RESEARCH ARTICLE

Development and Evaluation of Taste Masked Azithromycin by Crystal Engineering

Kanchan P. Upadhye*, Chetana S. Dhakate, Gouri R. Dixit, Suparna S. Bakhle

Department of Pharmaceutics, Priyadarshini J. L. College of Pharmacy, Nagpur, Maharashtra, India

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ABSTRACT

Purpose: Cocrystallisation is a promising technique for altering important physicochemical properties of drugs such as solubility and dissolution. The present study thus aims to utilize this technique to improve the drug solubility and study its effect on taste masking.

Method: Azithromycin co-crystals were formulated by solvent evaporation technique utilizing a synthetic sweetener neotame as the coformer. The study of microscopic characters characterized the formulated co-crystals, Fourier transforms infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Scanning electron microscopy (SEM), and Xray diffraction studies (XRD). Other evaluation parameters included taste evaluation, drug content determination, solubility, angle of repose, Carr's index, Hausner's ratio, and dissolution studies.

Results: The study revealed that the prepared co-crystals showed a marked improvement in taste and physicochemical properties. Co-crystals prepared in the ratio of 1:1 of drug and neotame displayed a nearly two-fold increase in solubility, improvement in flow properties, and a tremendous improvement in the taste as compared to the pure drug.

Conclusion: Thus, co-crystallization can be effectively used for solubility improvement and taste masking of poorly soluble bitter drugs such as azithromycin.

Keywords: Azithromycin, Bitter taste, Co-crystals, Neotame, Taste masking.

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INTRODUCTION

Drug administration by the oral route is the most popular due to the ease of self-administration, manufacturing, and good stability on storage compared to the other dosage forms. However, a major drawback of the oral dosage form is the difficulty in swallowing and bitter taste, leading to a severe pediatric and geriatric patient in compliance. Taste arises from the stimulation of taste buds present on the surface of the tongue.[1,2] Taste masking is necessary for an active ingredient with an unpleasant taste for increased patient compliance.

Taste masking has been done by various techniques like adding flavoring and sweetening agent,[3] ion-exchange resin complex,[4] micro-encapsulation,[5] prodrug approach,[6] inclusion complexation,[7] granulation, multiple emulsion technique, gel formation. However, very little work has been done using co-crystallization as a method of taste masking. This method not only improves the bitter taste but also improves the physicochemical properties of the drug.[8] Co-crystals are coordination types of molecular complexes involving noncovalent interaction between the drug and coformer and their complementary functional groups.[9] Thus, it involves drug and coformer that self-assemble by noncovalent interactions such as electrostatic interactions and hydrogen bonding in a well-defined stoichiometry. Such a development of co-crystal of an API leads to improved properties such as stability, solubility, drug release rate and taste.[10-12]

Azithromycin, with an IUPAC name 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin, belongs to the *azalide* subclass of macrolides. It consists of a 15-membered ring, and methyl-substituted nitrogen at the 9a position on the aglycone ring, which is responsible for preventing its metabolism. Such a type of structure makes azithromycin different from other types of macrolides azithromycin is a broad-spectrum macrolide antibiotic having a long half-life and a high degree of tissue penetration.[13]

Azithromycin is structurally related to erythromycin[14] and is commonly used for the treatment of infections of the respiratory and genitourinary tract as well as for enteric infections and may be used for sexually transmitted infections. It is a BCS Class II drug with an extremely bitter taste and a poor water solubility. Therefore the present work was aimed at using the co crystallisation technique of crystal engineering for the purpose of masking the bitter taste of the drug.

*Author for Correspondence: upadhyekanchan@gmail. com

MATERIALS AND METHODS

Materials

Azithromycin (AZI) was a gift sample from Zim Laboratories Pvt. ltd, Kalmeshwar, India. Neotame was received as gift samples from New Drug & Chemicals, Mumbai, and Ethanol from Loba Chemicals, Mumbai.

