Enhanced oral bioavailability of Ibrutinib encapsulated poly (lactic-co-glycolic acid) nanoparticles: Pharmacokinetic evaluation in rats

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Abstract

Background: The poor oral bioavailability of newly discovered chemical entities and marketed formulations are usually related with poor aqueous solubility or poor permeability, leading to failure of drug either in drug development phases or therapeutic failure in clinical setting. However, advancement in drug formulations and drug delivery technologies has enabled the scientists to improve the bioavailability of formulations by enhancing solubility or permeability.

Objective: This study reports the enhancement of oral bioavailability of ibrutinib (IBR), a poorly soluble anticancer drug in Wistar Albino rats.

Method: Ibrutinib loaded nanoparticles were formulated by nanoprecipitation method by utilizing poly lactide co-glycolide (PLGA) as a safe biodegradable and biocompatible polymer and poloxamer or pluronic 127 as stabilizer. Animals were administered with 10 mg/kg of IBR suspension or equivalent amount of IBR loaded nanoparticles. Plasma samples were extracted and analyzed by state of the art UPLC-MS/MS technique. Pharmacokinetic (PK) parameters and bioavailability were calculated by non-compartmental analysis.

Results: There was approximately 4.2-fold enhancement in the oral bioavailability of ibrutinib-loaded nanoparticles as compared with pure ibrutinib suspension. The maximum plasma concentration (Cmax; 574.31 ± 56.20 **Vs** 146.34 ± 5.37 ng/mL) and exposure (AUC; 2291.65 ± 263.83 **Vs** 544.75 ± 48.33 ng* h/mL) of ibrutinib loaded nanoparticles were significantly higher than those exhibited by pure ibrutinib suspension.

Conclusion: The outcomes of present study suggested the potential of PLGA nanoparticles in the enhancement of in bioavailability and therapeutic efficacy of ibrutinib.

Keywords: Ibrutinib; PLGA; nanoparticles; solubility; bioavailability; pharmacokinetics; area under curve; UPLC-MS/MS

1. Introduction

The performance of any administered drug or dosage form depends on consistency in bioavailability. Poor or inconsistent bioavailability often leads to therapeutic failure. Among the various reasons of poor bioavailability, the water insolubility or poor water solubility is known to be the most common culprit for bioavailability issues. As per an estimate, approximately 90% of drug molecules suffer with poor water solubility at early stage, while approximately 40% of the marketed drugs have bioavailability problems mainly due to poor water solubility [1-2]. There are several nano fabrication techniques for improving aqueous solubility of drugs that have bene employed to enhance bioavailability of formulations. Among various nano fabrication techniques, polymeric nanoparticles have been investigated extensively, owing to high drug encapsulation, high stability, ease in surface functionalization and versatility in route of administration [3-6]. Polymeric nanoparticles composed of biodegradable polymers such as poly lactic acid (PLA), poly glycolic acid (PGA) and poly lactic acid-poly glycolic acid (PLGA) copolymers have added advantage of being indigenous hence non-toxic or less toxic comparatively. PLGA copolymer is approved by US FDA for various applications. Furthermore, it is biocompatible, biodegradable and has a tunable physicochemical property that depends on ratio of monomers used in its synthesis [7-8]. Therefore, PLGA based nanoparticles have widely been studied for the enhancement of bioavailability of challenging drugs [9-12].

This study has been undertaken to investigate the oral bioavailability and pharmacokinetics of PLGA nanoparticles loaded with ibrutinib (IBR), a poorly soluble small synthetic molecule. IBR is an anticancer drug recently approved by USFDA to treat B lymphocyte cancers such as chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and Waldenström's macroglobulinemia (WM). It acts by inhibiting Bruton’s tyrosine kinase (BTK), an enzyme responsible for maturation/proliferation of B cells. It makes an irreversible covalent bond with BTK thus inhibits proliferation and survival of B

cells [13-17]. The chemical name of IBR is 1 ((3R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo(3,4d] pyrimidin-1-yl]-1-piperidinyl]-2-propen-1-one. It's empirical formula is $C_{25}H_{24}N_6O_2$ with a molecular weight of 440.5 [2]. IBR is a weak base having pKa of 3.74. It is freely soluble in dimethyl sulfoxide, soluble in methanol, however practically insoluble in water. It has been reported to exhibit pH dependent solubility as it is slightly soluble at pH 1.2 while practically insoluble at pH 3 to 8 as per USP and European Pharmacopoeia nomenclature [2,15].

