International Journal of Drug Delivery 7 (2015) 32-43
http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Development, characterization and in vivo evaluation of Tolvaptan solid dispersions via solvent evaporation technique

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Abstract

Investigation on in vitro and in vivo behavior of solid dispersions containing Tolvaptan is the focus of the present research work. The effect of various hydrophilic polymers on the aqueous solubility was studied. Kleptose HPB was selected as carrier and solid dispersions were prepared by solvent evaporation technique. Evaluation of solid dispersion for percentage yield, drug content and solubility was most appropriate. Solid dispersions of drug: Kleptose HPB: SLS (1:2:1 ratio) (SE8) shown higher dissolution rate i.e. 96.8 % compared with and pure drug (39.6%) and other formulations. Differential scanning calorimetry and powder X-ray diffraction performed on solid dispersion showed that Tolvaptan existed in the amorphous form within the solid dispersion formulation fabricated using the solvent evaporation process. Additionally, scanning electron microscopy studies suggested the conversion of crystalline Tolvaptan to an amorphous form. In vivo studies of pure drug and optimized formulation (SE8) were carried out in male Wistar rats and pharmacokinetic parameters calculated using Kinetica software 2000. In vivo studies revealed that a marked increase in dissolution and bioavailability was exhibited by optimized Tolvaptan solid dispersion (SE8). AUC (0-t) was increased more than 2.11 folds, Cmax increased about 2.67 folds and tmax reduced by 1 hour, when compared to the pure drug. Thus, the study has illustrated the potential use of a solid dispersion system for the delivery of a very poorly soluble drug tolvaptan with a better bioavailability. Therefore, the solid dispersions prepared by solvent evaporation method using Kleptose HPB as hydrophilic carrier can be successfully used for improvement of dissolution of Tolvaptan and resulted in faster onset of action as indicated by in vivo studies.

Keywords: Tolvaptan, hydrophilic carriers, solvent evaporation technique, solubility, solid dispersions.

Introduction

More than 40 percent of the drug coming from high throughput screening are poorly water soluble in water with poor permeability are increasingly posing challenges in the development of new drugs, since a large number of drugs coming directly from synthesis or from high throughput screening have a very poor solubility. It is well known that drug efficacy can be severely limited by poor aqueous solubility, leading to low dissolution rate and thus results in low absorption in the gastrointestinal tract after oral administration hence comprising oral bio-availability. The ability to increase aqueous solubility is thus a valuable aid to increase the efficacy for certain drugs [1].

One of the major challenges of pharmaceutical formulation scientists is to develop the oral dosage forms of poor aqueous solubility drugs, hence, two areas of pharmaceutical research that focus on improving the oral bioavailability of active agents include enhancing solubility and dissolution rate of poorly water-soluble drugs and enhancing permeability of poorly permeable drugs [2].

Solubility is one of the important physic-chemical property to be considered for formulation development and it is a predetermined and rate limiting step for drug absorption. There are many methods like salt formation, solubilisation and particle size reduction to enhance oral bioavailability of poorly soluble drugs. However, techniques such as size reduction increases dissolution and bioavailability but, micronization often leads to aggregation and agglomeration, which leads to poor wettability of particles [3].

This problem is rectified by preparation of solid dispersion of poorly water soluble drugs by using water soluble carriers. Poorly soluble drugs when formulated as tablet or capsule dosage forms it disintegrate into large solid particles in GI tact, which leads to poor dissolution and less absorbed into the body system. Significantly, solid dispersion disintegrates into colloidal particle of particle size less than 5 microns which enhances the dissolution rate of the

drug. Among all the methods, solid dispersion has been widely used to increase oral bioavailability, solubility and dissolution rate of the drug [4].

Tolvaptan is relatively a new chemical and pharmacologic class of drug known as aquaretic sorvaptans [5].

Tolvaptan (INN) is a selective, competitive vasopressin receptor 2 antagonist used to treat hyponatremia (low blood sodium levels) associated with congestive heart failure, cirrhosis, and the syndrome of inappropriate antidiuretic hormone (SIADH). Tolvaptan was developed by Otsuka Pharmaceutical Co. and approved by the U.S. Food and Drug Administration in 2009. It is the first and acquire V2-receptor antagonist in Chinese market [6].

Tolvaptan vasopressin antagonist role by increasing the excretion of urine sleep, enhance free water clearance, reduced urine osmolality, and increased serum sodium values, while not changing the urine and serum potassium, sodium and potassium secretion content. Tolvaptan is mainly metabolized by CYP3A4 [7].

Materials and Methods

Materials

SAMSCA® (Tolvaptan) 30 mg conventional tablets were obtained from Otsuka Pharmaceutical Ltd, Germany. Tolvaptan was gifted by Hetero drugs limited, Hyderabad, India. Kolliphor P 407 and Kolliphor P188 were obtained from BASF, US. Kolliwax GMS II, Kolliphor RH-40, Kolliphor EL, Kolliphor HS-15, Kolliphor TPGS, Kollidon 30, Kollidon CL, Soluplus and Kollidon® VA 64 was procured from BASF, Germany. Kleptose® HPB was gifted from Roquette Pharma, France. HPMC AS, MG Grade and Methocel HPMC 2.5cPs ~~was~~ were gifted by Dow Chemicals, USA. All other chemicals used were of analytical grade.

Methods

Preliminary solubility studies of Tolvaptan

Solubility measurements of Tolvaptan were performed according to a published method [8]. An excess amount of Tolvaptan was added to 25ml of aqueous solution of water soluble carriers like Kolliphor RH-40, Kolliphor EL, Kolliphor TPGS, Kolliphor HS-15, Kolliphor P 188, Kolliphor P 407, Kolliwax GMS II, Soluplus, DSS 100%, Kleptose HPB, HPMC AS, Kollidon 30, HPMC 2.5 cPs and mixture of Kolliphor P407& P188 in 1:1 and then drug to polymer to SLS in 1:1:1 were taken in screw capped bottles. Samples were shaken for the 48 hours at room temperature. Subsequently, the suspensions were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Tolvaptan in UV/Visible spectrophotometer at 269 nm.

Preparation of solid dispersions of Tolvaptan by solvent evaporation method

Tolvaptan solid dispersions of fourteen formulations were prepared by using various carriers Summarized in Table 1 (i.e. Kolliphor P407, Kolliwax GMS - II, Kleptose HPB, HPMC AS, Soluplus, Kolliphor P188 etc.,) with SLS in proportions viz. 1:1:1, 1:2:1 (Drug: Carrier: Surfactant). The drug and carrier along with SLS was dissolved in Methanol and triturated in dry mortar until the solvent is evaporated and a clear film of drug and carrier was obtained. Then the dispersion was subjected to Methanol solvent evaporation by placing in 50 C chamber for 30 min period. The resultant solid dispersion was scraped out with a spatula. Solid dispersions were pulverized in a mortar and pestle and passed through a 420 μm (ASTM #40 mesh) mesh before packing in an airtight container [9].

