Development, Characterization and Analysis of Azelnidipine Co-Crystals

Patel Ekta C.*, **Patel Hemangi S., Patel Mehul N., Christian Jenee R. and Suhagia B.N.**
Faculty of Pharmacy, Dharmsinh Desai University, Nadiad-387001, Gujarat, INDIA
*patelekta1807@gmail.com

Abstract
Azelnidipine, an anti-hypertensive drug of dihydropyridine class, has low solubility and high permeability drug (class-2) and thus often shows dissolution rate-limited oral absorption and high variability in pharmacological effects. The purpose of study was to prepare co-crystals of AZL to enhance its solubility. Co-crystals were prepared by solvent evaporation method using different co-crystal formers. The prepared co-crystals were evaluated for solubility. The solid state property was characterized by microscopy, differential scanning calorimetry (DSC) and Fourier transfer infrared spectroscopy (FTIR).

It was observed that the solubility of AZL co-crystals was significantly more than AZL. Co-crystal using succinic acid and saccharine gave maximum solubility. The microscopy, FTIR and DSC and studies of co-crystal confirmed the formation of co-crystals and indicated that new interactions were formed. In conclusion, co-crystals of AZL lead to the solubility enhancement and this can be further explored to study the impact of increased solubility on the bioavailability of AZL.

Keywords: Co-crystal, Azelnidipine, Co-crystal former, solubility.

Introduction

Major hindrance in drug development presently is solubility of novel drug candidates which make their oral administration challenging. Azelnidipine(AZL) which is vasodilator and has also been confirmed for its antioxidative, cardio-protective, neuro protective and anti-atherosclerotic properties and preventing insulin resistance, belongs to BCS class II and has a high hydrophobicity with 60–80% oral bioavailability. An important goal of solid state pharmaceutical development is to increase drug solubility while maintaining a stable form. Hence, it was thought of interest to increase its solubility by preparing co-crystals with pharmaceutically acceptable cocrystal formers.

Co-crystals, multi-component molecular complexes, are emerging solid-state form to change physicochemical and biopharmaceutical drug properties. Co-crystals can be formulated with acidic, alkaline, neutral or ionic solid drug and a co-former, that interacts through non-covalent interactions in a definite stoichiometric ratio without compromising the structural integrity but improving the solubility. In line with the notion, AZL co-crystals were prepared and characterized.

Material and Methods

Chemicals: AZL was supplied by J.B Chemicals, Ankleshwar. Succinic acid, saccharine and benzoic acid were supplied by S.D. Fine Chem Ltd. Methanol was purchased from Merk.

List of Instruments used in research work:
1. Digital weighing balance Shimadzu Aux 220.
2. UV-Visible double beam spectrophotometer Shimadzu 1800.
3. FTIR spectrophotometer of Shimadzu Corporation Kyoto, Japan.
4. Differential scanning Calorimetry of Mettler Toledo.
5. Sonicator of Shimadzu.
6. Microscope of Janki (Eclipse E200).

Estimation of AZL: Accurately weighed 10mg of AZL was transferred to a 10 ml volumetric flask and it was diluted up to mark with methanol to obtain a standard stock solution (1000µg/ml) and further dilution was made with methanol to obtain the working standard solution (100µg/ml). From this working, standard appropriate aliquots were taken and volume was made up with methanol to get final concentration of 2, 4, 6, 8, 10, 12, 14 µg/ml. Co-former (Succinic acid/Saccharine/Benzoic acid) (10 mg) was accurately weighed and transferred into 10 ml of volumetric flask.

Methanol was added to dissolve and prepare standard stock solution of 100µg/ml. From Standard stock solution, a series of 0.1 ml was accurately transferred into 10 ml volumetric flasks. The volume was made up with methanol to get final concentration of 10µg/ml respectively. The solutions of AZL alone and with co-formers were scanned at 400 – 200 nm wavelength.

Validation of UV method

Linearity (Calibration curve): Aliquots of standard solutions of AZL (1, 2, 3, 4, 5, 6 ml) were transferred in a separate series of 10 ml volumetric flask and diluted up to the mark with Methanol. The calibration curves were plotted over a concentration range of 10-60 µg/ml for AZL using methanol. The absorbance of solution was measured at 255 nm and 341 nm for AZL. The validation was done by taking the absorbance of AZL alone in methanol at 255 nm and AZL and co-former in methanol at 341 nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equation was calculated.

For intraday precision, 20, 40 and 60µg/ml AZL were analyzed three times on the same day. The results were reported in terms of relative standard deviation. For inter-day precision, test solutions containing 20, 40 and 60µg/ml AZL were analysed three times on three different days. The absorbance was measured at 341 nm.

The results were reported in terms of relative standard deviation. Limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of response (σ) and slope (S) of the calibration curve. Robustness of the proposed method was determined by changing the wavelength ± 0.5 nm and % RSD was calculated. The accuracy was done by standard addition method where known amounts of standard solutions of AZL were added at 50, 100 and 150 % level to pre quantified sample solutions of AZL (10µg/ml, 20µg/ml and 30µg/ml) and the % recovery was calculated.

Solubility study: Excess amount of AZL was added into conical flask containing 20 ml of distilled water and it was shaken for 72 hours using an agitator shaker. Then the solution was filtered. Calculate the concentration of dissolved AZL using spectrophotometry UV-Vis method at wavelength of 255 nm. Excess amounts of AZL and succinic acid, saccharine and benzoic acid were added into conical flask containing 20 ml of distilled water, later shaken for 72 hours using an agitator shaker. Then the solution was filtered and the concentration of dissolved AZL was estimated using verified spectrophotometry UV-Vis method at wavelength of 341 nm.

Preparation of Co-Crystals: Accurately weighed AZL and Succinic acid/Saccharine/Benzoic acid equivalent to a molar ratio (1:1), (1:2), (2:1) were diluted by methanol as a solvent, shaken for 10 minutes, later stored in a water bath at 30 ^{0}C for 24 hours for drying and next keep in to vial in room temperature(25 ^{0} C). The compositions of co-crystals are shown in table 1.