IBR belongs to class II drugs, as per biopharmaceutical classification system (BCS). Class II drugs suffer with low solubility therefore oral formulations of such drugs are challenging, for instance, slow dissolution rate or precipitation due to decreased solubility at the site of absorption impede the oral absorption therefore oral bioavailability is minimum and often irregular. IBR has been marketed by Janssen in capsule dosage form as Imbruvica capsules 140 mg. Absolute oral bioavailability of Imbruvica capsules has been reported to be 2.9%. Food is known to increase oral bioavailability of IBR (approximately 2 fold) when compared with fasted state (Zhu et. al, 2017). It is metabolized primarily by CYP3A4 enzyme therefore inhibitors of this enzyme such as grapefruit juice and others may increase the exposure of IBR. Researchers have evaluated the effect of grapefruit juice on the oral bioavailability of IBR capsules of 140 mg in healthy human volunteers. They reported oral bioavailability of IBR as 15.9% after consumption of grapefruit juice as compared to 3.9% and 8.4% in fasting and fed state respectively [18,19].

There are very few investigations on the enhancement of bioavailability of IBR [6, 19]. Moreover, there is no report on the use of PLGA nanoparticles for the enhancement of bioavailability of IBR, to the best of our knowledge. In the present study, we report the enhanced oral bioavailability of IBR loaded in PLGA nanoparticles compared with IBR suspension in the rats.

2. Materials and Methods

IBR was purchased from Beijing Mesochem Technology Co Ltd (Beijing, China). PLGA polymer (75:25), Pluronic acid 127 and dicloromethane was purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile and methanol were of HPLC-grade and were purchased from Winlab Pty. Ltd (Australia) and Avonchem Ltd (UK) respectively. Analytical grade formic acid and ammonium acetate were purchased from BDH Laboratory (UK). Type 1 water was obtained from Milli-Q, Millipore, Massachusetts USA.

The animal study was approved by ethical committee of Prince Sattam bin Abdulaziz university (Approval number-PHARM-1-25-06-2018), conducted according to the science and ethical principles for animal care and use at college of pharmacy, Prince Sattam bin Abdulaziz university.

2.1. Formulation of IBR-PLGA nanoparticles

Nanoparticles were formulated by nanoprecipitation technique wherein slow addition of organic phase containing drug and polymer in aqueous phase containing stabilizer causes precipitation of drug [20-23]. Organic phase was prepared by dissolving 150 mg of PLGA polymer and 20 mg of IBR in 25 mL of dichloromethane by vortexing and sonication. Aqueous solution was prepared by dissolving 100 mg of Pluronic acid-127 in 50 mL of deionized water. The prepared organic phase was added slowly to aqueous phase kept on magnetic stirrer set at 400 RPM. Organic solvent was then removed by evaporation under reduced pressure at 45°C, followed by separation of nanoparticles from aqueous phase by centrifuging at 5000 rpm for 60 min (Centurion-Scientific,UK). Obtained nanoparticles were then freeze dried by freeze dryer (Mill rock technology, Kingston, NY), after washing with cold distilled water.

