IJPSR (2018), Volume 9, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 January, 2018; received in revised form, 02 June, 2018; accepted, 13 July, 2018; published 01 October, 2018

DEVELOPMENT OF MESOPOROUS SILICA NANOPARTICLES OF RITONAVIR WITH ENHANCED BIOAVAILABILITY POTENTIAL: FORMULATION OPTIMIZATION, *IN-VITRO* AND *IN-VIVO* EVALUATION

Mohit Mahajan and Sadhana Rajput *

Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Kalabhavan Campus, Vadodara - 390001, Gujarat, India.

Keywords:

Ritonavir, MCM-41NPs, MCM-48NPs, Drug loading, Dissolution, Bioavailability

Correspondence to Author:
Prof. (Mrs) Sadhana J. Rajput

Faculty of Pharmacy,
The Maharaja Sayajirao University of Baroda, Kalabhavan Campus, Vadodara - 390001, Gujarat, India.

E-mail: sjrajput@gmail.com

ABSTRACT: The objective of the study was to develop mesoporous silica nanoparticles for the poorly water soluble drug ritonavir (RTV) for enhancement of *in-vitro* dissolution and corresponding *in-vivo* bioavailability. A comparative assessment between 2D-hexagonal silica nano-structured MCM -41NPs and 3D cubic pore system MCM -48NPs on drug release rate was also investigated. RTV (BCS class II drug), was loaded in the synthesized MCM-41NPs and MCM-48NPs by the solvent evaporation technique. The obtained MCM-41NPs, MCM-48NPs and RTV loaded mesoporous nanoparticles were characterized by different analytical techniques like UV spectrophotometry, differential scanning calorimetry, thermogravimetric analysis, FTIR, N_2 adsorption-desorption technique, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and powder-XRD. The *in-vitro* release profile of RTV was studied in 900 mL 0.1N hydrochloric acid (HCl) medium using USP apparatus-II at 50 rpm. Further; *In-vivo* studies were performed in Wistar rats where drug loaded mesoporous nanoparticles were compared with pure RTV. In dissolution study MCM-48NPs showed better and fast release of RTV than the MCM-41NPs. In pharmacokinetics study, the maximum peak plasma concentrations of RTV, R- MCM-41NPs and R- MCM-48NPs reached 3.8 ± 0.85 µg/ml, 5.5 ± 0.72 µg/ml and 9.2 ± 0.77 µg/ml by 1 h. The AUC_{0-t} values of the R- MCM-41NPs and R- MCM-48NPs were found 1.34-fold and 1.94-fold higher respectively, as compared with pure RTV. The results demonstrated superiority of MCM-48NPs against MCM-41NPs in enhancing dissolution and improving the bioavailability of RTV.

INTRODUCTION: Human Immunodeficiency Virus (HIV) is the major cause of Acquired Immune Deficiency Syndrome (AIDS) which was the leading cause of mortality globally [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(10).4127-37

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(10).4127-37

Antiretroviral therapy has controlled the mortality rate significantly HAART recognizes as the most effective treatment and protease inhibitor play an important role in this.

But most of the anti-HIV drugs are administered orally and very few are delivered through parenteral or topical route. Most of these drugs show poor solubility when administered orally and thus affecting their bioavailability. Because of low and variable oral bioavailability antiretroviral drugs require a high dose to produce the desired effect [2, 3].

Ritonavir (RTV), a HIV protease inhibitor comes under a BCS class II category drug, indicating that it has low solubility and high permeability. RTV is highly lipophilic as its log-D value is 4.3, at 25 ºC at pH 6.8. RTV is poorly water soluble and it shows an extremely slow dissolution rate 0.03mg/cm^2-min in 0.1 N HCl and pH-dependent solubility which could exhibit limited absorption. This may be the reason for the variation in solubility and bioavailability [4, 5].

RTV is reported to boost up the circulating concentration of other PI resulting in less dosing frequency though its higher dose could lead to higher toxicity effect in patients and because of their high dose and side effects [6, 7] there is a need for an innovative formulation approach to enhance the bioavailability.

It is widely accepted that formulation development helps BCS class II drugs to achieve much higher bioavailability by enhancing their solubility. As per literature, there are several techniques that have been utilized to increase the solubility, dissolution rate and bioavailability of RTV by using various approaches such as solid dispersion [8, 9], solid self-micro emulsifying drug delivery [10], solid self nano emulsifying drug delivery system [11], nanoparticles [12, 13], inclusion complex [14, 15] *etc*. From last decade, mesoporous silica nanoparticles are considered to be the best possible and unique approach for poorly water-soluble drugs to increase dissolution as well as the bioavailability.

Literature survey shows that a number of poor soluble drugs such as atrovastatin [16], celecoxibe [17, 18], itraconazole [19], carbamazepine [20] and dasatinib [21] could be successfully loaded into various types of mesoporous silica nanoparticles for various applications like solubility and bioavailability enhancement, controlled drug/gene release and targeted delivery carriers. Mesoporous silica nanoparticles have several attractive features like high surface area, high adsorption capacity of drug, ability to convert the crystallinity of the drug to an amorphous state, reduction of the particle size to nanometre range, the stability of loaded drugs within pores and pores can be easily modified as per drug delivery kinetics, that makes it useful for drug delivery. MSNs surface has free hydroxyl groups which easily interact with the particular functional group of drug molecules. Due to this feature, MSNs have opened new possibilities in drug delivery system [22, 23].

In the present study, different structural and pore size mesoporous silica nanoparticles have been used with the objective for improving the solubility and dissolution rate of RTV an antiretroviral drug, which ultimately affects its bioavailability. MCM -41NPs and MCM-48NPs (MSNs) belong to the M41S family of mesoporous material; MCM-41NPs is a 2D-hexagonal structure [24], whereas MCM -48NPs having a 3D cubic structure [25], both having high surface area and tunable pore size. Apart from this, no literature is available for RTV loaded in mesoporous silica nanoparticles till date.

The major aims of this study were: (i) synthesis and characterization of different mesoporous silica nanoparticles such as MCM-41NPs and MCM-48NPs (ii) loading of RTV in MSNs by solvent evaporation method (iii) preparation and evaluation of tablet formulation of R-MSNs as per IP (iv) performing *in-vitro* dissolution study of prepared formulation and (v) performing *in-vivo* pharma-cokinetic (PK) study of prepared formulation in Albino Wistar rats.