Solubility studies of Co-crystals: Accurately weigh dried co-crystal equivalently to AZL 10 mg, then input into the vial and reconstitute with 20 ml of distilled water, later shaken for 72 hours using an agitator shaker, afterward calculate the concentration of dissolved AZL using spectrophotometry UV-Vis method. The same procedure was repeated for pure SV and physical mixture (Azel: Sacch).

Characterizations of Co-Crystals

Microscopy: Microscopic characteristics of prepared co-crystals were observed co-crystals by light microscope.

FTIR (Fourier Transform Infrared Spectroscopy): FT-IR spectra of AZL excipients and co-crystals were recorded on the sample prepared in ATR disks, whereas sample is taken in 2 mg using Shimadzu Fourier Transform Infra-Red spectrometer. The samples were scanned over a frequency range 4000-400 cm^{-1}.

DSC (Differential Scanning Calorimetry): The DSC thermograms of pure drug and selected samples were recorded on a DSC (Mettle Instruments). The samples were weighed and hermetically sealed in aluminium pans. Thermal analysis system instrument Mettlar DSC with intra cooler and a refrigerated cooling system was used. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The system was purged with nitrogen gas at a flow rate of 80 ml/min. Initially samples were held at 50^{0}C for 1 min and the heating was performed from 20^{0}C- 200 °C at a rate of 10°C/min.

Results and Discussion

Estimation of AZL at wavelength: The standard solution of AZL was prepared in methanol and scanned in the wavelength range of 200-800nm and overlain spectrum was recorded. The absorption maxim for the drug AZL was taken at wavelength at 255 nm (Fig. 1). Method validation was done at 255nm and solubility study was also performed at wavelength of 255nm but the selected co-former in the formulation was interfering the results (Fig. 2) because of which the UV spectra of co-former were examined at wavelength 341nm, where no interference was observed (Fig. 3).

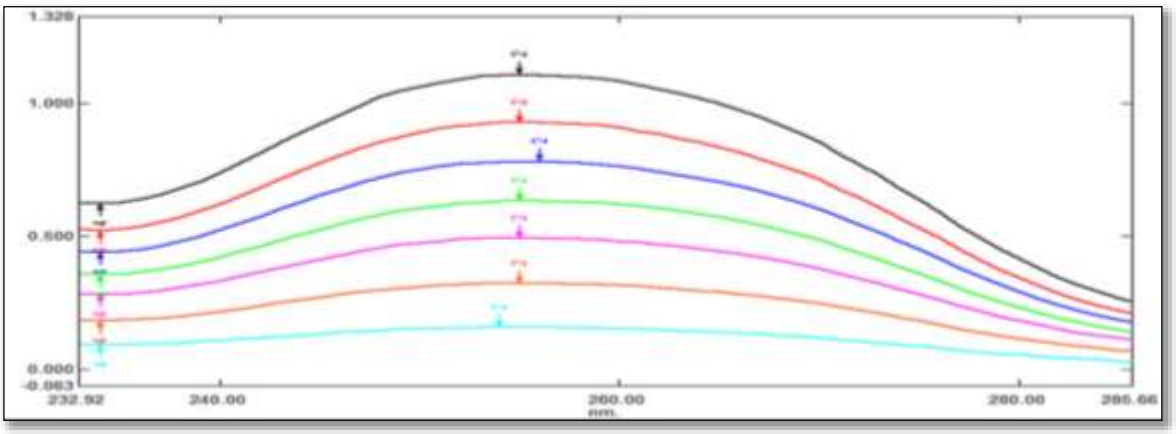


Figure 1: Overlain UV spectra of standard AZL (4-18µg/ml) in Methanol at 255 nm wavelength.

