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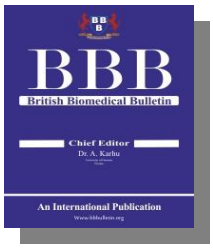
BRITISH BIOMEDICAL BULLETIN



Original

Development of Ezogabine Co Crystal Formation: An Efficient Approach to Enhance Aqueous Solubility

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ARTICLE INFO

Received 25 July 2015
Received in revised form 07 Aug. 2015
Accepted 14 Aug. 2015

Keywords:
Ezogabine,
Co crystal,
DSC,
XRPD,
FTIR,
Solubility.

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ABSTRACT

Purpose: The bioavailability of a development candidate active pharmaceutical ingredient (API) was very low after oral dosing. In order to improve bioavailability, we sought to increase the dissolution rate of the solid form of the API.

Methods: A crystal engineering approach was used to design, develop a co crystal of the API. Hydrogen bonding between the API and carboxylic acids were used as a coformer for associating multiple components in the solid state. A number of carboxylic acid guest molecules were tested for co crystal formation with the API.

Results: A co crystal containing the API and *p*-Amino benzoic acid or Benzoic acid in a 1:5 or 1:3 molecular ratio was identified and the crystal structure is reported. Physical characterization of the co crystal showed that it is unique regarding thermal, spectroscopic, X-ray, and dissolution properties. The co crystal solid is nonhygroscopic, chemically and physically stable to thermal stress. Use of the co crystal increased the aqueous dissolution rate by 11 times as compared to the pure form of the drug.

Conclusions: APIs that are non-ionizable or demonstrate poor salt forming ability traditionally present few opportunities for creating crystalline solid forms with desired physical properties. In this case, a co crystal of Ezogabine with *p*-Amino benzoic acid or Benzoic acid was observed and used to demonstrate an improvement in the solubility and micromeritics properties of the API.

Introduction

Most of the chemical entities that are being discovered are lipophilic and have poor aqueous solubility. Currently number of techniques addresses the problem of poor solubility and dissolution rate of poorly soluble drugs and is usually classified as BCS Class II or Class IV, where solubility is the limiting step for absorption. There are many strategies are available to increase the solubility and bioavailability of BCS Class

II includes particle size reduction, formation of salt, formation of co crystal, inclusion complex with cyclodextrin, amorphization and formation of solid dispersion.[1-3] Solubility is the property of a solid, liquid, or gaseous chemical substance called solute to dissolve in a liquid, or gaseous solvent to form a homogeneous solution of the solute in the solvent.[4] Co-crystals are those that are formed between an active pharmaceutical ingredient (API) and a co-crystal former (CF), which is a solid under ambient conditions, and is not limited to two components. The components of the crystal interact by hydrogen bond or other noncovalent and non-ionic interactions.[5] Co crystals are multi-component molecular crystals; are considered unique solid dosage form has many advantages over other traditionally known solid forms. Researchers demonstrated that through cocrystallization with different co crystal formers, solubility of drug could be greatly modified.[6]

Pharmaceutical co crystals represent a strategic opportunity for the optimization of key physiochemical properties of an API whilst retaining its molecular structure, and hence its physiological activity as there are no making or breaking of the API's covalent bonds.[7] Co crystals distinguishes from other types of multicomponent crystals such as salts and solvates is that drug and coformer are solids at ambient temperature and that the intermolecular interactions are nonionic in nature; the physicochemical properties of the co crystals can vary depending on the characteristics of its constituent molecules.[8] Pharmaceutical co-crystals provide new opportunities to enhance the physic-chemical properties, dissolution rate, and bioavailability of APIs, also create new opportunities for the pharmaceutical companies to address the intellectual property and new patent of APIs for extending their life cycle.[9-10]

Ezogabine (**Figure 1**) is anticonvulsant drug used in treatment of partial epilepsies. It acts as a potassium channel opener by activating a certain voltage-gated potassium channels in the brain.[11]

Materials and Methods

Materials

Ezogabine was a gift sample obtained from M/s Lupin Laboratory Ltd., Aurangabad, Maharashtra, India. *p*-Amino benzoic acid and Benzoic acid (Loba Chemie) were purchased from commercial sources and all other material used were of analytical grade. Microscopic evaluation was observed under Motic BA 210 microscope, drug and synthesized cocrystals were analyzed by Differential Scanning Calorimeter (Detector 60, Mettler-Toledo DSC 821e) over the range of 0-200°C at the rate of 10°C per minute, XRPD spectra were taken on a sample stage PW 1729, Philips, Netherland, the surface characteristic of prepared crystal was studied by SEM on JSM 6360 LV, Joel, Japan, FT-IR spectra were recorded in an Alpha-E Bruker FT-IR spectrophotometer with potassium bromide pellet method and angle of repose, bulk density, tapped density, Hausner's Ratio and Carr's index were determined by standard methods.