Table 1: Formulation plan of Tolvaptan solid dispersions

S. No	Ingredients	SE 1	SE 2	SE 3	SE 4	SE 5	SE 6	SE 7	SE 8	SE 9	SE 10	SE 11	SE 12	SE 13	SE 14
		1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2
1.	Tolvaptan (gm)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2.	HPMC AS, (MG Grade) (gm)	1	2	-	-	-	-	-	-		-	-	-	-	-
3.	Kolliwax GMS II (gm)	-	-	1	2	-	-	-	-	-	-	-	-	-	-
4.	Kolliphor P407 (gm)	-	-	-	-	1	2	-	-	-	-	-	-	-	-
5.	Kleptose HPB (gm)	-	-	-	-	-	-	1	2	-	-	-	-	-	-
6.	Kolliphor P188	-	-	-	-	-	-	-	-	1	2	-	-	-	-
7.	Mixture of Kolliphor P407& P188 in 1:1(gm)	-	-	-	-	-	-	-	-	-	-	1	2	-	-
8.	Soluplus (gm)	-	-	-	-	-	-	-	-	-	-	-	-	1	2
9.	SLS (gm)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10.	Methanol (mL)	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

Solubility studies of Tolvaptan solid dispersion by solvent evaporation method

A solubility measurement of Tolvaptan was performed according to a published method [10]. Solid dispersion samples were shaken for the 48 hours at room temperature. Subsequently, the suspensions

were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the tolvaptan by UV/Visible spectrophotometer at 269 nm.

Evaluation of Tolvaptan solid dispersions

Solid dispersions obtained from the above methods were tested for the % Practical yield, drug content and *in-vitro* release studies.

% Practical Yield

% of practical yield was calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production. SDs were collected and weighed to determine practical yield (PY) from the following equation.

$$\% \text{ Practical Yield} = \frac{\text{Practical weight (Solid dispersion)}}{\text{Theoretical weight (Drug + Polymer + Surfactant)}} \times 100$$

Drug content

Accurately weighed quantity of Tolvaptan solid dispersion which is equivalent to 30mg of Tolvaptan was taken in volumetric flask and the volume is made to 100ml with methanol. From this 1ml of solution is taken in a 10ml volumetric flask and is made up to 10ml with methanol. This solution is diluted to 10µg/ml and absorbance was measured at λ_{max} 269 nm against blank. The actual drug content was calculated using the following equation as follows:

$$\% \text{ Drug content} = \frac{\text{Actual amount of drug in solid dispersion}}{\text{Theoretical amount of drug in solid dispersion}} \times 100$$

In vitro release studies

The dissolution test was performed using USP type 2 (paddle type) dissolution apparatus (Electro lab) with 900 ml of 0.22% Sodium Lauryl Sulfate in water as a dissolution medium at an temperature of 37±0.5^{0}C with a paddle speed of 50 rpm. The solid dispersion equivalent to 30 mg of Tolvaptan was added and the sample of 10ml were withdrawn at 5, 10, 15, 30, 45 and 60 minutes time intervals and replaced with the same volume of the dissolution medium. The obtained samples were analyzed for cumulative percentage release by using UV-Visible spectrophotometer at 269nm.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The IR spectrum of the samples was prepared using KBr (spectroscopic grade) disks by means of hydraulic pellet press at pressure of seven to ten tons [11].

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Accurately weighed samples were placed on aluminium plate, sealed with aluminium lids and heated at a constant rate of 5ºC/min, over a temperature range of 0 to 250ºC [12].

Powder X-ray diffraction

A Bruker D8 diffractometer was used to perform powder X-ray diffraction (PXRD) of all samples. A Cu K- 1 tube was the source, set at 40 KV and 50mA. A scan from 2 to 60^0 2 θ was carried out at a rate of 0.01220^0 2θ/s. The diffractometer was calibrated using powdered -alumina. Hot-melt extruded samples were ground before analysis [13].

Scanning electron microscopy

The shape and surface morphology of the Tolvaptan and optimized formulation of solid dispersion prepared by solvent evaporation was examined using XL 30 model JEOL 6800 scanning electron microscope (Japan) [14].

Stability studies

Prepared solid dispersions were placed inside sealed 40cc HDPE container with child resistant cap and charged at an accelerated stability conditions of 40 ±2^{0}C / 75 ±5% RH. Samples were unloaded after 1, 2, 4 and 6 months, evaluated for % drug content and in vitro dissolution study and compared with those SD tested immediately after preparation [15].

In vivo bioavailability studies

Male Wister rats (weighing approximately 250±25 g) were procured from institutional animal house. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC NO: P28/VCP/IAEC/2014/3/DBP/AE12). Twelve healthy Wister rats were used for this study weighing 250±25 g. Rats were divided in to two groups at random each group containing six animals. First group was administered Tolvaptan (as such) suspension was prepared in 0.5% w/w HPMC 2.5cPs, second group was administered optimized preparation of solid dispersion suspension (Formulation SE8) was prepared in 0.5% w/w HPMC 2.5cPs by oral route at an equivalent dose of 30 mg/kg body weight. About 500 µl of blood was withdrawn from retro orbital plexus at different time intervals such as 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 12.00 and 24.00h. Blood samples were transferred into eppendorf tubes containing heparin in order to prevent blood clotting. The samples were centrifuged immediately at 4000 rpm and the plasma was stored in light-protected container at -20 ^{0}C till analysis. The concentration of Tolvaptan from plasma was measured by using reversed phase HPLC. The chromatographic system consisted of RP-C18 chromatographic column, Phenomenex Kinetex (150 mm 4.6 mm i.d) and a mobile phase consisting of Water: Acetonitrile (40:60) as eluent at flow rate 1.0 ml / min. UV detection was at 254nm. Retention times of

Tolvaptan and internal standard Metronidazole was 4.50 and 5.45min respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters employed to evaluate were maximum plasma concentration (C_{max}), time to attain C_{max} i.e., T_{max} and t ½ values, area under plasma concentration–time curve from zero to the last sampling time (AUC_{0-t}), area under plasma concentration–time curve from zero to infinity (AUC_{0-}) and mean residence time (*MRT*). AUC_{0-t} was calculated by the linear trapezoidal rule and AUC_{0-} from the following formula.