Formulation of Taste-masked Co-crystals

Azithromycin taste-masked co-crystals were formulated using the solvent evaporation method.[15] Azithromycin and the co-former neotame were taken in different ratios, and they were dissolved separately in ethanol. The drug solution and co-former solution were then mixed and stirred for few minutes. The solvent was evaporated, and the dried co-crystals were collected and stored in glass vials in a cool and dry place at room temperature.

Co-crystal Characterization Physicochemical Properties

Solubility Studies

The aqueous solubility was determined by carrying out saturation solubility studies[16] in distilled water in triplicate. About 20 mL of distilled water was added to an excess amount of the formulated co-crystals of azithromycin, and it was shaken for 24 hours in rotary flask shaker at room temperature to achieve equilibrium. The required aliquots were withdrawn, filtered through Whatman filter paper no. 41 and analyzed spectrophotometrically at 284 nm. The results so obtained from these studies were validated statistically**.**

Microscopic Properties

Microscopic properties of the formulated co-crystals were studied under light microscope.[16]

Micromeritic Studies

The micromeritic properties of the formulated co-crystals were studied with the parameters such as angle of repose, bulk density, tapped density, Carr's Index and Hausner's ratio.[17]

Drug Content Determination

To determine drug content, co-crystals formulations equivalent to 20 mg of azithromycin were dissolved in ethanol, and the volume was made up with distilled water.[20] After filtering the solution through Whatman filter paper no. 41 and a suitable dilution, it was analyzed at 284 nm using a spectrophotometer.

In-vitro Drug Release Studies

The dissolution studies were conducted in a USP Type II dissolution apparatus using 900 mL of the buffer of pH 6. 8 at a stirring speed of 100 rpm maintained at 37 ± 0.5°C.[16] A suitable amount of aliquots were withdrawn every 10 minutes, immediately filtered using a membrane filter, diluted as required, and its absorbance was determined spectrophotometrically at 284 nm.

Sensory Evaluation

In this method, taste evaluation was done on 11 healthy human volunteers trained for taste evaluation .[18] The volunteers were made to sign a written consent form before the test. They were told to keep 1 mg of azithromycin in the pure form in the mouth for 10seconds and then were asked to spit it out. Similarly, they were told to keep the co-crystals (1 mg) in the mouth until they were completely soluble. The degree of bitterness was immediately scored at several intervals for 15 minutes as per the bitterness intensity scale from 0-3 where 0, 0.5, 1, 2, and 3 indicate no, threshold, slight, moderate, and strong bitterness.

Solid-state Characterization

Fourier Transform Infrared Spectroscopy (FTIR) Studies

The study was performed to check the compatibility of coformer with the drug,[19] and the suitability of coformer for new species formation. The samples were scanned in the range of 4000 to 500 cm^{-1}

Differential Scanning Calorimetry (DSC) Studies

Figure 4 depicts DSC studies were carried out to study the interaction of co-former with the azithromycin as can be observed from the changes in endothermic peaks of the drug and co-crystals.[20] Accurately weighed samples were placed in aluminum pans, heated from 36 to 500°C at a rate of 15°C/min in an air atmosphere. For reference, an empty sealed aluminum pan was used.

Powder X-ray Diffraction (PXRD) Studies

The XRD studies were carried out to determine the crystal formation and changes occurring in their nature. These studies also help to determine the %crystallinity of co-crystals.[21]

Scanning Electron Microscopy (SEM) Studies:

The SEM studies were used to determine the surface morphological properties of co-crystals. Samples were mounted on a double-faced adhesive tape sputtered with gold. Scanning electron photographs were taken at an accelerating voltage of 17 kV, and obtained micrographs were examined magnification of ×1000.[21]

Stability Studies

The formulation that was optimized based on the above studies was subjected to stability studies every 10 days up to one month. The optimized batch was studied concerning its micromeritic properties, drug content and drug release.

RESULTS AND DISCUSSION

Formulation of Taste masked Co-crystals

Thus the taste-masked co-crystals of azithromycin were prepared using neotame as the coformer, as shown in Table 1.

