Research Article

Development of non-covalent derivative of sulfasalazine with theobromine for its properties optimization

Madhu Bala, Manoj K. Gautam, Renu Chadha

Department of Chemistry, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

Correspondence:
Prof. Renu Chadha,
University Institute of Pharmaceutical Sciences, Panjab University,
Chandigarh - 160 014,
India. Phone: +91-9316015096.
E-mail: renukchadha@rediffmail.com

How to cite this article:
Bala M, Gautam MK, Chadha R. Development of non-covalent derivative of sulfasalazine with theobromine for its properties optimization. Innov Pharm Pharmacother 2017;5(3):154-158.

Source of Support: Nil,
Conflict of Interest: None declared.

ABSTRACT

To customize the biopharmaceutical parameters of a poorly soluble, disease modifying anti-rheumatic drug, sulfasalazine (SSZ) by preparing its novel non-covalent derivative (NCD). For this theobromine (TBR) was used as coformer. The novel solid form prepared by liquid assisted grinding of SSZ with TBR using catalytic amount of solvent and termed as SSZ-TBR. The ground product (SSZ-TBR) was characterized with various techniques such as differential scanning calorimetry, powder X-ray diffraction (PXRD), and spectrometric methods involving Fourier transform infrared and solid state nuclear magnetic resonance. The structure of the SSZ-TBR was determined from its PXRD pattern using Material Studio® by BIOVIA. The crystal structure also helped to understand the nature of complex and type of interactions involved in its formation and stabilization. The SSZ-TBR was further evaluated for *in vitro* solubility and intrinsic dissolution rate (IDR) profile. This NCD was characterized to be cocrystal of SSZ with TBR in 1:1 stoichiometry. Its crystal packing as well as nature of interactions was established by crystal structure determination. Further, the fulfillment of objective was witnessed byE increased solubility as well as IDR in comparison to the pure SSZ. The evaluation of this novel cocrystal of SSZ a poorly soluble drug with TBR exhibit potential to enhance the option of material phase in comparison to pure active pharmaceutical ingredient without disturbing its chemical structure.

Keywords: Characterization, cocrystal, intrinsic dissolution rate, solubility, sulfasalazine

Introduction

Recent decades have evidenced a tremendous growth of interest by various research groups in the design as well as synthesis of multicomponent crystals particularly pharmaceutical cocrystals.[1] Cocrystals are multicomponent single phase crystalline complexes in which two or more neutral molecular constituents (active pharmaceutical ingredient [API]-1 and coformer, and/or solvent of crystalliztion) in the crystal lattice bound together through non-covalent interactions, primarily hydrogen bonding.[2] To modulate physical as well as mechanochemical properties of drugs, namely, stability, hygroscopisity, solubility, dissolution rate, and compressibility while maintaining their pharmacological behavior, pharmaceutical cocrystallization is one of the most reliable and reproducible method.[1,3] During cocrystallization in the crystal lattice of cocrystal consists of API, coformer, and/or solvent of crystallization while maintaining the pharmacological activity of API.[4-6] The coformer can be either drug or inert excipient of generally recognized as safe status which comprises food additives, pharmaceutical excipients, and preservatives.[7,8] Pharmaceutical cocrystals offers an alternative materials to pure drug substance and has the advantage over established drug modifications such as polymorphic and amorphous form, salt, solvate, and conventional inclusion complexes, each of which have associated limitations in their application.[9,10] In addition, for neutral drugs, lacking ionisable group, cocrystals proved a viable method for optimizing the properties of such molecules. Moreover, the availability of cocrystal ligands is far than suitable counterions for developing salts.[11]

Sulfasalazine (SSZ) is a poorly water soluble drug with low aqueous solubility used to treat rheumatoid arthritis.[12] Pharmaceutical cocrystal of SSA was prepared with theobromine (TBR) with an objective to modulate its physicochemical properties, especially solubility and intrinsic dissolution rate (IDR). Both SSZ and TBR contain functional groups that are donor and acceptor of hydrogen bond, which is a pre-requisite for cocrystals formation. The prepared cocrystal was characterized thoroughly using various analytical techniques and further evaluated for solubility and IDR in simulated gastric and intestinal fluid.