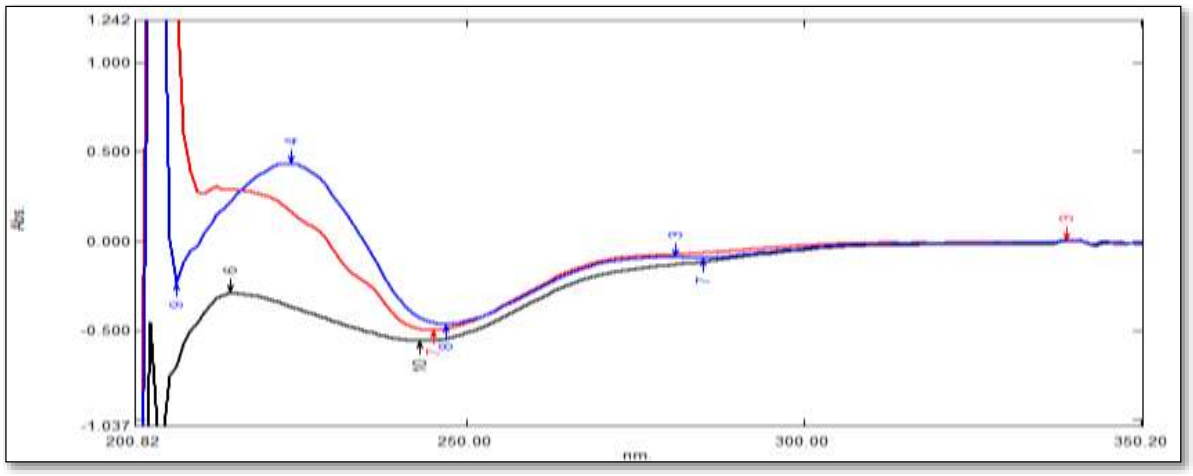


Figure 2: Effect of addition of co-formers

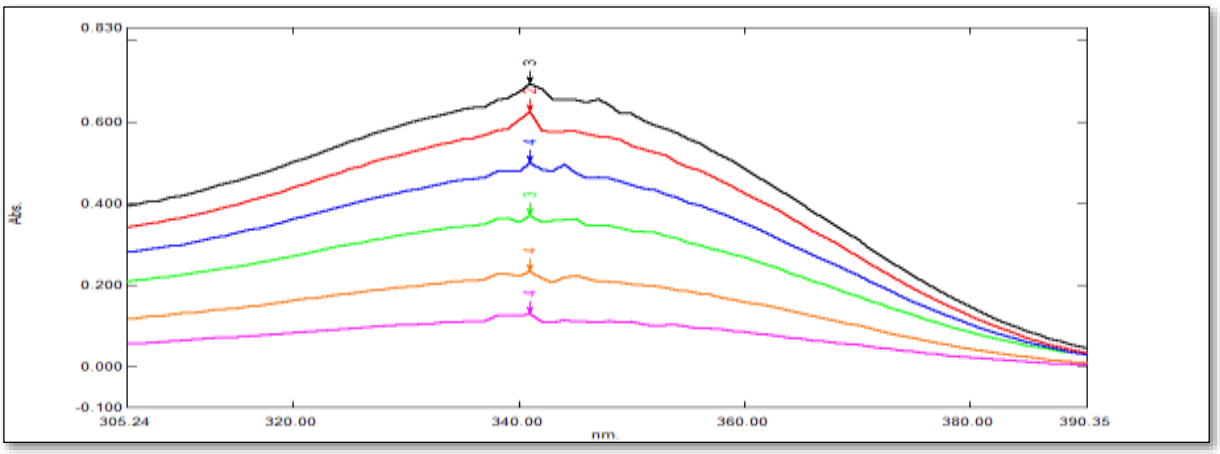


Figure 3: Overlain UV spectra of standard AZL (10-60µg/ml) with co-former in methanol at 341 nm wavelength.

Table 1
Different batches of Co-formers.

Batch	Molar ratio	Amount of drug (mg)	Succinic acid (mg)	Methanol (ml)
Sa1	(1:1)	582.6	118.09	10
Sa2	(1:2)	582.6	236	10
Sa3	(2:1)	1165	118.09	10
Sc1	(1:1)	582.6	183.18	10
Sc2	(1:2)	582.6	366.36	10
Sc3	(2:1)	1165	183.18	10
Ba1	(1:1)	582.6	122.24	10
Ba2	(1:2)	582.6	244.48	10

*Sa co-former of succinic acid, *Sc co-former of saccharine, *Ba co-former of benzoic acid

Method validation: The developed method was validation and the results are as shown in the table 2 where the method at 255 nm and 341 nm was found to be linear, accurate, precise, sensitive and robust.

Solubility Studies: Solubility study was done by addition of excess amount of drug in distilled water and was analyzed by UV spectrophotometric and results were found to be 9.74 µg/ml or 9.74 × 10^{-6} g/ml. From the calculation of the solubility criteria, it was found that the drug AZL was insoluble in water. To increase its solubility, effect of co-formers was assessed where excess amount of drug and the co-formers were added in distilled water and then the solution was analysed by UV spectrophotometric. The solubility of AZL alone in 20 ml water was found to be 0.10 mg while incorporation of co-former like succinic acid and saccharine improves solubility more of Azl in water (4.12 mg and 3.43 mg respectively) than benzoic acid co-former (1.85 mg). And thus on the basis of solubility studies, for the

preparation of co-crystal, succinic acid and saccharine were selected as co-former.

Preparation of Co-Crystals: Co-crystals were prepared by solvent evaporation method with different co-formers such as succinic acid and saccharine by molar ratio of (1:1), (1:2) and (2:1). % yield obtained is shown in table 3.

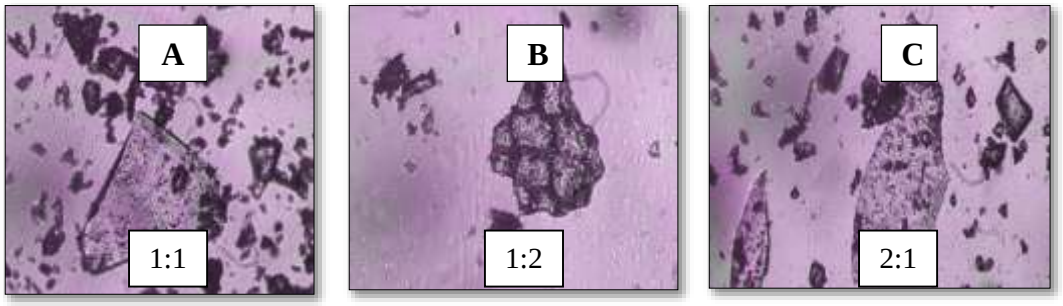
Solubility study of Co-Crystals: Co-crystallization technique shows significant improvement in the aqueous solubility of AZL. Solubility of the Azel + succinic acid and Azel + saccharine co-crystal was higher in molar ratio of (1:2) than those recorded for AZL and also higher in (1:1) and (2:1) molar ratio than native AZL. This is because of the affinity of the solvent towards AZL being stronger in the presence of the co-former, which led to a decrease in the energy of crystal lattice by the formation of co-crystal. This indicates the successful interaction of AZL with coformer and formation of cocrystals. Based on the results, cocrystals were further characterized (Table 4).

Table 2
Validation of UV method

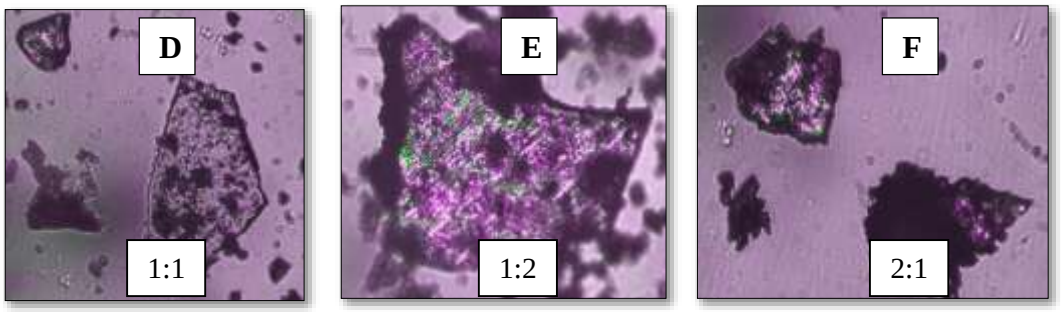
Wave length (nm)	Linearity (µg/ml)	Regression Equation	Co-relation coefficient (r2)	Precision (%RSD)		LOD (µg/ml)	LOQ (µg/ml)	Robustness (n=3)
				Intrady precision (n=3)	Interday precision (n=3)			
255	2-18	Y=0.0538X-0.0808	0.9989	0.170-1.853	0.170-0.408	0.0515	0.155	0.295-1.085
341	10-60	Y=0.0122X-0.0111	0.9978	0.432-1.455	0.1883-1.258	1	3.015	0.959-1.798

Table 3
Percentage yield for prepared co-crystals.