$AUC_{0-} = AUC_{0-t} + C_t / K_E$

Results and Discussion

Preliminary solubility studies of Tolvaptan

In case of solid dispersions initially preliminary solubility analysis were carried out to select the suitable water soluble carriers for the preparation of solid dispersion in which pure drug solubility was found to be 0.04 mg/ml (Table 2).

From the solubility results physical mixtures Shown in Table 2 of drug substance and Kleptose HPB with SLS in the ratio of 1:1:1 shown the highest drug solubility i.e. 0.11 mg/ml almost 3 fold increased as compared to that of pure drug. For all the water soluble / hydrophilic carriers used in preliminary solubility studies, except Kolliphor P407, Kleptose, Kolliwax, HPMC 2.5 cPs, mixture of Kolliphor P407 and P188 in 1:1 ratio, Kolliphor P188 and Soluplus, gave turbid solutions. The results are tabulated in Table 2, and graphical representation was is shown in Figure 1.

Table 2: Preliminary Solubility studies of tolvaptan in different polymers with SLS

S. No	Sample (Physical mixtures)	Drug :Polymer: Surfactant ratios	Solubility(mg/ml)*
1.	Pure drug	-	0.04±0.02
2.	Drug: Kolliphor RH-40:SLS	1:1:1	0.09±0.01
3.	Drug: Kolliphor EL:SLS	1:1:1	0.08±0.02
4.	Drug: Kolliphor TPGS:SLS	1:1:1	0.08±0.02
5.	Drug: Kolliphor HS-15:SLS	1:1:1	0.09±0.01
6.	Drug : Kolliphor P188:SLS	1:1:1	0.10±0.03
7.	Drug : Kolliphor P 407:SLS	1:1:1	0.10±0.04
8.	Drug : Kolliwax GMS II:SLS	1:1:1	0.09±0.01
9	Drug : Soluplus:SLS	1:1:1	0.10±0.01
10.	Drug : DSS 100%:SLS	1:1:1	0.08±0.03
11.	Drug : Kleptose HPB:SLS	1:1:1	0.12±0.04
12.	Drug : HPMC AS:SLS	1:1:1	0.10±0.02
13.	Drug: Kollidon 30:SLS	1:1:1	0.09±0.03
14.	Drug: Poloxamers (mixture of Kolliphor P407 and P188 in 1:1 :SLS	1:1:1	0.10±0.03

*Mean±SD, range, n=3

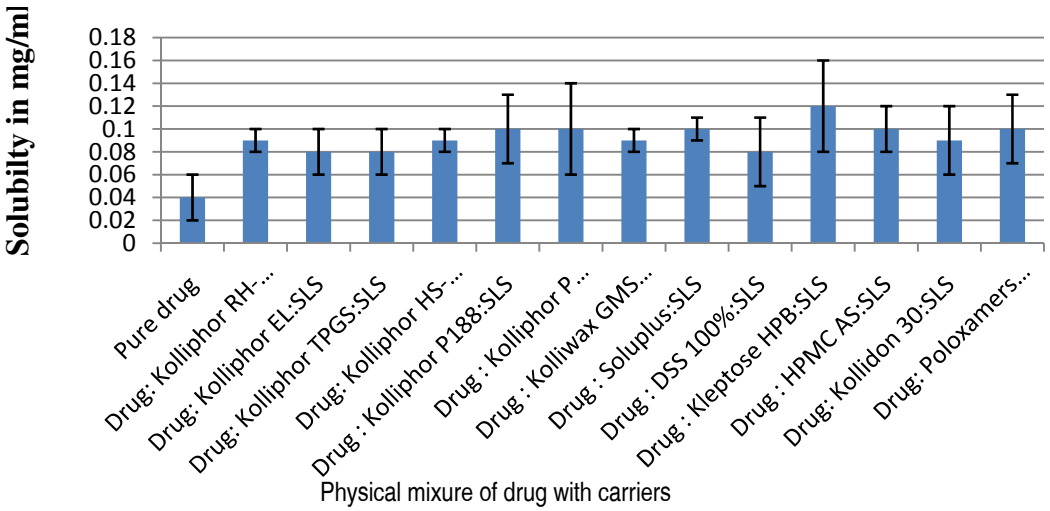


Figure 1: Solubility studies of Tolvaptan physical mixture

Preparation of Tolvaptan solid dispersions

Solid dispersions of Tolvaptan were prepared by using Kolliphor P407, Kleptose, Kolliwax GMS II, HPMC 2.5 cPs, mixture of Kolliphor P 407 and P188 in 1:1 ratio, Kolliphor P188 and Soluplus in 1:1 and 1:2 with equal proportion of SLS (to that of drug substance). In the present investigation 14 formulations were prepared and their complete composition is shown in Table 3. All the solid dispersions prepared were found to be fine and free flowing powders. Solid dispersions SE8 is shown in Figure 2.

Figure 2: Formulation SE 8

Evaluation parameters

Solubility studies of Tolvaptan solid dispersions

Fourteen formulations of solid dispersions were prepared by solvent evaporation method with their respective carriers. After preparation of solid dispersion solubility analysis was carried out, this is compared with pure drug. The formulation with Kleptose HPB and SLS in the ratio of 1:2:1 (drug to carrier to surfactant) which had increased the solubility by 8 fold as compared to that of the pure drug (Pure drug solubility is 0.04 mg / ml and Drug with carriers in SE8 is 0.33 mg/ml,). The results are tabulated in Table 3.

Table 3: Solubility studies for solid dispersions

S. No.	Formulation	Solubility (mg/ml)*
1	Pure drug	0.04±0.02
2	SE1	0.22±0.01
3	SE2	0.24±0.01
4	SE3	0.20±0.03
5	SE4	0.23±0.02
6	SE5	0.24±0.01
7	SE6	0.25±0.03
8	SE7	0.30±0.01
9	SE8	0.33±0.02
10	SE9	0.24±0.04
11	SE10	0.26±0.02
12	SE11	0.23±0.01
13	SE12	0.25±0.02
14	SE13	0.24±0.01
15	SE14	0.27±0.03