2.2. Bioavailability study protocol

The bioavailability study of optimized formulation of IBR was carried in healthy, male, Wistar-Albino rats with a weight range of 180-250 g. The study was conducted according to the science and ethical principles for animal care and use at college of pharmacy, Prince Sattam bin Abdulaziz university. Rats were obtained from Animal house of College of Pharmacy, Prince Sattam bin Abdulaziz university, Alkharj, Saudi Arabia. The rats were adapted to standard laboratory conditions at a temperature of 25 ± 1◦C before the commencement of study with free access to water. Single dose, fasting state parallel design was followed for this study, therefore, six rats were randomly selected and separated into two groups of three each. The rats were kept on fasting overnight before the commencement of study to rule out effect of food or any other confounding factor. The rats in Group one were gavaged with IBR suspension (1 mg/mL) in aqueous sodium carboxy methyl cellulose at a fixed dose of 10 mg/kg body weight according to weight of animals. Therefore, a rat weighing 220 g received 2.2 mL of IBR suspension. Likewise, rats in the Group two received appropriate volumes of IBR loaded nanoparticle suspension at equivalent doses (10 mg/kg body weight). Rats were anaesthetized by ether inhalation and approximately 0.5 mL of blood was withdrawn from retro-orbital plexus into heparinized tubes before administration of dose (pre-dose sample), then at 0.5, 1, 1.5, 2, 3, 4, 12, 24 and 48 hours after drug administration (post-dose). All the samples were centrifuged at 4000 rpm for 10 min to separate the plasma. Plasma samples were then kept in deep freezer at -70 °C till analysis of IBR and further evaluations of PK parameters and bioavailability.

2.3 Analysis of IBR concentration in rat plasma samples

The analysis of IBR concentration in rat plasma samples was carried out by using a newly developed and validated UPLC-MS/MS method [24-25]. The separation and analysis module consisted of ACQUITY^{TM} UHPLC coupled with triple–quadruple tandem mass spectrometer Micromass Quattro micro^{TM} (TQDMS) from Waters Corp., Milford, MA.

Briefly, 200 µL of plasma sample was taken in 1.5 mL centrifuge tubes, 20 µL of IS (vilazodone, 80 ng/mL in acetonitrile) was added and mixed by vortexing for 30 s. Plasma proteins were precipitated and cleaned by addition of 400 µL of acetonitrile followed by centrifugation at 10500g at 4 °C for 10 min. The supernatants were taken and evaporated to dryness, that followed reconstitution with 200 µL of acetonitrile then transferred to UPLC vials for analysis. Five microliter of cleaned samples were injected on Acquity BEHTM C18 column (50 mm x 2.1 mm i.d with a pore size of 1.7 µm, Waters, USA), maintained at 40 °C temperature. Separation of IBR was achieved by mobile phase, a mixture of acetonitrile:10 mM ammonium acetate: formic acid (80: 20:0.1, v/v/v), flowing at a rate of 0.25 mL/min. The detection was achieved by electrospray ionization in positive mode with selected reaction monitoring. The precursor to product ion transitions of m/z were 441.16>84.02 and 442.17 >155.02 for IBR and vilazodone, respectively. Data was handled and controlled by Target LynxTM and Mass Lynx respectively (version 4.1, SCN 714).

2.4 Evaluation of oral bioavailability and Pharmacokinetic parameters of IBR

The concentrations of IBR as found in plasma samples were plotted against time to construct plasma concentration-time curve. The oral bioavailability and PK parameters of IBR were calculated by using non-compartmental pharmacokinetic analysis model (NCA). The Microsoft office excel 2010 was used for calculation of PK parameters. Excel program was opened in NCA extravascular input mode to enter the plasma concentreation and time data indivisually for one animal at a time. After entering the further details such as dose and units, linear trapezoidal method was selected to calculate the PK parameters such as Cmax, the maximum IBR concentration in plasma; Tmax, the time of Cmax; exposure, the area under the curve of plasma concentration and time; MRT, the mean residence time; t1/2, the terminal half-life, λz, the elimination rate constant; Vz, the apperant volume of distribution after oral administration and Cl/f, the total clearance after oral administration. The relative bioavaiabilty (F_{rel}) of the IBR in PLGA loaded nanoparticles was calcuated with respect to

the pure IBR suspension by comparing their exposures observed in the PK analyses using the follwing equation.

$$Frel = \frac{AUC(IBR\ loaded\ nanoparticles) * dose\ of\ IBR\ suspension}{AUC\ (IBR\ suspension) * dose\ of\ nanoparticles}$$

2.5 Statistical analysis

The plasma concentration-time data were entered to microsoft excel 2010 to calculate the descrptive parametrs such as mean concentration and standard deviation (SD). The PK parameters were subjected to inferential statistical evaluations to draw a valid conclusion. PK parameters were presented as mean ± SEM (standard error of mean). The differences between the means were statistically tested with the help of Graphpad by applying unpaired two samples students t-test. The differences in the mean were considered as significant or highly signicant for P values lesser than 0.05 and 0.01 respectively .