Access this article online	
Website: www.innpharmacotherapy.com	**e-ISSN:** 2321-323X **p-ISSN:** 2395-0781

Materials and Methods

Materials

Sulfasalazine (USP) was purchased from Sigma Aldrich. Methanol, ethanol, sodium dihydrogen orthophosphate dihydrate, disodium hydrogen orthophosphate dihydrate (AR grade), and orthophosphoric acid were obtained from different commercial suppliers.

Preparation of SSZ-TBR cocrystal

SSZ-TBR was prepared by grinding SSZ (390.0 mg) with TBR (180.12 mg) using solvent (methanol) drop grinding method in a pestle and mortar for 120 min. The solid powder was then scratched from walls of mortar and stored in vial. The solid obtained was then characterized using various analytical techniques.

Characterization of SSZ-TBR

Differential scanning calorimetry (DSC)

DSC thermograms of SSZ, TBR, and SSZ-TBR were obtained on DSC, Q20, and TA instruments - Waters and LLC USA. The instrument was calibrated for temperature and heat flow accuracy using the melting of pure indium (mp 156.6°C and ΔH of 25.45 J/g). The temperature range was from 25°C to 400°C with a heating rate of 10°C per min.

X-ray powder diffraction

Powder diffraction patterns of SSZ, TBR, and SSZ-TBR were recorded on an X-ray diffractometer (XPERT-PRO, Netherlands, Holland) with Cu as tube anode the diffractograms were recorded under following conditions: Voltage 35 kV, 20 mA, angular range 5, divergence slit 10, receiving slit 0.15 mm.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of SSZ, TBR, and SSZ-TBR were obtained on spectrum RX I, FTIR spectrometer (Perkin Elmer, UK) over the range 400-4000/cm. Dry KBr (50 mg) was finely ground in mortar and samples (1-2 mg) were subsequently added and gently mixed to avoid trituration of the crystals. A manual press was used to form the pellets.

Solid state nuclear magnetic resonance (SSNMR)

The solid state ^{13}C NMR spectra of SSZ, TBR, and SSZ-TBR were acquired using a Joel Resonance JNM-ECX400II Instrument from IISC, Bengaluru, India.

Crystal structure determination

Powder X-ray diffraction (PXRD) patterns of SSZ-TBR were subjected to Material Studio® software by BIOVIA system to determine its structure. Four steps are involved in the overall structure determination: (1) indexing, (2) pawley fitting, (3) structure solution, and (4) rietveld refinement. The indexing utilizing X-cell module is done to obtain appropriate crystal lattice from the peak positions in the observed powder diffraction pattern. Further, to prepare suitable cell, the unit cell with maximum figure of merits was selected. The optimization of prepared unit cell was done through Pawley refinement. Moreover, the space groups search was done to produce an appropriate cell. The structures of SSZ and TBR were sketched and optimized geometrically in DMOL3 module. Into the unit cell generated by powder indexing, these optimized structures were imported and with full-profile comparison method in Powder Solve module with 10 simulated annealing cycles and 2100000 iterations in each cycle, the atomic arrangement in the asymmetric unit was determined. Rietveld refinement gave the final R_{wp} value (similarity between calculated and the experimental diffraction patterns).

Equilibrium solubility study

Equilibrium solubility studies of SSZ and SSZ-TBR were performed by introducing an excess amount of sample in both simulated gastric and intestinal fluid which was shaken in water bath shaker (MSW-275 Macro scientific Works, New Delhi) at 37°C. The aliquots were withdrawn from the slurry after 24 h. These were filtered through a 0.45 µm membrane filter paper (millipore), diluted suitably, and concentration determined by measuring drug content by high-performance liquid chromatography (HPLC).