Solubility Studies

The solubility study of co-crystals is shown in Table 2. Pure azithromycin drug shows poor solubility in water of

Table 1: Co-crystals of azithromycin

	Batches of co-crystals (AZI: Neotame)			
Ingredients (mg)	*C_1 (1:0.5)*	*C_2 (1:0.75)*	*C_3 (1:1)*	*C_4 (1:1.25)*
Drug(AZI)	500	500	500	500
Neotame	250	375	500	625

about 3.56 µg/mL. However, the formulated co-crystals showed a remarkable improvement in the water solubility of AZI, and there was a 1.59 times increase in saturation solubility, as is evident from Table 2.

Microscopic Characterization of Co-crystals

Microscopic properties of the formulated co-crystals were studied by a light microscope, as shown in Figure 1. The image reveals a semi-crystalline nature of the formulated co-crystals

Micromeretic Properties

The flow properties of co-crystals are shown in Table 3. The flow characteristics of co-crystals expressed in terms of angle of repose, Carr's index, and Hausnar's ratio were significantly improved as compared to those of the original drug.

Drug Content Determination

The drug content of the formulated co-crystals is shown in Table 4. The drug content was found to be satisfactory and ranged from 74.33% to 83.52%

***In-vitro* Drug Release**

The results of the *in-vitro* dissolution studies of the formulated co-crystals are represented by Figure 2 in which the cumulative percent drug released is plotted as a function of time. Batch C_3 of co-crystals showed a significant improvement in the % drug release (88.32%) over pure AZI (57.38%)

Sensory Evaluation

Taste evaluation study were performed in 11 healthy human volunteers. Azithromycin shows a strong bitter taste, whereas the co-crystals show 0 or 0.5 (no or threshold) bitter taste, as shown in Table 5.

Thus, based on all the above parameters, the batch C_3 of the co-crystals was optimized and was subjected further to microscopic characterization, solid state characterization and stability studies

Solid State Characterization

Fourier Transform Infrared Spectroscopy (FTIR) Studies

Figure 3 depicts the FTIR spectra of AZI, Neotame, physical mixture, and co-crystals. The pure AZI spectrum shows sharp peaks at 731.05, 1084.04, 1251.86, 1379.16, 1721.54, 3246 cm^{-1}. All these sharp drug peaks are also seen in the spectra of the physical mixture at the same wave number, which indicates that there is no interaction and the drug and the co-former are compatible with each other. In the case of co-crystals there is a little shifting of peaks observed at 700.19, 1170.84, 1691.64, 3323.49, 1514.19 cm^{-1}. This observation suggests that azithromycin shows no significant change in its characteristics in its physical mixture, but there is a shift of peaks in the co-crystals due to interaction between the drug and conformer forming the cocrystals.[22]

Table 2: Solubility studies

Sr. No.	*Drug*	*Solubility (µg/mL)*
1	AZI	3.56 ± 0.011
2	C_1	4.40 ± 0.062
3	C_2	4.89 ± 0.097
4	C_3	5.20 ± 0.16
5	C_4	5.10 ± 0.17

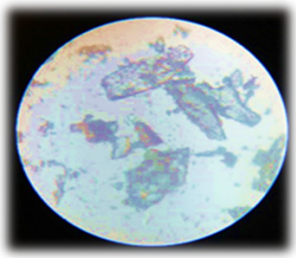

Figure 1: Microscope image of co-crystals

Table 3: Micromeritic studies

Formulation	*Hausnar's ratio*	*Carr's index (%)*	*Angle of repose (θ°)*
AZI	1.24 ± 0.21	19.27 ± 0.017	38.49
C_1	1.26 ± 0.40	21 ± 0.016	35.00
C_2	1.13 ± 0.37	11.87 ± 0.019	37.14
C_3	1.16 ± 0.43	13.98 ± 0.13	33.22
C_4	1.13 ± 0.24	12.15 ± 0.19	38.88

Table 4: Drug content

Formulation	*% Drug content (w/w)*
C_1	74.38 ± 0.37
C_2	78.79 ± 0.26
C_3	83.52 ± 0.52
C_4	76.77 ± 0.55