Batch No	Ratio	Co-former	% yield
Sa1	(1:1)	Succinic acid	98.45
Sa2	(1:2)	Succinic acid	91.08
Sa3	(2:1)	Succinic acid	97.66
Sc1	(1:1)	Saccharine	95.85
Sc2	(1:2)	Saccharine	90.46
Sc3	(2:1)	Saccharine	99.3



Azel – succinic acid



Azel-saccrine

Figure 4: Microscopy of Co-crystals of (a) succinic acid and (b) Saccharin

Characterization of prepared co-crystals

Fourier transform infrared (FTIR): FTIR measurements were carried out to gain insight into the molecular level of interaction between AZL and succinic acid/saccharine and the spectra of AZL, succinic acid, saccharine and the complexes. FTIR spectra of AZL showed characteristic peaks of cyclic alkanes C-H stretching at 2990 cm^{-1}, N-H stretching at 3442 cm^{-1}, C=O stretching at 1681 cm^{-1}, C-N stretching at 1246 cm^{-1} and N-H bending at 1515 cm^{-1}. FTIR spectra of succinic acid showed characteristic peaks of C-H stretching at 2538 cm^{-1}, C=O group at 1692 cm^{-1}, C=O stretching at 1307 cm^{-1}, C-O-H in plane bending at 909 cm^{-1}.

FTIR spectra of saccharine contained characteristic peaks of C=O ketone at 1723 cm^{-1}, O-H stretching at 3613 cm^{-1}, O=S=O sulfones at 1055 cm^{-1} and C-N group at 1337 cm^{-1}. The individual FTIR spectra confirmed the identity of AZL, succinic acid and saccharine.

FTIR spectra of AZL and Succinic acid co-crystals showed broadening of IR absorption peaks and new peaks were observed for all the (1:1), (1:2) and (2:1) molar rations. The different peaks observed were 3000 and 2990 cm^{-1} Aromatic (C-H) stretching, (N-H) stretching at 3300 cm^{-1}, C-O stretching at 1250 and 1690 cm^{-1} and (C-H) bending at 740 and 730 cm^{-1}.

In FTIR spectra of AZL and Saccharin co-crystals, the peaks were observed to be broad for (1:1), (1:2) and (2:1) molar ratios. The different peaks observed indicated aromatic (C-H) stretching which is at 2990, 3250 and 3300 cm^{-1}, (N-H) stretching at 1650 and 1690 cm^{-1} and (C-H) bending at 650 cm^{-1}

The FTIR spectra showed that AZL was compatible with coformer succinic acid and saccharine and the formation of co-crystal was confirmed from the formation of hydrogen bond between AZL and co-former.