Synthesis of co-crystals

Various methods like grinding, ultrasound assisted co-crystallization and solvent evaporation were available for the synthesis of co crystals of *p*-Amino benzoic acid and Benzoic acid with Ezogabine. Solvent evaporation method was selected for the synthesis of co-crystals in the present study.[12-14]

Method of synthesis

Solvent evaporation method

Co-crystals of Ezogabine with *p*-Amino benzoic acid and Benzoic acid

The co-crystals of Ezogabine were prepared by co-former method. Equimolar or different molar quantities of Ezogabine and co-formers such as *p*-Amino benzoic acid or Benzoic acid were dissolved in 20 ml ethanol by keeping in water bath maintained at a temperature at 80°C to obtain clear solution as shown in **Table 1**. The solution was allowed to cool in ice bath for about 5 h for thorough crystallization to occur. The crystals were collected by filtration through a whatman filter paper, dried in air for 24 h and finally stored in desiccators until further investigated by microscopic, melting point, SEM, XRPD and DSC study.[15]

Characterization

The obtained co-crystals were investigated by different techniques; which include:

1. Melting Point (Veego, Mumbai)
2. Solubility of Ezogabine and Co crystals with *p*-Amino benzoic acid and Benzoic acid
3. Microscopic evaluation (Motic, BA-210, Hong Kong)
4. Differential Scanning Calorimetry (Detector 60, Mettler-Toledo DSC 821e, USA)
5. X-ray Powder Diffraction (PW 1729, Philips, Netherland)
6. SEM (JSM 6360 LV, Joel, Japan)
7. FT-IR (Alpha-E Bruker, Germany)

Microscopic Evaluation

Microscopic evaluation was used as a primary investigation tool to confirm the formation of co-crystals visually and to observe the crystal habit of the prepared co-crystals, compared the shape of co-crystals with the pure drug. Crystallization of the pure drug was also carried out in the same solvent which was used for the synthesis of co-crystals; differences in crystal habit of co-crystals with that of pure drugs were in observed.

Solubility of Ezogabine and its Co crystals with *p*-Amino benzoic acid and Benzoic acid

The solubility study was carried out first in distilled water; excess amount of Ezogabine or its co crystals was added in 10 mL distilled water and the bottle was screw capped with stopper. The bottle was kept shaking for about 24 h and then centrifuge for 15 min than filtered and then filtrate 1.2 mL diluted up to 10 mL with distilled water; finally the absorbance of sample was taken at 232nm.[16]

Differential Scanning Calorimetric (DSC)

DSC analysis is a thermo analytical tequnique used to identify the difference in the amount of heat required to increase the temperature of a sample and reference as a function of temperature. DSC, thermo analysis gave characteristic and comparable results for the pure drug Ezogabine and the synthesized co-crystals as shown in **Figure 2 D** and **E**.[17]

X-Ray Powder Diffraction (XRPD)

X-ray powder diffraction was done for the pure drug Ezogabine and prepared co-crystals; result reveals the information about the crystal structure, physical properties of the material as shown in **Figure 3**.[18]

Scanning Electron Microscopy (SEM)

The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode. SEM analysis has

been performed for the pure drug Ezogabine and co-crystals of Ezogabine with *p*-Amino benzoic acid or Benzoic acid as shown in **Figure 4**.[18]

Fourier Transform Infrared (FT-IR)

Fourier Transform Infrared (FT-IR) spectra were recorded for the Ezogabine, co-former *p*-Amino benzoic acid or Benzoic acid and co-crystals of Ezogabine with *p*-Amino benzoic acid or Benzoic acid as shown in **Figure 5**.[19]

Study of micromeritics properties of Ezogabine and its co-crystals

Angle of repose of Ezogabine, its co-crystal with *p*-Amino benzoic acid and Benzoic acid

The frictional force in powder can be measured by the angle of repose. It is the maximum angle possible between the surface of pile of powder and the horizontal plane. The blend that has angle of repose in between 20^0-30^0 is best for compression as it has good flow property. Angle of repose was calculated by fixed funnel method, in which funnel was fixed to a stand in such a way that the lower tip of funnel was 2.5 cm above the surface. A graph paper was placed on the flat surface. The blend was allowed to fall freely on the graph paper through the funnel, till the tip of heap formed just touched the funnel. The radius of heap was noted and from this angle of repose was determined using following equation and are depicted in **Table 2.**

$\theta = \tan^{-1}(h/r)$

Where, h = height of pile r = radius of pile

Bulk density and Tapped density

Bulk density was determined by pouring preweighed and presieved bulk drug into a graduated cylinder via a large funnel and the volume was measured and recorded as bulk volume. The cylinder was tapped until powder bed volume reached a minimum volume and the volume was recorded as tapped volume. The bulk density and tapped density were calculated using following equation and shown in **Table 3**.

Bulk density = Mass / Bulk volume and Tapped density = Mass / Tapped volume

Hausner's Ratio

Hausner's found that the ratio of tapped density/bulk density was related to inter particle friction as such, and could be used to predict powder flow properties. Hausner's ratio shows that the powders with low inter particle friction had ratio of approximately 1.2, whereas more cohesive less free flowing powders have Hausner's ratio greater than 1.6. Hausner's ratio less than 1.25 indicates good flow. It is the ratio of tapped density to the bulk density. It is given by the formula.

Hausner's ratio = Tapped density / Bulk density

Carr's index

This property is also known as percent compressibility, indirectly related to the flow rate, cohesiveness and particle size. Compressibility is the ability of powder to decrease in volume under pressure, is obtained from density determinations. The compressibility index of the powder was determined by Carr's compressibility index. It is simple, fast and accurate method of predicting powder flow characteristics. It is given by the formula,

Carr's Index of Ezogabine

Carr's Index = Tapped Density – Bulk Density x 100

Carr's index is the measure of the potential strength that the powder could build up in its arch in a hopper and also the ease with which such an arch could be broken.

