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Formulation development, In-vitro and In-vivo evaluation of novel solid oral dosage form containing Quetiapine nanoparticles.

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Abstract

Poorly water soluble drugs such as quetiapine fumarate (QF) offer challenges in developing a solid dosage form such as tablets with adequate bioavailability. The objective of the present work is to develop a solid dosage form for quetiapine nanoparticles in order to increase the saturation solubility, rate of dissolution so that the oral bioavailability is enhanced. Quetiapine fumarate is a BCS class II drug, hence its oral bioavailability is dissolution limited. To enhance the oral bioavailability a nanoparticle formulation of QF was prepared by using high pressure homogenization. The nanosuspension prepared was converted into dry powder by using spray drying. The nanosuspension and spray dried nanoparticles are characterized for particle size, polydispersity index, zeta potential, saturation solubility, drug content, dissolution rate, solid state characterization such as X-ray diffraction(XRD), Differential scanning calorimetry(DSC), infrared(IR), scanning electron microscopy(SEM), transmission electron microscopy(TEM). The spray dried nanoparticles were blended with excipients to convert into solid dosage form such as tablets. The compressed tablets were evaluated for physical parameters,assay anddissolution was compared with the commercial QF formulation. Solid state characterization data showed loss of drug crystallinity after homogenization. The novel dosage form has shown significant increase in the rate of dissolution when compared to microparticle formulation in discriminating medium. In-vivo studies have shown that the rate and extent of absorption of nanoparticle formulation was significantly high when compared to its microparticleformulation when administered in rats.

Keywords: Quetiapine Fumarate, Nanoparticles, high pressure homogenization, Particle size.

Introduction

Poor aqueous solubility is major challenge in achieving the required oral bioavailability. A major hurdle that has prevented commercialization of many promising poorly soluble drugs is dissolution rate limited bioavailability [1].The major limitations of using a poorly soluble drug are high dose and higher administration frequency that may results in the side effects of the drugs [[1].It has been reported in the literature that drug solubility and rate of dissolution have impact on the rate and extent of the bioavailability [2].Thus there is need for improvement of the bioavailability of such poor soluble drugs. Increasing drug solubility and stability through appropriate formulation approaches can lead to increased therapeutic efficacy of the drug. Many approaches are used to improve the solubility and dissolution properties of poorly soluble active ingredients including salt formation, formation of nanoparticles, pH adjustment, use of surfactants, inclusion complexes, use of oily formulations, use of self-emulsifying drug delivery systems, formation of co-precipitates with hydrophilic polymers, co-milling with hydrophilic excipients, to name a few. Among these the pharmaceutical particle size technology appear to be mostly employed to improve the poor aqueous solubility of poorly soluble drugs that limits in vivo bioavailability owing to its low dissolution rate in the gastrointestinal fluid after oral administration [3].Apart from conventional micronizing methods to reduce the particle size, nanotechnology appears to be promising method to enhance solubility and thereby the bioavailability because the particle size reduction increases the surface area [4].There are various ways in which nanoparticles of poorly water-soluble molecules are generated [5-8]. Alternatively, nanoparticles can be successfully generated using drug-fragmentation processes such as homogenization [9,10],microfluidation [11] or milling [12]. The main disadvantage of jet mill, bead or pearl millare the polydispersity of the product requiring subsequent removal of microparticles or abrasion of pearls in a mill. In the field of nanotechnology, polymer based nanoparticles have become prominent area of current research and development due to their ease of preparation [13].In this study high pressure homogenization technique is used as it is simple, fast and cost effective reproducible technique [14]. To prepare an effective solid dosage form from a drug nanosuspension, it is important that the nanosuspension is first dried and the dried nanoparticles should go back to their original particle size when reconstituted in an aqueous system [15]. Reported literature shows that the nanoparticles

improve the oral bioavailability of poorly bioavailable drugs due to their specialized uptake mechanism which may also prevent first pass metabolism [16] Quetiapine fumarate) is a psychotropic agent belonging to a chemical class, the dibenzothiazepine derivatives. Quetiapine fumarate is a BCS class II drug having poor solubility and having only 9% bioavailability [ii]. The chemical designation is 2-[2-(4-dibenzo [*b,f*] [1,4]thiazepin-11-yl-1-piperazinyl) ethoxy]-ethanol fumarate (2:1) (salt) and used to treat schizophrenia and bipolar disorder. The US FDA [United States Food and Drug Administration] approved label shows two major boxed warnings such as a) Increased Mortality in Elderly Patients with Dementia-Related Psychosis and b) Suicidal Thoughts and Behaviors.The bioavailability of quetiapine is marginally affected by administration with food, with Cmaxand AUC values increased by 25% and 15%, respectively. The main objective of the current work is to evaluate the effect of particle size reduction on solubility, dissolution and bioavailability of quetiapine fumarate so that there can be a possibility of reducing the dose and address the dose related side effects.

Materials and Methods

Materials

Quetiapine fumarate was procured from Dr Reddys Laboratories limited (Hyderabad, Inida). Mannitol was purchased from Roquette, Polyvinyl pyrrolidone [PVP K-30] was purchased from BASF (Germany); sodium lauryl sulfate was purchased from JRS, crospovidone and sodium starch glycolate were obtained from ISP and Grain Processing corporation. All other chemicals were of analytical grade.

Methods

Preparation of nanosuspension

High pressure homogenization technique was used to produce the nanoparticles. Different batches of nanosuspension of 100 ml batch size were prepared by using combination of steric and electrostatic stabilizers. The drug suspension was prepared by dissolving the stabilizers in purified water followed by dispersing the drug quetiapine fumarate with constant stirring to prevent the lump formation. The drug suspension was first subjected to high shear homogenization process for about 15 minutes so that a homogeneous drug suspension was obtained. By subjecting to high shear homogenization it has been observed that there is reduction of the particle size of the drug to submicron level. This drug suspension at this level (i.e. before subjecting to high pressure homogenization process) is referred to a drug microparticle suspension and used to compare with the nanosuspension in the characterization studies. This homogenous drug suspension was subjected to high pressure homogenization process, wherein the drug suspension is passed through a narrow gap in the homogenizer at high pressure such as 750 – 1000 bar pressure. The drug suspension was continuously subjected to high pressure homogenization in a recirculation mode for about 90 minutes. This process converts submicron particles into nanoparticles. To produce stable nanosuspension a combination of steric and electrostatic stabilizers has been used. The concentration of the stabilizers used and process of the homogenization process was optimized. The stability of the nanosuspension was evaluated upon storage at 2-8 C and room temperature for a period of one week. Based on the particle size data, physical stability in terms of zeta potential, poly dispersity index at initial and on stability the formulation NS-04, NS-08 and NS-11 were selected for scaleup. These selected formulations were scaled to 1 L batch size wherein the drug suspension was prepared in the same manner as described above and subjected to high pressure homogenization for a period of 90 minutes. After homogenization the suspension was collected and stored at below temperature of 25 C until further processing.