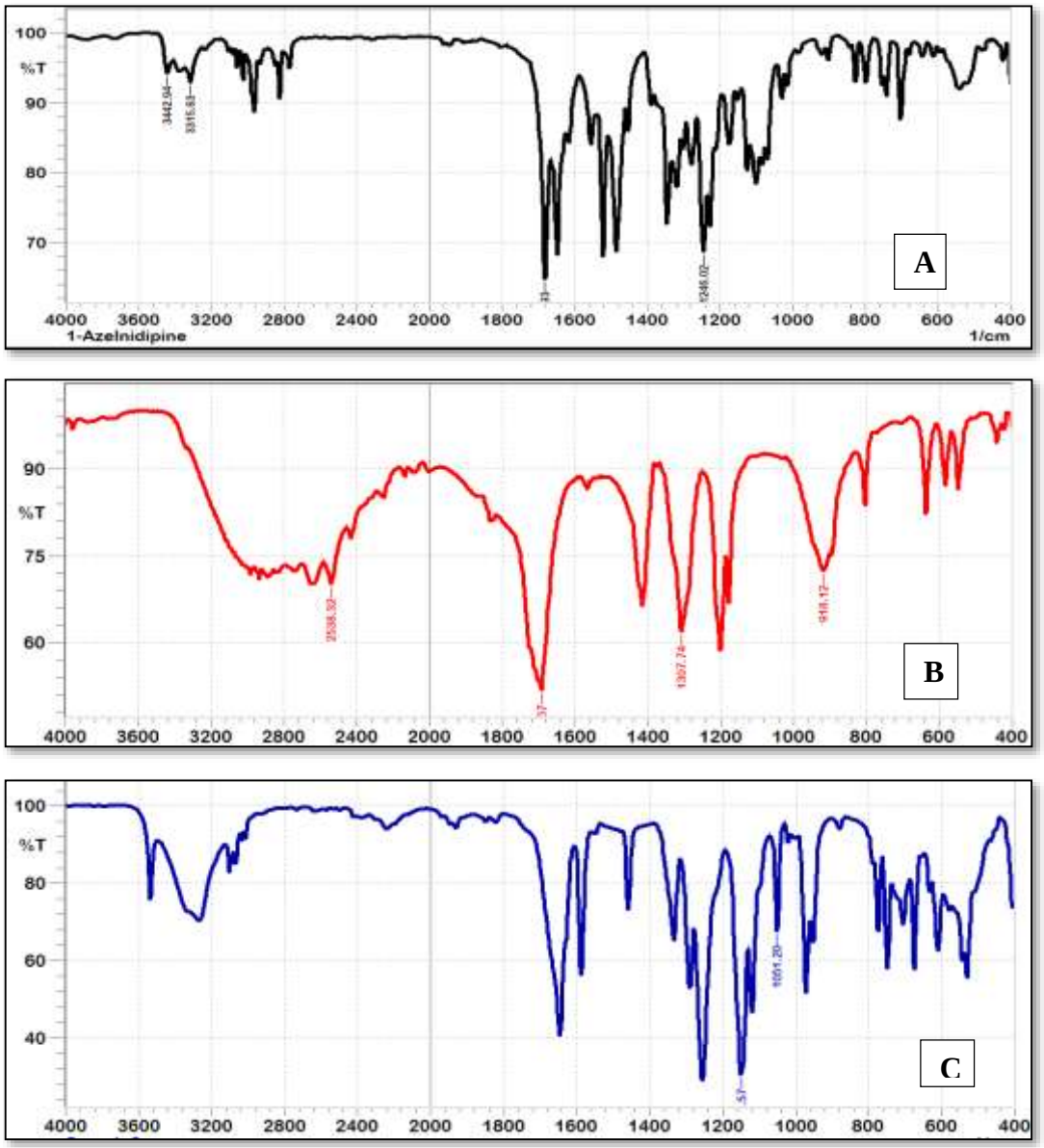


Figure 5: FTIR Spectra of: (a) AZL (b) Succinic acid (c) Saccharine

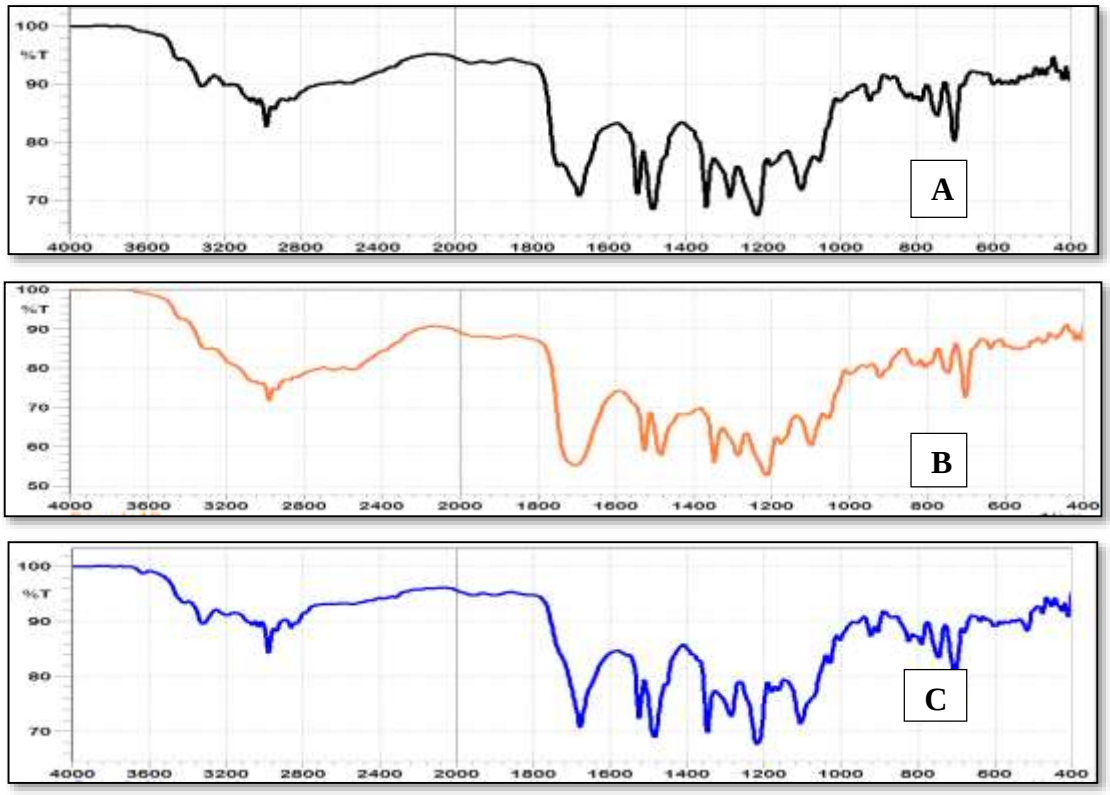


Figure 6: FTIR Spectra of (A) (1:1) Azl + Succinic acid co-crystals (B) (1:2) Azl + Succinic acid co-crystals (C) (2:1) Azl + Succinic acid co-crystals.