IDR

The IDR was performed with a rotating disk dissolution test apparatus (DS 8000, Laboratory India Analyticals) in simulated gastric fluid, and simulated intestinal fluid at 37°C and 100 rpm for 2 h, respectively. A pellet of the sample was formed using a die and punch, compressed with a tablet press and attached to a dissolution apparatus holder and immersed in dissolution medium. 10 mL of media with replacement was withdrawn at different intervals of time and after filteration through a 0.45 µm nylon filter were assayed for drug content by HPLC.

Results and Discussion

As discussed in the experimental section that SSZ-TBR was prepared by solid-state grinding method. Now to establish the identity of the ground product whether it is salt, cocrystal or eutectic, SSZ-TBR subjected to DSC, FTIR, and PXRD analysis. It is very well established that distinct thermal as well as spectroscopic behavior and PXRD patterns are observed for salts and cocrystals while the latter shows only a lowering in the melting endotherm in comparison to the parent materials as identification characteristic. In Figure 1, the molecular structures of SSZ and TBR are depicted.

Thermal analysis

The DSC is a fundamental technique to determine any solid state alterations. The DSC thermogram of SSZ and TBR showed single

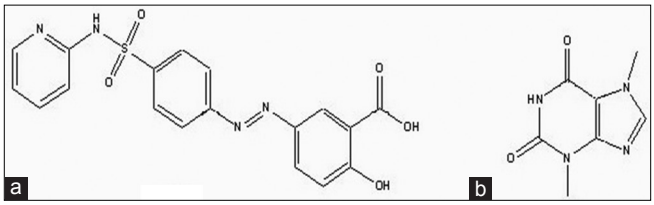

Figure 1: Molecular structures of (a) sulfasalazine, (b) theobromine

melting endotherm at 261.64°C and 356.89°C, respectively (Figure 2). Whereas in the DSC scan of their physical mixture, two endotherms were observed, one at 255.98°C followed by another at 313.56°C depicting that melting endotherms of both SSZ and TBR shifted to lower temperatures. The lowering in melting temperatures of individual components might be due to functioning of one component to other as impurity. However, the DSC scan for SSZ-TBR shows sharp single peak at 293.17°C. The appearance of this peak at different temperature value from that of individual starting components and their physical mixture revealed the formation of new crystalline solid single phase. The single endothermic event signifies the absence of any absorbed or unbound molecules of solvent. Along with indicates the stability of newly formed solid phase until its melting.

PXRD analysis

PXRD is kind of a fingerprint characterization method for solid phases such as cocrystals and salts since a different pattern of products implies the formation of a new phase. The characteristic reflections at 2Θ value of 13.5°, 19.9°, and 27.9° in PXRD pattern of SSZ-TBR (Figure 3c) are absent in SSZ (Figure 3a) and TBR (Figure 3b). Therefore, on the basis of appearance of novel PXRD pattern for SSZ-TBR in comparison to their individual parent compounds confirms the existence of new solid crystalline phase.

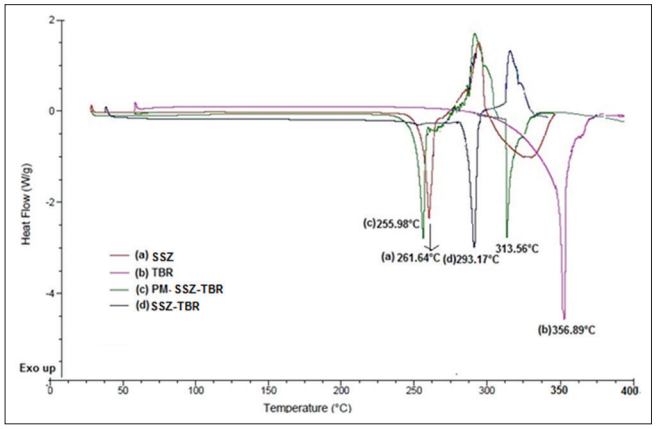

Figure 2: Differential scanning calorimetry thermogram of (a) sulfasalazine (SSZ), (b) theobromine (TBR), (c) physical mixture of SSZ and TBR, (d) SSZ-TBR