Table 5: Sensory evaluation

	Bitterness score					
Formulation	*10 seconds*	*1 minutes*	*2 minutes*	*5 minutes*	*10 minutes*	*15 minutes*
AZI	3	3	3	3	3	2
C_1	0	0	0	0	0. 5	1
C_2	0	0	0	0	0	0. 5
C_3	0	0	0	0	0	0. 5
C_4	0	0	0	0	0	0. 5

(Note: 0- no, 0. 5- threshold, 1- slight, 2- moderate, 3- strong bitterness)

Differential Scanning Calorimetry (DSC) Studies:

Figure 4 depicts DSC thermographs of pure drug, co- former, physical mixture, and co-crystals. Azithromycin showed a sharp melting endotherm at 126.23˚C, co-former showed a melting endotherm at 76.53˚C, whereas the co-crystals showed a melting endotherm peak at 197.10˚C, indicating formation of a newer crystalline material.

Powder X-ray Diffraction (P-XRD) Studies

The presence of polymorphs, crystal habit modification in drug crystals and generation of new crystals form during co-crystallization can be very well studied using the *P-XRD* technique. Figure 5 represents the XRD pattern of AZI, Neotame and the co-crystal. The XRD pattern of the formulated co-crystals indicated a decrease in the number and intensity of peaks compared to pure AZI thus indicating a decrease in the crystallinity. From the literature, it is evident that such a type of X-ray diffraction pattern can be observed with semi-crystalline solids.[23] Hence it can be concluded that the co-crystals formed may be semi-crystalline in nature.

Scanning Electron Microscopy (SEM) Studies:

Figure 6 represents the SEM images of AZI, Neotame and co-crystal. The SEM images of azithromycin show absence of crystalline structure, thereby indicating its amorphous nature. Similarly, the SEM image of coformer also indicates its non-crystalline nature. However the SEM images co-crystals show crystal aggregates. An examination of the surface morphological properties of the drug and co-crystals confirm that azithromycin co-crystals have crystallized from ethanol system containing neotame as co-former.

Stability Studies

Optimized batch C_3 of the co-crystals was further subjected to stability studies. It was observed that there were no significant changes in the micromeritic properties, drug content and drug release in the duration of 1 month, as shown in Table 6.

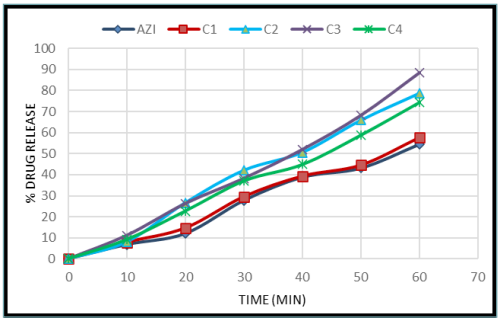

Figure 2: *In vitro* drug release

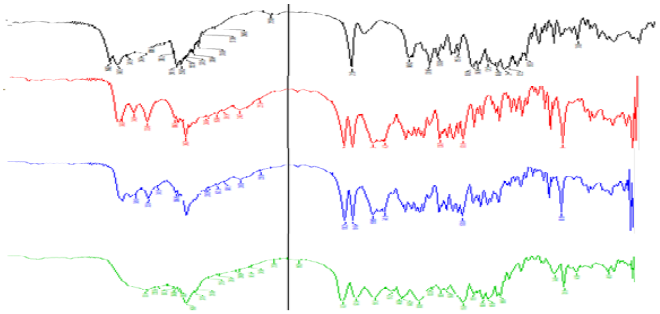

Figure 3: FTIR studies, A: AZI, B: Coformer (Neotame), C: Cocrystal, D: Physical mixture