MATERIALS AND METHODS:

Materials: The active pharmaceutical agent RTV was received as gift sample from Hetero drug limited, Hyderabad, India. Polyoxyethylene 10 lauryl ether (PLE), Cetyltrimethylammonium bromide (CTAB; ≥ 98 %), tetraethyl ammonium hydroxide (TMAOH; ≥ 98 %), tetraethyl orthosillicate (TEOS; ≥ 99 %) and fumed silica were purchased from Sigma-Aldrich (USA). Hydrochloride acid (HCl) and methanol (HPLC and AR grade) were purchased from Rankem (India). All other solvents and material were of AR grade and were used without further purification. Deionized water was utilised in the synthesis of mesoporous silica nanoparticles.

Synthesis of Mesoporous Silica Nanoparticles (MSNs):

Synthesis of MCM-41NPs: MCM-41NPs was synthesized as per the procedure given in the literature [26]. Accurately weighed 4.42 g of CTAB was added in 36 g of deionized water and stirred for 15 min. Then 3.46 g TMAOH was added drop

wise in the surfactant solution of CTAB with constant stirring. The mixture was stirred for 30 min. Then 3 g of fumed silica was added slowly and stirred continued for 1.5 h.

The obtained gel was treated by hydrothermal crystallization technique for 48 h at 110 °C in a reactor. The solid product was recovered by filtration, washed with deionized water and was kept at room temp overnight. Further removal of surfactant was carried out by calcination of the product in a muffle furnace at 550 °C for 6 h. The recovered final product was named as MCM-41NPs.

Synthesis of MCM-48NPs: The MCM-48NPs synthesis was carried out as per the procedure given in literature [27]. Firstly, 0.4 g CTAB was added in 30 mL of methanol-water (1:2) solution and stirred the solution for 15 min. Then 5.4 mL ammonium hydroxide (NH_4OH) and 1.7 mL ethyl acetate were added in the surfactant solution and stirred the solution for 10 min. afterward, 0.9 mL TEOS was added drop wise into the solution with continuous stirring.

Further; 150 mL water was added into the solution and kept the solution overnight under stirring at room temperature. The pH of the solution was around 11.5. Then the resulting solid sample was filtered and washed with methanol and finally dried at 60 ºC in an oven. Then the final product was calcined in a muffle furnace for removing of surfactant at 550 °C for 6 h. Recovered material was labelled as MCM-48NPs.

Loading of RTV in Mesoporous Silica Nanoparticles (R-MSNs): A solvent evaporation method was used to load RTV in MCM -41NPs and MCM-48NPs (MSNs). MCM-41NPs was added in the methanolic solution of RTV (10 mg/ml); at drug to carrier ratio was 1:1.5 (w/w). Afterwards, the mixture was stirred for 2 h at room temperature for achieving maximum drug loading in the MCM-41NPs. Finally, the methanol was evaporated at 50 °C on a Bucchi rotary evaporator until completely dry. The recovered solid was dried at room temperature, which was named as R- MCM -41NPs. The same solvent evaporation method was used for loading RTV in the MCM-48NPs and after drug loaded it was designated as R-MCM-48NPs.

Physical Mixture of RTV and Mesoporous Silica Nanoparticles: The physical mixtures (PM) were prepared by mixing of pure RTV with MCM-41NPs and RTV with MCM-48NPs in a mortar and pastel with gentle triturate which were designated as R-MCM-41NPs-PM and R- MCM-48NPs-PM respectively.

Characterization of MSNs and RTV Loaded MSNs (R-MSNs):

Scanning Electron Microscopy (SEM): The morphology of synthesized MSNs was determined by SEM operated at an acceleration voltage of 15 kV. The MSNs samples were affixed to an aluminium stubs with double side adhesive carbon tape and gold coated with ion sputter MC1000. The samples were examined using a Hitachi-SU 1510 scanning electron microscope.

Transmission Electron Microscopy (TEM): The porous structure and particle size of MSNs was confirmed by TEM Analysis. A TEM image of MSNs was taken with a TECHNAI-G2 Spirit-Biotwin, operated at 120 kV. The MSNs samples were dispersed in deionized water in an ultrasonic bath for ten minutes. Several drops were deposited on 200 mesh, copper grid coated with a holey carbon film. The electron micrographs images were recorded on electron negative films and digital PC system attached to the TEM system.

UV-VIS Spectroscopy and Thermogravimetric Analysis for Evaluation of Drug Loading Efficiency: UV-VIS spectroscopy (UV-1700, Shimadzu) was used for determining the loading efficiency of synthesized MSNs at 240 nm wavelength. A small amount of RTV loaded MSNs were dispersed and mixed in a certain volume of methanol respectively; so that the RTV gets easily solubilised into the preferred solvent and subsequent filtration of sample was carried out. The amount of drug was calculated with the help of the standard calibration curve. The loading efficiency was confirmed by using thermogravimetric analysis (TGA) on Shimadzu thermogravimeter TGA-50. Around 3-10 mg sample of RTV and R-MSNs were kept into the platinum pan respectively, then heated up to 500 °C at a scanning rate of 10 °C/min under a nitrogen gas flow of 50 mL/min. The thermo-grams were analyzed using the TA-60 software.

The % entrapment efficiency for RTV and % loading efficiency for MSNs were calculated by using a formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Weight of RTV in nanoparticles}}{\text{Weight of RTV initially added}} \times 100$$

$$\% \text{ Loading Efficiency} = \frac{\text{Weight of RTV in nanoparticles}}{\text{Total Weight of sample}} \times 100$$

FT-IR Analysis: FT-IR spectra of RTV, MCM -41NPs, MCM-48NPs, R-MCM-41NPs- PM, R-MCM-48NPs-PM R- MCM-41NPs and R- MCM-48NPs were recorded on a BRUKER ALPHA-T (GERMANY) FT-IR Spectrophotometer at room temperature. The samples were gently mixed with KBr powder in mortar and pestle. Then pallets were prepared under 5000 psi pressure. The IR spectras of samples were taken in the spectral region 4,000 to 700 cm^{-1} using the resolution $1cm^{-1}$.

Differential Scanning Calorimetry (DSC): To study the physical state of RTV, MCM-41NPs, MCM-48NPs, R-MCM-41NPs-PM, R-MCM-48NPs-PM R- MCM-41NPs and R- MCM-48NPs were examined by DSC on a Shimadzu DSC-60. Around 3-5 mg of samples were put into an aluminium pan, crimped it with an aluminium lid to provide an adequate seal and heated under nitrogen purging (flow rate 40 mL/min) from room temperature to 300 °C. The temperature rise rate was fixed at 10 °C per min. The data were analyzed using TA 60-WS software.