In vitro dissolution study

UV-VIS measurement

The samples were analyzed on UV spectrophotometeter; the absorbance was recorded at 232nm against a dissolution medium as a blank. *In vitro* dissolution study of co-crystal of Ezogabine with *p*-Amino benzoic acid or Benzoic acid offers a convenient and inexpensive means of predicting absorption and bioavailability of co crystal of Ezogabine with *p*-Amino benzoic acid or Benzoic acid. The release profile of co crystal predicts how a delivery system might function and gives valuable insight into its *in vivo* behavior. *In vitro* release profile for co crystals as well as pure drug was performed using USP XXIV type II (paddle) dissolution apparatus. Took co crystal sample equivalent to 400 mg of Ezogabine was added to 1000 ml 0.01N HCl solution at 37± 0.5ºC and stirred at 75 rpm. Aliquot of 5 ml was withdrawn at time intervals of 5, 10, 15, 20, 25, and 30 min. The withdrawn volume was replaced with the same volume of dissolution medium in order to keep the total volume constant. The absorbance of the samples was measured at λ max 232nm as shown in **Table 4 and Figure 6**.[20]

Discussion

Synthesis of co-crystals of Ezogabine with *p*-Amino benzoic acid or Benzoic acid was carried out by solvent evaporation method. Different solvents were employed for the synthesis of co-crystals but ethanol was proved to be the best. Other solvents suffered from drawbacks like upon inspecting visually the crystal pattern of co-crystals and comparing them with crystals of pure drugs using Optical microscope Motic, BA-210. Microscopic analysis of prepared co-crystals of Ezogabine with *p*-Amino benzoic acid or Benzoic acid reveals visual difference between the co-crystals and pure drugs Ezogabine. In Aqueous solubility study of Ezogabine and its Co crystals result reveal that Ezogabine 17.46 µg/mL, Ezogabine co-crystal with *p*-Amino benzoic acid (1:5)192.25µg/mL and Ezogabine co-crystal with Benzoic acid (1:3): 188.88µg/mL, which suggest 10-11 fold improvement in solubility of co crystals than pure drug. The DSC data of the co-crystals reveals single sharp endotherms at 112.57 as shown in **Figure 2 D**, and 173.38 °C as shown in **Figure 2 E**, for products **A** (Co crystals of Ezogabine with *p*-Amino benzoic acid) and **B** (Co crystals of Ezogabine with Benzoic acid) respectively. These endotherms, which correspond to the melting point of the solids, occur at significantly different temperature to those of Ezogabine (143.90°C) or *p*-Amino benzoic acid (193.24°C) and Benzoic acid (136.22°C), indicating the formation of stable co crystals and not simple physical mixtures. The co-crystals described here showed large variation in melting temperature from that of Ezogabine, suggesting that the cohesive energy of co crystals **D** and **E** is decreased and increased from that of pure Ezogabine form. In analysis of the X-ray Powder Diffraction data of the polycrystalline materials arising from solvent evaporation experiments reveals that for co-crystal formation of Ezogabine with *p*-Amino benzoic acid or Benzoic acid, reflections arising from the starting materials are absent, indicate and support the presence of new phase when they were taken in proportion of 1:5 for co-crystals of Ezogabine with *p*-Amino benzoic acid and 1:3 for co-crystals of Ezogabine and Benzoic acid (**Figure 3 A**, **B** and **C**). In

SEM results reveals that a pure drug Ezogabine exhibited irregular shape with smooth surface and the co-crystals of Ezogabine with *p*-Amino benzoic acid or Benzoic acid also exhibited irregular shape but the shape was different with those of the pure drug as shown in **Figure 4**.In FT-IR spectroscopy for Ezogabine and its co crystals shows various FT-IR frequency bands are present in spectra's, which reveals that drug is compatible with co formers as well as formation of co crystals as shown in **Figure 5**. Also in micromeritics property evaluation and dissolution study for Ezogabine and co-crystals of Ezogabine with *p*-Amino benzoic acid or Benzoic acid results as shown in **Table 3** and **4**, indicate improvement than pure drug, which is essential for development of formulations.

Conclusion

In the present study demonstrate low solubility drug Ezogabine has improved its solubility as well release profile along with improved micromeritics properties upon formation co crystals with *p*-Amino benzoic acid or Benzoic acid. Synthesis of co crystals of Ezogabine with *p*-Amino benzoic acid or Benzoic acid was established by advance techniques like DSC, XRPD, SEM and FT-IR.

Acknowledgment

The authors thank to Shri. Prashant Patil Gadakh, President, Mula Education Society's, Sonai and Dr. V. K. Deshmukh, Principal, MES`s College of Pharmacy, Sonai for providing all laboratory facilities, Mr.Hemant Kansagra, Manager, Biodeal Pharmaceuticals Pvt.Ltd.,for gift sample of Ezogabine, UDCT Dr. BAMU, Aurangabad for recording FT-IR Spectra, Government College of Pharmacy, Aurangabad for recording DSC, Diya Labs, Mumbai for recording XRPD.

