ORIGINAL ARTICLE

Oral Bioavailability Enhancement of Sertraline Hydrochloride by Nanoprecipitation and Solvent Diffusion Techniques for Stable Nanosuspension

Anil Raosaheb Pawar[1,2], Pravin Digambar Choudhari[3], Ashwini Balasaheb Nidhane[2]

[1]Department of Pharmacy, PRIST University, Thanjavur, Tamil Nadu, India, [2]Department of Pharmaceutics, College of Pharmacy, Sonai, Maharashtra, India, [3]Department of Pharmaceutics, Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India

Abstract

Aim: The enhancement of oral bioavailability of sertraline (SRT) hydrochloride (HCl) using the nanoprecipitation and solvent diffusion techniques for stable nanosuspension. **Materials and Methods:** There have been still few of published researches on the formulation of SRT HCl using solid dispersion, fast dissolving tablet, and self-microemulsifying drug delivery systems. Nanosuspension was prepared separately by using different techniques and different polymers (Eudragit RL 100 and PVP K 25). The *in vitro* dissolution study was performed as per the USP paddle method in 0.1N HCl. The crystalline structure of the drug, the molecular interaction, morphology, and particle size of nanosuspension were also investigated. **Results and Discussion:** The nanoprecipitation method with drug and PVP K 25 (1:1) showed its potential in the enhancement of the drug release rate (99% in 45 min). The synergistic effects of reduction of drug crystallinity and particle size could increase the dissolution rate of SRT HCl by providing a stable nanosuspension. The *in vivo* study demonstrated that the maximum plasma concentration and area under the curve values of selected nanosuspension in rabbits were found greater than that of the commercial tablets (tablet potiga, 50 mg) and standard SRT HCl. **Conclusion:** This work may contribute to a new strategy for enhancement oral bioavailability of SRT HCl.

Key words: Bioavailability, nanosuspension, sertraline hydrochloride

INTRODUCTION

Currently, more than 40% of drugs are poorly water-soluble, leading to the poor bioavailability. Therefore, one of the major current challenges of the pharmaceutical industry is related to strategies that improve the water solubility of drugs.[1] A number of studies have been conducted with the aim to enhance solubility and dissolution rate of poorly water-soluble drugs. Different hydrophobic drugs have already been formulated successfully in this way, for instance, naproxen clofazamine, bupravaquone, nimesulid, mitotane, amphotericin B, omeprazole, nifedipine, and spironolactone.[2] Nanoprecipitation method is one of the promising approaches for the formulation of poorly water-soluble drug compounds because nanoprecipitation and solvent diffusion techniques have been proved to be an effective method for breaking down particles into nanoparticles as well as conversion of crystalline form to amorphous form on precipitation.[3] Sertraline (SRT) hydrochloride (HCl) is chemically (1S, 4S)-4-(3,4 dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronapthalene-1amine HCl. SRT belongs to a class of antidepressant agents known as selective serotonin-reuptake inhibitors and was chosen as a model drug in this research. It is virtually insoluble in water (BCS Class II drug).[4,5] In previous studies, successfully enhanced the dissolution rate of SRT HCl using solid dispersion techniques, fast dissolving tablet, and self-nanoemulsifying drug delivery system.[6-14] Herein, this study

Address for correspondence:
Anil Raosaheb Pawar, PRIST University, Thanjavur, Tamil Nadu, India. Phone: +91-9881969052.
E-mail: anilpharma123@gmail.com

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was aimed to develop nanosuspension for enhancing oral bioavailability of SRT HCl by enhancing drug dissolution. Different polymers (Eudragit RL 100 and PVP K 25) were used to investigate the enhancement of oral bioavailability. Morphology was conducted through transmission electron microscope (TEM). The potential molecular interaction between drug and polymer was also investigated by Fourier transform infrared (FTIR) spectroscopy.

MATERIALS AND METHODS

Material

SRT HCl was provided by Dr. Reddy's lab, Hyderabad. Poloxamer 188, PVP K 25, Eudragit RS 100, Ethanol, and Tween 80 were purchased from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India. The solvents for high-performance liquid chromatography (HPLC) were purchased from Rajesh Chemicals, Mumbai, Maharashtra, India. All other chemicals were of analytical grade and used without further purification.