3. Results and Discussions

In the present study we used PLGA as a polymer to prepare biocompatible polymeric delivery system to enhance the bioavailability of IBR. PLGA is one of the most widely used polymers owing to its biocompatibility, biodegradability and tunable physicochemical and formulation characteristics. Once administered, it is bio transformed to its monomers, lactic acid and glycolic acid, which are endogenous materials and hence don't initiate any immunogenic reactions. Moreover, monomers are easily metabolized by the body [26]. PLGA (50:50) contains equal proportions of both monomers. By varying the proportion of monomers, we can modify the rate of decomposition of the polymer thus control the release rate of encapsulated drugs. In the present study we used PLGA composed of 75% lactic acid and 25% glycolic acid (75:25), as higher percentage of lactic acid will cause slower degradation of nanoparticles and subsequent sustained release of drug comparatively (Guo et. al, 2017).

3.1. Analysis of IBR concentration in rat plasma samples

The IBR concentration in the rat plasma was analyzed by UPLC-TQDMS, which is state of the art analytical technique currently. The method adopted for the analysis of IBR was developed, validated and reported recently [25]. The calibration plot was linear between the conc. range of 1.81–2000 ng/ mL (r2 ≥0.991). The lower limit of quantification was 0.35 ng/mL. All the calibrators passed the acceptance criteria of accuracy (within ±15%) and precision (≤15%) as these were back calculated by using regression equation obtained for the calibration plot (Y= 0.0202 X – 3214). Fig. 1

The run time of analysis of each sample was two minutes only with a retention time of 0.66 min and 0.52 min for ibrutinib and IS, respectively. The selected chromatograms of ibrutinib and IS have been shown in Figure 2.

sample at 2 h after oral adminstration of 10 mg/kg IBR-PLGA nanoparticles. (b) chromatogram of plasma sample at 2 h after oral adminstration of 10 mg/kg IBR-suspension.

The plasma concentarion versus time curves for both treatments (IBR suspension and IBR-PLGA nanoparticles) were constructed by using microsoft excel 2010 as showmin Figure 3. Every points on the curve represents the mean plasma concentration ± SD (as error bar) of three animals for a particlular time point.

The PK profile curves (Figure 3) of both IBR suspension and nanoparticles showed rapid absorption phase after administration of dosage, however, nanoparticles exhibited enhanced absorption that may be due to enhanced contact surface area of the nanoparticle formulation.

3.2 Calculation of PK parameters oral bioavailability of IBR

The PK parameters were calculated by using non-compartmental pharmacokinetic analysis model (NCA). The Microsoft office excel 2010 was opened in NCA extravascular input mode to enter the plasma concentration-time data to calculate the PK parameters. The PK parameters were then subjected to inferential statistical evaluations by calculating mean and standard error of mean. Moreover, hypothesis testing was performed to check whether the differences in the calculated means are significant or not. The PK parameters for treatments, IBR suspension and IBR-nanoparticles are presented in Table 1.

Cmax- Peak plasma concentration, Tmax-Time to reach peak plasma concentration, AUC0-48- Area under the plasma concentration-time curve from zero to 48 hours, AUC0-∞ - total area under the plasma concentration time curve, λz- Terminal rate constant, t1/2- Elimination half-life, MRT 0-t- Mean residence time from zero to 48 hours, MRT 0-inf_obs- Mean residence time from zero to infinity, Vz/Fobs - Apparent volume of distribution during terminal phase, Cl/F_obs- Apparent total clearance of the drug from plasma after oral administration. (* $P < 0.05$, indicated that the differences in the means were statistically significant; ** $P< 0.001$ indicated that the differences in the means were statistically very significant)