Powder X-ray Diffraction (PXRD): The crystalline arrangements and the nature of pure RTV, MCM-41NPs, MCM-48NPs, R-MCM-41NPs and in R- MCM-48NPs were studied using a powder X-ray diffractometer (EMPYREAN, PAN alytical) using CuKa radiation beam operating at 40 kV and 30 mA. The samples (both MSNs and R-MSNs) were scanned at a low angle from 1 to 10 degrees in continuous mode at scanning speed 0.02 2θ/5s and also RTV and R-MSNs scanned from 5 to 50 degrees in continuous mode.

N_2 Adsorption-Desorption Analysis: N_2 adsorption and desorption analysis is a most reliable method to study the porosity of mesoporous material and was carried out to get information regarding BET surface area, pore size (nm) and pore volume of plain MSNs and R-MSNs by using micromeritics ASAP 2010. Earlier to characterization, plain MSNs samples were degassed under vacuum at 200 °C for 5 h, while the R-MSNs samples were degassed at 40 °C for overnight in order to avoid sublimation of RTV. The BET specific surface area was calculated by application of the BET method to the isotherm. The pore volume and pore diameter of both plain MSNs and R-MSNs were calculated by application of Barrett-Joyner-Halenda (BJH) method to the isotherm.

Formulation of Tablet and Evaluation: R- MCM -41NPs and R- MCM -48NPs were formulated in tablets by direct compression method. Nano-particles equivalent to 100 mg RTV and different excipients like low- hydroxypropyl cellulose (L-HPC), microcrystalline cellulose, cross-povidone, lactose monohydrate (SUPERTAB 11SD) and magnesium stearate were blended and punched in single punch tablet machine having 12 mm diameter punches with flat faced beveled edges. Prepared tablets were characterized by various parameters such as weight variation, hardness, friability and disintegration time *etc*.

***In-vitro* Dissolution Study:** *In-vitro* dissolution and release study was performed in dissolution apparatus (Veego dissolution test apparatus). Six dissolution units were studied for *in-vitro* dissolution of the RTV pure drug and R-MSNs. R-MCM-41NPs and R- MCM-48NPs equivalent to 100 mg tablets of RTV and pure drug RTV tablets were taken for the *in-vitro* dissolution study. *In-vitro* dissolution studies were conducted in the 0.1 N HCl media using USP dissolution apparatus II, 900 mL media volume at 37± 0.5 °C temperature and the rotation speed of 50 rpm.

At predetermined time intervals of 10, 20, 30, 45 and 60 min, five mL of dissolution sample was removed from the vessels with the help of cannula, replacing the same amount with fresh dissolution medium. Samples were filtered through 0.22 µm syringe filter and RTV content was determined by UV spectro-photometry (λ = 240 nm) method.

***In-vivo* Pharmacokinetic Study:** The *in-vivo* studies were carried out for comparing the plasma profile of the pure RTV, R- MCM -41NPs and R-MCM-48NPs. In order to establish that the improved bioavailability was achieved with a preparation of R-MSNs as compared with the pure RTV. In this study, either sex of Albino Wistar rats (250-300 g) were used having oral dose administration. The research protocol for the animal studies was approved by the Institutional Animal Ethics Committee (IAEC, file No. 1404), The Maharaja Sayajirao University of Baroda, Vadodara, India. All the experiments were performed in triplicates and all the results are given in the mean ± standard deviation (SD).

The bioavailability of R- MCM-41NPs and R-MCM-48NPs was compared to the pure RTV. The pure RTV and R-MSNs equivalent to 10 mg/kg dose of RTV were dispersed in 2 mL of CMC solution (0.5 % w/v) and administrated orally to Wistar rat. The rats were anesthetized by using ether before blood withdrawing. Blood samples (0.3 mL) were collected through the retro-orbital vein into 60 µL EDTA (0.5% w/v) containing micro centrifuge tubes at 0, 0.5, 1, 1.5, 2, 4, 6, 8 and 12 h after administration. Collected blood samples were mixed with the anticoagulant by properly shaking and centrifuged at 5000 rpm for 10 min at 4 ºC using a high-speed centrifuge machine and then plasma samples were collected and stored at -20 °C.

A simple protein precipitation method was used for extraction of RTV from collected plasma samples. Acetonitrile was used as a protein precipitating solvent. 100 µL of drug contain plasma samples were piped into a micro centrifuge tubes and 400 µL of acetonitrile was added into it and mixed onto vortex for 2 min. Further, the samples were centrifuged at 10000 rpm at 4-5 °C for 15 min. The supernatants of the centrifuged samples were transferred into a sample loading vials and which were injected into the HPLC system. In the PK study, parameters like C_{max}, t_{max}, $t_{1/2}$ and Area Under Curve (AUC) were calculated from plasma concentration *vs.* time profile curve and results were showed as mean ± SD.

Storage Stability Studies: Storage stability study were performed by following the European Agency for the Evaluation of Medicinal Products guidelines for solid dosage forms which prescribes to keep the samples at 40 ºC ± 2 and 75% ± 5 of relative humidity (RH) for 6 months [28]. R-MSNs was kept into a glass vial respectively and then thermo stated at 40 ºC ± 2 and 75% ± 5 RH. The samples were withdrawn at established time 1, 3 and 6 month and changes in R-MSNs were observed by DSC and P-XRD.

RESULTS AND DISCUSSION:

SEM and TEM Study: The morphology, pore structure and particle size of MSNs were confirmed by SEM and TEM analysis respectively.