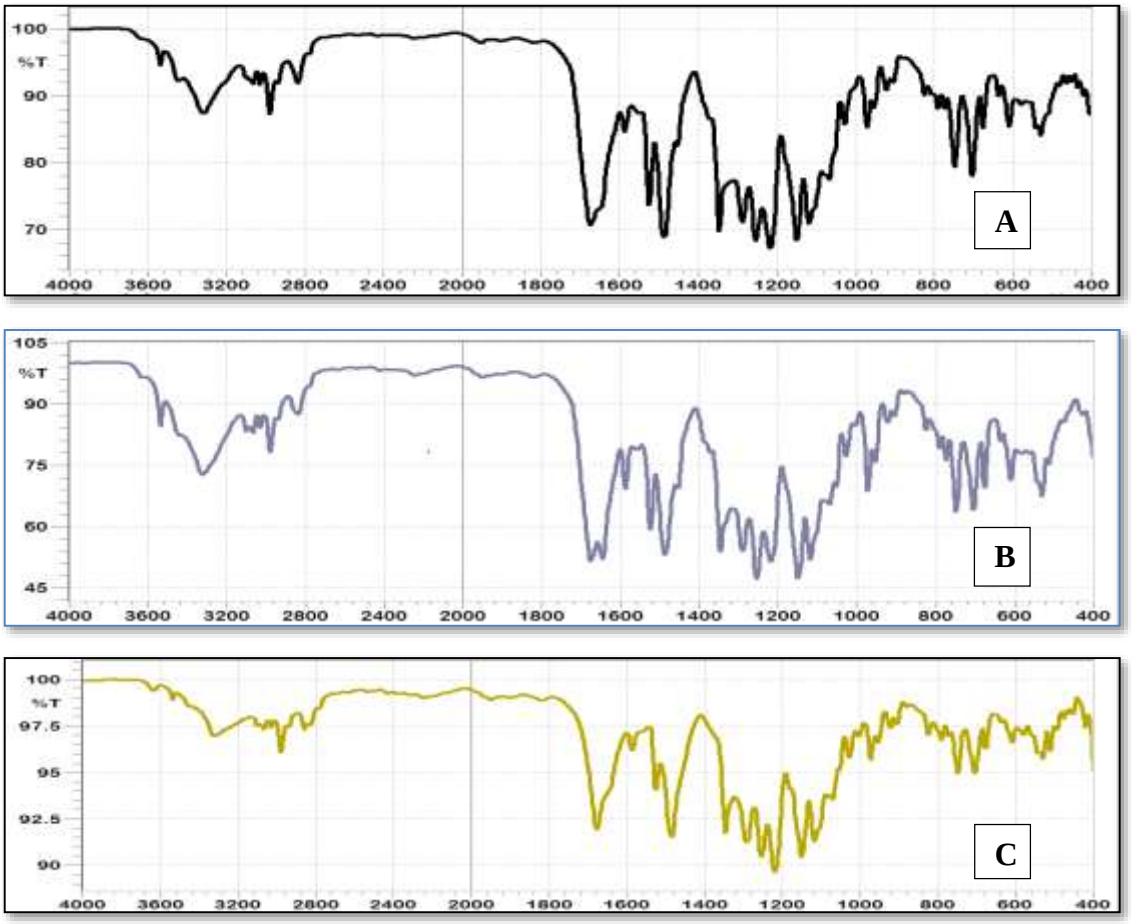


Figure 7: FTIR Spectra of (A) (1:1) Azl + Saccharine co-crystals (B) (1:2) Azl + Saccharine co-crystals (C) (2:1) Azl + Saccharine co-crystals.

Differential scanning Calorimetry: DSC studies are undertaken to observe the co-crystal formation from the difference in the melting point of co-crystal as compared to its own constituent by the endotherm phase. DSC has been used for rapid screening of the co-crystal. The thermogram figure 6 showed the melting point (endotherm phase) of AZL. Individual native AZL showed melting point peaks at 199 (Fig. 8) and the melting points of succinic acid and Saccharine were observed to be 183 °C and 228.8 °C respectively.

The melting points of the AZL and succinic acid co-crystals were found to be 352°C and also at 67°C far different from the native individual drug AZL and succinic acid. The thermogram figure 7 showed the melting point (endotherm phase) of Azel + Succinic acid co-crystals and figure 8 showed the melting point (endotherm phase) of Azel + Saccharine co-crystals.

The melting points of the AZL and succinic acid co-crystals were found to be 298°C and also at 86°C. The melting point peaks for the individual native AZL and succinic acid were not observed in the co-crystals, whereas new intact endothermic peaks were due to the fusion of the co-crystals. Hence the present investigation denotes the formation of co-crystal.

Analysis of Co-crystals: The proposed UV spectrophotometric method was successfully applied for determination of AZL in formulated Co-Crystals.