Table 1: Composition of Quetiapine Fumarate Nanosuspension.

Composition No.	Composition (% w/v)					Batch size/ Purified water in mL
	Drug	SLS	PVP	HPC	HPMC	
NS-01	2.5	1	3	-	-	100
NS-02	5	1	3	-	-	100
NS-03	5	0.5	3	-	-	100
NS-04	5	0.75	5	-	-	100
NS-05	5	1	-	2.5	-	100
NS-06	5	0.5	-	5	-	100
NS-07	5	0.75	-	5	-	100
NS-08	5	0.75	-	5	-	250
NS-09	5	1	-	-	2.5	250
NS-10	5	0.5	-	-	5	250
NS-11	5	0.75	-	-	5	250
NS-12	5	0.75	5	-	-	1000
NS-13	5	0.75	-	5	-	1000
NS-14	5	0.75	-	-	5	1000

Characterization of the nanosuspension

The nanosuspension obtained was characterized for particle size, zeta potential and polydispersity index.The particle size, which is represented in terms of d10, d50, d90 affects the solubility of the poorly soluble drug. The mean particle size (d50, ie 50 % of particles are having size less than this value) of the nanosuspension before drying was estimated in triplicate by using Zeta sizer- Nano ZS, Malvern Instruments UK) at room temperature. A refractive index of 1.65 was used for particle size analysis. Nanosuspension was added to the sample dispersion unit (deionized water) and stirred at 2000 rpm to reduce the interparticulate aggregation and laser obscuration range was maintained between 10-20 %. The samples were adequately diluted with deionized water and placed in electrophoretic cell and measurement was carried out with help of software. The particle size was measured after performing the experiments in triplicates.
The particle size of drug in dried nanoparticles was analyzed by adding water to dried nanoparticles (so that the surface stabilizers, re-dispersants are in dissolved state and only drug in dispersed form) followed by dilution with water to obtain suitable concentrations for measurement in the same manner as carried out for the nanosuspension.

Zeta Potential

A prerequisite to achieve an enhancement of oral bioavailability with drug nanocrystals is that crystals are finely dispersed in the gut and do not aggregate. In case they start aggregation, the bioavailability decreases with increasing aggregate formation. This is attributed to the fact that they lose special properties of nanoparticles such as their adhesive property to the mucosal wall. Therefore it is necessary to prepare nanosuspensions with a physical stability as high as possible. Surface charge properties of the nanosuspensions are studied through zeta potential. The value of particle surface charge indicates the stability of nanosuspensions at the macroscopic level. A minimum zeta potential of ±30 mV is required for electrostatically stabilized nanosuspensions [17,18] and a minimum of ±20 mV for steric stabilization [19]. The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential [20].
Zeta potential of the nano suspension has been analyzed in Malvern zeta sizer after diluting nanosuspension with water to obtain suitable concentration for measurement. Further the zeta potential of the dried nanoparticles is obtained by adding water to the dried nanoparticles and diluted with water to obtain suitable concentration for measurement. The diluted sample was added in specialized zeta cell and the same procedure as that of particle size was carried out.

Production of dried nanoparticles

Spray drying process was used to convert the nanosuspension into dried nanoparticles. Buchi mini spray dryer B-290 is used for this process. Spray drying is a process where in dry powder is obtained from a drug solution or suspension by rapidly drying with a hot gas such as nitrogen or oxygen etc.
The nanosuspension (batch no. NS-12) prepared as above is spray dried using lactose as bulking agent. The resultant suspension was spray dried at inlet temperature of 140 C, nitrogen pressure 5 kg/cm2and liquid suspension feed rate 6-10 ml/minute. Prior to spray drying process, lactose is dissolved in the drug suspension as bulking agent and the composition is shown in table 2. For the comparison study, the drugmicroparticle suspension also subjected to spray drying process in similar manner as carried out for drying the nanosuspension. The difference between microparticle suspension and nanosuspension is that microparticle suspension is not subjected to particle size reduction in high pressure homogenizer.

Table 2: Composition of the dried nanoparticles*

Ingredients	Quantity /Tablet (mg)
Quetiapine Fumarate	25.00
Polyvinyl pyrrolidone	12.5
Sodium lauryl sulfate	5
Water equivalent to	0.5 mL
Bulking agent/Redispersant	
Lactose	50

*the dried nanoparticles means the spray dried nanosuspension after incorporating lactose as the bulking agent or redispersants.

Characterization of dried nanoparticles

The dried nanoparticles and microparticles are characterized for particles size, zeta potential, poly dispersity index. Further the dried nanoparticlesare further subjected to solid state characterization such as X-ray powder diffraction, differential scanning calorimeter, Fourier transform infra-red spectroscopy, Scanning electron microscopy, drug content, saturation solubility, dissolution profile.

X-ray Powder diffraction (XRD)

The dried nanoparticles were characterized for X-ray powder diffraction to study the change in the crystallinity of the drug after subjecting to particle size reduction by high pressure homogenization process. X-ray diffraction pattern was recorded using over a 2-theta range of 3 – 45 using the X-ray diffraction model X'Pert MPD Model, Phillips, Holland using the Cu-target X-ray tube and Xe-filled detector. The operating conditions were voltage 40 kV; current 30 mA; scanning speed 1/min.

Differential Scanning Calorimetry (DSC)

DSC is a method wherein the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. DSC analysis give add on to the observation of the XRD data for any change in the crystallinity of the drug after processing in high pressure homogenizer. DSC scans of the Quetiapine fumarate drug, the physical mixture of quetiapine, PVP, SLS and lactose and dried nanoparticles obtained by spray drying,have been studied using DSC- Shimadzu 60 with TDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 20 C/min under dry air flow (100 ml/min) between 25 C and 200 at 10 C/minute.

Fourier Transform Infra-Red (FTIR) Spectroscopy

Generally there are two regions in the infra-red region one is frequency region (1500 -4000 cm-1) and the second is finger print region (1500-400 cm-1), In the frequency region the corresponding peaks of the functional groups is being observed. And in the finger print region a series of the absorption bands are observed due to bending vibrations within the molecules and this region is very unique to each compound. The infra-red (IR) spectroscopy is an identification test generally used to study the compatibility of the drug with different excipients used while preparing the formulation, because it has been observed that when there is incompatibility there is shift in the characteristic peaks of different functional groups. In this analysis, the spectra corresponding to different functional groups is captured and if the IR spectra of the test formulation is identical to that of the drug and excipients, then it is understood that the drug is compatible with the excipients used in the formulation.