*Range n=3

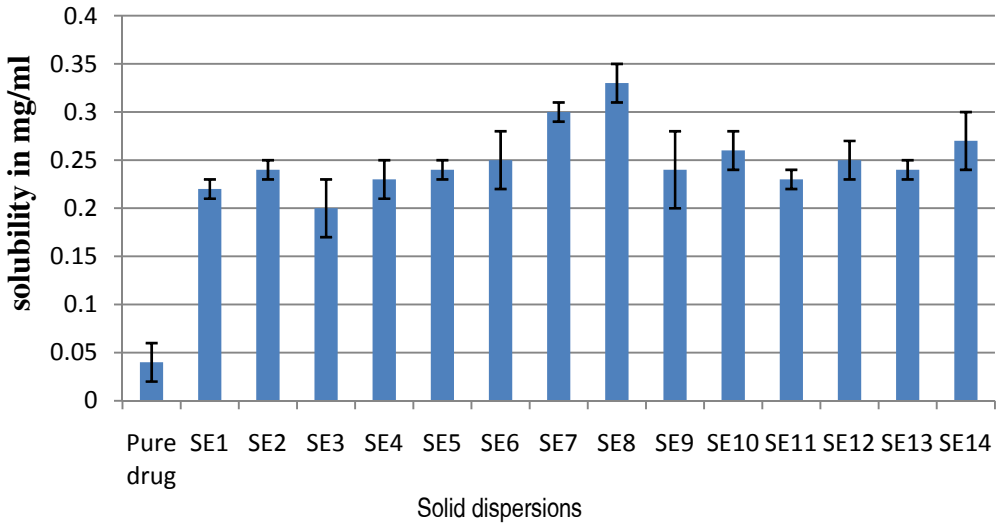


Figure 3: Solubility studies of Tolvaptan solid dispersions

Practical yield and Drug content

The results of % practical yield for all formulations of solid dispersions found to be 86.48% - 97.01%. Maximum yield was found to be 97.01% in formulation SE8. The drug content of the prepared solid dispersions was found to be in the range of 84.56 - 97.90%. Maximum % drug content i.e. 97.90% was founds in the formulation SE8. The results of % practical yield and drug content are shown in Table 4.

Table 4: % practical yield and drug content for different formulations of Tolvaptan Solid dispersions

S. No	Formulations	% Yield*	Drug content (%)**
1	SE1	95.12±4.15	95.19±2.70
2	SE2	96.15±3.90	96.24±2.45
3	SE3	88.15±4.50	85.62±2.20
4	SE4	89.54±3.45	87.52±3.30
5	SE5	86.48±5.06	84.56±1.25
6	SE6	87.42±2.70	85.45±3.20
7	SE7	96.7±4.40	97.48±1.40
8	SE8	97.01±3.35	97.90±1.20
9	SE9	93.25±4.18	92.56±3.85
10	SE10	92.02±5.24	91.45±2.84
11	SE11	92.27±4.74	87.62±2.24
12	SE12	91.16±3.08	88.52±2.12
13	SE13	94.14±2.92	96.03±3.45
14	SE14	96.15±1.94	97.80±4.10

* Mean±SD, n=20 **Mean±SD, n=3

In vitro dissolution studies

The drug release data obtained for formulations SE1-SE14 are tabulated in Table 5 and 6. The Table shows the cumulative percent drug released as a function of time for all formulations. Cumulative percent drug released after 90 min was 91.1%, 93.6%, 76.8%, 77.1%, 65.4%, 66.4%, 94.5%, 96.8%, 80.2%, 82.6%, 78.2%, 80.2%, 87.2%, & 89.6 % for SE1-SE14 respectively and was 39.6 % in 90 min for pure drug.

In vitro studies reveal that there is marked increase in the dissolution rate of Tolvaptan from all the solid dispersions when compared to pure Tolvaptan itself. From the *in vitro* drug release profiles, it can be seen that formulation SE8 containing Kleptose HPB (1:2:2 1:2:1 ratio of drug: Kleptose HPB:SLS) shown higher dissolution rate i.e. 96.8 % compared with other formulations. This may be attributed to the increase in drug wettability, conversion to amorphous (conformed through DSC /XRD, please present them) form and solubilization of the drug due to hydrophilic carrier. The increase in dissolution rate is in the order of Kleptose-HPB> HPMC AS> Soluplus > Kolliphor P188> Poloxamers (mixture of Kolliphor P407 & P188 in 1:1 ratio) > Kolliwax GMS> Kolliphor P407. The graphical representation of solid dispersions of SE1-SE4, SE5-SE8, SE9-SE11 and SE12-SE14 was depicted in Figure 4 and 5 respectively.

Table 5: In vitro drug release of pure drug, Innovator product and prepared solid dispersions by solvent evaporation method from SE1 to SE7

Time in min	Cumulative % drug release*								
	Pure drug	SAMSCA®	SE1	SE2	SE3	SE4	SE5	SE6	SE7
0	0	0	0	0	0	0	0	0	0
5	12.2±2.5	32.4±2.6	34.5±1.2	31.9±0.7	36.7±1.8	35.6±1.4	34.4±1.5	36.5±1.5	36.3±1.5
10	18.6±3.1	49.4±2.2	54.2±2.4	49.6±1.2	46.7±0.6	44.6±1.4	43.9±1.5	45.7±1.4	46.7±2.4
20	22.1±3.6	72.8±1.6	67.8±1.5	59.5±1.5	56.4±1.8	56.4±1.5	56.6±1.2	58.2±1.2	59.8±1.5
30	28.9±3.3	79.2±1.9	77.5±1.4	70.0±1.4	63.7±1.3	66.5±1.8	60.8±1.4	62.4±2.3	66.3±1.7
45	32.5±2.6	89.8±1.2	86.1±1.6	78.3±1.5	70.2±1.4	73.8±1.2	62.4±1.4	63.7±2.4	74.6±2.3
60	39.6±2.7	94.2±0.9	91.1±1.5	93.6±1.3	76.8±0.6	77.1±1.1	65.4±0.4	66.4±2.1	94.5±1.5

*Mean±SD, n=3

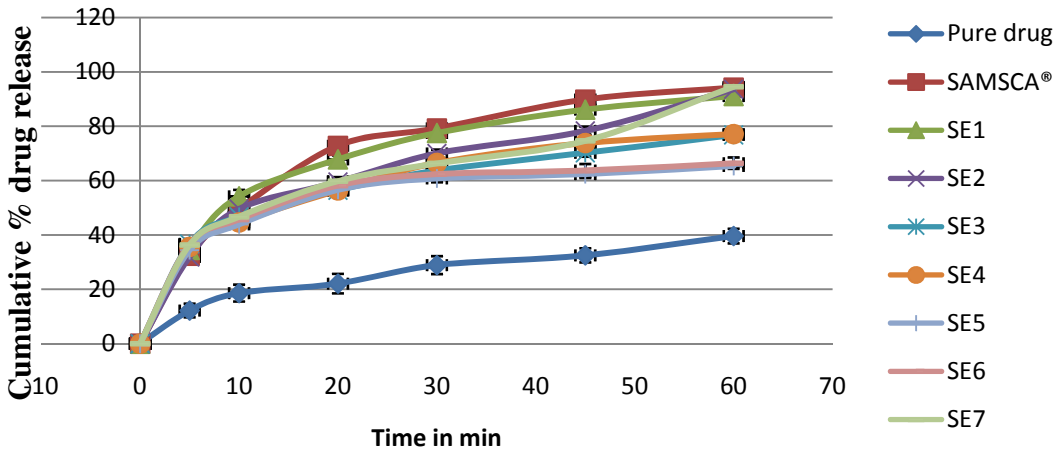


Figure 4: In vitro dissolution profile of pure drug, innovator and Tolvaptan solid dispersions (SE1-SE7)