References

1. Choudhary NH, Kumbhar MS, Dighe DA, Mujgond PS and Singh MC.Solubility enhancement of escitalopram oxalate using hydrotrope., *International Journal of Pharmacy and Pharmaceutical Sciences*., 2013,5(1),121-125.
2. Siok-Yee C, Yin-Ying C, Xin-Zi C, Eryn Yen-Ling T and Joan Q. The characterization and dissolution performances of spray dried solid dispersion of ketoprofen in hydrophilic carriers., *Asian Journal o f Pharmaceutical Sciences* xxx (2015),1-14.In press.
3. Maleki Dizaj S, Vazifehasl Zh, Salatin S, Adibkia Kh and Javadzadeh Y. Nanosizing of drugs: Effect on dissolution rate., *Research in Pharmaceutical Sciences*., 2015,10(2),95-108.
4. Lachman L, Lieberman H, and Kanig JL. The Theory and practice of industrial pharmacy, Lea & Febiger, 3rd edition, 1986.
5. Altaf AN and Yasser A. Pharmaceutical co-crystals: A new paradigm of crystal engineering., *Journal of the Indian Institute of Science*., 2014,94(1),45-67.
6. Bhupinder Singh S.Nutraceutical co crystals: An overview., RGUHS J Pharm Sci., 2012,2 (2).
7. Nizar I. Towards more efficient screening of pharmaceutical co crystals., Ph.D. thesis submitted to the University of London.2011,1-232.
8. Neal CH. Engineering co crystal solubility and stability via ionization and micellar solubilization., Ph.D. thesis submitted to the University of Michigan., 2011,1-260.
9. Hong-Liang L, Po-Chun H and Shan-YL. Theophylline-citric acid co-crystals easily induced by DSC-FTIR micro spectroscopy or different storage conditions., *Asian Journal of Pharmaceutical sciences*., 2013, 8,19-27.
10. Thakkar H, Patel B. and Thakkar S. A review on techniques for oral bioavailability enhancement of drugs., *International Journal of Pharmaceutical Sciences Review and Research*., 2010,4(3),203-223.
11. Celene MA and Arvind V. Ezogabine: A novel antiepileptic for adjunctive treatment

of partial-onset seizures., *Pharmacotherapy*., 2013,33(2),187-194.
12. Sreya M. Crystal engineering of pharmaceutical co crystals, *Graduate thesis. University of South Florid.,*2011.
13. Kermit GD. The Use of *p*-amino bemzoic acid in amebiasis: Preliminry report, *Bull N Y Acad Med.,* 1948,24(6),391–393.
14. Amended final safety assessment benzyl alcohol, and benzoic acid and its salts and benzyl ester, October 17, 2011.
15. Sevukarajan M, Thanuja B, Riyaz S, and Rahul Nair. Synthesis and characterization of a pharmaceutical co-crystal: (Aceclofenac: Nicotinamide), *J. Pharm. Sci. & Res*.,2011,3(6), 1288-1293.
16. Cheney ML, Weyna DR, Shan N, Hanna M, Wojtas L and Zaworotko MJ. Supramolecular architectures of meloxicam carboxylic acid co-crystals, a crystal engineering case study. Crystal Growth Des., 2010,10(10),4401-4413.
17. McNamara DP, Scott LC, Jennifer G, Anthony I, James C, Manjunath SS, Richard M, Ed O'Donnell, and Aeri P. Use of a glutaric acid cocrystal to improve oral bioavailability of a low solubility API, *Pharmaceutical Research*., 2006, 23(8), 1888-1897.
18. Suresh Kumar GS, Seethalakshmi PG, Sumathi D, Bhuvanesh N and Kumaresan S. Syntheses, structural characterization, and DPPH radical scavenging activity of co crystals of caffeine with 1- and 2-naphthoxyacetic acids, *Journal of Molecular Structure*.,2013, 1035,476-482.
19. Laszlo B. and Jhon KH. Cocrystal polymorphism of pharmaceuticals, *Acta Pharm. Jogosl*., 1990,40,71-94.
20. Pritish K, Sunil D, Inayat Bashir P and Harsha S. Preparation of spherical crystal agglomerates via crystallo-co-agglomeration technique, *Digest Journal of Nanomaterials and biostructures*., 2012,7(3),1223-1236.

Table 1. Preparation of Co-crystals of Ezogabine with *p*-Amino benzoic acid and Benzoic acid (1:1to1:5)

Sr. No.	Ratio	Ezogabine with PABA in gm	Ezogabine with Benzoic acid in gm
1	1:1	0.15 and 0.067	0.15 and 0.061
2	1:2	0.15 and 0.135	0.15 and 0.120
3	1:3	0.15 and 0.203	0.15 and 0.181
4	1:4	0.15 and 0.271	0.15 and 0.241
5	1:5	0.15 and 0.339	0.15 and 0.301

Table 2. Angle of repose of Ezogabine, its co-crystal with *p*-Amino benzoic acid and Benzoic acid

	Ezogabine	Ezogabine Co crystal with	
		p-Amino benzoic acid (1:5)	Benzoic acid (1:3)
h	2.8cm	2 cm	2 cm
r	3 cm	4.4 cm	4.3cm
tan θ^{-1}	42.93	27.21	27.71
Type of flow	Very Poor	Good	Good
Angle of flow: < 25- Excellent, 25 – 30 Good, 30 – 40- Passable and > 40- Very Poor			

Table 3. Bulk density, Tapped density, Carr's index and Hausner's ratio of Ezogabine, its co-crystal with *p*-Amino benzoic acid and Benzoic acid

Parameter	Ezogabine	Ezogabine Co crystal with	
		p-Amino benzoic acid (1:5)	Benzoic acid (1:3)
Mass	10 gm	10 gm	10 gm
Bulk volume	18vol	14 vol	14vol
Bulk density	0.62gm/cc.	0.76gm/cc.	0. 76gm/cc.
Tapped volume	12vol	12 vol	12 vol
Tapped density	0.83gm/cc.	0. 83gm/cc.	0. 83gm/cc.
Hausner's Ratio	1.33	1.09	1.09
Carr's index	25.30%	8.43%	8.43%
% Compressibility-5 to 15- Excellent,12 to 16- Good,18 to 21- Fair,23 to 28- Poor,35 to 38- Very poor and > 40- Extremely poor			