The pure drug quetiapine, physical mixture of quetiapine, PVP, SLS and lactose and dried nanoparticles obtained by spray drying have been characterized for IR. The sample for IR analysis has been prepared by using disks of drug and Potassium Bromide (KBr) in 1:100 ratio, using Shimadzu Fourier Transform Infra-Red spectrometer over wave number range of 400 to 4000 cm-1.

Scanning Electron Microscopy [SEM]

The scanning electron microscopy is used to study the morphology of the drug and the nanoparticles prepared. In this analysis the images of the sample are obtained by scanning the sample with the focused beam of the scattered electrons. The sample for the SEM analysis is prepared by applying the sample on the carbon rape stuck to the aluminium stub followed by applying a 5nm gold coating onto the sample. The sample prepared is subjected scanning electron microscopy, using model QUANTA 250 FA867operated at the low vacuum with a LFD detector and 60 Pa pressure

The morphology of the raw quetiapine fumarate, nanosuspension and dried nanoparticles was examined with the scanning electron microscopy.

Transmission electron microscopy [TEM]

Transmission electron microscopy is used to study the internal composition of the sample as the sample is scanned with the focused beam of transmitted electrons and has higher resolution. Hence TEM gives information like nanoparticle size information, crystallization, stress in addition to morphology. TEM analysis of the nanosuspension prepared has been carried out in FEI Tecnai™ transmission electron microscope, G2 series.A droplet of sample diluted 1:100 in deionised water was dried on a carbon on copper TEM grid and were imaged in the TEM

Saturation Solubility

Saturation solubility gives an idea about the maximum amount of drug dissolved in the media. This study is useful particularly for poor soluble drugs. Saturation solubilityof Quetiapine Fumarate, spray dried microparticles and nanoparticle was carried out in 0.1 N HCl, 4.5 acetate buffer, 6.8 phosphate buffer and purified water. For determination of the saturation solubility, excess amount of the drug or dried nanoparticles have been added to 100 ml of media maintained at 37 C and shaken onrotary shake flask for a period of 24 hours. The samples are centrifuged for about 10 minutes at 4000 rpm. The supernatant was collected and filtered through 0.22 µm nylon membrane filter, diluted with diluents (methanol and acetonitrile in 1:1 ratio) and analyzed using HPLC (waters Alliance HPLC system USA) method same as that used for determination of drug content.

Preparation of the Quetiapine Fumarate tablet

The spray dried nanoparticles were mixed with the extragranular materials to form the final blend for compression. The extragranular materials include the disintegrant, glidant, lubricant. Four different disintegrants such as sodium starch glycolate, pregelatinized starch, crospovidone, croscarmellose sodium. Colloidal silicon

dioxide is used as a glidant and magnesium stearate is used as a lubricant. Firstly the spray dried nanoparticles blended with disintegarnt, glidant in double cone blender for about 25 minutes to form unlubricated blend. Finally the magnesium stearate is added to the unlubricated blend and blended for about 5 minutes to form final lubricated blend. This lubricated blend is compressed into tablets using 8 mm round punches using 8-station cadmach compression machine.

Table 3: Composition of the tablets

Ingredients	Composition mg/tablet				
	*NF-1	NF-2	NF-3	NF-4	MF-1**
Spray dried nanoparticles$	92.5	92.5	92.5	92.5	92.5
Croscarmellose sodium	10	-	-	-	-
pregelatinized starch	-	10	-	-	-
Crospovidone	-	-	10	-	-
Sodium starch glycolate	-	-	-	10	10
Colloidal silicon dioxide	1	1	1	1	1
Talc	1	1	1	1	1
Magnesium stearate	0.5	0.5	0.5	0.5	0.2
Total weight	105	105	105	105	105

*NF:represents the tablet formulation prepared by using the spray dried nanoparticles with different disintegrants.
**MF: represent the tablet formulation prepared by using the spray dried nanoparticles with sodium starch glycolate as disintegrant.
$92.5 mg of the spray dried nanoparticles or microparticles contain 25 mg of quetiapine fumarate.

Evaluation of Quetiapine tablets

The physical parameters of the tablet such as tablet hardness, thickness, disintegration time, friability have been measured. The tablet hardness was measured using a hardness tester (Model: 8M, Dr. SchleunigerPharmatron, USA). Each hardness value reported is an average of ten measurements. The disintegration time was measured in purified water at 37 ± 0.5 C, using a disintegration tester (Model: ED2L, Electrolab, India). The disintegration time reported is an average of six measurements. Tablet friability was measured as the percentage weight loss of 20 tablets after 100 revolutions using a friabilator (Model: EF2, electrotab, India).

Drug content

The tablet formulation prepared as above was evaluated for drug content by HPLC. The drug content of the tablet formulation prepared by using spray dried nanoparticles and microparticles was determined to know if there is any loss of drug during spray drying process. The drug content was determined by high pressure liquid chromatography (HPLC) method. Each tablet formulation was taken in volumetric flask and dissolved in diluents comprising water: acetonitrile in 20:80 ratiowith the help of sonication. Then the solution is subjected to centrifugation at the speed of 3000 rpm for about 10 minutes. Then this solution was filtered throughDurapore0.45 µm PVDF membrane filter and was analyzed by HPLC. The mobile phase consists of mixture of potassium di hydrogen orthophosphate, acetonitrile, tetrahydrofuran, triethyl amine pH adjusted to 6.4 with KOH solution. Chromatographic separation was accomplished using an Xterra Column RP8 3.5 µm; 4.6X150 mm column. The mobile phase was pumped isocratically at a flow rate of 1.5 ml/minute during analysis and maintained at a column temperature of 50 C and detection wavelength of 217 nm.

In-Vitro Dissolution Study

Quetiapine fumarate is shown to have pH dependent solubility with highest solubility in acidic medium and decreasing the solubility as the pH is increasing. The tablets prepared above using nano and microparticles are tested for rate of dissolution. The media chosen is pH 6.8 phosphate buffer as this pH was found to be discriminating media. The dissolution profiles of tablet formulation prepared using nanoparticles, microparticles and commercial tablets were determined in a USP apparatus II in 900ml phosphate buffer pH 6.8, at 37±0.5 C with a paddle rotation speed at 50 rpm. Samples were filtered through a 0.22µm nylon membrane filter (Millipore, Bedford, MA) and assayed for drug content in the same manner as carried out for drug content.