Table 6: *In vitro* drug release of pure drug, Innovator product and prepared solid dispersions by solvent evaporation method from SE8 to SE14

Time in min	Cumulative % drug release*								
	Pure drug	SAMSCA®	SE8	SE9	SE10	SE11	SE12	SE13	SE14
0	0	0	0	0	0	0	0	0	0
5	12.2±4.9	32.4±2.6	36.7±1.2	45.6±2.3	35.6±1.4	34.8±2.7	46.7±2.1	46.7±1.8	31.9±1.6
10	18.6±4.1	49.4±2.2	56.7±2.4	54.5±2.2	44.6±1.4	42.6±2.4	56.7±2.4	56.7±2.3	42.6±1.5
20	22.1±3.6	72.8±1.6	76.4±2.3	64.4±2.1	54.6±1.8	48.7±2.6	64.6±2.1	64.6±2.4	52.5±1.7
30	28.9±3.3	79.2±1.9	83.7±1.7	68.5±2.1	62.1±1.7	52.2±2.7	67.3±2.1	67.3±2.1	60.0±1.4
45	32.5±2.6	89.8±1.2	90.2±1.4	73.6±1.4	69.3±1.6	66.7±2.4	74.2±2.2	74.2±2.3	68.3±1.5
60	39.6±2.7	94.2±0.9	96.8±1.4	80.2±1.8	82.6±1.2	78.2±2.6	80.2±2.4	87.2±2.4	89.6±1.4

*Mean±SD, n=3

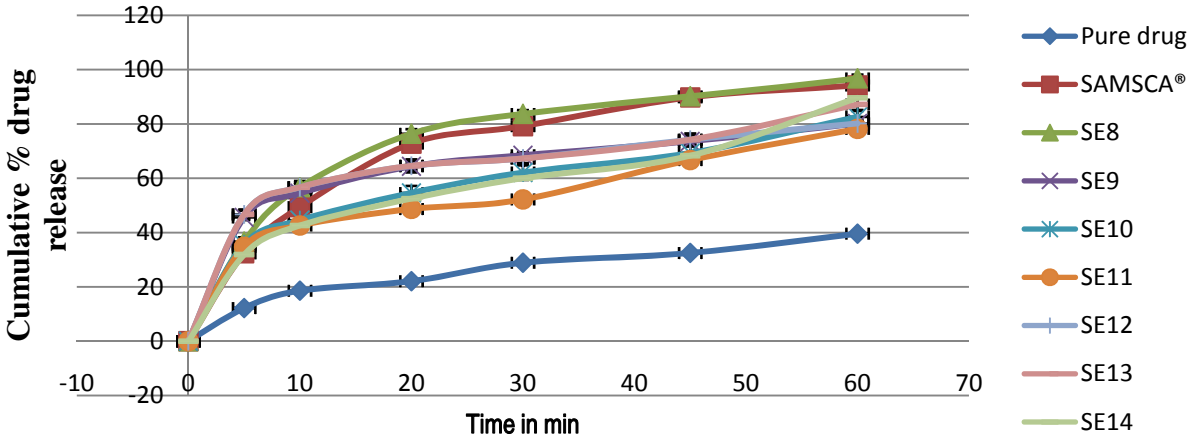


Figure 5: In vitro dissolution profile of pure drug, innovator and Tolvaptan solid dispersions (SE8-SE14)

FTIR studies

The prominent peaks of Tolvaptan was observed (Figure 6) the region of 3410.26 cm^{-1} due to the (Polymeric OH stretching), a peak at 3263.66 cm^{-1} due to (aromatic H(-C=C-)H stretching) and a peak at 3086.21 cm^{-1} due to (aromatic C-H stretching). At the lower frequencies 741 cm^{-1} (C-Cl), 1195.91 cm^{-1} (C-N stretching), 1357.93 cm^{-1} (N-H stretching), 1681.98 cm^{-1} (C=O stretching) observed. Kleptose HPB (Figure 7) shows the prominent peak at 3410.26 cm^{-1} due to polymeric OH stretching, a peak at 2978.19 cm^{-1} due to the (aliphatic CH_3 stretching) a peak at 3086.21 cm^{-1} due to (aromatic C-H stretching) and a peak at 1188.19 cm^{-1} due to (C-O-C stretching). Physical mixture (Figure 8) of the drug and Kleptose HPB shows summation of the spectra of the drug and Kleptose HPB equivalent to the addition of the spectrum of polymer and drug. This indicates that interaction has occurred with simple physical mixture of drug and polymer. In case of solid dispersion (Figure 9) of the drug and Kleptose HPB shows overlapping of O-H and N-H group and broadening of peak was observed. However other peaks related to C-H stretching remains unchanged. This indicates that overall symmetry of the molecule might not be significantly changed.