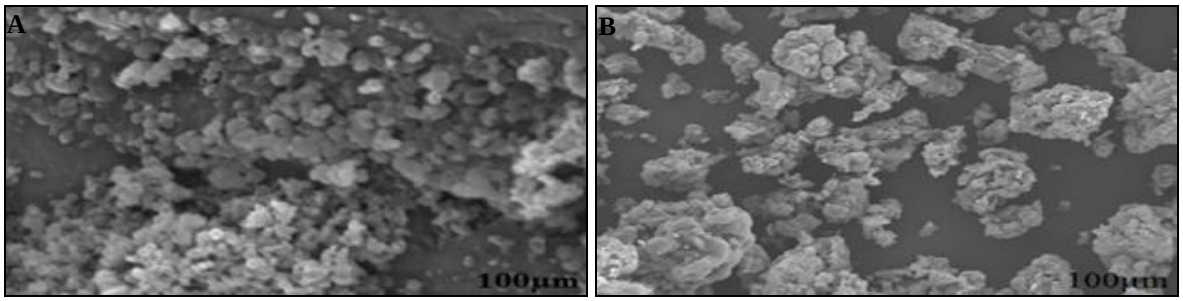

FIG. 1: SEM IMAGE OF (A) MCM-41NPs AND (B) MCM-48NPs

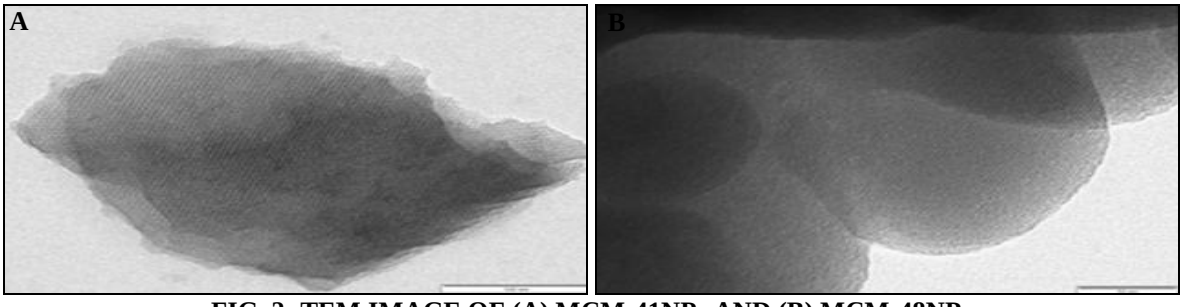

FIG. 2: TEM IMAGE OF (A) MCM-41NPs AND (B) MCM-48NPs

Fig. 1A and **B** showed the morphology of MCM-41NPs and MCM-48NPs were uniform in shape and having a smooth surface. **Fig. 2A** and **B** illustrated pore structure of MCM-41NPs and MCM-48NPs and it clearly showed regular 2D hexagonal honeycomb like structure arrangement and the cylindrical 3D cubic network formed by MCM-41NPs and MCM-48NPs respectively. Well structured MCM-41NPs and MCM-48NPs shows mean particles size 150 nm.

Analysis of Drug Loading Methods: After RTV loading in MSNs by solvent evaporation method, % loading efficiency and % entrapment efficiency were determined by UV spectrophotometry using above formulas (1 and 2). Alike, the thermal analysis was also carried out for the same samples for confirmation of % drug loading. TGA thermograms are shown in **Fig. 3**. Both analyses showed comparable results for % loading and % entrapment efficiency *i.e.* 88.5% and 38% for R-MCM-41NPs and 98.5% and 45% for R-MCM 48-NPs respectively.

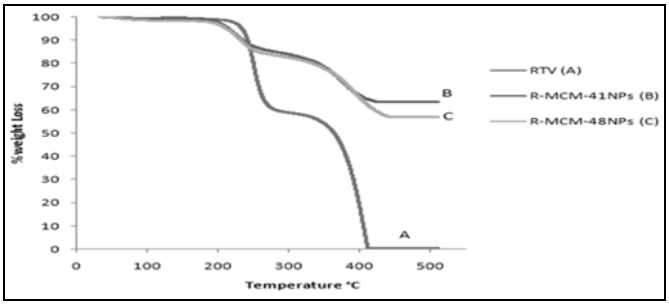

FIG. 3: TGA THERMOGRAM OF (A) RTV, (B) R-MCM-41NPs AND (C) R-MCM-48NPs

Fourier Transforms-Infrared Spectroscopy (FT-IR): For functional group identification and confirming the compatibility between RTV and silica nanoparticles, FT-IR study was carried out. FT-IR spectra of pure RTV, MCM-41NPs, MCM-48NPs, R- MCM-41NPs-PM, R-MCM-48NPs-PM, MCM-41NPs and R-MCM-41NPs are shown in **Fig. 4**. The RTV spectrum shows peaks at 3327 cm^{-1} relative to the N-H stretching of an amide group, 2962 cm^{-1} relative to hydrogen-bonded acid within the molecule, 1706 cm^{-1} relative to the ester group, 1660, 1611 and 1540 cm^{-1} relative to –C=C– stretching aromatic carbons.

The FT-IR spectrum of MCM-41NPs and MCM-48NPs **Fig. 4B** and **C** gave a broad peak between 3350-3500 cm^{-1} which proving the presence of isolated terminal silanol groups. The Si-O-Si and Si-OH stretching vibrations were shown at 1084 and 801 cm^{-1} respectively. In R-MCM-41NPs-PM spectrum showed characteristic peaks of RTV and MCM-41NPs which proves compatibility between both drug and silica nanoparticles. Similar results were obtained for R-MCM-48NPs-PM. **Fig. 4D** and **E**.

On the other hand, in case of R- MCM-41NPs and R- MCM-48NPs spectrum **Fig. 4F** and **G** showed a remarkable decrease of the peak at 2962 cm^{-1}, 1706 cm^{-1} and slight shifting of –C=C– stretching aromatic carbons with the disappearance of other peaks indicating that the complete uptake of the drug by MSNs. These changes suggested that the isolated terminal silanol group present in MSNs have some interactions with RTV functional groups.

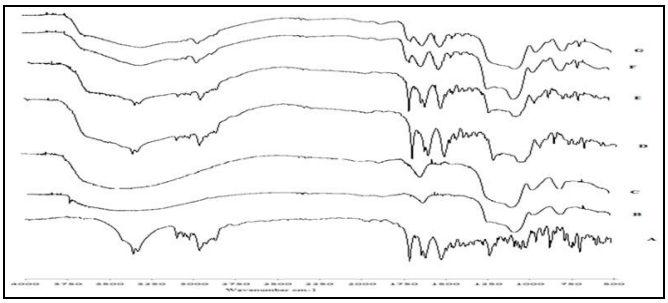

FIG. 4: FT-IR (A) RTV, (B) MCM-41NPs, (C) MCM-48NPs, (D) R-MCM-41NPs-PM, (E) R- MCM-48NPs-PM, (F) R-MCM-41NPs AND (G) R- MCM-48NPs

Differential Scanning Calorimetry (DSC): The DSC thermogram of crystalline pure RTV, MCM-41NPs, R-MCM-41NPs-PM, R-MCM-48NPs-PM, R-MCM-41NPs and R-MCM-48NPs are shown in **Fig. 5**.