The maximum absorption (Cmax) for IBR nanoparticles was found as 574.31 ± 56.20 ng/mL, which was approximately four fold higher as compared to maximum absorption exhibited by IBR suspension (146.34 ± 5.37 ng/mL). The difference in maximum absorption of IBR from nanoparticle and suspension was statistically very significant ($P < 0.01$). The lower absorption for suspension may be attributed to poor solubility or precipitation at the site of absorption. It is noteworthy that absorption from suspension was faster as compared to nanoparticles as shown by time of the maximum absorption of 1.33 ± 0.17 h and 2 ± 0.00 h for IBR suspension and IBR-nanoparticles respectively. However, the

difference in the time of absorption was not very significant ($P < 0.05$). The difference in the rate of absorption may be due to the polymer, PLGA, used in the nanoparticle formulation. PLGA has been known to reduce and sustain the release of drug encapsulated (Anwer et. al, 2018). The exposure of IBR from nanoparticles, the time-averaged concentration of drug circulating in the animal for the 48 hours, AUC_{0-48}, was found as 2175.68 ± 224.92 ng.h/mL, while the total exposure, $AUC_{0-\infty}$, (drug circulating in the body for infinite time) was calculated as 2291.65 ± 263.83 ng.h/mL. The exposure to the last measurable time-point (AUC_{0-48}) and total exposure ($AUC_{0-\infty}$) of IBR from suspension was calculated as 511.75 ± 54.21 and 544.75 ± 48.33 ng.h/mL respectively. Therefore, it is evident that IBR exposures from nanoparticles were approximately over four times higher than those calculated for suspension, and these enhancements in the exposures were statistically very significant ($P < 0.01$). The oral bioavailability of IBR loaded PLGA nanoparticles was compared with those achieved after oral administration of IBR suspension at similar dose. Based on the exposures of the formulations, nanoparticles exhibited approximately 4.2-fold higher bioavailability than suspension.

Scientists have evaluated PK parameters of IBR after oral administration of 10 mg/ kg of IBR suspension prepared in methyl cellulose, in rat model [27]. They reported Cmax, Tmax, AUC0-48, $AUC_{0-\infty}$, $t_{1/2}$ and Kel as 0.7 ± 0.16 µg/ml, 0.5 h, 1.42 ± 0.22 µg/ml, 1.43 ± 1.35 h, and 0.49 ± 0.30 h^{-1} respectively. Differences in the values of parameters we reported here could be attributed to differences in time points of withdrawal of blood samples. Moreover, differences in the analytical methods could also give different results. There is another report of PKs parameters of IBR suspension and IBR loaded in self nanoemulsifying drug delivery system (SNEDDS) alongwith relative bioavailability [6]. They reported PK parameters for IBR suspension as Cmax, Tmax, AUC_{0-48}, $AUC_{0-\infty}$, Kel, $t_{1/2}$ and MRT as 266 ± 74 ng/mL, 0.75 h, 1180 ± 403 ng/mL, 1217 ± 480 ng.h/mL, 0.1±0.07 h^{-1}, 8.39 ± 2.99 h and 9.5 ± 3.7 h respectively. Furthermore, they reported comparative

bioavailability of IBR loaded SNEDDS with IBR suspension with 2.64-fold enhancement in bioavailability due to advanced drug delivery method. In the present study, we observed 4.2 fold enhancements in the oral bioavailability of IBR-loaded PLGA nanoparticles in comparison to IBR suspension which could be attributed to better absorption and higher exposure of the formulation.

4. Conclusions

The oral bioavailability of the IBR loaded nanoparticles of PLGA, a safe, biocompatible, biodegradable polymer approved by USFDA, was found significantly higher than the pure IBR, which suffers with low bioavalibity due to poor solubility. PLGA is a promising polymeric material for the fabrication of advanced drug delivery systems and can be used for enhancement of oral bioavailability of challenging drugs. Further studies involving different animal species are suggested to explore the therapeutic benefit of this formulation. For instance, reduction of the therapeutic dose required for the treatment owing to enhanced bioavailability.

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Figure Caption:

Figure 1: Plasma calibration curve of ibrutinib and IS

Figure 2. Sample chromatograms of ibrutinib and IS (a) chromatogram of plasma

Figure 3. Mean plasma concentartion-time curves of IBR in rats (n=3, ± SD) after oral adminstration of 10 mg/kg IBR-suspension (▲) and IBR-PLGA nanoparticles (◆).

Table 1: Mean PK parameters (± SEM, standard error of mean) of IBR after oral administration of IBR suspension and IBR-PLGA nanoparticles 10 mg/kg body weights in rats (n=3).

