OF SELECTED FORMULATIONS AT 900 TIMES DILUTION WITH VARIOUS DISPERSANT MEDIA

Formulation	Dispersant media	Droplet size (nm)	PDI	Zeta potential (mV)
D	Water	173.2 ± 2.691	0.450 ± 0.087	-20.10 ± 0.2
		107.4 ± 0.666	0.430 ± 0.013	-0.45 ± 0.186
		143.7 ± 4.325	0.417 ± 0.010	5.11 ± 0.537
E	Water	87.23 ± 1.19	0.468 ± 0.031	-16.90 ± 1.57
		65.85 ± 2.298	0.526 ± 0.017	-2.80 ± 1.11
		111.2 ± 1.473	0.557 ± 0.045	3.98 ± 0.71

H. Transmission Electron Microscopy (TEM)

Microscopy analysis revealed well-defined circular globules with the size of less than 100nm (Fig. 5).

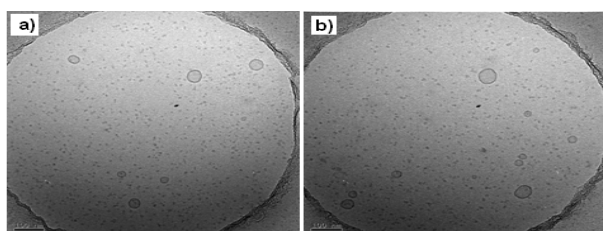


Fig. 5 TEM image of formulation E upon a) 500 times and b) 900 times dilution with water

I. In Vitro Dissolution Studies

Formulation E, Diovan tablet and pure valsartan powder demonstrated more than 80% of drug release within 5 minutes in pH 6.80 ± 0.05 PBS and the differences were insignificant (p>0.05) (Fig.6.). As in pH 1.20 acid buffer, it was found that both Diovan tablet and pure valsartan powder had less than 13% of drug release even by 30 minutes, which was considered poor and undesirable. On the contrary, formulations E achieved significantly (p<0.05) better DRUG release, with 80.0% of drug release by 30 minutes (Fig. 7).

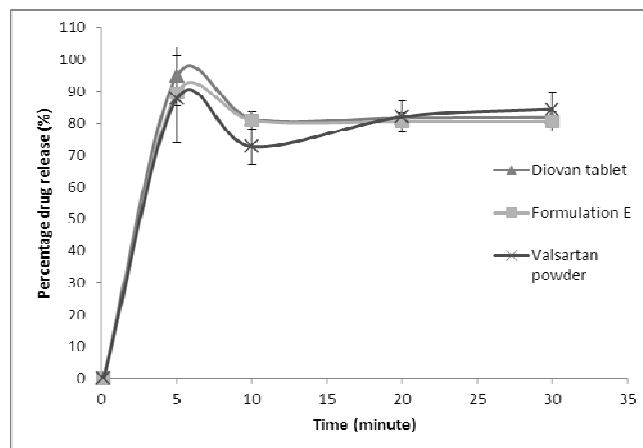


Fig. 6 In vitro dissolution profile using pH 6.80 ± 0.05 PBS as dissolution medium (n=3)

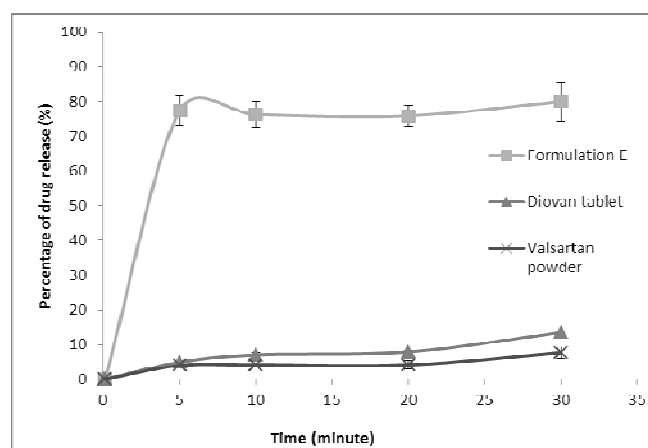


Fig. 7 In vitro dissolution profile using pH 1.20 ± 0.05 acid buffer as dissolution medium (n=3)

J. Emulsification Time

Formulation E was considered to have good emulsification time as it took less than 1 minute (i.e. 15.3 ± 0.6 s and 14.0 ± 1.7 s in PBS and acid buffer respectively) to form homogenous mixture with dissolution media.

K. Dispersibility Testing

Upon contact with dissolution media, formulation E produced translucent mixtures (as represented by the centre glass vials in Fig. 3). This hinted the formation of stable nanoemulsions with the approximate droplet size of 100 nm.

L. Accelerated Stability Testing

There was no observation of phase separation, drug precipitation or colour change in formulation E at the end of a 4-week accelerated stability study. Zeta size and potential measurement also revealed similar droplet size (p>0.05) and relatively stable droplets. However, at least 6 months of accelerated stability study is required to clearly confirm the stability.

V. CONCLUSION

SNEDDS of valsartan was successfully prepared using Labrafil M 1944 CS (23%), Tween 80 (58%) and Transcutol HP (19%). This formulation showed significant improvement in dissolution rate in pH1.20 acidic buffer (more than 6-fold of drug release) and there is a possibility of improved drug absorption across stomach linings. However, more studies need to be carried out in order to affirm the bioavailability.