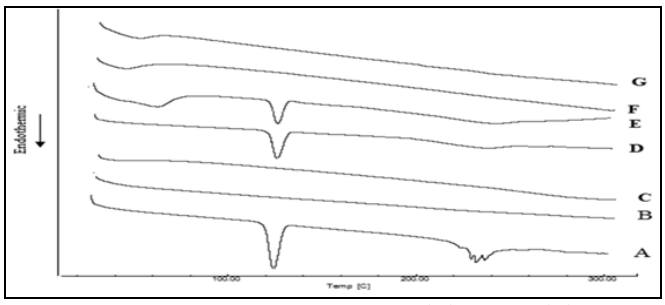

FIG. 5: DSC THERMOGRAM OF (A) RTV, (B) MCM-41NPs, (C) MCM-48NPs, (D) PHYSICAL MIXTURE OF RTV AND MCM-41NPs, (E) PHYSICAL MIXTURE OF RTV AND MCM-41NPs, (F) R- MCM -41NPs AND (G) R-MCM-48NPs

Crystalline RTV thermogram exhibited a sharp endothermic peak at 123 °C which corresponds to its fusion temperature point **Fig. 5 A**. MCM-41NPs and MCM-48NPs thermogram did not show any transition because the fusion point of silica is very high **Fig. 5B** and **C**. In both physical mixtures, the sharp endothermic peak of RTV was present indicating the compatibility between MSNs and pure RTV **Fig. 5D** and **E**. The R-MCM-41NPs and R-MCM-48NPs thermogram did not show any sharp endothermic peak of RTV, it suggested that no drug was present on the outer surface of nanoparticles **Fig. 5F** and **G** confirmed successful loading of RTV.

Powder XRD (PXRD): Low-angle powder XRD (LPXRD) patterns of MCM-41NPs and MCM-48NPs are shown in **Fig. 6**. LPXRD patterns of R-MCM-41NPs and R- MCM-48NPs are shown in **Fig. 6A (b)** and **6B (b)** respectively, in that the intensity of the nanoparticles peak was slightly decreased that confirmed the drug load in MSNs and no structural changes in the structure of nanoparticles. The high angle powder XRD pattern of plain RTV is shown in **Fig. 6C (a)** showed several characteristic peaks at region 5-40° in the 2θ/5s region, which confirmed the crystalline nature of the drug.

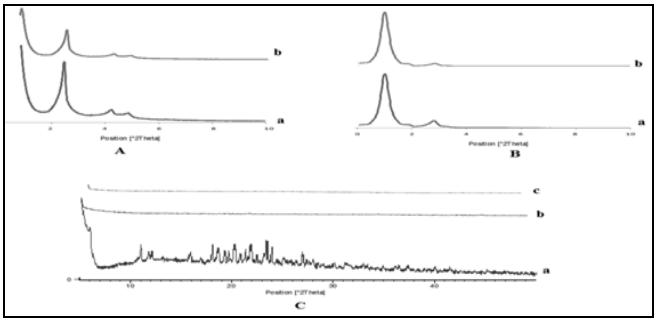

FIG. 6: LOW ANGLE POWDER-XRD (A) (a) MCM-41NPs, (b) R-MCM-41NPs; (B) (a) MCM-48NPs, (b) R-MCM-48NPs. HIGH ANGLE POWDER XRD (C) (a) RTV, (b) R-MCM-41NPs, (c) R-MCM-48NPs

Whereas in **Fig. 6C** (**b** and **c**) showed that the drug characteristic peaks were completely disappeared in R-MCM-41NPs and R-MCM-48NPs respectively. That confirmed the RTV was completely loaded and no crystalline drug remains on the outer surface of MSNs respectively. This also shows that MSNs can stabilize the amorphous state due to confinement.

N_2 Adsorption Analysis: N_2-adsorption-desorption isotherms relative to MCM-41NPs, MCM-48NPs and R-MSNs are shown in **Fig. 7**. The N_2 adsorption-desorption isotherms gave data related to specific surface area, pore volume and pore size of nanoparticles.

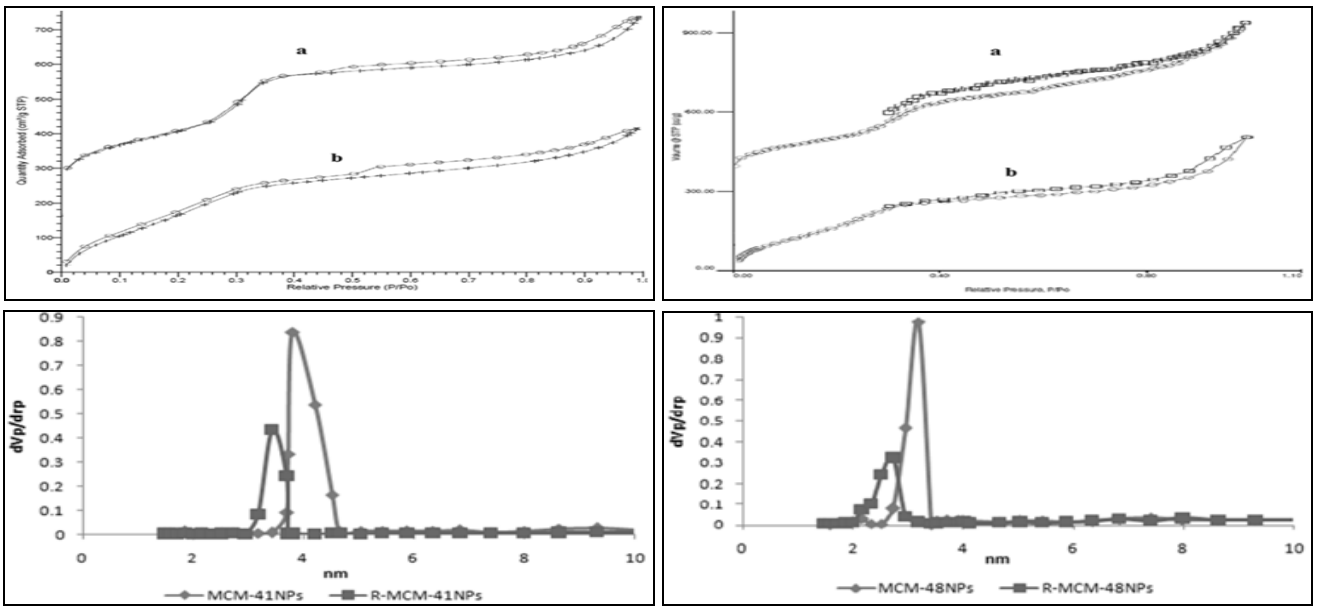

FIG. 7: BET ISOTHERM OF (A) (a) MCM-41NPs, (b) R-MCM-41NPs (B) (a) MCM-48-NPs, (b) R-MCM-48NPs. PORE SIZE DISTRIBUTION OF (C) MCM-41NPs AND R- MCM-41NPs, (D) MCM-48-NPs AND R-MCM-48NPs

All the N_2 adsorption-desorption isotherms showed typical type IV isotherms and hysteresis loop (according to IUPAC) which confirmed that the nanoparticles have mesoporous property. After the drug loading, in R-MCM-41NPs, the type IV pattern and hysteresis loop of isotherm remain

intact, reduction in surface area, pore volume and pore size as compared with MCM-41NPs **Fig. 7A** and **C**. The similar results were obtained for MCM-48NPs and R-MCM-48NPs **Fig. 7B** and **D**. However, MCM-48NPs having the higher surface area, cubic structure and smaller pore size compared to MCM-41NPs which could possibly have a significant impact on *in-vitro* and *in-vivo* profile of RTV. N_2- adsorption- desorption parameters of MCM-41NPs, MCM-48NPs and R-MCM-41NPs, MCM-48NPs are given in **Table 1**.