Method

Preparation of nanosuspension by nanoprecipitation method

Nanosuspension of SRT HCl was prepared by the Nanoprecipitation method with various ratios of polymers and surfactants [Table 1]. The surfactant was dissolved in water and labeled as mixture 1. This mixture was kept on a magnetic stirrer for uniform mixing. Drug and polymer were dissolved using defined volume of a suitable solvent. This drug solution was slowly added drop wise to mixture 1 and continues the stirring on a magnetic stirrer until complete evaporation of the solvent. After 15 min, the solution was kept in an ultrasonicator (CD-4820, Citizen) for 1 h. Thus, the nanosuspension was formed and preserved for further use.

Preparation of nanosuspension by solvent diffusion method

Nanosuspension of SRT HCl was prepared according to the modified procedure of the solvent - diffusion method. Drug and polymer in various ratios [Table 2] were accurately weighed and dissolved in ethanol with continuous stirring. This solution is considered as organic phase since it contains organic solvent ethanol. The surfactants were added in distilled water. It was then stirred separately with a magnetic stirrer and considered this solution as an aqueous solution. The organic solution was transferred drop-by-drop to the aqueous phase under continuous stirring on magnetic stirrer at room temperature. Thus, the nanosuspension of SRT HCl was formulated and preserved for further use.

Characterization of nanosuspension

Drug content

About 1 ml of nanosuspension was taken and diluted appropriately with 0.1N HCl and the drug content of the samples was estimated by UV-visible spectrophotometer (JASCO V-630, Japan) at 274.1 nm.

Density

The density of the SRT HCl nanosuspension was determined using the specific gravity bottle.

pH

The pH of the formulation was measured using a digital pH meter (Equip-Tronics, EQ-610) with a glass electrode as a reference.

Solubility of drug

An excess amount of drug was added to 5 ml distilled water in a volumetric flask with cap. The volumetric flasks were kept in a shaker at 37°C ± 0.5°C for 24 h. The solutions were filtered through 0.45 μm Millipore filter and the filtrate was analyzed spectrophotometrically at 274.1 nm.

FTIR spectroscopy

An infrared study was carried out to rule out the interaction between drug and carrier used in the formulation of nanosuspension by a potassium bromide disc method using an infrared spectrophotometer (Shimadzu, 8300, Japan). The scanning range was 500-4000/cm.

FTIR studies were carried out at Government college of Pharmacy, Aurangabad, Maharashtra, India.

Particle size and size distribution

Particle size analysis and size distribution of prepared nanosuspension were carried out using Beckman coulter particle size analyzer (Delsa™ Nano Common). The dried nanoparticles were dispersed in double distilled water before running the sample in the instrument, to ensure that the light scattering signal, as indicated by particle count per second was within instrument's sensitivity range. The polydispersity index (PDI) was studied to determine the narrowness of the particle size distribution.

Particle size analysis and size distribution of prepared nanosuspension were carried out at Diya Lab, Mumbai, Maharashtra, India.

Surface charge

Zeta potential measurements were performed in distilled water with conductivity adjusted to 0.0504 mS/cm to determine the surface charge. To estimate the long-term stability properties, zeta potential was also measured in the original dispersion medium (water). The analysis was performed using the Beckman Coulter (Delsa™ Nano Common).

Table 1: Preparation of nanosuspension by nanoprecipitation technique

Formulation code	Drug- polymer ratio	Surfactant			Ethanol (ml)	Water (ml)
		SLS (%)	Poloxamer 188 (mg)	Tween 80 (ml)		
KA1	1:1	0.01	-	-	3	10
KA2	1:3	0.01	-	-	3	10
KA3	1:5	0.01	-	-	3	10
KA4	1:1	-	50	-	3	10
KA5	1:3	-	100	-	3	10
KA6	1:5	-	150	-	3	10
KA7	1:1	-	-	0.1	3	10
KA8	1:3	-	-	0.2	3	10
KA9	1:5	-	-	0.3	3	10
KA10	1:1	0.01	-	-	3	10
KA11	1:3	0.01	-	-	3	10
KA12	1:5	0.01	-	-	3	10
KA13	1:1	-	50	-	3	10
KA14	1:3	-	100	-	3	10
KA15	1:5	-	150	-	3	10
KA16	1:1	-	-	0.1	3	10
KA17	1:3	-	-	0.2	3	10
KA18	1:5	-	-	0.3	3	10

Polymer Eudragit RS 100 was used for preparation KA1-KA9 and PVP K 25 was used for KA10-KA18