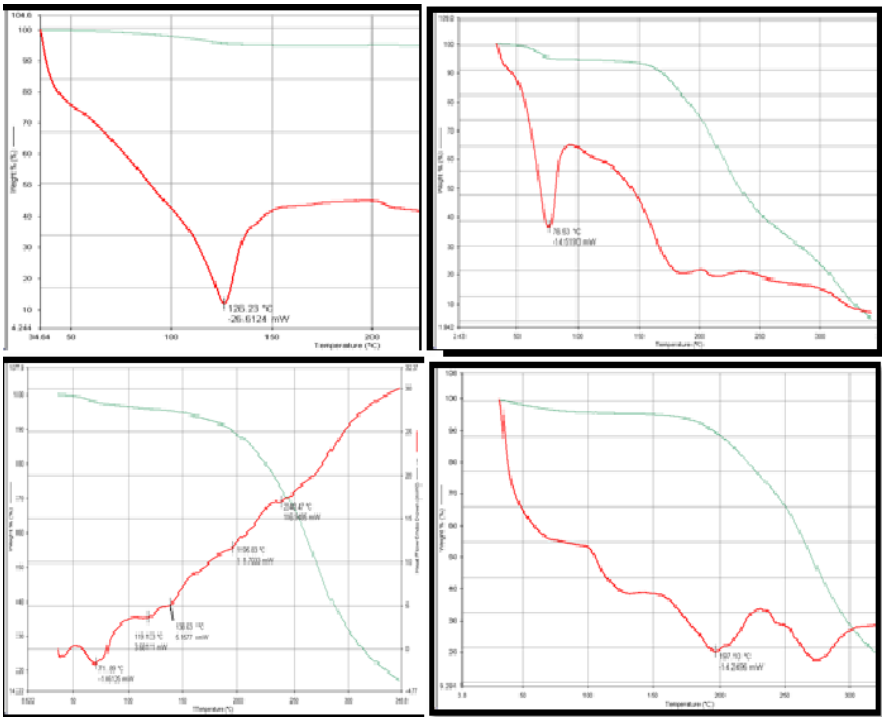

Figure 4: DSC studies, A: AZI B: Neotame C: Physical mixture, D: Co-crystals

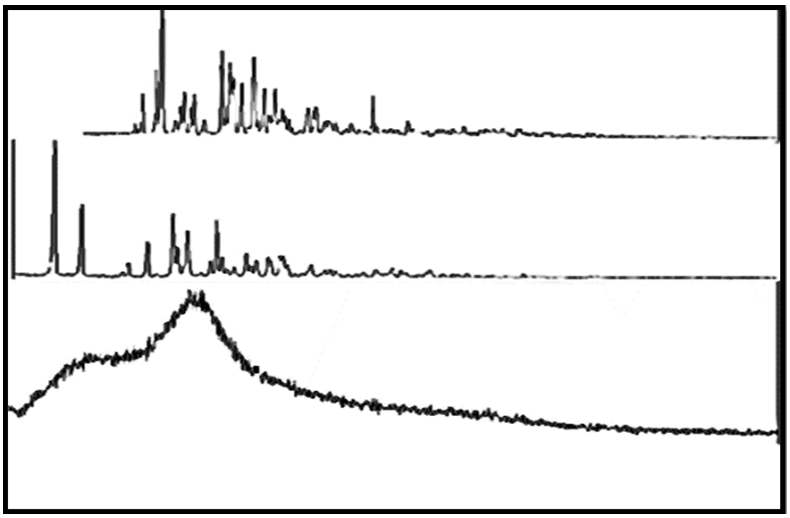

Figure 5: PXRD Diffraction studies A: AZI, B: Neotame, C: Co-crystal

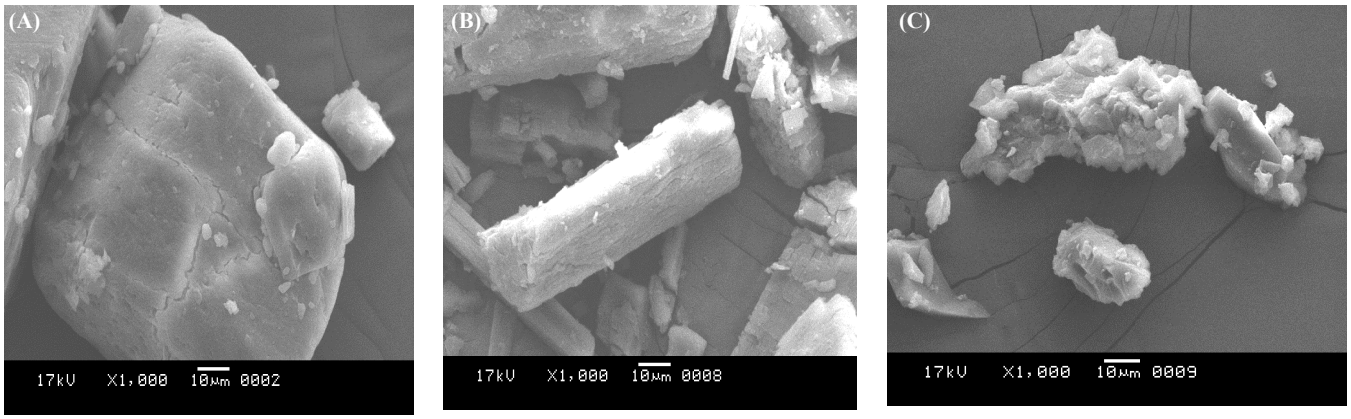

Figure 6: SEM images (A) AZI (B) Neotame (C) Co-crystals

Table 6: Stability studies of optimized batch C_3

PERIOD PARAMETERS	*INITIALLY*	*10 DAYS*	*20 DAYS*	*30 DAYS*
Bulk density (g/cc)	0.443 ± 0.032	0.443 ± 0.023	0.442 ± 0.019	0. 441 ± 0. 043
Tapped density (g/cc)	0.515 ± 0. 026	0.51 ± 0.023	0.51 ± 0.019	0. 50 ± 0. 034
Angle of repose	33.22°	33.22°	33.22°	33. 22°
Drug content (%)	83.52 ± 0.28	83.50 ± 0.38	83.50 ± 0.36	83. 49 ± 0. 24
Drug release (%)	88.32 ± 0.52	88.31 ± 0.47	88.29 ± 0.39	88. 28 ± 0. 56

CONCLUSION

Thus, in the present work, attempts have been made to prepare taste masked formulation of bitter drug azithromycin using co-crystallization technique. For this purpose, sweetening agent neotame was selected as co-former. From XRD pattern, it may be concluded that the co-crystals exhibit semi-crystalline nature. The prepared co-crystals showed a marked improvement in taste as well as solubility. Thus, co-crystallization can be effectively used for taste masking of bitter drugs apart from other taste masking techniques. It also has the advantage of solubility enhancement for poorly soluble drugs.

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REFERENCES

1. Sastry SV, Nyshdhan JR, Fix JA. Recent technological advances in oral drug delivery: A Review. Pharmaceutical science and technology today 2000; 3: 138-145.