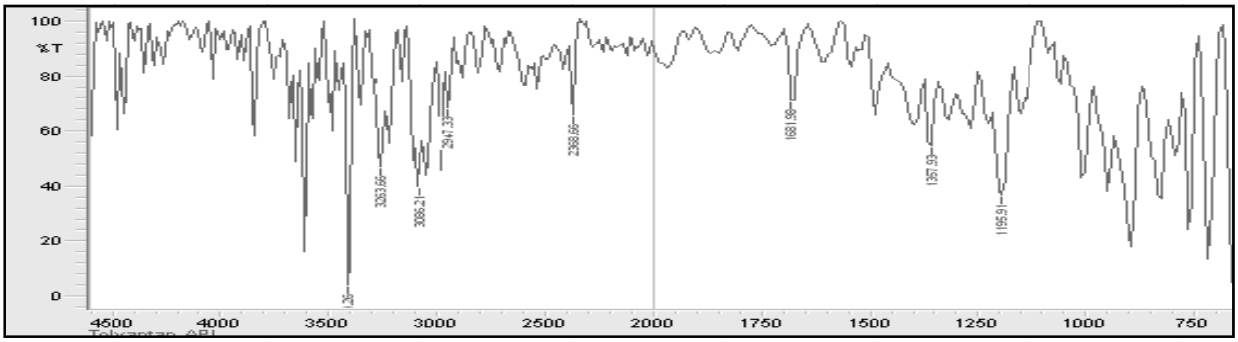


Figure 6: FTIR spectra of pure drug

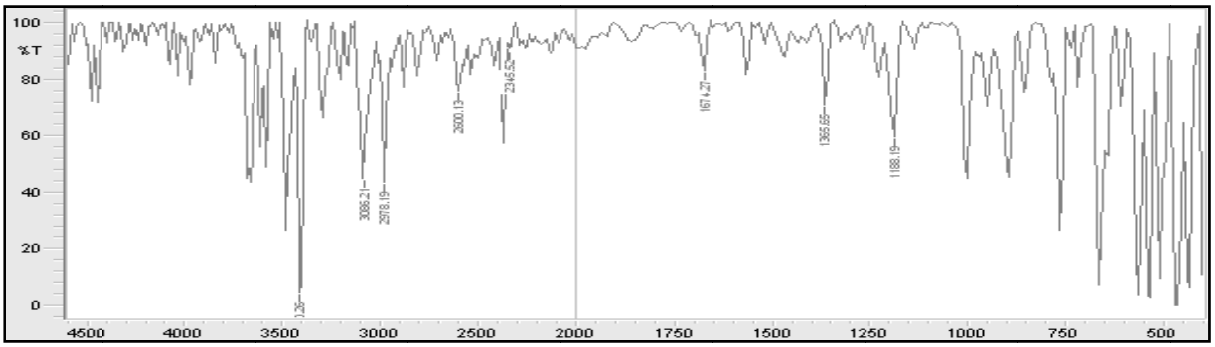


Figure 7: FTIR spectra of Kleptose HPB

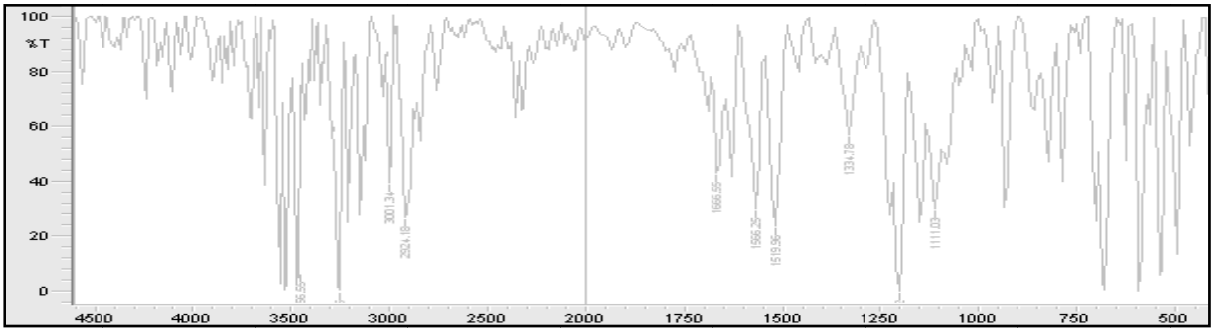


Figure 8: FTIR spectra of physical mixture of Tolvaptan + Kleptose HPB

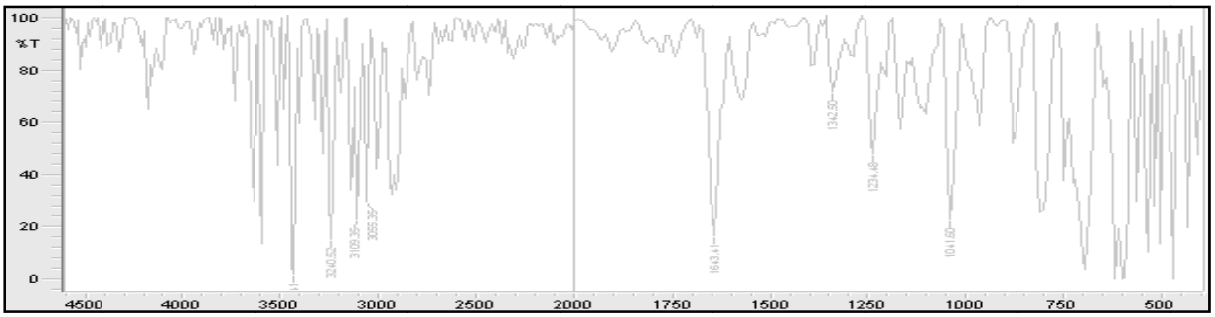


Figure 9: FTIR spectra of formulation SE8 solid dispersion prepared by solvent evaporation method

X-Ray Diffraction studies

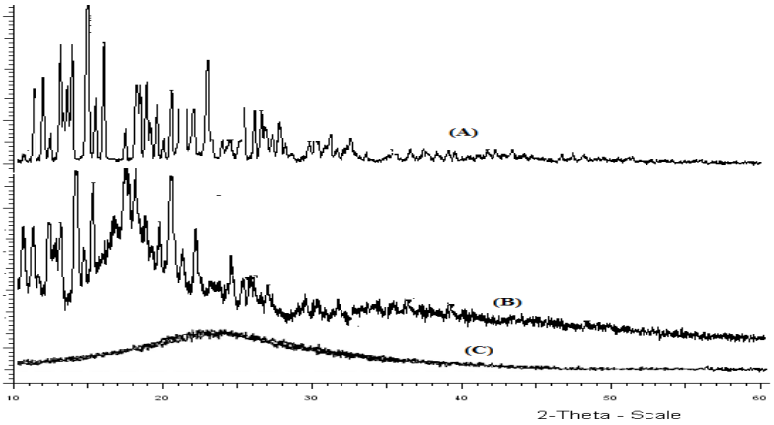


Figure 10: X-Ray powder diffractograms of Tolvaptan pure drug (A), Physical mixture (B) and optimized formulation SE8 (C)

The Tolvaptan pure drug, physical mixture and solid dispersions were analyzed in Bruker A6 advanced PXRD instrument to find out whether the solid dispersions of various drug polymer ratios are crystalline or amorphous. The presence of numerous distinct peaks in the XRD spectrum indicates that Tolvaptan was present as a crystalline material. The XRD pattern depicted by physical mixture reveals a decrease in the number of peaks which probably represents decrease in crystallinity. The spectrum of optimized formulation SE8 of solid dispersion was characterized by the complete absence of any diffraction peak, which is characteristic of an amorphous compound (Figure 10). The enhancement in the dissolution rate of the drug from the drug-kleptose HPB solid dispersion is ascribed to the marked reduction in the crystallinity of the drug.