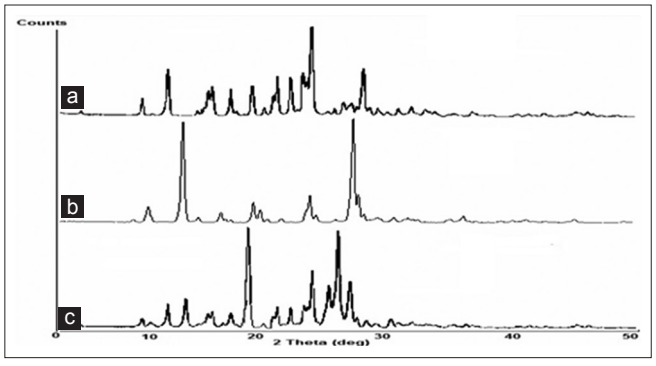

Figure 3: Powder X-ray diffraction pattern of (a) sulfasalazine (SSZ), (b) theobromine (TBR), (c) SSZ-TBR

FTIR

In FTIR spectra, the change in the intensity and location of characteristic peaks due to alteration in physical state of the solid occurs which conclude the formation of new crystalline forms. After cocrystallization of SSZ with TBR, the FTIR spectrum (Figure 4) of SSZ-TBR showed the shifts in the hydroxyl peak of SSZ from 3132.26/cm to 3141.02/cm. Whereas that of TBR shifted from 3112.51/cm and 1547.97/cm to 3103.09/cm and 1553.14/cm corresponding to –NH and $C=N_{aromatic}$ stretching, respectively.

SSNMR

^{13}C SSNMR signals of SSZ, TBR, and SSZ-TBR in Figure 5 are assigned according to the chemical shifts. The small changes in SSZ-TBR ^{13}C chemical shifts compared to the spectra of individual starting materials are ascribed to the change in the chemical environments associated with the generation of new solid phase. In the SSZ-TBR spectra, the signal from C25 is found to be at 168.9 ppm which has shifted from 170.5 ppm in SSZ. Whereas chemical shifts from 150.1 to 148.9 ppm and 155.1 to 154.0 ppm for carbons C2 and C6, respectively, adjacent to –NH and from 148.4 to 147.1 ppm and 143.3 to 142.3 ppm for carbons C4 and C8, respectively, adjacent to $N_{aromatic}$ of TBR were observed. These shifts can be explained as a result of the involvement of the carboxylic hydroxyl group of SSZ in hydrogen bonding with neighboring TBR molecules through $HO_{(SSZ)}\cdots NH_{(TBR)}$ and $OH_{(SSZ)}\cdots N_{aromatic(TBR)}$.

Crystal structure determination

The SSZ-TBR crystallizes in P-1 space group with two molecules of each SSZ and TBR in its single unit cell consists (Figure 6b). The pertinent crystallographic parameters and hydrogen bond distances are tabulated in Tables 1 and 2, respectively. In the prepared SSZ-TBR cocrystal, interaction between SSZ and TBR molecule is through generation of H bond (Fig. 6c) between oxygen atom O26 of carboxylic hydroxyl of SSZ and –NH group of six membered ring

Table 1: Crystallographic parameters for SSZ-TBR

Parameters	SSZ-TBR
Chemical formula	$C_{25}H_{22}N_8O_7S$
Stoichiometry	1:1
Temperature (K)	298 (2)
Crystal system	Triclinic
Space group	P-1
a (Å)	18.1123
b (Å)	12.2155
c (Å)	8.2993
α (°)	64.02
β (°)	117.09
γ (°)	100.64
Z	1
Vol. (Å^3)	1469.34
R-factor (%) or R_{wp}	8.12