Table 4. Dissolution studies of Ezogabine co-crystal with *p*-Amino benzoic acid and Benzoic acid

Sr.No.	Time (min)	% Cumulative drug release									
		Ezogabine	Ratio of Ezogabine : *p*-Amino benzoic acid (1:5)					Ratio of Ezogabine : Benzoic acid (1:3)			
			1:1	1:2	1:3	1:4	1:5	1:1	1:2	1:3	1:4	1:5
1	0	0	0	0	0	0	0	0	0	0	0	0
2	5	09.62	16.25	22.70	11.33	23.33	40.34	11.14	15.45	38.00	10.32	18.33
3	10	11.38	22.45	30.95	15.74	31.12	48.56	13.27	20.27	45.71	16.25	24.33
4	15	14.66	29.14	36.87	21.54	35.51	56.22	18.33	25.52	53.65	24.54	31.36
5	20	19.41	34.85	40.52	27.17	39.22	64.55	23.33	30.64	61.70	30.69	37.47
6	25	22.50	41.36	44.61	31.12	42.14	78.74	27.63	35.98	75.33	34.16	42.32
7	30	25.54	51.25	48.33	37.45	44.30	89.69	33.47	38.12	82.82	41.54	46.12

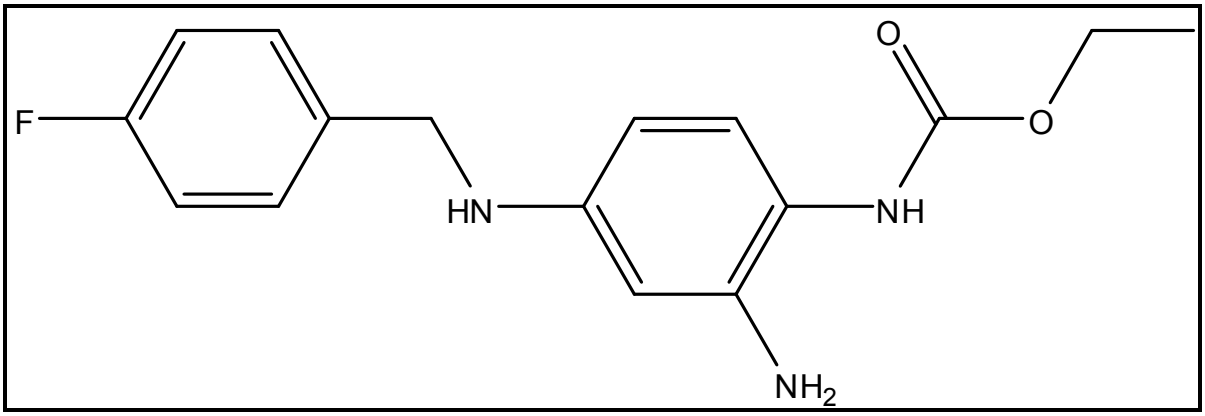


Figure 1. Chemical structure of Ezogabine

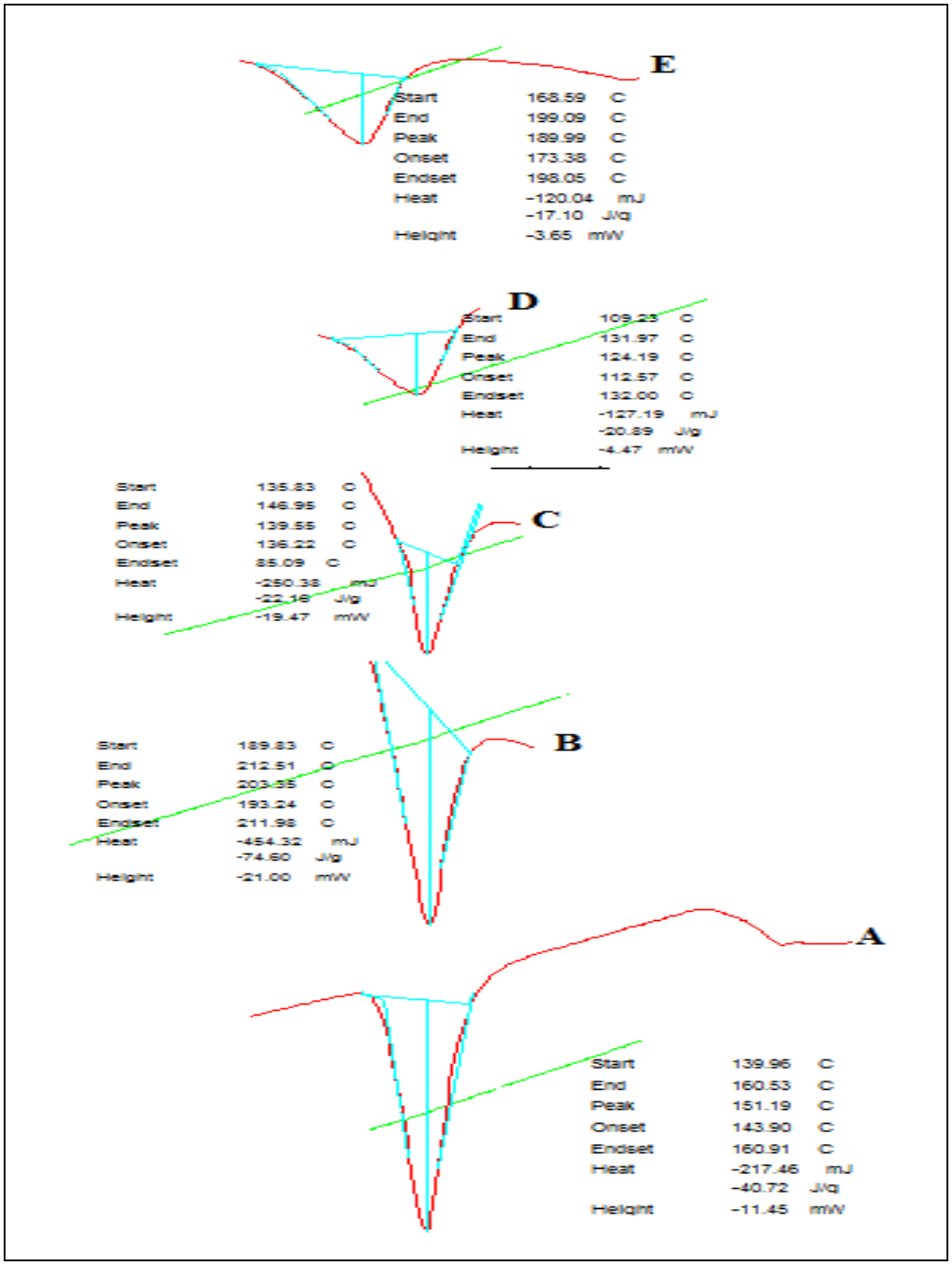


Figure 2. Differential Scanning Calorimetric analysis of Ezogabine A, *p*-Amino benzoic acid B, Benzoic acid C, Co-crystals of Ezogabine with *p*-Amino benzoic acid D and Co-crystals of Ezogabine with Benzoic acid E

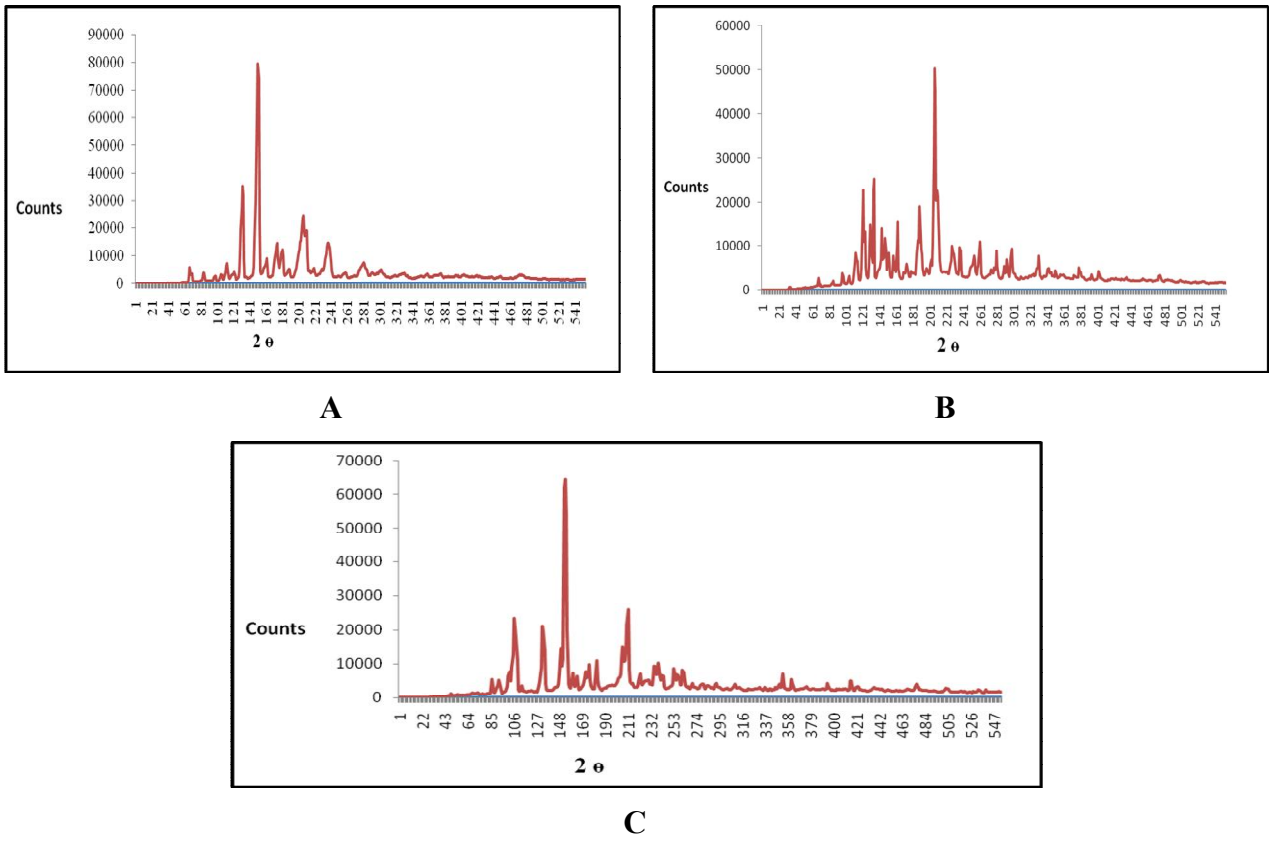
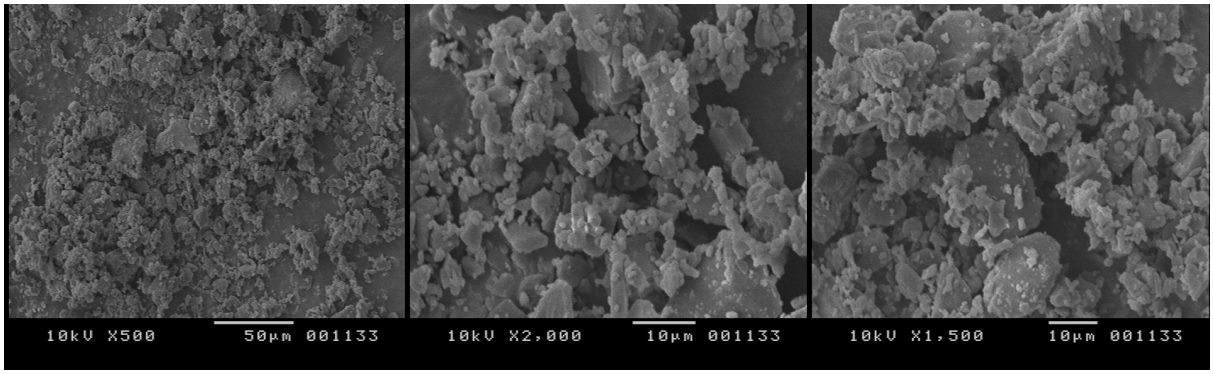
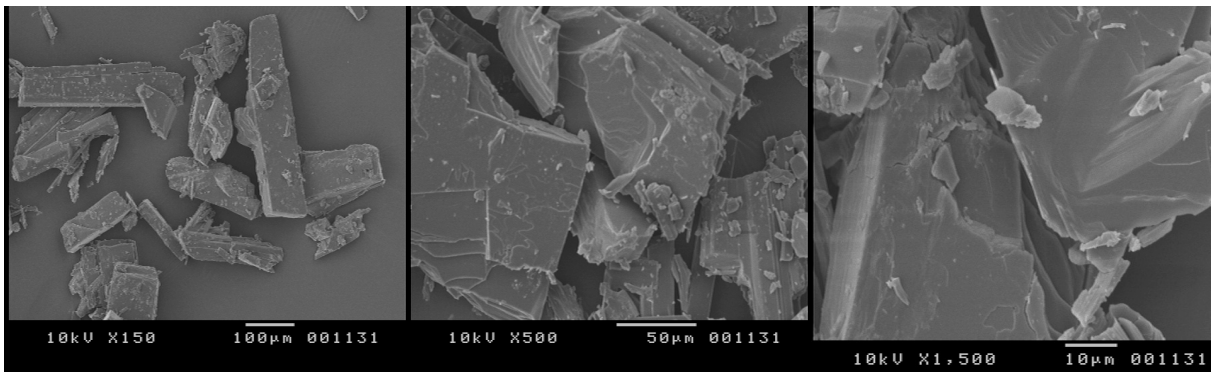