In-vivo pharmacokinetic study

Male wistar rats weighing about 250 g were used for the pharmacokinetic study. The rats were fasted overnight with the free access to water. The animal experiments were carried out in accordance with the guidelines provided by institutional Animal Care and Ethics committee.All animalexperiments were approved by CPCSEA and IAEC. Eight rats were divided into two groups one is test and other group is reference. The test group received the suspension comprising spray dried nanoparticles and the reference group received the suspension comprising drug microparticles using oral gavage at a dose level of 10 mg/kg. Rodent feed was

returned after 3 hours post dosing. Blood samples have been collected from retro orbital vein at predetermined time intervals 0, 0.5,1, 3, 5, 8, 10 and 24 hours post dosing. The blood samples were collected into centrifuge tubes upto 0.5 ml. Collected blood samples were immediately centrifuged at 9000 rpm for about 15 minutes. The serum separated at top with theblood cells depositing at thebottom of the tube. The supernatant pale yellow color plasma is collected using micropipette. Approximately 0.3 -0.5 ml of the plasma has been collected into polypropylene vials and stored in freezer temperature at -20 C until the analysis.

Plasma Analysis/ Bioanalysis

From the plasma the proteins should be separated. For the extraction of the proteins, to 250 µl of plasma, 50 µl of internal standard, 10 µl of lamotrigine was added into a centrifuge tube. To this 2 ml of acetonitrile is added and subjected to cyclomixer for about 15 seconds. Then the tubes are subjected to vertex for about 2 minutes and finally centrifuge for about 3 minutes at 3200 pm speed. After the centrifugation the organic layer is collected. About 10µl of the organic layer is injected into HPLC and analysis was carried out.

Chromatographic conditions:

Column	: KROMOSIL 250 4.6µ 5mm
Mobile phase composition	: Buffer : Acetonitrile 55:45
Flow rate	: 1 ml/min
Injection volume	: 20 µl
Run time	: 8 min
Detection wavelength	: 254 nm
Column temperature	: 30 ºc
Sample temperature	: 5 ºc
Diluent	: water : methanol 50:50

Buffer was prepared by dissolving potassium dihydrogen phosphate in water to make 0.02 N KH2PO4. Analytical data was acquired by Empower 2 software. Standard curves were obtained from linear square regression analysis of drug/internal standard peak area ratio as a function of plasma concentration versus time data was analyzed, and the oral pharmacokinetic data were developed.

Results and Discussion

Particle size, Polydispersity index and Zeta potential

The quetiapine nanosuspension with different steric stabilizers and electrostatic stabilizers have been prepared and characterized. Selection of stabilizers is very important because these nanoparticles try to form aggregate and destabilize. From the particle size data as shown in table 4 it has been observed that among different concentrations of electrostatic stabilizers ie compositions NS-02, NS-03, NS-04 which are at concentration of 1%, 0.5 % and 0.75% of electrostatic stabilizers, the composition with 0.75 % concentration has shown least particle size of d50 as 112 nm and d90 as 220 nm. Hence with these concentrations of SLS, three different steric stabilizers at concentration of 5% have been evaluated. From the particle size data of compositions NS-04, NS-08, NS-011 has shown mean particle size (d50) as 112, 145 and 127 nm respectively. Hence these three compositions has scaled up to 1 litre batch size and again characterized for particle size wherein d50 was 107,176,154 nm and was matching with the small batch size. All the compositions have been exposed to stability conditions of 2-8 C and 25/60 % RH for about 1 week duration and similar trend was observed on stability. The formulation with PVP and SLS as stabilizers at concentration of 5% and 0.75 % has shown mean particle size as 210 and 198 at 2-8 C and 25/60 %^ RH conditions respectively.

Table 4: Particle size (nm) of nanosuspensions

Formulation code	Initial			1 week 2-8 C			1 week 25 C/60 % RH		
	d10	d50	d90	d10	d50	d90	d10	d50	d90
NS-01	10	224	375	108	399	607	119	653	1350
NS-02	12	173	564	74.4	355	516	98	521	687
NS-03	258	484	821	88	210	345	101	417	569
NS-04	51	112	220	88	210	245	136	198	286
NS-05	63.5	368	490	139	366	1460	480	612	743
NS-06	12.5	249	845	27	339	793	89	417	1210
NS-07	19.6	161	427	103	532	775	108	520	1050
NS-08	71	145	594	51	176	314	89.5	357	542
NS-09	56	181.5	496	133	594	843	135	616	1000
NS-10	55	392	517	169	205	242	66	271	360
NS-11	64	127	287	136	198	286	191	339	503
NS-12	49	107	206	44	104	214	53	108	210
NS-13	79	176	641	71	891	1510	64	1348	1547
NS-14	70	154	314	68	216	674	93	545	1310

The polydispersity index (as shown in table 5) of the compositions with PVP and HPC as steric stabilizers have shown the PDI as 0.27 and 0.283 respectively owing to the narrow particle size distribution, however on stability it has been observed that there is aggregation in the compositions with HPC thus showing higher PDI. And the composition with PVP was found to stabilize the nanoparticles, thus it retained narrow particle size distribution with PDI of 0.3 and 0.4 at 2-8 C & 25 C/60% RH respectively. This was also represented by the zeta potential data wherein the ZP of the compositions with HPC and HPC was reduced on stability when compared to initial but the ZP of the composition with PVP was retained owing to its stability. Based on the above data, the composition with PVP and SLS as primary and secondary stabilizers is selected for further processing.

Table 5 Polydispersity index and Zeta potential of nanosuspensions

Formulation code	Initial		1 week 2-8 C		1 week 25 C/60 % RH	
	PDI	ZP	PDI	ZP	PDI	ZP
NS-01	0.353	14.4	0.7	10.7	0.565	8.22
NS-02	0.518	9.34	0.61	16.7	0.71	16.9
NS-03	0.566	10.9	0.51	11.7	0.7	14.6
NS-04	0.27	34	0.3	36.7	0.4	35.9
NS-05	1	29.4	0.6	16.7	1	8.61
NS-06	0.86	22	0.43	8.61	0.6	10.7
NS-07	0.4	34	0.53	30.1	0.736	25
NS-08	0.283	34.2	0.365	28.8	0.655	21
NS-09	0.54	21.9	0.61	14.1	0.627	9
NS-10	0.93	21	1	18.5	0.8	10.9
NS-11	0.48	31.9	0.4	32.1	0.4	21
NS-12*	0.25	42.5	0.28	34	0.4	32.5
NS-13	0.36	31.4	0.5	27.1	1	25.2
NS-14	0.31	32.1	0.7	21.2	0.89	20.5

It is very important that the drug nanoparticles prepared should retain their particle size on stability as well as after converting into powder form or when dispersed in the water or physiological fluid. Spray drying technique was used to convert suspension into powder form using lactose as redispersant or bulking agent and characterized. From the particle size data as shown in table 6 of the spray dried nanoparticles was observed that after drying also the particles retained its particle size and are stable wherein d50 is 103 nm and PDI as 0.29 and ZP as 36 mV.