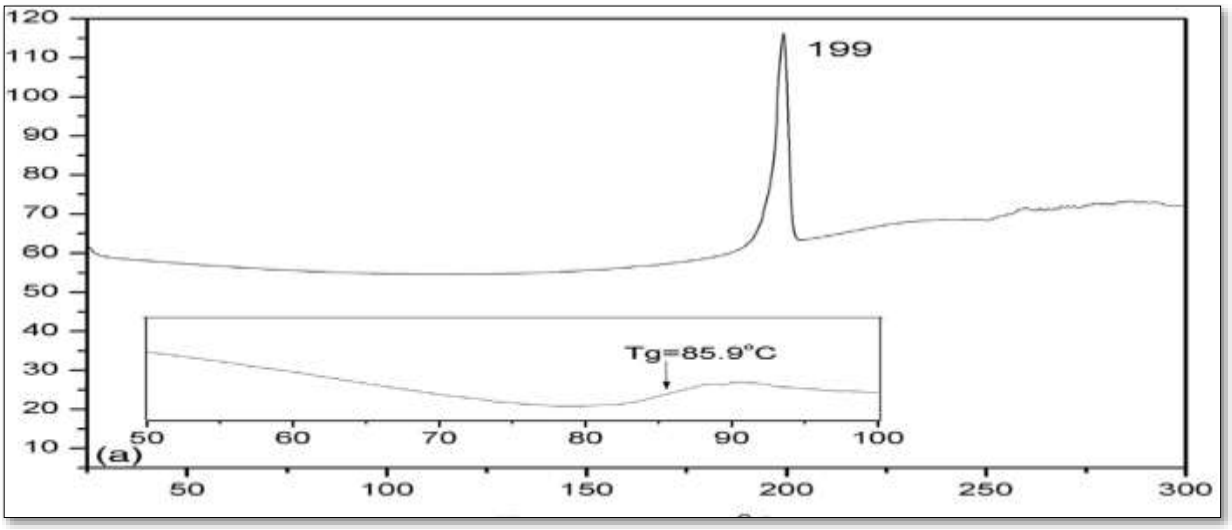


Figure 8: DSC spectra of AZL

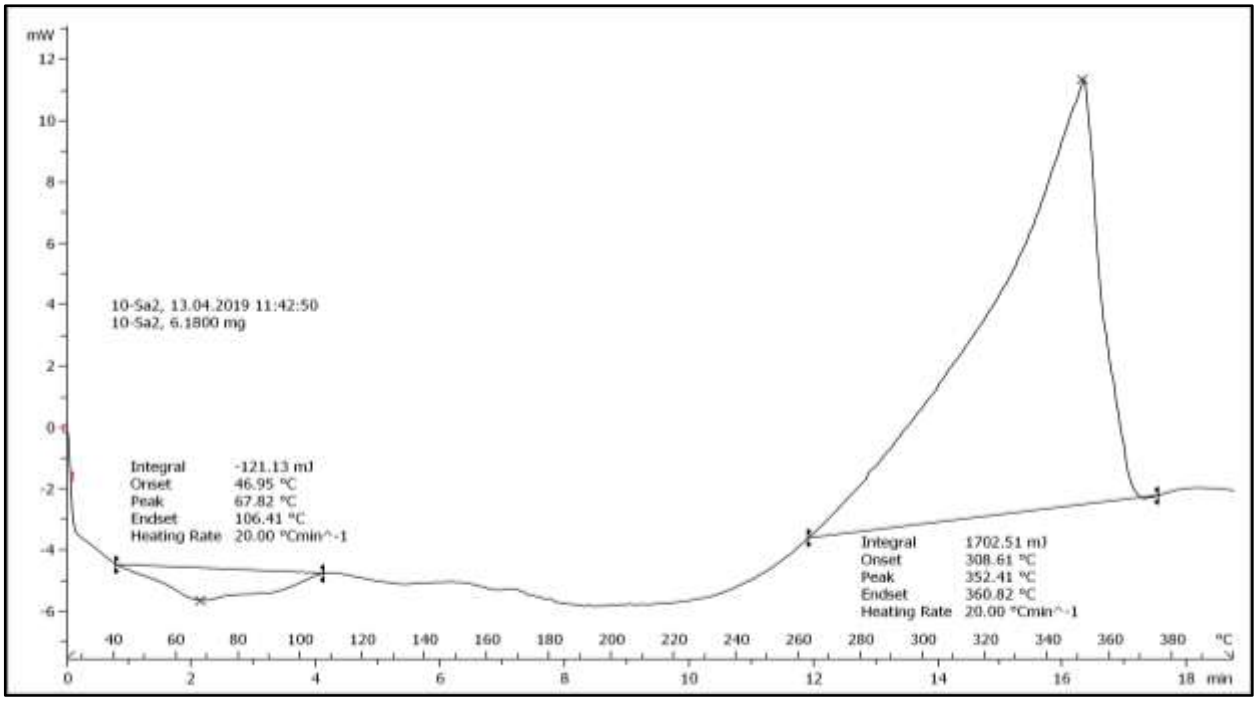


Figure 9: DSC spectra of (1:2) Co-crystal in presence of succinic acid

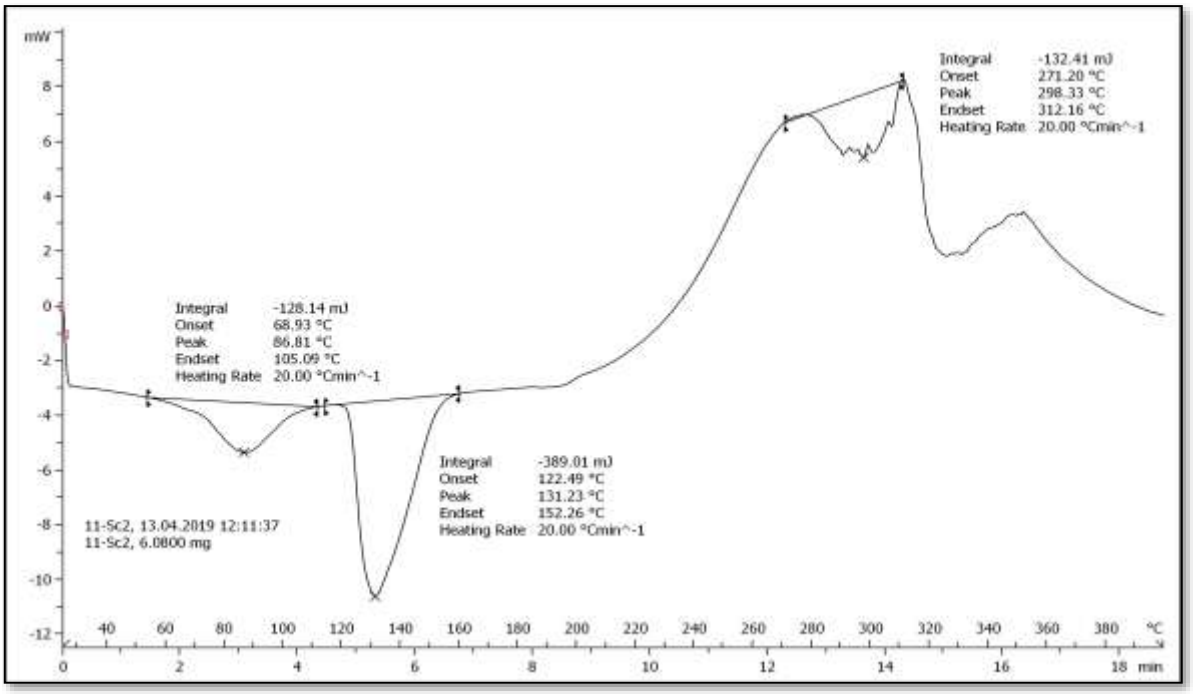


Figure 10: DSC spectra of (1:2) Co-crystal in presence of Saccharine

Table 4
Solubility study of prepared Co-Crystals

Batch	Ratio	Co-former	Solvent	Theoretical value (Azel)	Solubility (mg) solubilized in 20ml	%
Sa1	(1:1)	Succinic acid	Water	10	2.15	21.49
Sa2	(1:2)	Succinic acid	Water	10	4.53	45.26
Sa3	(2:1)	Succinic acid	Water	10	3.17	31.66
Sc1	(1:1)	Saccharine	Water	10	3.10	31.00
Sc2	(1:2)	Saccharine	Water	10	4.20	41.98
Sc3	(2:1)	Saccharine	Water	10	2.80	28.05

Table 5
Analysis data of Co-crystals

Formulation	Co-former	Ratio	Amount present (mg)	% Mean Amount found	SD	%RSD
Co-Crystals	Succinic acid	(1:1)	30	102.9	0.002	0.569
	Succinic acid	(1:2)	30	98.7	0.002	0.436
	Succinic acid	(2:1)	30	95.6	0.001	0.295
	Saccharine	(1:1)	30	104.0	0.002	0.413
	Saccharine	(1:2)	30	100.1	0.004	0.988
	Saccharine	(2:1)	30	97.1	0.004	1.174
	Benzoic acid	(1:1)	30	90.7	0.002	0.623
	Benzoic acid	(1:2)	30	95.2	0.002	0.453

Conclusion

The purpose of study was to prepare co-crystals of AZL to enhance solubility and method validation of AZL.Co-crystals were prepared by solvent evaporation method by use of three different co-crystal formers which included saccharin, succinic acid and benzoic acid. % yield was found for molar ratio (1:1) with 95%, (1:2) with 90%, (2:1) with 98.9%. The solubility significantly increased by preparation of co-crystal, co-crystal using succinic acid as co-crystal formers (molar ratio 1:2) gave maximum solubility than two other molar ratio and also the co-crystals using saccharine as a co-former with molar ratio (1:2) gave maximum solubility than two molar ratio. The solid state property was characterized by microscopy, differential scanning calorimetry (DSC) and Fourier transfer infrared spectroscopy (FTIR), The microscopy, FTIR and DSC and studies of co-crystals confirmed the formation of co-crystals.