Figure 3. XRPD spectra of Ezogabine A, Co-crystals of Ezogabine with *p*-Amino benzoic acid B and Co-crystals of Ezogabine with Benzoic acid C



A



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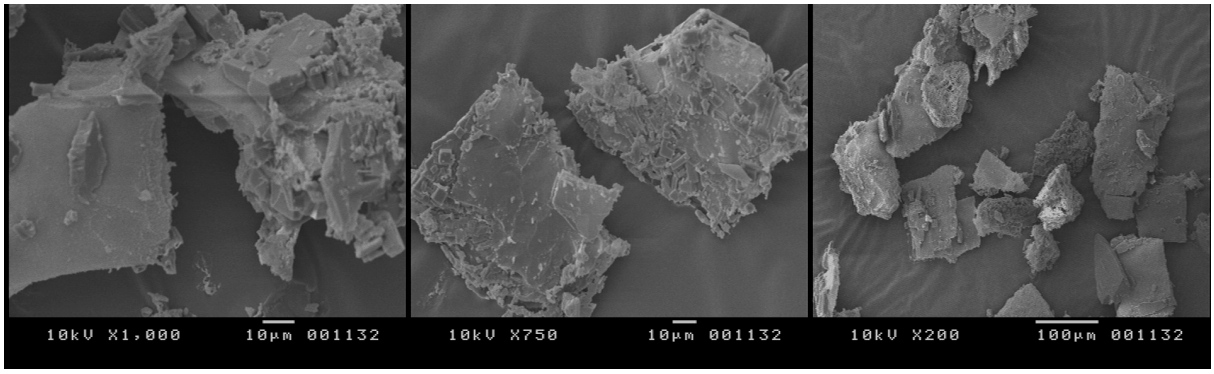


C

Figure 4. SEM images for Ezogabine A, co-crystals of Ezogabine with *p*-Amino benzoic acid B and Ezogabine with Benzoic acid C