Table 6: Characterization of spray dried nanoparticles and spray dried drug microparticles

	Particle size distribution			PDI	ZP
	d10	d50	d90		
Nanoparticles	54	103	198	0.29	36
Microparticles	313	1070	4970	1	21

Solid state characterization

The change in the solid state of the drug after homogenization and drying has been evaluated using XRD, DSC. The X-ray diffraction pattern of the drug quetiapine fumarate, dried nanoparticles, physical mixture (of quetiapine fumarate, polyvinyl pyrrolidone, sodium lauryl sulfate) are compared in Figure 1. The XRD pattern of the formulation shows absence of the characteristic peaks of the drug, thus drug lost its crystallinity. This is further confirmed by the DSC data, wherein the characteristic endotherm of the drug has been lost in the formulation owing to loss of its crystallinity.

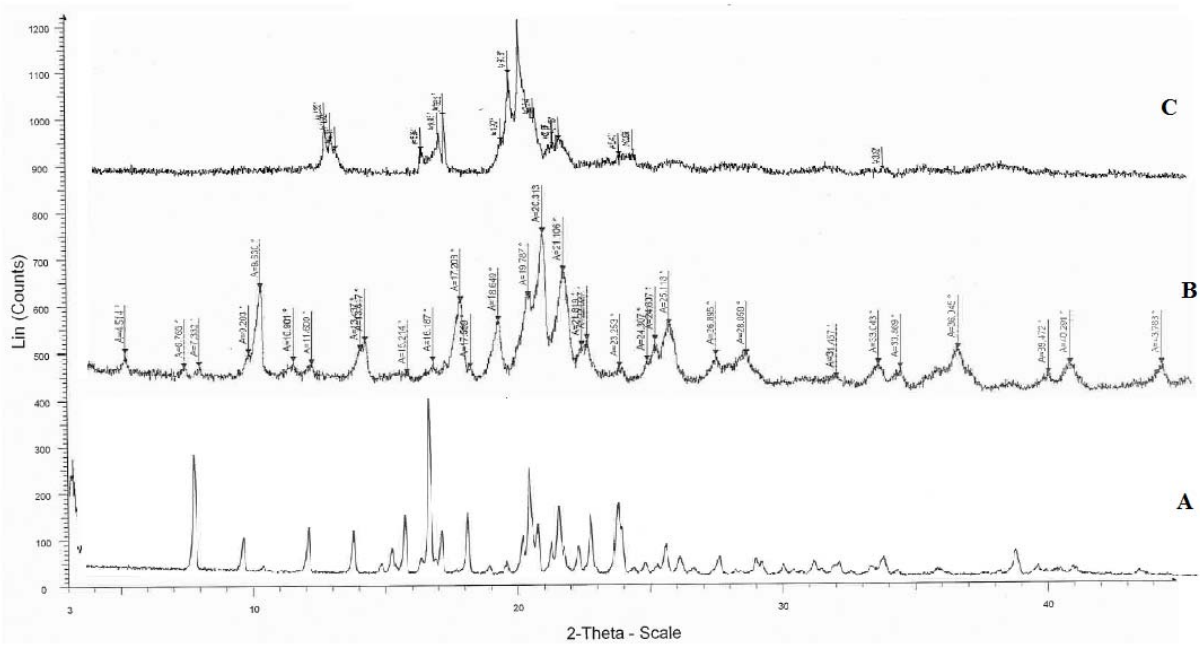


Figure 1: XRD diffractograms for A: API Quetiapine fumarate, B: Physical mixture of quetiapine fumarate, PVP, SLS and lactose C: spray dried nanoparticles.

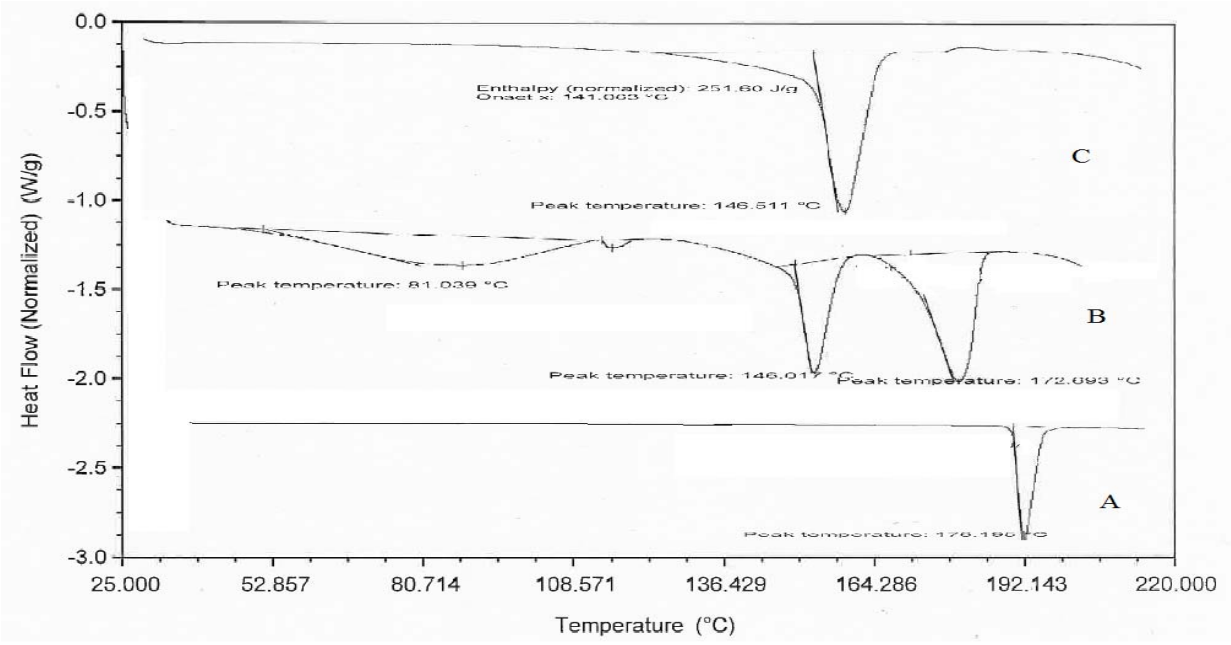


Figure 2: DSC curves of A: Quetiapine Fumarate; B: Physical Mixture of quetiapine fumarate, PVP, SLS and lactose and C: spray dried nanoparticles.