Table 2: Preparation of nanosuspension by solvent diffusion technique

Formulation code	Drug-polymer ratio	Surfactant			Ethanol (ml)	Water (ml)
		SLS (%)	Poloxamer 188 (mg)	Tween 80 (ml)		
AK1	1:1	0.01	-	-	3	10
AK2	1:3	0.01	-	-	3	10
AK3	1:5	0.01	-	-	3	10
AK4	1:1	-	50	-	3	10
AK5	1:3	-	100	-	3	10
AK6	1:5	-	150	-	3	10
AK7	1:1	-	-	0.1	3	10
AK8	1:3	-	-	0.2	3	10
AK9	1:5	-	-	0.3	3	10
AK10	1:1	0.01	-		3	10
AK11	1:3	0.01	-		3	10
AK12	1:5	0.01	-		3	10
AK13	1:1	-	50	-	3	10
AK14	1:3	-	100	-	3	10
AK15	1:5	-	150	-	3	10
AK16	1:1	-	-	0.1	3	10
AK17	1:3	-	-	0.2	3	10
AK18	1:5	-	-	0.3	3	10

Polymer Eudragit RS 100 was used for preparation KA1-KA9 and PVP K 25 was used for KA10-KA18

Zeta potential measurements were carried out at Diya Lab, Mumbai, Maharashtra, India.

Surface morphology

Morphology of nanosuspension was examined by TEM (FEI Tecnai G2 F20 S-twin/EDAX). The prepared nanosuspension was dropped onto the carbon coated grid; the extra solution was removed using a blotting paper. The grid was allowed to dry for 5 min. The TEM micrograph was taken by applying an accelerating voltage of 80 kV.

Morphology of nanosuspension was examined at IIT, Mumbai, Maharashtra, India.

In vitro drug release (dissolution) studies

The *in vitro* drug release study was performed for all the formulations and pure drug powder using USP type I dissolution apparatus under the suitable conditions like dissolution medium: 900 ml of 0.1N HCl, RPM: 50, Temperature: 37°C ± 0.5°C, and Sampling time: 5, 10, 15, 30, 45, 60 min. At predetermined time intervals, aliquot samples (5 ml) were collected and replaced with the same volume of fresh medium. The aliquot samples (5 ml) were filtered through 0.45 µm membrane filter, and the filtrate was diluted appropriately and was estimated using UV-Visible spectrophotometer at λ_{max} 274.1 nm.

In vivo study (pharmacokinetics parameter)

The *in vivo* studies were performed to compare the plasma profile of nanosuspension and the pure drug to establish that the enhanced bioavailability was obtained with the preparation of nanosuspension when compared with the drug. The study was conducted in New Zealand White male rabbits which weighed 900-1500 g following oral administration. The research protocol of the animal experimentation was approved by the Institutional Animal Ethics Committee, MESCOP, Sonai, Maharashtra, India. The animals were housed in animal house, Faculty of Pharmacy, MESCOP, Sonai, Maharashtra, India. The animals were housed in polypropylene cages with free access to standard laboratory diet and water. The routine animal handling was performed according to Good Laboratory Practices. The animal dose was calculated as 2.09 mg/kg for rabbit for the current study. The selected formulation, marketed formulation, and drug were dissolved in 1 ml of gum acacia solution (2% w/v) and given orally using oral feeding sonde. The rats were anesthetized using ether and blood samples (1-1.5 ml) were withdrawn from the ear vein at predetermined time intervals. The samples were collected in microcentrifuge tubes which were the first rinsed with EDTA followed by addition of small quantity (4-8 mg) of powdered EDTA to the tube. Blood collected was mixed with the anticoagulant properly by shaking and then centrifuged at 4,000 rpm for 20 min. The plasma was separated and stored at −20°C until drug analysis by HPLC. The Pharmacokinetic parameters such as area under the curve (AUC), maximum plasma concentration (C_{max}), t_{max}, $[AUC]^{0-t}$, and $[AUC]^{0-inf}$ were calculated from plasma profile curve. All pharmacokinetic parameters were calculated individually for each subject, and the values were expressed. The area under concentration time curve was calculated according to log trapezoidal method.