PK Parameters	**IBR suspension Mean ± SEM**	**IBR PLGA nanoparticles Mean ± SEM**
Cmax (ng/mL)	146.34 ± 5.37	**574.31 ± 56.20
Tmax (h)	1.33 ± 0.17	*2 ± 0.00
AUC 0-48 (ng/mL*h)	511.75 ± 54.21	**2175.68 ± 224.92
AUC 0-inf_obs (ng/mL*h)	544.75 ± 48.33	**2291.65 ± 263.83
λz (h^{-1})	0.05 ± 0.01	0.02 ± 0.01
t1/2 (h)	13.37 ± 1.62	11.76 ± 1.74
MRT 0-48 (h)	8.78 ± 0.71	8.78 ± 0.38
MRT 0-inf_obs (h)	12.60 ± 1.34	11.62 ± 1.10
Vz/F_obs (mg/kg)/(ng/mL)	0.37 ± 0.08	*0.07 ± 0.01
Cl/F_obs (mg/kg)/(ng/mL)/h	0.02 ± 0.00	0.01 ± 0.00
Relative oral bioavailability (%)	100	421

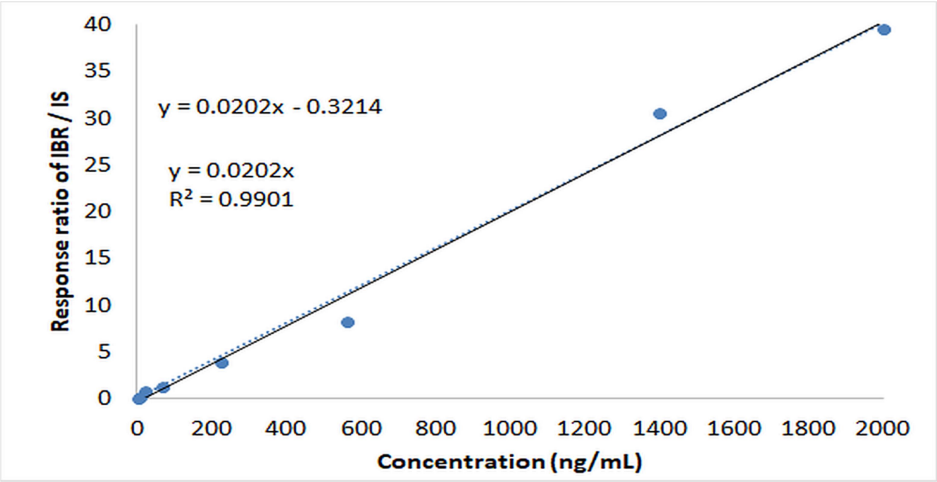
Response ratio of IBR / IS
Concentration (ng/mL)
y = 0.0202x - 0.3214
y = 0.0202x
R² = 0.9901
0
5
10
15
20
25
30
35
40
0
200
400
600
800
1000
1200
1400
1600
1800
2000