SSZ: Sulfasalazine, TBR: Theobromine

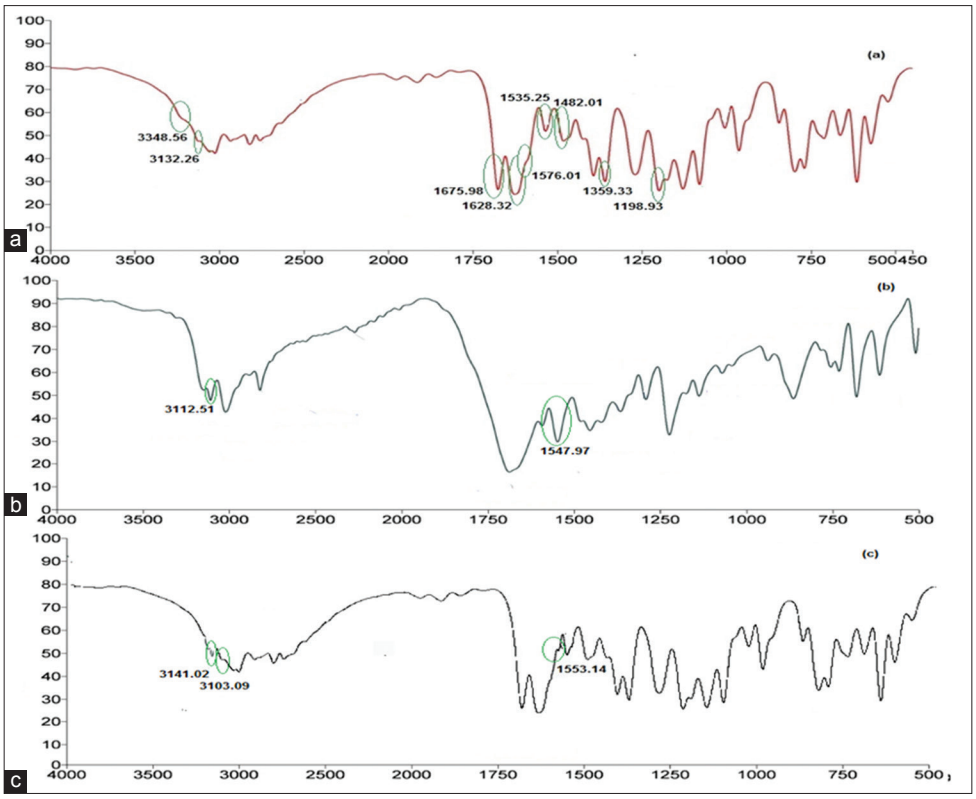

Figure 4: Fourier transform infrared spectra of (a) sulfasalazine (SSZ), (b) theobromine (TBR), (c) SSZ-TBR