Figure 3 shows the infrared spectra of quetiapine fumarate, physical mixture and the spray dried nanoparticles. From the IR spectra it has been observed that there is no change in the nature and position of the characteristic bands in the formulation or physical mixture, hence there is no chemical interaction of the drug with the stabilizers used.

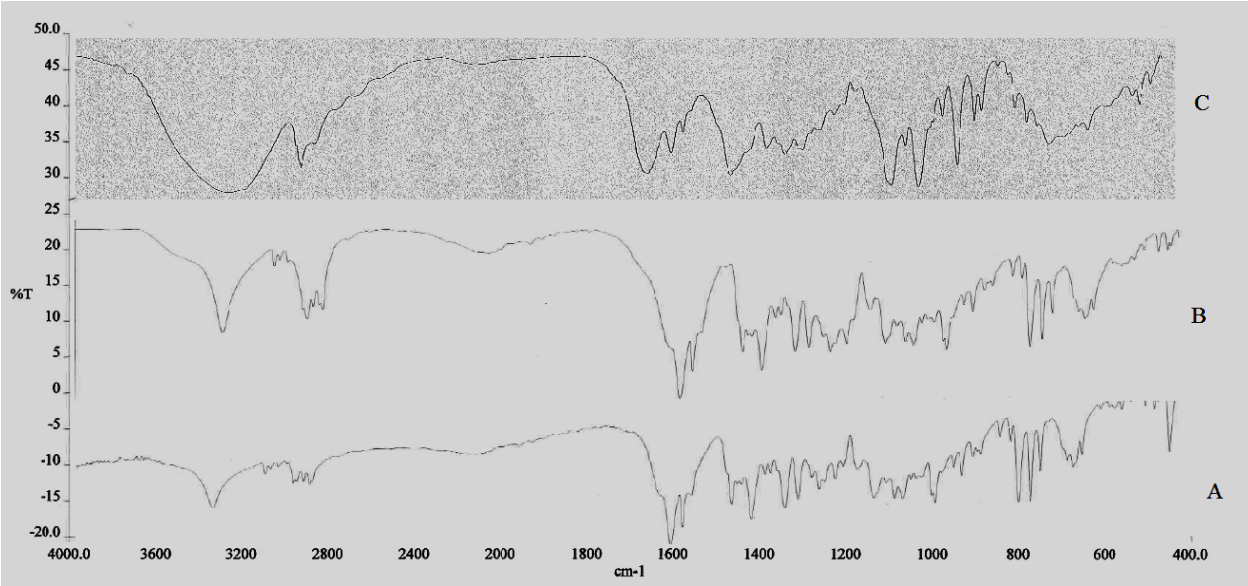
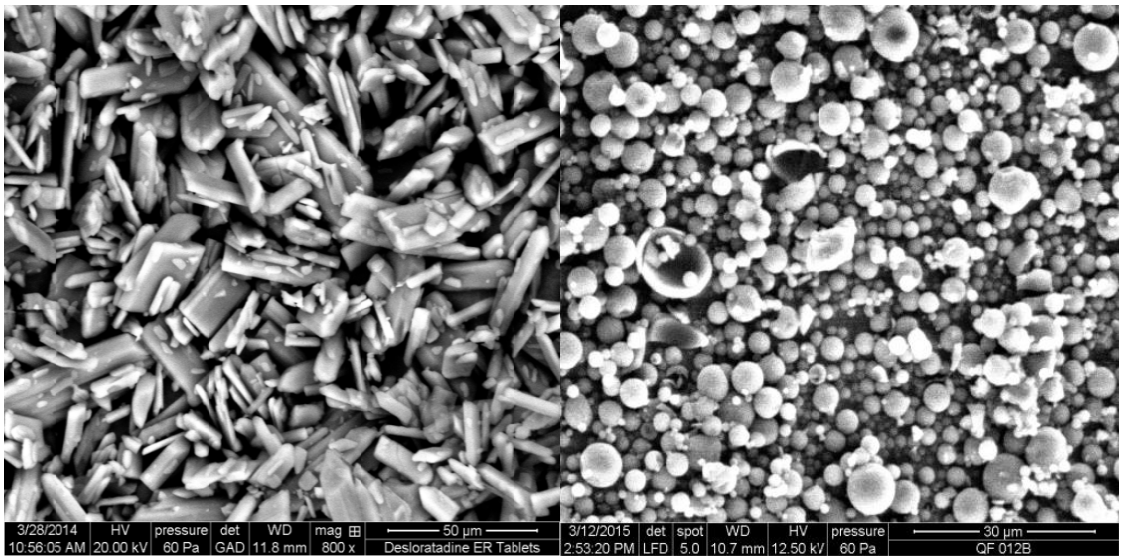


Figure 3: Infra-red spectra for A: Quetiapine Fumarate; B: Physical Mixture of quetiapine fumarate, PVP, SLS and lactose and C: spray dried nanoparticles.

On comparing the scanning electron microscopy picture of the pure API and the spray dried nanoparticles as shown in Figure 4, it has been observed that the drug crystals has been converted into spherical particles by deposition of the stabilizers and/or bulking agent (redispersant) around the drug nanoparticle, thus creating the hydrophilic microenvironment and aiding in improvement of dissolution rate.

Figure 4: SEM images: of A: Quetiapine fumarate, B: Spray dried nanoparticles.



A **B**

The TEM image of the nanosuspension for the composition NS-12 as shown in Figure 5 indicated that the nanosuspension has irregular shaped particles of almost uniform size not showing any aggregation, this is supported by the low polydispersity index of the nanosuspension. TEM further confirms the nanoparticle size.