2. Seager H. Drug delivery products and the Zydis fast- dissolving dosage form. Journal of pharmacy and pharmacology 1998;50(4): 375-382.
3. Choi du H, Kim NA, Nam TS, Lee S, Jeong SH. Evaluation of taste-masking effects of pharmaceutical sweeteners with an electronic tongue system. Drug Dev Ind Pharm 2014;40(3):308-317.
4. Bidkar SJ, Bidkar RJ, Dama GY, Todkar VD, Bhanoji Rao ME, Ravikumar BVV. A review: Taste masking of bitter pharmaceutical agents by using ion exchange resins. World Journal of Pharmacy and Pharmaceutical Sciences 2018;4(9): 464-482.
5. Basheer Al-kasmi, Mhd Bashir Alsirawan, Mais Bashimam, Hind El-zein. Mechanical microencapsulation: The best technique in taste masking for the manufacturing scale - Effect of polymer encapsulation on drug targeting. Journal of Controlled Release 2017; 20:134-141.
6. Gupta P, Tiwari A, Mishra M. Taste masking of drugs: an extended approach. International Journal of Current Advanced Research 2017; 6(3):2571-2578.
7. Chay SK, Keating AV, James C, Aliev AE et al. Evaluation of the taste-masking effects of (2-hydroxypropyl)-β-cyclodextrin on ranitidine hydrochloride; a combined biosensor, spectroscopic and molecular modelling assessment, RSC Adv 2018;8:3564-3573
8. Dhakate CS, Upadhye KP, Dixit GR, Bakhle SS, Umate RN. Taste masking by cocrystallisation: A review. World Journal of Pharmaceutical research 2017;6(7):1531-1548.
9. Ning Q, Mingzhong L, Angela D, Gray T. Pharmaceutical co-crystal: An overview. International journal of Pharmaceutics 2006;419:1-11.
10. Yadav AV, Shete AS, Dabake, AP, Kulkarni PV and Sakhare SS. Co-Crystals: A Novel Approach to Modify Physicochemical Properties of Active Pharmaceutical Ingredients. Indian J Pharm Sci 2009;71(4):359-370.
11. Yadav, A. V, Shete, A. S and Dabake, A. P. Crystal engineering an approach to modify physicochemical characteristics of Mefloquine Hydrochloride. Drug Dev Ind Pharm, 2010; 36 (09):1036-1045.
12. Sekhon, B. S. Pharmaceutical co-crystals- A review. ARS Pharmaceutica 2009;50(3):99-117.
13. McMullan BJ, Mostaghim M. Prescribing azithromycin. Aust Prescr 2015; 38(3):87-9. Epub 2015 Jun 1. [PubMed:26648627]
14. Peters DH, Friedel HA, McTavish D: Azithromycin. A review of its antimicrobial activity, pharmacokinetic properties and clinical efficacy. Drugs 1992 ;44(5):750-99. doi: 10.2165/00003495-199244050-00007. [PubMed:1280567]
15. Dwi S, Retno S, Helmy Y, Riesta P. Preparation and characterization of astesunate-nicotinamide co-crystal by solvent evaporation and slurry method. Asian journal of pharmaceutical and clinical research 2014;7(1):62-65.
16. Sopyan I, Fudholi A, MuchtaridiM, Puspitasari I. A simple effort to enhance solubility and dissolution rate of simvastatin using co-crystallization. International journal of Pharmacy and Pharmaceutical sciences 2016;8(6):342-346.
17. Subrahmanyam CVS. Micromeretics. Textbook of physical pharmaceutics. Edn 2, Vallabh Prakashan, Delhi, 2000;221-227.
18. Amin F, Khan S, Shah SMH, Rahim H, Hussain Z et al. A new strategy for taste masking of azithromycin antibiotic: development, characterization, and evaluation of azithromycin titanium nanohybrid for masking of bitter taste using physisorption and panel testing studies. Drug design, development and therapy 2018;12:3855-3866
19. Nugrahani I, Bahari MU. The dynamic study of co-crystal formation between anhydrous and monohydrate theophylline with sodium saccharin dihydrate by FTIR. Journal of chemistry and biochemistry 2014;2(2):117-137.
20. Londhe V, Shah K, Borhade S. Research article- Utilization of co-crystallization for solubility enhancement of poorly soluble antiretroviral drug- ritonavir. International journal of Pharmacy and Pharmaceutical sciences 2014;6(2):556-558.
21. Bagde SA, Upadhye KP, Dixit GR, Bakhle SS. Formulation and evaluation of co-crystals of poorly water soluble drug. International Journal of Pharmaceutical sciences and research 2016;7(12):4988-4997
22. Shete AS, Kumbhar B, Yadav AV, Korpale S, Sakhare SS et al. Amorphous Mixtures of Albendazole with Carboxylic Acids By Cogrinding Technique: Solid State Characterizations and *In Vitro* Efficacy Study. International journal of Drug Delivery and Technology 2019;9(4):509-516
23. Kasargod SN, Shivanna S, Subramani NK, Siddaramaiah H. Revisiting Powder X-ray Diffraction Technique: A powerful tool to characterize polymers and their composite films, Journal of material science 2016;4(4):1-5