Differential Scanning Calorimetry

The DSC thermo grams of pure Tolvaptan showed in Figure 11, sharp endothermic peak at melting point 225 ^{0}C, indicating that the drug is highly crystalline. The absence of drug peak in the solid dispersion formulation SE8 indicating the drug was in amorphous form. As the intensity of the endotherm was markedly decreased in the drug- Kleptose HPB solid dispersion, the faster dissolution rate of the drug from the solid dispersion is attributed to the reduction in the crystallinity of the drug. Crystallization inhibition is attributed to the entrapment of the drug molecules in the polymer matrix during solvent evaporation.

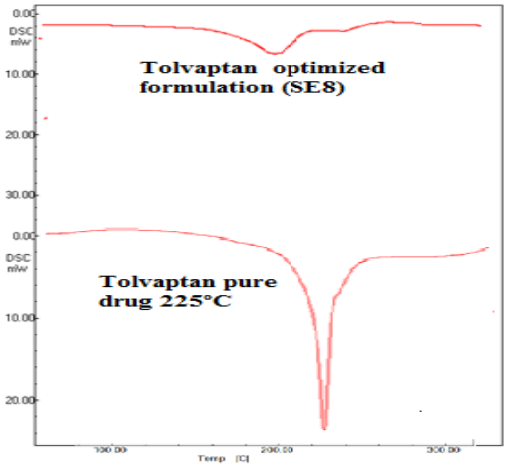
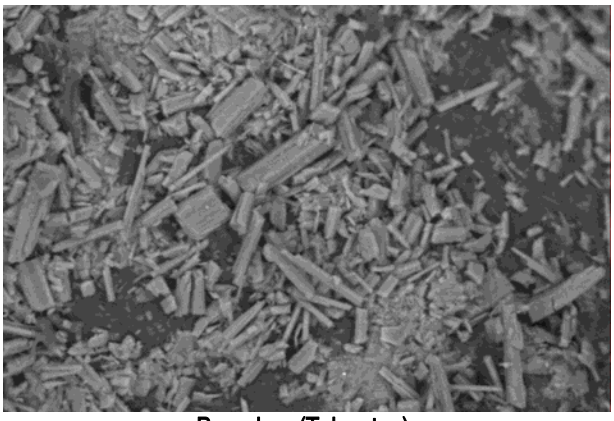


Figure 11: DSC thermograms of Tolvaptan pure drug and optimized formulation SE8.

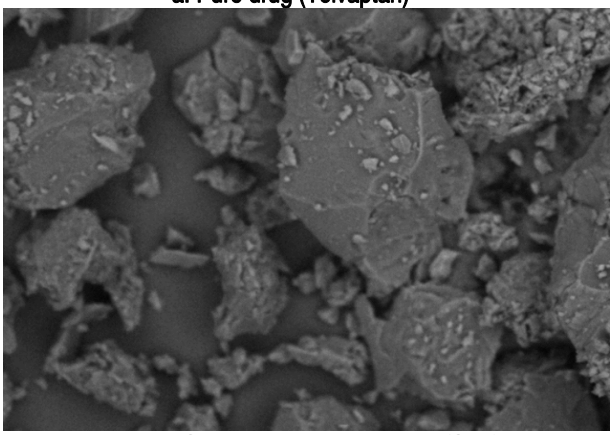
SEM Studies

Figure 12 displayed SEM photographs for pure drug (a) and formulation SE 6 (b). The drug crystals seemed to be smooth-

surfaced, irregular in shape and size. In case of Solid dispersions, it was difficult to distinguish the presence of drug crystals. The drug surface in solid dispersion seems to be more porous in nature. Solid dispersions appeared as uniform and homogeneously mixed mass with wrinkled surface. Drug crystals appeared to be incorporated into the particles of the polymers. The solid dispersion looked like a matrix particle. The results could be attributed to dispersion of the drug in the molten mass of the polymer.



a. Pure drug (Tolvaptan)



b. Optimized solid dispersion (SE8)

Figure 12: SEM pictures of drug and formulation SE8

Stability studies

Tolvaptan optimized formulation (SE8) was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted for 6 months according to ICH guidelines. To evaluate the physical state of the drug, the samples were evaluated for drug content, In vitro drug release profile and characterized by XRD after storage for 6 months (Figure 13). The samples were found to be stable during a 6-month period. From these results it was concluded that, optimized formulation is stable and retained their original properties with minor differences which depicted in Table 7.

Table 7: Drug content and %drug release of optimized formulation stored at 40 ±2^{0}C /75 ±5%RH

Stability period for optimized formulation	% Drug content	*In-vitro* drug release (%)
Initial	97.90	96.8
1 Month	96.60	95.70
2 Months	96.15	95.15
3 months	95.55	94.50
6 Months	95.10	93.05

Pharmacokinetic parameters comparison for pure drug suspension and optimized solid dispersions formulation

The Tolvaptan plasma concentrations in rats treated with optimized solid dispersions formulation (SE8) were significantly higher than those treated with pure drug suspension. Plasma pharmacokinetic parameters of Tolvaptan after oral administration of the formulation to Wister rats are shown in Table 8. Based on the results, it was clearly evident that Tolvaptan from an optimized solid dispersions formulation was significantly increased in comparison with that of the pure drug (Tolvaptan suspension). C_{max} of the optimized solid dispersions formulation was 0.696 µg /ml ($p<0.05$) and was significantly higher as compared to C_{max} of the pure drug suspension formulation 0.261µg/ml. T_{max} of optimized solid dispersions formulation and pure drug suspension was 1.00 and 2.00 h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\ inf}$ for optimized solid dispersions formulation was higher 6.05 µg h/ml than the pure drug suspension 2.85 µg h/ml. Statistically, AUC_{0-t} of the optimized solid dispersions formulation was significantly higher ($p<0.05$) as compared to pure drug suspension formulation. Higher amount of drug concentration in blood indicated better systemic absorption of Tolvaptan from optimized solid dispersions formulation as compared to the pure drug suspension.