RESULTS AND DISCUSSION

Drug content, density, pH, solubility

The drug content of the diluted samples of prepared nanosuspension was found to be in the range of 97.1-100.36%. The density of the SRT HCl nanosuspension was found to be in the range 0.981-1.0983 g/ml. The pH of the SRT HCl nanosuspension prepared by nanoprecipitation method was found to be in the range of 4.19-6.49. Saturation solubility of prepared nanosuspension by nanoprecipitation and solvent diffusion method was found to be in the range of 9.29-12.28 mg/ml. Saturation solubility of the pure drug was found to be 2.80 ± 0.055 mg/ml. This great increase in saturation solubility of SRT HCl was due to a reduction in particle size and conversion of crystalline to an amorphous structure. From the graphical representation of solubility [Figures 1 and 2], the formulation KA13 prepared by nanoprecipitation method showed the highest solubility than the others.

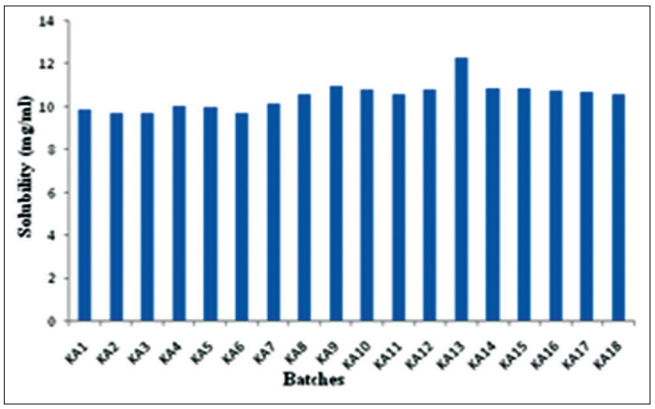

Figure 1: Graphical representation of solubility study data of KA1-KA18 (Nanoprecipitation technique)

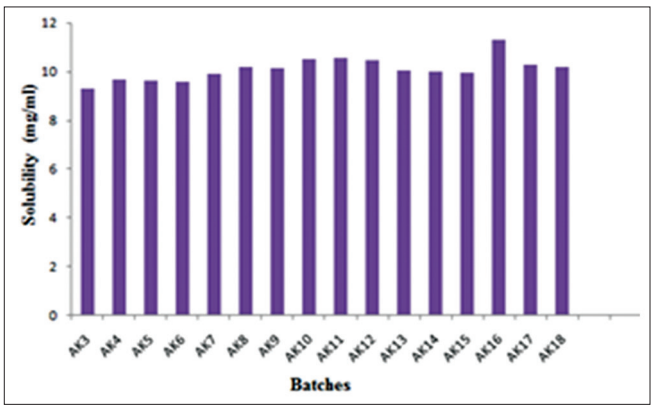

Figure 2: Graphical representation of solubility study data of AK1-AK18 (Solvent evaporation technique)

FTIR spectroscopic studies

For saturation solubility study, KA13 was selected (1:1 ratio of drug and polymer [PVP K 25]) and 50 mg of Surfactant (poloxamer 188) for further studies. FTIR spectra of the pure SRT HCl and selected formulation KA13 were characterized to investigate if there was any interaction between drug molecules and PVP K 25 that may affect the dissolution rate of drug [Figures 3 and 4]. The FTIR spectra of pure drug SRT HCl with polymers and surfactants were obtained which shows no chemical interaction between pure drug and excipients. The interpretation of FTIR data is shown in Table 3. The results indicate that there was no significant change in principle peak of the pure drug in selected formulation.

Table 3: FTIR interpretation data

Compound	Frequency (cm^{-1})	Type of vibration
Sertraline hydrochloride	3010	Ar-CH str.
	1582, 1468	–C=C str.
	789	C-Cl str.
	3430	C-NH str.
	2810	CH str. of tetrahydronaphthalene
	1428	CH bending of tetrahydronaphthalene
Selected batch (KA13)	3178	Ar-CH str.
	1712, 1450	–C=C str.
	650	C-Cl str.
	3450	C-NH str.
	2819	CH str. of tetrahydronaphthalene
	1450	CH bending of tetrahydronaphthalene

FTIR: Fourier transform infrared

Particle size and size distribution

The selected formulation (KA13) showed better size reduction than the others [Table 4]. The particle size of selected formulation (KA13) was found to be 60.6 nm. PDI is the measure of size-distribution and found to be 0.222 [Figure 5]. The closer the PDI value to zero, the more homogenous is the nanosuspension.