TABLE 1: EVALUATION PARAMETER OF R- MCM-41NPs AND R- MCM-48NPs BY N_2 ADSORPTION-DESORPTION

Name of compound	BET surface area	Pore volume	Pore diameter
MCM-41NPs	935.76 m²/g	0.82cm³/g	3.9nm
MCM-48NPs	1220.29 m²/g	0.96cm³/g	3.2nm
R- MCM -41NPs	380.15 m²/g	0.46cm3/g	3.3nm
R- MCM -48NPs	440.60 m²/g	0.31cm3/g	2.7nm

Evaluation of RTV Tablets: Evaluations of formulated RTV tablets were carried out by using several parameters like weight variation, hardness, friability, disintegration time and drug content (%). The results of all parameters are shown in **Table 2**. The Hardness for both prepared tablet formulations were in ranging from 6.8 to 7.8 kP, indicating that the hardness of tablet was good enough to withstand the external pressure. Tablets were prepared using standard excipients exhibited disintegration time of 1 ± 0.3 min; friability was less than 1% and % drug content values were obtained in the range of 98.12 - 101.56%.

TABLE 2: EVALUATION OF PREPARE R-MCM-41NPs AND R- MCM-48NPs TABLETS

Parameters	R- MCM-41NPs	R- MCM -48NPs
Hardness kP	6.8-7.8 kP	6.9-7.5 kP
Friability (%)	< 1 %	< 1 %
Disintegration time (min)	1 ± 0.3 min	1 ± 0.2 min
Weight variation (mg)	505.35 ± 5.88	503.62 ± 6.88
Drug content (%)	98.55-100.56%	98.12-101.56%

***In-vitro* Dissolution Study:** Drug release studies were performed to see the release pattern of pure RTV and drug loaded nanoparticles in 0.1 N HCl. The dissolution rate was significantly enhanced in the R-MCM-41NPs and R-MCM-48NPs as compared to pure RTV. The augment in dissolution rate in silica nanoparticles was observed due to the conversion of RTV into amorphous form after loading into mesoporous nanoparticles.

The RTV release profiles are illustrated in **Fig. 8**. R-MCM-48NP showed more than 95% drug release in dissolution media within 45 min, whereas pure RTV and R-MCM-41NP showed almost 39% and 72% drug release respectively in 0.1 N HCl. The reason for MCM-48NPs showing better dissolution profile than MCM-41NPs is might be due to the high surface area, small pore size and 3D-cubic pore structure of MCM-48NPs which adsorbed the drug molecule very efficiently.

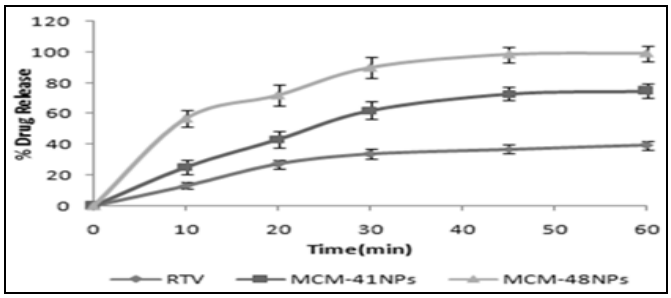

FIG. 8: *IN-VITRO* DISSOLUTION STUDY IN 0.1 N HCl

The RTV molecules adsorbed in the high surface of the 3D interconnected MCM-48NPs gave faster dissolution and more rapid diffusion in the dissolution medium while, MCM-41NP has 2D independent long channels, appeared to prevent the drug molecules in the pore channels from diffusing into the dissolution medium resulting slow drug release.

***In-vivo* Study:** RTV is a typical BCS II drug; whose absorption will be rate limited through the dissolution process. In the present study, the results of *in-vitro* dissolution studies were confirming the enhanced dissolution of RTV by R- MCM-48NPs and R-MCM-41NPs. To study the silica nano-particles effect, the *in-vivo* studies were performed in which the drug suspension was given orally to Wistar rat.

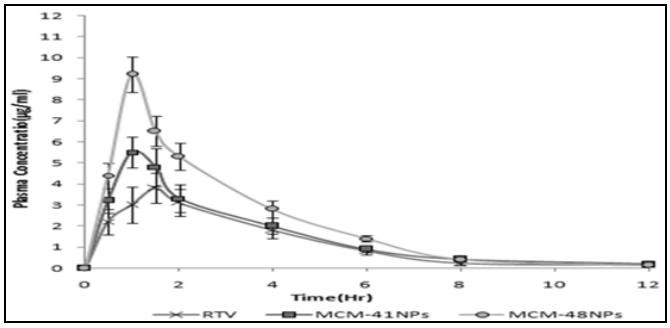

FIG. 9: PLASMA CONCENTRATION–TIME PROFILES OF RTV AFTER ORAL ADMINISTRATION AT A DOSE OF 10 mg/kg IN MALE RATS (n=3)

The results of plasma concentration-time profiles and the PK parameters of RTV are shown in **Fig. 9** and **Table 3**, respectively. In **Fig. 9**, it is clearly shown that the absorption rate of R-MCM-48NPs was higher than R-MCM-41NP and pure RTV; that exhibited maximum plasma concentration was 9.21µg/mL by 1 h. Additionally, compared with the pure RTV the C_{max} and AUC_{0-t} values of the R-MCM-48NPs were increased 2.48-fold and 1.94-fold respectively.

TABLE 3: PHARMACOKINETIC PARAMETERS OF PURE DRUG AND PREPARED FORMULATION

Parameter	Pure RTV	MCM-41NPs	MCM-48NPs
C_{max}	3.83 ± 0.35 µg/mL	5.54 ± 0.72 µg/mL	9.21 ± 0.77 µg/mL
T_{max}	1.5 h	1 h	1 h
AUC_{0-t}	13.64 ± 1.14 µg/mL*h	18.29 ± 1.77 µg/mL*h	26.55 ± 1.84µg/mL*h
$T_{1/2}$	2.18 h	2.16 h	1.80 h

However, regarding the R-MCM-41NPs, the C_{max} and the AUC_{0-t} values were increased 1.44-fold and 1.34-fold respectively. The PK profile clearly showed improvement in the bioavailability of R-MSNs as compared to pure drug. Maximum RTV concentration in plasma was achieved by the R-MCM - 48NPs formulation which was approximately 1.67 - fold more than the R-MCM-41NPs formulation.