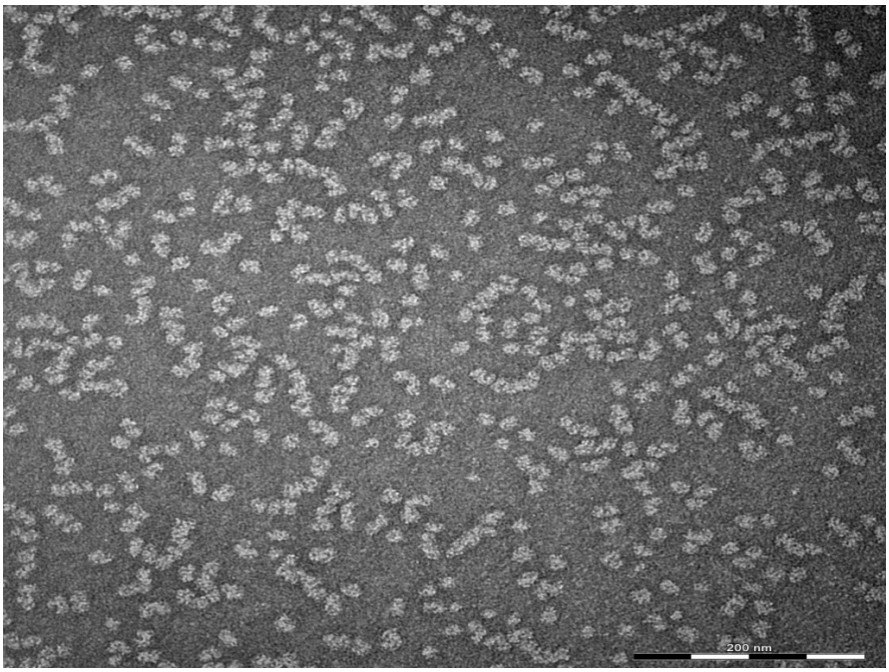


Figure 5 :TEM image of the nanosuspension.

Saturation solubility

The saturation solubility of the drug quetiapine fumarate, spray dried microparticles and spray dried nanoparticles has been evaluated in different pH media i.e 0.1 N HCl, 4.5 acetate buffer, 6.8 phosphate buffer and water. Solubility data has been captured in table 7. The results indicated that the drug the solubility of drug quetiapine fumarate decreases with the increase in the pH, this is because the drug is showing pH dependent solubility. The saturation solubility of the spray dried nanoparticles was significantly higher when compared to the pure drug or the spray dried microparticles.

Table 7: Saturation solubility of drug quetiapine fumarate and spray dried microparticles and nanoparticles.

Solvents	Solubility mg/ml		
	Drug quetiapine fumarate*	spray dried microparticles	Spray dried nanoparticles
0.1 N HCl	9	20	44
4.5 acetate buffer	4	6	15
6.8 phosphate buffer	3.1	3.2	6
Water	2.6	3.1	5.4

*solubility was analyzed in respective solvents comprising PVP and SLS.

Conversion of spray dried nanoparticles into tablet dosage form

To enhance the tabletting properties of the spray dried nanoparticles, these nanoparticles were blended with the extragranular materials and compressed into tablets. Four different

disintegrants were evaluated. The physical properties of the tablets are shown in Table 4. For comparison the spray dried microparticles were also compressed and evaluated for physical properties. Out of all the four disintegrants, sodium starch glycolate showed fastest disintegration time i.e. 5 minutes and croscarmellose showed the slow disintegration time i.e 12 minutes. Whereas the tablets prepared by the microparticles along with sodium starch glycolate as disintegrant showed still slowest disintegration time ie> 15 minutes. Further all the tablets have shown uniform tablet weight, thickness and drug content, hardness in the range of 5-9 kp and friability less than 0.1 % showing good strength of the tablets.

Tablet 8: Physical properties of the tablets

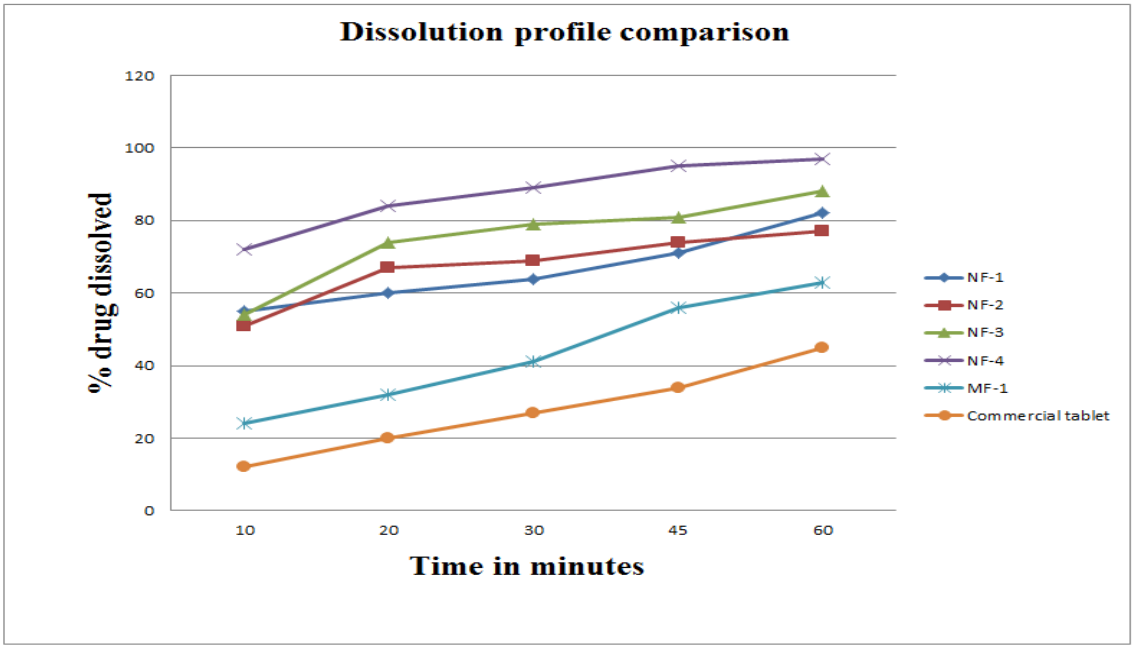
	Tablet weight(mg)*	Thickness (mm)@	Hardness (kp)$	Disintegration time minutes)**	Friability (%)	Drug content
NF-1	105 ± 5	2.0 ± 0.2	7 .0 ± 0.5	12± 1.6	0.09	98.2
NF-2	105 ± 5	2.0 ±0.2	6.5 ± 0.4	8 ± 1.0	0.06	97.3
NF-3	105 ± 4	2.0 ± 0.2	5.0 ± 0.7	9 ± 0.5	0.07	97.8
NF-4	105 ± 3	2.0 ± 0.2	9.0 ± 0.2	5 ± 0.4	0.02	99.1
MF-1	105 ± 4	2.0 ± 0.2	10 ± 0.5	> 15	0.03	98.3

* Tablet weight range is determined by weighing 20 tablets individually.
@ Thickness range is determined by taking thickness of 5 tablets individually.
$ Hardness range is determined by taking hardness of 5 tablets individually.
**Disintegration time range is determined on 6 individual tablets.