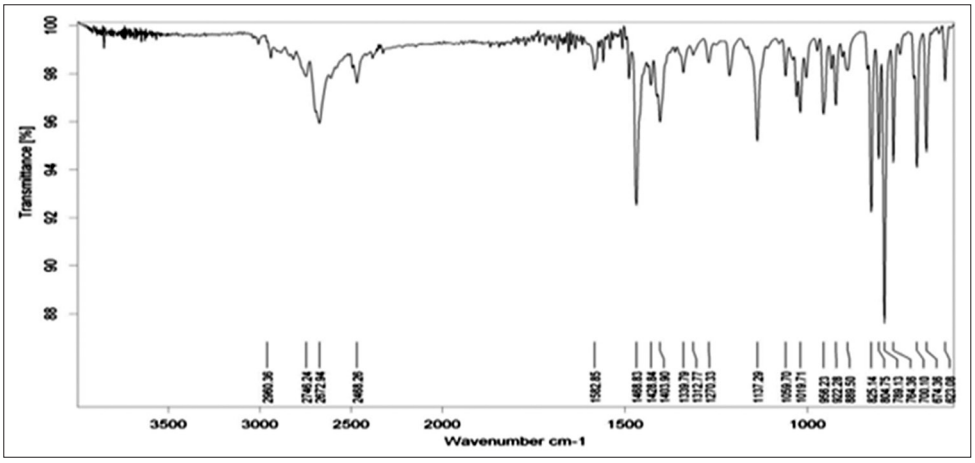

Figure 3: Fourier transform infrared spectrum of sertraline hydrochloride

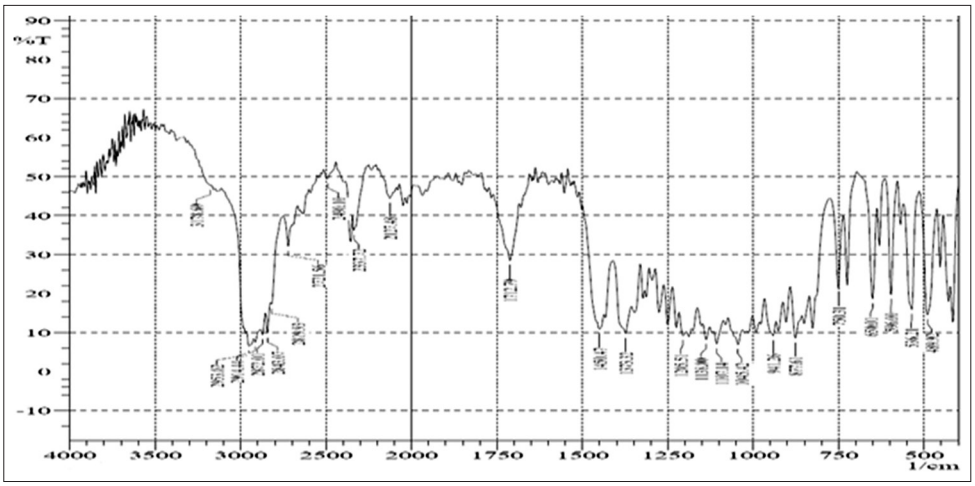

Figure 4: Fourier transform infrared spectrum of selected formulation (KA13)

Table 4: Particle size cumulants results

Cumulants results	Observation
Diameter (d)	60.6 nm
Polydispersity index (P.I.)	0.222
Diffusion const. (D)	8.122e-008 (cm^2/s)
Measurement condition	
Temperature	25 (°C)
Diluent name	Water
Refractive index	1.3328
Viscosity	0.8878 (cP)
Scattering intensity	8790 (cps)

Surface charge

The zeta potential of selected formulation (KA13) was found to be +8 which indicate stable formulation [Table 5 and Figure 6].

Surface morphology

The morphology of nanoparticles was determined by TEM. TEM gives information about the structure and size of nanoparticles. The prepared nanoparticles were found to be spherical in shape as shown in Figure 7. The average particle size observed by TEM was 50 nm.