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REFERENCES

- [1] Lipinski C: Poor aqueous solubility – an industry wide problem in drug discovery. [<http://americanpharmaceuticalreview.com/ViewArticle.aspx?ContentID=142>]
- [2] Di L, Kerns EH: Optimizing the physicochemical properties of clinical candidates during drug discovery. [<http://mediaserver.aaps.org/meetings/webinars/ppb-9/ppb-9.pdf>]
- [3] Stegemann S, Leveiller F, Franchi D, Jong HD, Linden H: When poor solubility becomes an issue: From early stage to proof of concept. [<http://capsugel.com/media/library/when-poor-solubility-becomes-an-issue-from-early-stage-to-proof-of-concept.pdf>]
- [4] Product Leaflet of Diovan. New Jersey: Novartis; 2011 Apr
- [5] Siddiqui N et al: Pharmacological and Pharmaceutical Profile of Valsartan: A Review. Journal of Applied Pharmaceutical Science 2011; 01(04): 12-9
- [6] Saydam M, Takka S: Bioavailability File: Valsartan. FABAD J. Pharm. Sci. 2007; 32: 185 – 196.
- [7] Venkates KK, Arunkumar N, Verma PRP, Rani C: Preparation and in vitro characterization of valsartan solid dispersion using skimmed milk powder as carrier. IJPRIF. 2009, 1(3):431-7.
- [8] Mahapatra AK, Murthy PN, Biswal S, Mahapatra APK, Pradhan SP: Dissolution enhancement and physicochemical characterization of valsartan in solid dispersions with β -CD, HP β -CD and PVP K-30. Dissolution Technologies 2011, 39-35.
- [9] Dixit AR, Rajput SJ, Patel SG: Preparation and bioavailability assessment of SMEDDS containing valsartan. AAPS 2010, 11(1):314-21
- [10] Jain CP, Naruka PS: Formulation and evaluation of fast dissolving tablets of valsartan. IJPS 2009, 219-26.
- [11] Nielsen FS, Petersen KB, Müllertz A: Bioavailability of probucol from lipid and surfactant based formulations in minipigs: influence of droplet size and dietary state. Eur J Pharm Biopharm 2008, 69:553-62.
- [12] Kumar GP, Rambhau D, Aapte SS: Oral bioavailability enhancement of carbamazepine in healthy human volunteers. J Pharm Research 2011, 4(2): 361-5.
- [13] Taha EI, Al-Saidan S, Samy AM, Khan MA: Preparation and in vitro characterization of self-nanoemulsified drug delivery system (SNEDDS) of all-trans-retinol acetate. I J Pharm. 2004, 285(2004):109-19.
- [14] Patel AR, Vavia PR: Preparation and in vivo evaluation of SMEDDS (Self-Microemulsifying Drug Delivery System) containing fenofibrate. AAPS 2007, 9(3):344-52
- [15] Shafiq-un-Nabi S et al: Formulation Development and Optimization Using Nanoemulsion Technique: A Technical Note. AAPS Pharm. Sci. Tech. 2007, 8(2):28
- [16] Mahmood EA, Bendas ER, Mohamed MI: Preparation and evaluation of self-nanoemulsifying tablets of carvedilol. AAPS 2009, 10(1):9192-7.
- [17] Kassem AA, Marzouk MA, Ammar AA, Elosaily GH: Preparation and in vitro evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) containing clotrimazole. Drug Discovery and Therapeutics 2010, 4(5):373-9.
- [18] Jeevana JB, Sreelakshmi K: Design and evaluation of self-nanoemulsifying drug delivery system of flutamide. J Young Pharm. 2011, 3(1):4-8.
- [19] Gupta AK et al: Preparation and in-vitro evaluation of self-emulsifying drug delivery system of antihypertensive drug valsartan. International Journal of Pharmaceutics and Live Sciences March 2011, 2(3): 633 – 639.
- [20] Patel J, Kevin G, Patel A, Raval M, Sheth N: Design and development of a self-nanoemulsifying drug delivery system for telmisartan for oral delivery. IJPI 2011, 1(2):112-8.
- [21] Date AA, Desai N, Dixit R, Nagarsenker M: Self-nanoemulsifying Drug Delivery Systems: Formulation Insights, Applications and Advances. Nanomedicine 2010, 5(10).
- [22] Pouton CW: Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 2006, 29:278-87
- [23] Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM: Nanoemulsions: Formation, structure and physical properties. J. Phys. 2006, 18(2006): R635-666.
- [24] Zetasizer Nano series technical notes. [<http://www.nbtc.cornell.edu/facilities/downloads/Zeta%20potential%20-%20An%20introduction%20in%2030%20minutes.pdf>]
- [25] Selection of emulsifiers. In Emulsion Science and Technology: A General Introduction. Edited by Tadros TF. UK: Wiley-VCH; 2009:25.
- [26] Parambi DGT, Mathew M, Ganesan V: A validated stability indicating HPLC method for the determination of valsartan in tablet dosage forms. Journal of Applied Pharmaceutical Science 2011, 01(04): 97-9.