Figure 1

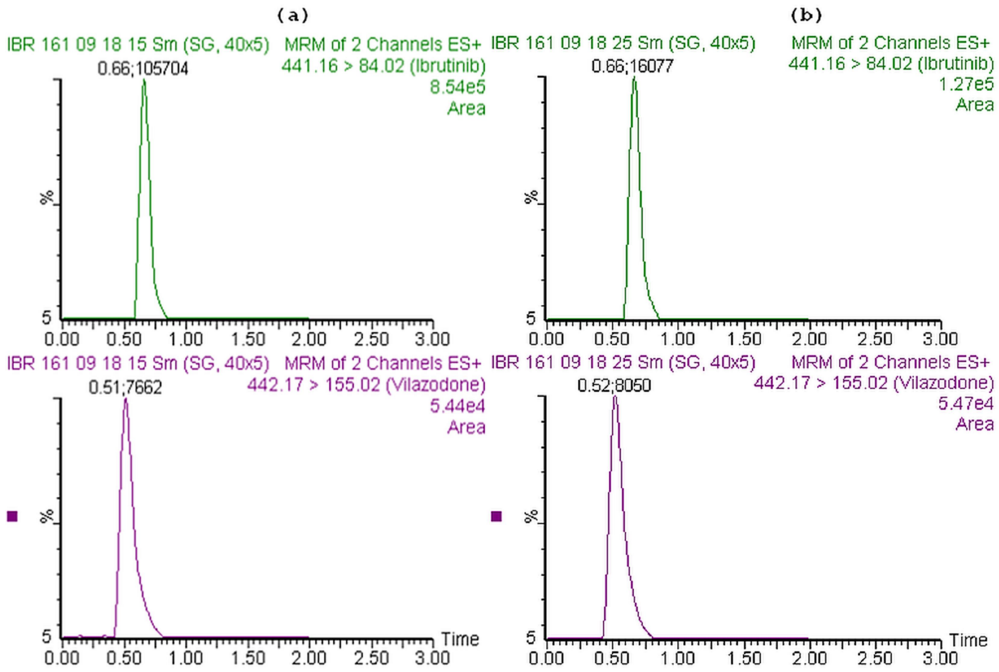
(a)
IBR 161 09 18 15 Sm (SG, 40x5) MRM of 2 Channels ES+
441.16 > 84.02 (Ibrutinib)
0.66;105704
8.54e5
Area
%
5
0.00 0.50 1.00 1.50 2.00 2.50 3.00
IBR 161 09 18 15 Sm (SG, 40x5) MRM of 2 Channels ES+
442.17 > 155.02 (Vilazodone)
0.51;7662
5.44e4
Area
%
5
Time
0.00 0.50 1.00 1.50 2.00 2.50 3.00
(b)
IBR 161 09 18 25 Sm (SG, 40x5) MRM of 2 Channels ES+
441.16 > 84.02 (Ibrutinib)
0.66;16077
1.27e5
Area
%
5
0.00 0.50 1.00 1.50 2.00 2.50 3.00
IBR 161 09 18 25 Sm (SG, 40x5) MRM of 2 Channels ES+
442.17 > 155.02 (Vilazodone)
0.52;8050
5.47e4
Area
%
5
Time
0.00 0.50 1.00 1.50 2.00 2.50 3.00

Figure 2

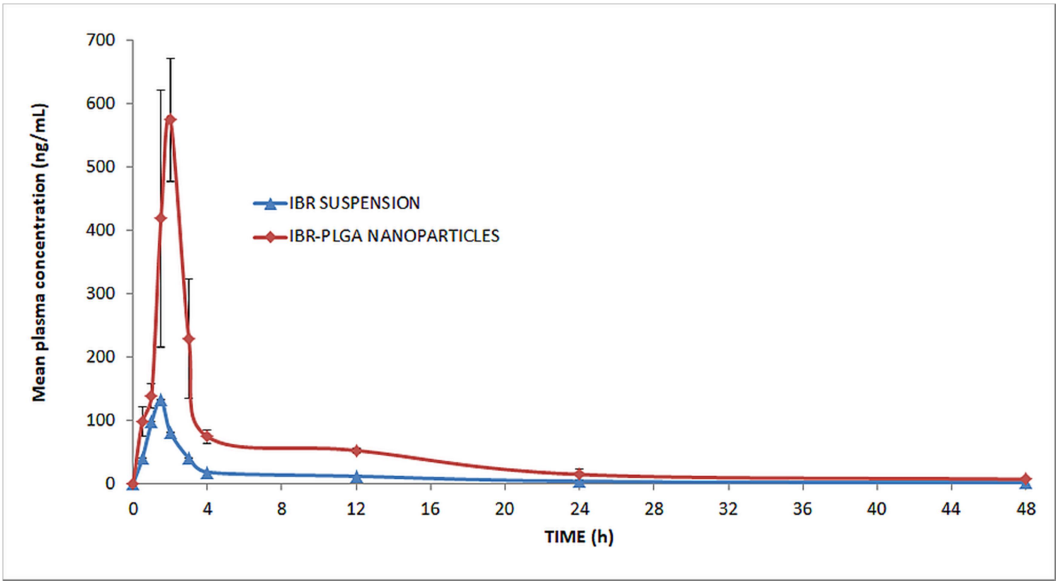
Mean plasma concentration (ng/mL)
700
600
500
400
300
200
100
0
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME (h)
IBR SUSPENSION
IBR-PLGA NANOPARTICLES

Figure 3