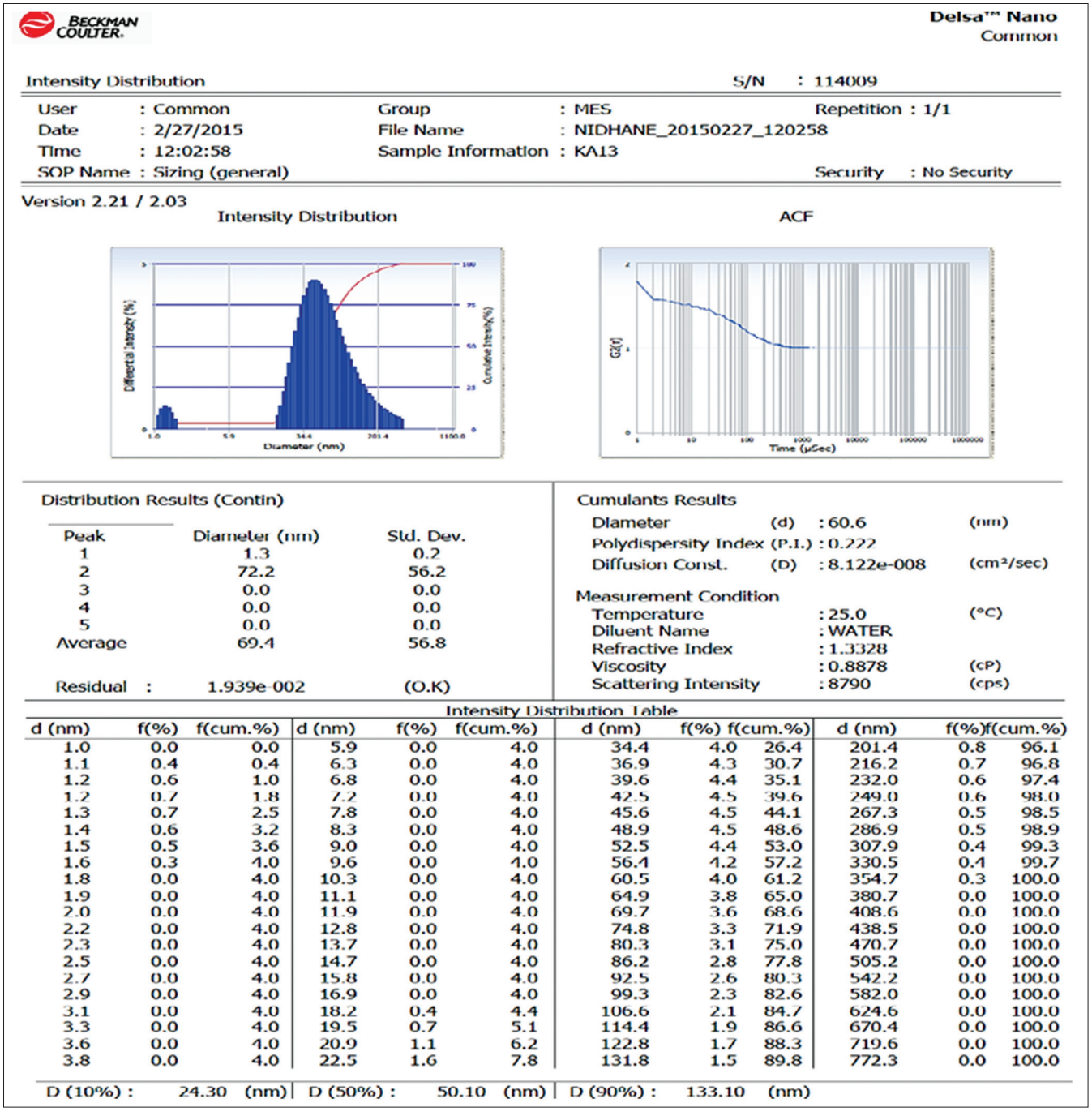

Figure 5: Particle size analysis of formulation KA13

In vitro dissolution study

In vitro dissolution study of KA1 to KA18 was carried out to see the release pattern [Figures 8 and 9]. The drug release was found to be 65.96-99.92% in 1. The selected batch (KA13) showed maximum drug release (99.92% in 45 min). *In vitro* drug release study of pure drug was found to be 64.72% in 1.