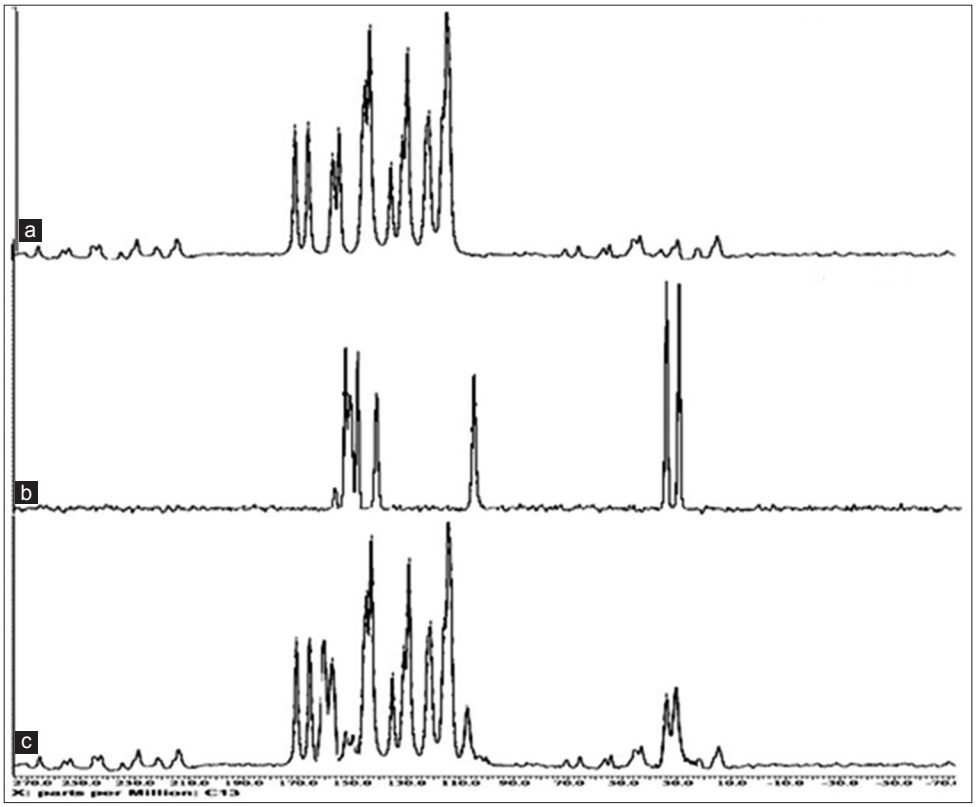

Figure 5: Solid state nuclear magnetic resonance spectra of (a) sulfasalazine (SSZ), (b) theobromine (TBR), (c) SSZ-TBR

of TBR leading to the formation of N1-H1 O26 heterosynthon. In addition, the carboxylic hydroxyl of SSZ forms H bond with N9 of TBR resulting into the O26-H26·N9 heterosynthon. The intramolecular bond between O28-H28$_{(\text{phenolic hydroxyl of SSZ})}$…O27$_{(\text{carboxylic carbonyl of SSZ})}$ also

Table 2: Geometrical parameters of SSZ-TBR

Compound	D-H…A	R (H…A)/Å°	r (D…A)/Å°	r (D…H)/Å°	∠D–H…A (°)
SSZ-TBR	N1-H1…O26	2.068	3.007	0.95	167.90
	O26-H26…N9	2.071	3.035	0.97	172.46