In-vitro Evaluation

Dissolution profiles were determined for the tablets prepared by spray dried microparticles; nanoparticles and the commercial tablets in pH 6.8 phosphate media (as this is the discriminating media). As shown in figure 6, it has been observed that rate of the dissolution of all the nanoformulations is significantly higher than the commercial tablet and also high than the microparticle formulation. Out of all the nanoformulations the formulation NF-4 showed fastest dissolution profile. The rate and extent of the dissolution of the drug from the tablet dosage of nanoparticles is more than 80 % and NF-4 is 97 % at end of the dissolution study whereas the commercial tablet showed only 45 % and microparticle formulation showed 63 % of dissolution. The formulation with sodium starch glycolate as disintegrant has shown almost complete release of drug at the end of dissolution but the tablets with other disintegrants has shown incomplete release of the drug.

Figure 6: Comparative dissolution profile



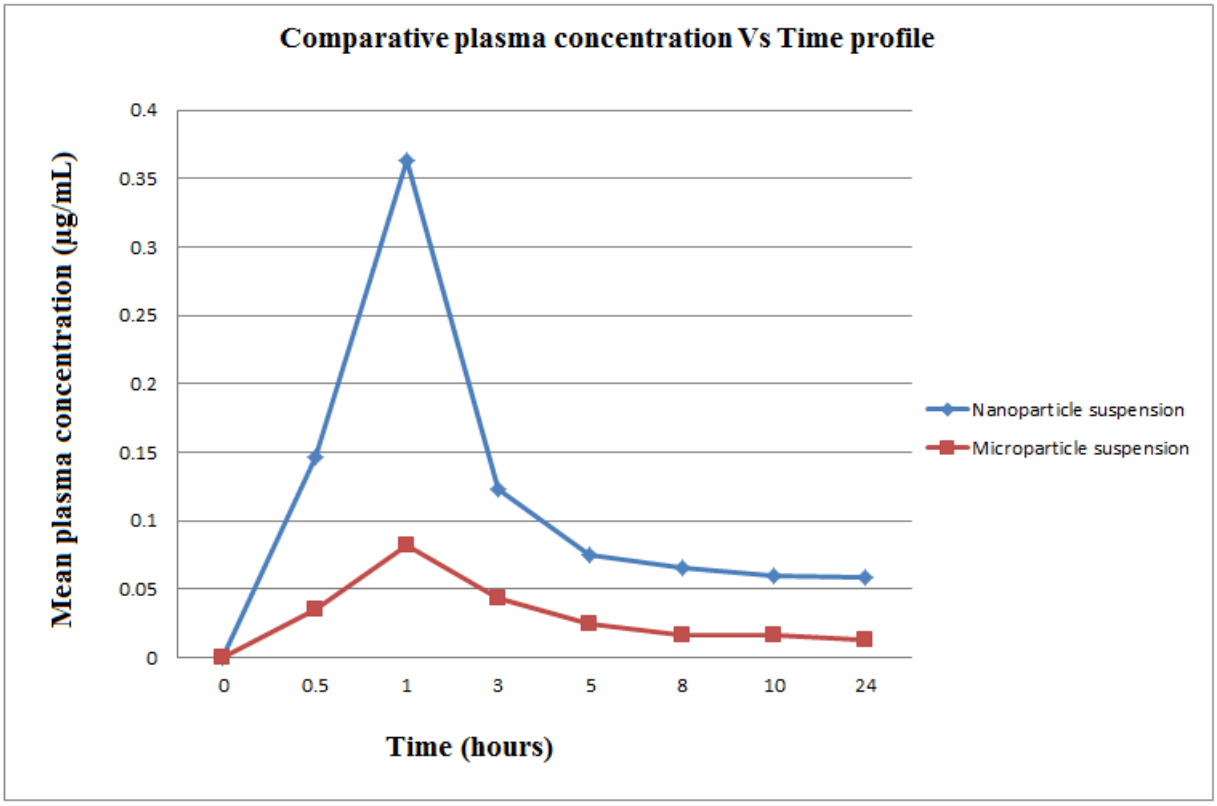
In-vivo evaluation

Both the microparticle and the nanoparticle formulation has been evaluated for the single dose parallel pharmacokinetic study in the male wistar rats. And the results have been shown in Table 9. From the data it has been observed that maximum plasma concentration for the microparticle and nanoparticle formulation is 0.0822 ± 0.005 and 0.3632 + 0.002 respectively. The results also has been represented in figure 7, which shows approximately 2.5 folds increase in the AUC (0-24 h) and 4.5 folds increase in the plasma concentration of quetiapine fumarate with reduction in time required to achieve the maximum plasma concentration from 1.32 hr to 1.0 hr. The significant increase in the plasma concentration and oral bioavailability can be attributed to increase in the saturation solubility and rate of dissolution due to particle size reduction to the nanoparticle size.

Table 9: Pharmacokinetic parameters (mean ± SD, n=4) of Quetiapine Fumarate.

Parameter	Microparticles formulation	Nanoparticle formulation
AUC (0-24)	0.6125 ± 0.005	1.5147 ± 0.005
Cmax (µg/mL)	0.0822 ± 0.005	0.3632 ± 0.002
Tmax (hr)	1.32	1.0

Figure 7: Comparative plasma concentration Vs time profile for the nanoparticle suspension and microparticle suspension in wistar rats.



Conclusions

From this study it has been observed that the formulation containing quetiapine nanoparticles has been successfully prepared by high pressure homogenization technique combined with spray drying technique. From the solid state characterization data ie XRD and DSC data, it has been observed that drug after processing for particle size reduction followed by spray drying the drug has lost its crystallinity. The significant enhancement in saturation solubility and rate of dissolution can be attributed to the particle size reduction which is further confirmed by TEM analysis. The in-vivo pharmacokinetic study in rats has shown 4.5 folds increase in the plasma concentration and 2.5 folds increase in the oral bioavailability with the reduction in time required to achieve the maximum plasma concentration. The increase in the rate of

dissolution can be correlated to improvement in absorption of the quetiapine fumarate in the gastro intestinal tract of the rats. The enhancement of the oral bioavailability of the nanoparticle formulation can be attributed to increase in the surface area obtained by particle size reduction. This enhancement in the oral bioavailability can be explored on the strong possibility of the dose reduction of quetiapine fumarate so that dose related side effects of this drug can be minimized.

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