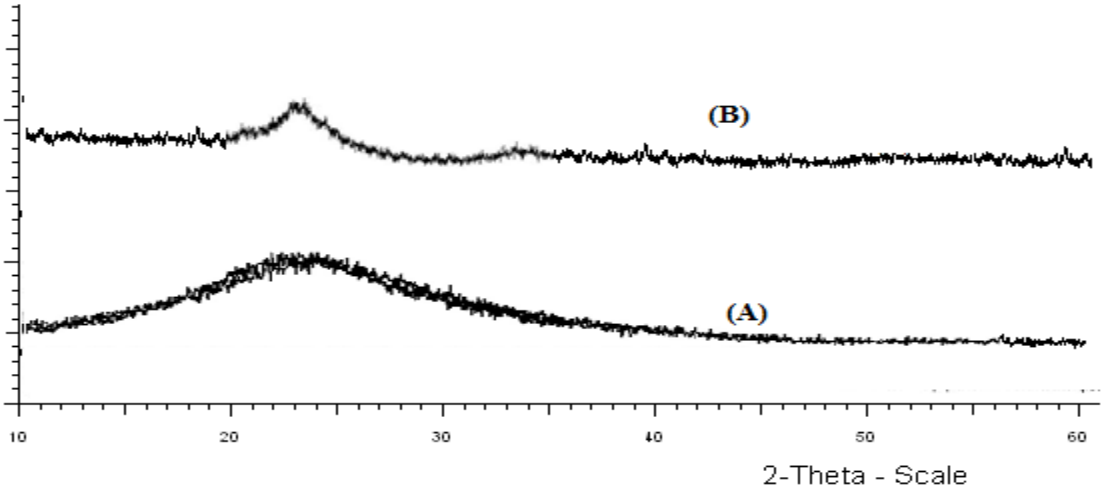


Figure 13: X-Ray powder diffractograms of optimized formulation SE8 (A), optimized formulation SE8 after stability studies (B)

Table 8: Pharmacokinetic Parameters of Tolvaptan from optimized formulation (SE8) and pure drug

Pharmacokinetic Parameters	Tolvaptan Pure drug	Tolvaptan free flowing solid dispersion (SE8)
C_{max} (µg/ml)	0.261	0.696
AUC_{0-t} (µg h/ml)	2.62	5.52
AUC_{0-inf} (µg h/ml)	2.85	6.05
T_{max} (h)	2.00	1.00
$t_{1/2}$ (h)	3.32	4.52
K el (h^{-1})	0.284	0.141

Conclusion

The dissolution rate of Tolvaptan was increased with solid dispersions prepared by solvent evaporation technique using Kleptose without any physical and chemical interaction. Solid dispersions of drug: Kleptose HPB: SLS (1:2:1 ratio) (SE8) shown higher dissolution rate i.e. 96.8 % compared with and pure drug (39.6%) and other formulations. Analysis by DSC and powder X-ray diffraction showed that Tolvaptan existed in the amorphous form within the solid dispersion formulation fabricated using the solvent evaporation process. Additionally, scanning electron microscopy studies suggested the conversion of crystalline Tolvaptan to an amorphous form. A marked increase in dissolution and bioavailability was exhibited by optimized Tolvaptan solid dispersion (SE8). In vivo studies revealed that AUC (0-t) was increased more than 2.11 folds, C_{max} increased about 2.67 folds and t_{max} reduced by 1 hr, when compared to the pure drug. Thus, the study has illustrated the potential use of a solid dispersion system for the delivery of a very poorly soluble drug tolvaptan with a better bioavailability. The in vivo studies clearly indicated that solid dispersion approach can be adopted for formulation of Tolvaptan in order to achieve a faster onset of action.

References

[1]. Wagh VT, Jagtap VA, Shaikh TJ, Nandedkar SY. Formulation and Evaluation of Glimepiride Solid Dispersion Tablets for Their Solubility Enhancement. J. Adv Sci Res. 2012; 3(4): 36-41.

[2]. Bhaskar D, Rama Rao T. Formulation and in vitro evaluation of flurbiprofen-polyethylene glycol 20000 solid dispersions. J. Applied Pharm Sci. 2014; 4 (07): 76-81.

[3]. Sameer Singh, Raviraj Singh B, Lalit Yadav. A review on solid dispersion. Int. J. pharm. & Life sci. 2011; 2(9): 1078-1095.

[4]. Deepthi Naidu K, Prasanna Lakshmi A, Ajay Kumar B, Narandra Reddy J.

Formulation and In-vitro evaluation of conventional tablets of Ezetimibe by using Solid Dispersion. . Int J Pharm Pharm Sci. 2013; 5 (2): 331-335.

[5]. Sree Giri Prasad B, Gupta VRM, Vijaya K, Tamilselvan A, Siva Subramanian N. Formulation and evaluation of fast dissolving tablet of Tolvaptan. JGTPS. 2015; 6(1): 2403 – 2410.

[6]. Dongqing J. Progress of clinical studies, tolvaptan. J Clinical Res Adva in Clinical Use of Drugs Abroad. 2014; 03: 176-178.

[7]. Xiaogao Z, Shengjun Z. Bioequivalence Study of Two 30 Mg Tolvaptan Tablets Formulations in Healthy Chinese under Fed Condition. J Bioequiv Availab. 2014; 6: 181-185.

[8]. Higuchi T, Connors KA .Phase solubility techniques. Adv. Anal. Chem. Instrum. 1965; 4: 117–122.

[9]. AppaRao B, Shivalingam M. R, Kishore Reddy Y. V, Soma sekhara Rao, Rajesh K, Sunitha N. Formulation and Evaluation of Aceclofenac Solid Dispersions for Dissolution Rate Enhancement. Int J Pharma Sci and Drug Res. 2010; 2(2): 146-150.

[10]. Higuchi T, Connors KA. Phase solubility techniques. Adv. Anal. Chem. Instrum. 1965; 4: 117–122.

[11]. Mayur Chaudhari D, Raju Sonawane O, Laxmikant Z, Sasmita Nayak, Sanjay Bari B. Solubility and dissolution enhancement of poorly water soluble Glimepiride by using solid dispersion technique. . Int J Pharm Pharm Sci. 2012; 4(5): 534-539.

[12]. Prasanna Kumari J, Ramarao T, Jayaveera K N, Bikshapathi D V R N, Madhusudan Rao Y. Design and In vivo evaluation of Metoprolol Tartrate bilayer floating tablets in healthy human volunteers. IJDD. 2014; 6: 14-23.

[13]. Ramesh K, Chandra Shekar B, Khadgapathi P. Formulation and evaluation of poorly soluble Etravirine by spray drying method. . Int J Pharm Pharm Sci. 2015; 7(4): 98 – 103.

[14]. Ritesh F, Purnima A. Development and evaluation of Lafutidine solid dispersion via hot melt extrusion: Investigating drug-polymer miscibility with advanced characterization. Asian J Pharma Sci. 2014; 9(2): 92-106.

[15]. Breitenbach J. Melt extrusion: from process to drug delivery technology. Eur J Pharm Biopharm. 2002; 54: 107–117.

[16]. Kalyana Chakravarthy V, Gowri Shankar D. Development and validation of RP- HPLC method for estimation of tolvaptan in bulk and its Pharmaceutical formulation. RJC. 2011; 4 (1): 165-171.