Stability Study: Improvement in the dissolution rates were occurred due to drug adsorption on silica nanoparticles which having high surface area and very small pore size that converted drug in to amorphous form. Therefore, it is mandatory to conduct physical stability study for adsorbed drug. The effect of humidity and temperature was observed. The 40 ºC ± 2 and 75% ± 5 relative humidity for six months accelerated stability test samples were analyzed by DSC and PXRD. PXRD of samples that stored at 40 ºC ± 2 and 75% ± 5 relative humidity for six months showed in **Fig. 10 C** and **D**, absence of characteristic crystalline peaks of RTV for the R-MSNs samples. The result shows the mesoporous material upkeep the RTV in an amorphous state. These results were again confirmed by DSC as well; where RTV fusion point could not be detected in **Fig. 10A** and **B**. The results showed that the silica nanoparticles can hold the drug in amorphous form for a longer duration of time.

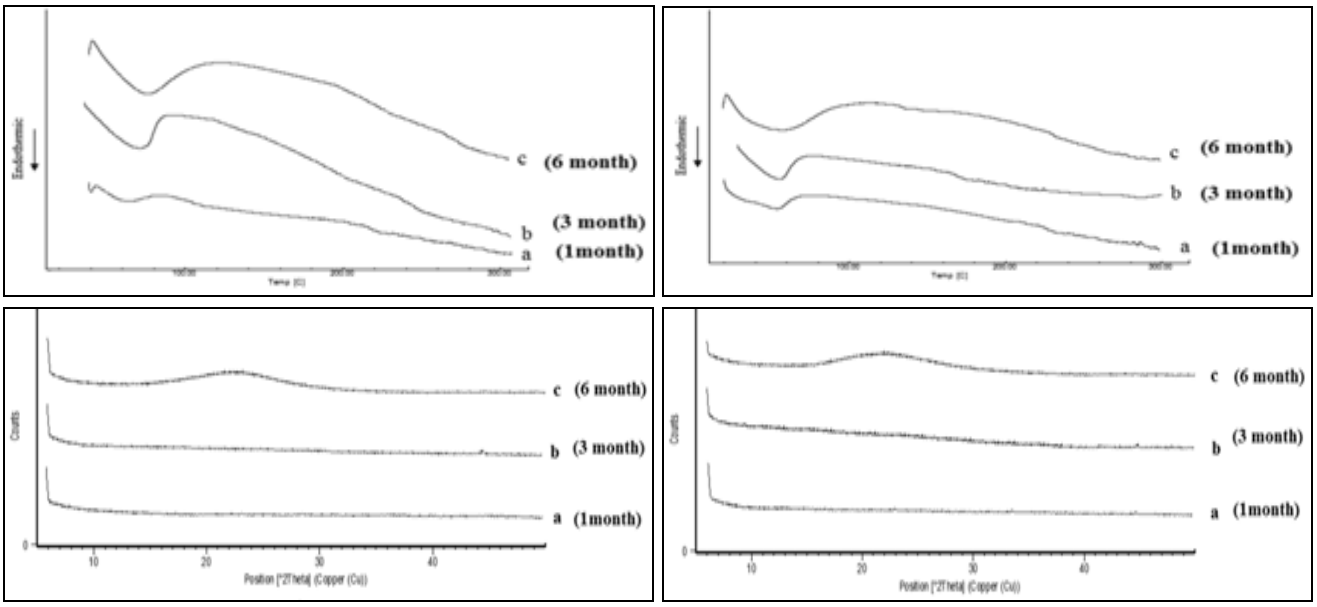

FIG. 10: DSC AND P-XRD OF STABILITY SAMPLES (A) AND (C) R-MCM-41NPs; (B) AND (D) R-MCM-48NPs RESPECTIVELY

CONCLUSION: In this study, the synthesized mesoporous silica nanoparticles MCM-41NPs and MCM-48NPs were suitable carriers for poorly soluble RTV drug. RTV was loaded in both the silica nanoparticles to examine the effect on solubility through the drug loading. To achieve maximum drug loading, solvent evaporation method was preferred with an appropriate ratio of drug and carrier (1:1.5). In addition, characterization results like DSC, PXRD and N_2 adsorption-desorption confirmed that the RTV was successfully loaded into the mesoporous

silica nanoparticles. In *in-vitro* drug dissolution study, both MCM-41NPs and MCM-48NPs showed several advantages as a carrier for drug delivery respectively. Both MCM-41NPs and MCM-48NPs could notably increase the dissolution rate of RTV as compared to the pure RTV, but R- MCM-48NPs showed better in the fast release.

The PK studies results affirmed the ability of the mesoporous silica nanoparticles, especially the MCM-48NPs enhancing the *in-vitro* dissolution rate and improve the bioavailability. The reason behind the above results is that MCM-48NPs having 3D cubic pores structure which offers easy drug diffusion from the interconnected pores into the dissolution media. From all the above facts revealed that MCM-48NPs contribute faster drug release as compared to MCM-41NPs with 2D hexagonal long channels. Thus, MCM-48NPs shows more propitious mesoporous carrier giving fast and maximum release compare with MCM-41NPs.

ACKNOWLEDGEMENT: Mr. Mohit Mahajan is highly thankful to the University Grants Commission, New Delhi, Government of India, for availing Senior Research Fellowship. The authors are thankful to the Hetero Pharmaceuticals Pvt., Ltd., Hyderabad, India for providing RTV gift sample.

CONFLICT OF INTEREST: There are no conflicts of interest.