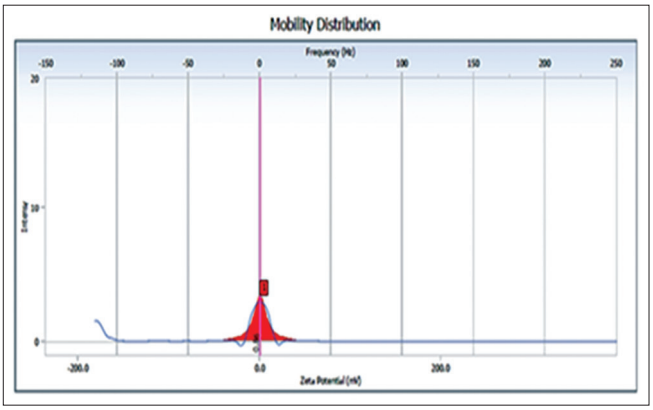

Figure 6: Zeta potential of optimized batch

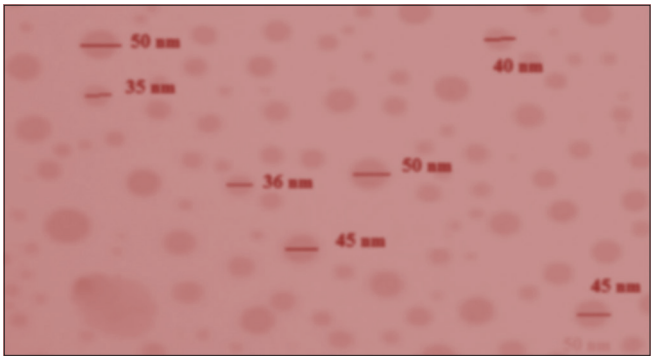

Figure 7: Transmission electron microscope of KA13 formulation size measurement at 50 nm

Table 5: Measurement result of zeta potential

Measurements results	Observation
Zeta potential	8 (mV)
Mobility	6.680e-006
Conductivity	0.0504 (mS/cm)
Doppler shift	0.55
Base frequency	115.7

In vivo bioavailability study

By comparing the selected formulation to the standard SRT HCl and marketed formulation (Tab. Potiga, 50 mg, GlaxoSmithKline), it can be concluded that selected formulation has a higher concentration, AUC. The t_{max} of selected formulation has lower than that of the pure SRT HCl and marketed formulation. The pharmacokinetic parameters [Table 6] and profile [Figure 10] of SRT HCl were determined after oral administration of pure SRT HCl and nanosuspension prepared by nanoprecipitation to New Zealand white male rabbits. In the process of absorption of a drug, dissolution is the rate-limiting step. Once the drug is available in the intestinal fluid as a dissolved form, it follows the transport across the gastrointestinal epithelium membrane. It was found that the formulation produced a C_{max} compared to standard SRT HCl and marketed formulation, values of 7.29, 5.49, and 5.95 μg/ml. The extent of absorption of standard SRT HCl from the formulation, as represented by the total AUC, was also higher as compared to standard Ezogabine and marketed formulation. AUC produced by the formulation was 230.68 μg/h/ml compared to 173.70 μg/h/ml for standard SRT HCl and 188.14 μg/h/ml for marketed formulation. The time to reach C_{max} (t_{max}) for the formulation was also lower where it was about 20.76 h compared to pure drug 22.31 h and marketed formulation 22.86 h. These values indicated C_{max} and AUC were achieved by selected formulation prepared by nanoprecipitation. Thus, it is suggested that prepared nanosuspension could exhibit enhanced bioavailability compared with pure Ezogabine and marketed formulation. HPLC chromatogram of KA13 was shown in Figure 11. The pharmacokinetic profile and parameters clearly show enhanced bioavailability of prepared nanosuspension of SRT HCl as compared to pure drug and marketed formulation. Maximum concentration in plasma was achieved by the formulation KA13 which was approximately 1.5 times more than pure drug indicating better release from formulation.

CONCLUSION

A nanoprecipitation technique was successfully employed to produce stable SRT HCl nanosuspension. The particle size was highly dependent on process parameters. With the optimized process parameters, nanosuspension with a diameter of about 60.6 nm could be obtained. The reduction

Table 6: Comparative study of the pharmacokinetic parameters of selected batch, standard SRT HCl and marketed formulation

Pharmacokinetic parameter	Standard SRT HCl	Marketed formulation	Optimized batch
T_{max}	22.31 h	22.86 h	20.76 h
C_{max}	5.49 μg/ml	5.95 μg/ml	7.29 μg/ml
$[AUC]^{0-t}$	173.70 μg/hr/ml	188.14 μg/hr/ml	230.68 μg/hr/ml
$[AUC]^{0-inf}$	333.54 μg/hr/ml	370.78 μg/hr/ml	412.10 μg/hr/ml

SRT: Sertraline, HCl: Hydrochloride, AUC: Area under the curve, C_{max}: Maximum plasma concentration