IN characterization of co-crystals observed some new characteristics peaks of FTIR from DSC. It shows the change in their crystalline property and melting point due to new peaks which show the interactions between the drug and co-formers and formation of co-crystals. From the study carried out, it was concluded that stable co-crystals of AZL were successfully prepared with increased solubility.The developed method was validated as per ICH guideline for their accuracy, linearity, precision, robustness, limit of detection and limit of quantification etc. was done. The method was shown to be specified, linear, precise and accurate and can be used for routine quality control in laboratories and in formulations without interference.

References

1. Aitipamula S., Chow P.S. and Tan R.B., Polymorphism in cocrystals: a review and assessment of its significance, *Cryst Eng Comm*, **16**, 3451-65 **(2014)**

2. Babu N.J. and Nangia A., Solubility advantage of amorphous drugs and pharmaceutical cocrystals, *Crystal Growth & Design*, **11**, 2662-79 **(2011)**

3. Bagde S.A., Upadhye K.P., Dixit G.R. and Bakhle S.S., Formulation and Evaluation of Co-Crystals of Poorly Water Soluble Drug, *International Journal of Pharmaceutical Research*, **7**, 4988-97 **(2016)**

4. Chauhan Nidhi, Mandale Drashti and Mistry Rujuta, An analytical apporoch of AZL: A review, *World Journal of Pharmacy & Pharmaceautical Sciences*, **10**, 682-692 **(2021)**

5. Duggirala N.K., Perry M.L., Almarsson Ö. and Zaworotko M.J., Pharmaceutical co-crystals: along the path to improved medicines, *Chemical Communications*, **52**, 640-55 **(2016)**

6. Gadade D.D. and Pekamwar S.S., Pharmaceutical cocrystals: regulatory and strategic aspects, design and development, *Advanced Pharmaceutical Bulletin*, **6**, 479 **(2016)**

7. Good D.J. and Rodriguez-Hornedo N., Solubility advantage of pharmaceutical co-crystals, *Crystal Growth and Design*, **9**, 2252-64 **(2009)**

8. Kotak U., Prajapati V., Solanki H., Jani G. and Jha P., Co-crystallization technique its rationale and recent progress, *World J Pharm Sci*, **4**, 1484-508 **(2015)**

9. Lachman L., Lieberman H.A. and Kanig J.L., The theory and practice of industrial pharmacy, Philadelphia, Lea & Febiger **(1976)**

10. Mohd Y., Mohd A., Kumar A. and Aggarval A., Biopharmaceutical Classification System: An Account, *International Journal of Pharm Tech Research*, **2**, 1681-1690 **(2010)**

11. Nalawade P., Patil S., Mote U., Haval P., Gatade S. and Phuke V., Pharmaceutical Co-Crystals: A Novel Approach and Techniques for Solubility Enhancement, *European Journal of Pharmaceutical and Medical Research*, **4**, 376-384 **(2017)**

12. Nalte Y., Nalte Y.K., Arsul V.A., Shep S.G. and Sunil Bothara D.B., Solubility enhancement of nevirapine by co-crystallisation technique, *J. Pharm. Res*., **99**, 556-61 **(2015)**

13. Nijhawan M., Babu P. and Subrahmanyam C., Co-crystals of Irbesartan with Hippuric acid, *Indo American Journal of Pharmaceutical Research*, **5**, 1323-1329 **(2015)**

14. Panzade P., Shendarkar G. and Rathi B., Pharmaceutical Co-Crystal of Piroxicam: dseign, formulation and Evaluation, *Advanced Pharmaceutical Bulletin*, **7**, 399-408 **(2017)**

15. Panzade P., Shendarkar G., Shaikh S. and Rathi P.B., Pharmaceutical cocrystal of piroxicam: Design, formulation and evaluation, *Advanced Pharmaceutical Bulletin*, **7**, 399 **(2017)**

16. Patel D.M., Shah H.R., Patel R.J. and Patel C.N., Preparation and characterization of lornoxicam co-crystals, *World Journal of Pharmacy and Pharmaceutical Sciences*, **3**, 713-32 **(2014)**

17. Patole T. and Deshpande A., Co-crystallization-a technique for solubility enhancement, *Int J Pharm Sci Res*, **5**, 3566 **(2014)**

18. Raskapur K.D., Patel M.M. and Captain A.D., UV-Spectrophotometric method development and validation for determination of Azelnidipine in pharmaceutical dosage form, *Toxicology*, **106**,135-43 **(2010)**

19. Sarangi M.K., A Brief Overview on Co-Crystals and Their Pharmaceutical Applications, *Chem Inform*, **47(13) (2016)**

20. Savjani J.K., Co-crystallization: An approach to improve the performance characteristics of active pharmaceutical ingredients, *Asian Journal of Pharmaceutics*, **9**, 147-51 **(2015)**

21. Savjani K.T., Gajjar A.K. and Savjani J.K., Drug solubility: importance and enhancement techniques, *ISRN Pharmaceutics*, doi:10.5402/2012/195727 **(2012)**

22. Schultheiss N. and Newman A., Pharmaceutical cocrystals and their physicochemical properties, *Crystal Growth and Design*, **9**, 2950-67 **(2009)**

23. Sharma D., Soni M., Kumar S. and Gupta G.D., Solubility enhancement–eminent role in poorly soluble drugs, *Research Journal of Pharmacy and Technology*, **2**, 220-4 **(2009)**

24. Sopyan I., Fudholi A., Muchtaridi M. and Sari I.P., Co-crystallization: A Tool to Enhance Solubility and Dissolution Rate of Simvastatin, *Journal of Young Pharmacists*, **9(2)**,183-186 **(2017)**

25. Swapna B., Maddileti D. and Nangia A., Cocrystals of the tuberculosis drug isoniazid: polymorphism, isostructurality and stability, *Crystal Growth & Design*, **14**, 5991-6005 **(2014)**.

(Received 16th January 2022, accepted 15th March 2022)