REFERENCES:

1. Fajardo-Ortiz D, Lopez-Cervantes M, Duran L, Dumontier M, Lara M, Ochoa H and Castano VM: The emergence and evolution of the research fronts in HIV/AIDS research. PLoS One 2017; 12(5): 1-13.
2. Ramana KV: Are we close enough to get rid of Aids: Insights in to impact of Human Immunodeficiency Virus (HIV) Infection Post Highly Active Antiretroviral Therapy (HAART) Era. International Journal of Molecular Medical Science 2013; 3: 25-29.
3. Ramana KV: Effect of Highly Active Antiretroviral Therapy (HAART) on Human Immunodeficiency Virus Disease Pathogenesis and Progression. American Journal of Public Health Research 2014; 2: 68-74.
4. Law D, Schmitt EA, Marsh KC, Everitt EA, Wang W, Fort JJ, Krill SL and Qiu Y: Ritonavir–Peg 8000 amorphous solid dispersions: *In-vitro* and *in-vivo* evaluations. Journal of Pharmaceutical Sciences 2004; 93: 563-570.
5. Law D, Krill SL, Schmitt EA, Fort JJ, Qiu Y, Wang W and Porter WR: Physicochemical considerations in the preparation of amorphous Ritonavir ± Poly (Ethylene Glycol) 8000 solid dispersions. Journal of Pharmaceutical Sciences 2001; 90: 1015-1025.
6. Guiard-Schmid JB, Poirier JM, Luc Meynard J, Bonnard P, Gbadoe AH, Amiel C, Calligaris F, Abraham B, Pialoux G, Girard PM, Jaillon P and Rozenbaum W: High variability of plasma drug concentrations in dual protease inhibitor regimens. Antimicrobial Agents and Chemotherapy 2003; 47: 986-990.
7. Shelton MJ, Wire MB, Lou Y, Adamkiewicz B and Min SS: Pharmacokinetic and safety evaluation of high-dose combinations of fosamprenavir and ritonavir. Anti-microbial Agents and Chemotherapy 2006; 50: 928-934.
8. Dashamukhi. R, Kanagala V and Chittimalla AK: Formulation development of ritonavir tablets containing solid dispersions employing montmorillonite: Dissolution rate enhancement. Asian Journal of Pharmaceutical and Clinical Research 2013; 6: 206-208.
9. Sinha S, Ali M, Baboota S, Ahuja A, Kumar A and Ali J: Solid dispersion as an approach for bioavailability enhancement of poorly water-soluble drug ritonavir. AAPS Pharm Sci Tech 2010; 11: 518-527.
10. Deshmukh A and Kulkarni SK: Solid self-micro-emulsifying drug delivery system of ritonavir. Drug Development and Industrial Pharmacy 2014; 40: 477-487.
11. Reddy S, Rudra R and Haq FMD: Formulation and Evaluation of Solid Self Nano Emulsifying Drug Delivery System (S-SNEDDs) of ritonavir drug. Indo American Journal of Pharmaceutical Research 2015; 5: 2010-2024.
12. Gawali PB, Kshirsagar SJ, Bhalekar MR and Madgulkar AR: Preparation and characterization of amorphous nanoparticles for solubility enhancement of ritonavir. International Journal of Pharmaceutical Science Invention 2012; 2: 27-35.
13. Guo S, Pham K, Li D, Penzak SR and Dong X: Novel in situ self-assembly nanoparticles for formulating a poorly water-soluble drug in oral solid granules, improving stability, palatability, and bioavailability. International Journal of Nanomedicine 2016; 11: 1451-1460.
14. Shankar KR, Chowdary KPR and Rao AS: A factorial study of formulation of ritonavir tablets employing B cyclodextrin, soluplus and pvp K30. World J. of Pharmacy and Pharmaceutical Sciences 2015; 4: 1191-1200.
15. Venkata DR and Chowdhary KPR: Formulation development of ritonavir tablets employing B cyclo-dextrin, solutol HS-15 and PVP-K30. Int. Journal of Pharm. Res. and Dev. 2012; 4: 45-50.
16. Maleki A and Hamidi M: Dissolution enhancement of a model poorly water-soluble drug, atorvastatin, with ordered mesoporous silica: comparison of MSF with SBA-15 as drug carriers. Expert Opinion on Drug Delivery 2016; 13: 171-181.
17. Günadin S and Yilmaz A: Improvement of solubility of celecoxib by inclusion in MCM-41 mesoporous silica: drug loading and release. Turkish Journal of Chemistry 2015; 39: 317-333.
18. Seda Eren Z, Tunçer S, Gezer G, Yildirim LT, Banerjee S and Yilmaz A: Improved solubility of celecoxib by inclusion in SBA-15 mesoporous silica: drug loading in different solvents and release. Microporous and Mesoporous Materials 2016; 235: 211-223.
19. Liu X and Che S: Enhanced release of the poorly soluble drug itraconazole loaded in ordered mesoporous silica. Science China Chemistry 2015; 58: 400-410
20. Ambrogi V, Marmottini F and Pagano C: Amorphous carbamazepine stabilization by the mesoporous silicate SBA-15. Microporous and Mesoporous Materials 2013; 177: 1-7.

21. Kjellman T, Xia X, Alfredsson V and Garcia-Bennett AE: Influence of microporosity in SBA-15 on the release properties of anticancer drug dasatinib. Journal of Materials Chemistry B 2014; 2: 5265-5271.
22. Zhao L, Qin H, Wu R and Zou H: Recent advances of mesoporous materials in sample preparation. Journal of Chromatography-A 2012; 1228: 193-204.
23. Vallet-Regí M, Colilla M, Izquierdo-Barba I and Manzano M: Mesoporous silica nanoparticles for drug delivery: Current insights. Molecules 2018; 23:1-19.
24. Lelong G, Bhattacharyya S, Kline S, Cacciaguerra T, Gonzalez MA and Saboungi ML: Effect of surfactant concentration on the morphology and texture of MCM-41 materials. Journal of Physical Chemistry C 2008; 112: 10674-10680.
25. Wang Y, Sun L, Jiang T, Zhang J, Zhang C, Sun C, Deng Y, Sun J and Wang S: The investigation of MCM-48-type and MCM-41-type mesoporous silica as oral solid dispersion carriers for water insoluble cilostazol. Drug Development and Industrial Pharmacy 2014; 40: 819-828.
26. Wouters BH, Chen T, Dewilde M and Grobet PJ: Reactivity of the surface hydroxyl groups of MCM-41 towards silylation with trimethylchlorosilane. Microporous and Mesoporous Materials 2001; 44: 453-457.
27. Schumacher K, Grun M and Unger K: Novel synthesis of spherical MCM-48. Microporous and Mesoporous Materials 1999; 27: 201-206.
28. Guideline on stability testing: stability testing of existing active substances and related finished products. European Medicines Agency; CPMP/QWP/122/02, rev 1; London: 2003.

How to cite this article:
Mahajan M and Rajput S: Development of mesoporous silica nanoparticles of ritonavir with enhanced bioavailability potential: formulation optimization, *in-vitro* and *in-vivo* evaluation. Int J Pharm Sci & Res 2018; 9(10): 4127-37. doi: 10.13040/IJPSR.0975-8232.9(10).4127-37.