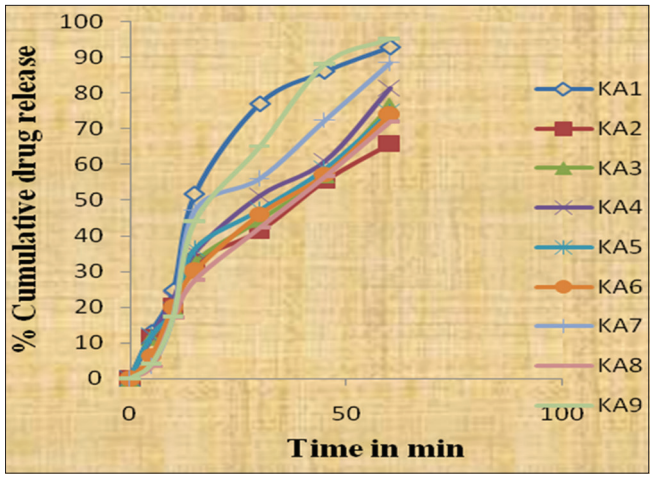

Figure 8: *In vitro* dissolution study of nanosuspension (nanoprecipitation method) of KA1-KA9 (Eudragit RS 100)

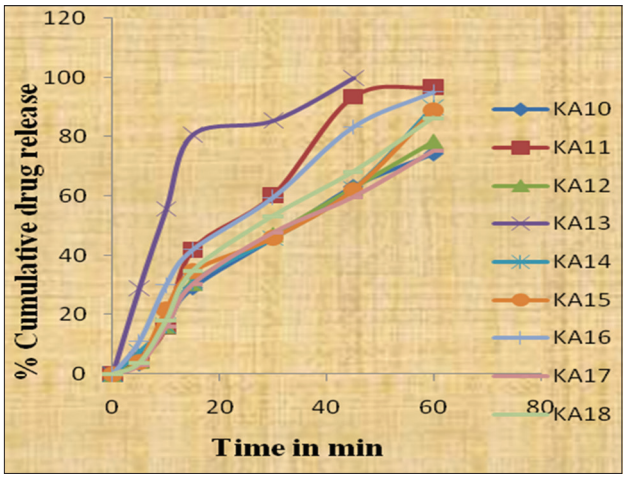

Figure 9: *In vitro* dissolution study of nanosuspension (nanoprecipitation method) of KA10-KA18 (PVP K 25)

of drug particle size and the change of crystalline form to amorphous form may attribute the main mechanism to the oral bioavailability enhancement. The oral bioavailability of SRT HCl in New Zealand white male rabbits resulted from nanosuspension was increased compared with the standard SRT HCl and marketed formulation. The best nanosuspension of SRT HCl (50 mg) can be obtained with 50 mg of PVP K 25 (polymer) and 50 mg of Poloxamer 188 (surfactant) using nanoprecipitation method. Hence, we concluded that nanosuspension represents a promising alterative to current drug delivery systems aiming to improve the biopharmaceutical performance of drugs with low water solubility.

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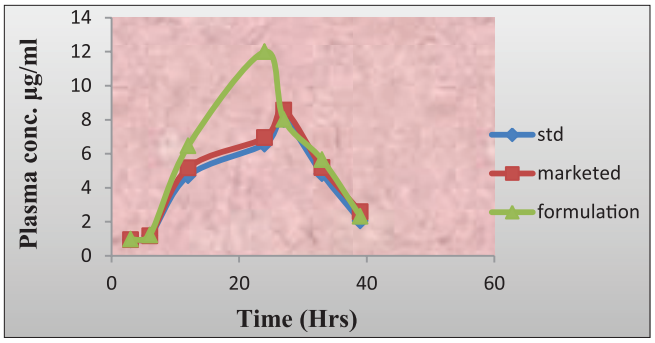

Figure 10: Plasma drug profile of standard sertraline hydrochloride, marketed formulation, and optimized batch

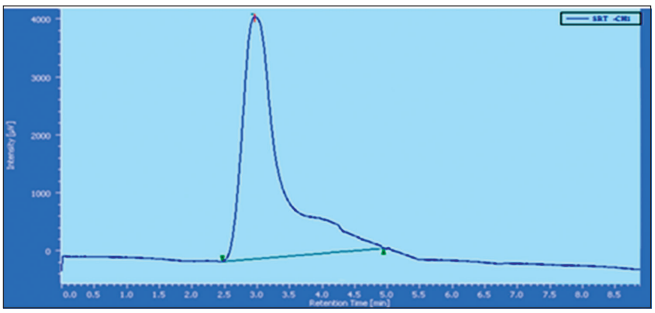

Figure 11: High-performance liquid chromatography chromatogram of KA13

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