SSZ: Sulfasalazine, TBR: Theobromine

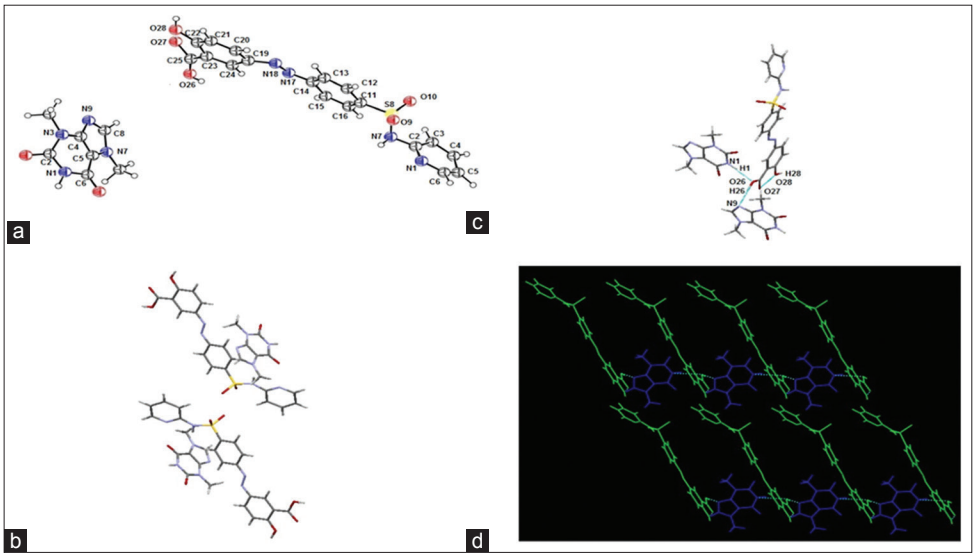

Figure 6: Sulfasalazine-theobromine (SSZ-TBR) (a) ORTEP diagram with 50% thermal ellipsoid probability of the asymmetric unit (b) asymmetric unit (c) unit cell representing hydrogen bond packing arrangement of SSZ-TBR

prevailed as in pure drug molecule. This hydrogen-bonded network lead to the formation of layers along b* axis (Figure 6d) which are held by van der Waal forces in crystal lattice of SSZ-TBR.

Solubility and IDR studies

The solubility and intrinsic dissolution profile of SSZ and SSZ-TBR were performed in simulated gastric and intestinal fluid. The results showed 1.5 folds improvement in both solubility as well as IDR in simulated intestinal fluid which in turn improved its bioavailability as it depends upon equilibrium solubility.

Conclusion

The preliminary screening of SSZ-TBR was done by melting point followed by systematic characterization and structure analysis. The present study established the usefulness of mechanochemical approach to develop cocrystal of SSZ with enhanced *in vitro* activity.

References

1. Almarsson O, Zaworotko MJ. Crystal engineering of the composition of pharmaceutical phases. Do pharmaceutical co-crystals represent a new path to improved medicines? Chem. Communications 2004;17:1889-96.
2. Jayasankar A, Somwangthanaroj A, Shao ZJ, Rodríguez-Hornedo N. Cocrystal formation during cogrinding and storage is mediated by amorphous phase. Pharm Res 2006;23:2381-92.
3. Schultheiss N, Newman A. Pharmaceutical cocrystals and their physicochemical properties. Cryst Growth Des 2009;9:2950-67.
4. Trask AV. An overview of pharmaceutical cocrystals as intellectual property. Mol Pharm 2007;4:301-9.
5. Basavoju S, Bostrom D, Velaga SP. Indomethacin-saccharin cocrystal: Design, synthesis and preliminary pharmaceutical characterization. Pharm Res 2008;25:530-41.
6. Stanton MK, Bak A. Physicochemical properties of pharmaceutical co-crystals: A case study of ten AMG 517 co-crystals. Cryst Growth Des 2008;8:3856-62.
7. Rodríguez-Hornedo N, Nehm SJ, Jayasankar A. Cocrystals: Design, Properties and Formation Mechanisms, in Encyclopedia of Pharmaceutical Technology. 3rd ed. London: Taylor & Francis; 2007. p. 615-35.
8. McNamara DP, Childs SL, Giordano J, Iarriccio A, Cassidy J, Shet MS, *et al.* Use of a glutaric acid cocrystal to improve oral bioavailability of a low solubility API. Pharm Res 2006;23:1888-97.
9. Mathew D. A study on suitability of nimesulide-betacyclodextrin complex in oral and topical dosage forms. Int J Pharm Pharm Sci 2009;1:193-8.
10. Srikanth MV, Babu GV, Rao NS, Sunil SA, Balaji S, Ramanamurthy KV. Dissolution rate enhancement of poorly soluble bicalutamide using β-cyclodextrin inclusion complexation. Int J Pharm Pharm Sci 2010;2:191-8.
11. Childs SL, Hardcastle KI. Cocrystals of piroxicam with carboxylic acids. Cryst Growth Des 2007;7:1291-304.
12. Genc H, Cakit BD, Nacir B, Saracoglu M, Kacar M, Erdem HR. The effects of sulfasalazine treatment on enthesal abnormalities of inflammatory rheumatic diseases. Clin Rheumatol 2007;26:1104-10.