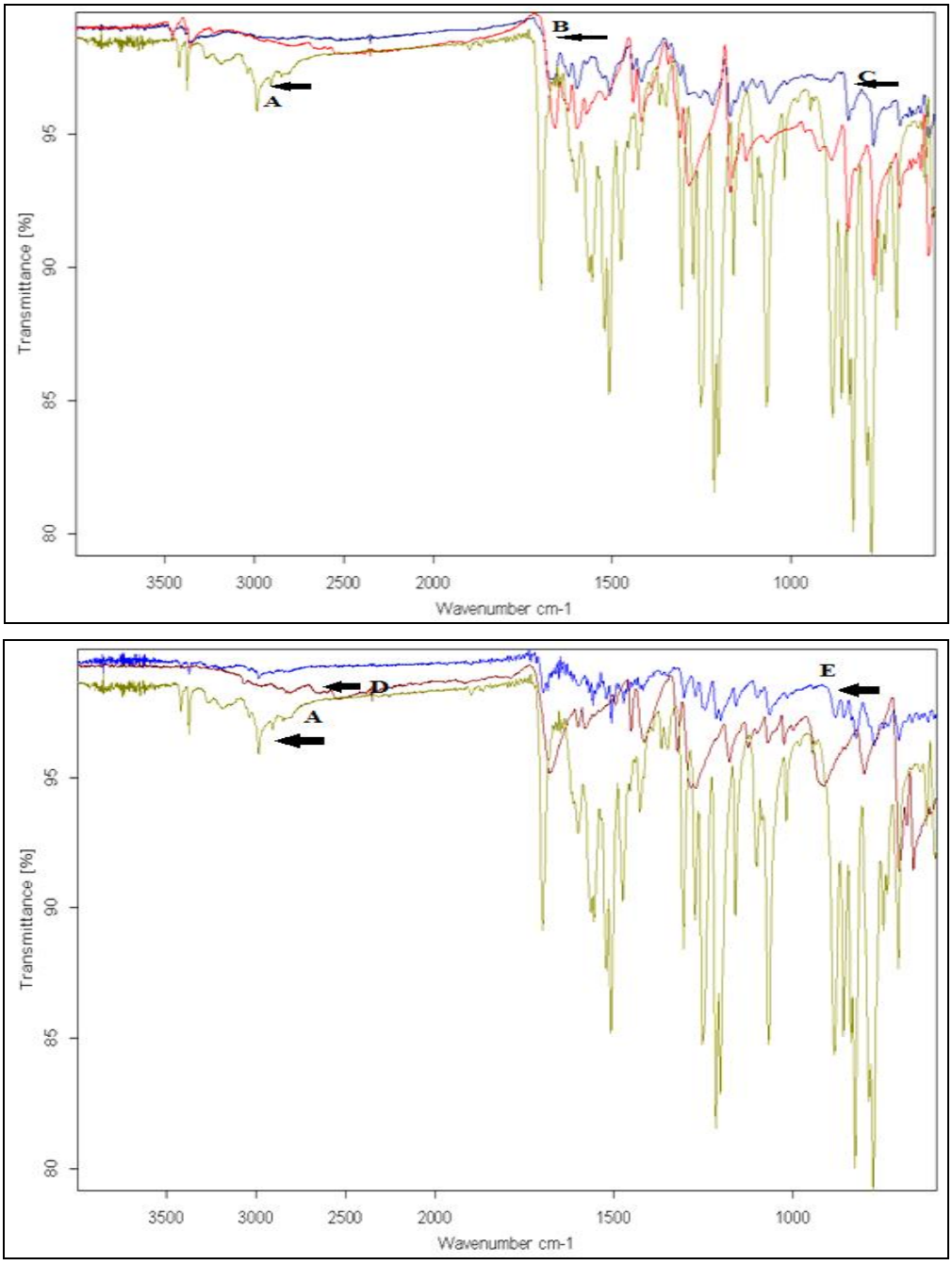
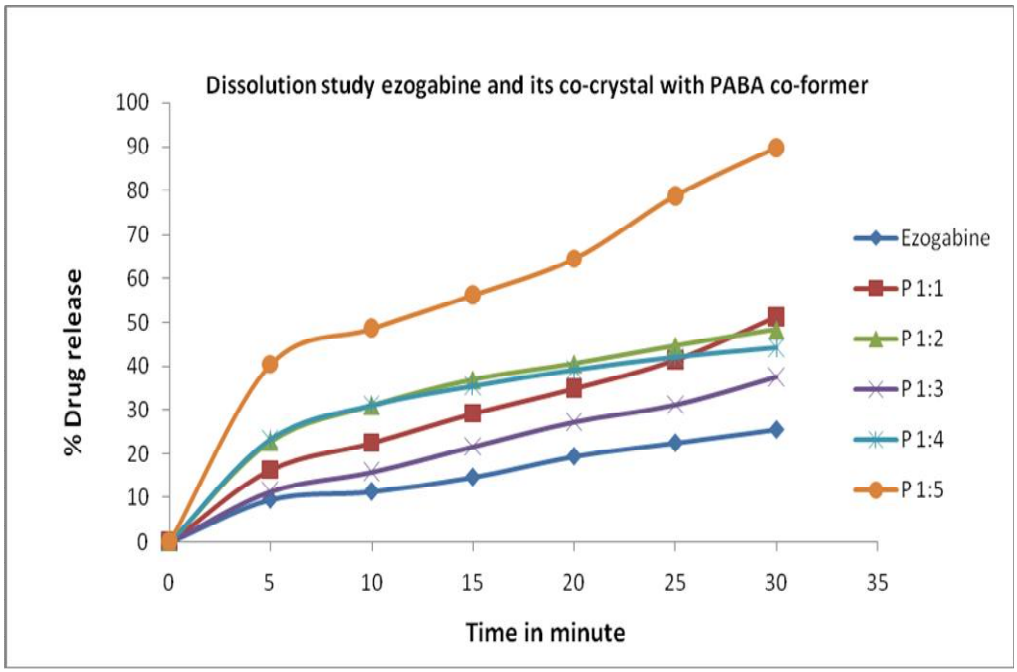


Figure 5. FT-IR spectra for Ezogabine A, *p*-Amino benzoic acid B, co-crystals of Ezogabine with *p*-Amino benzoic acid C, Benzoic acid D and Co-crystals of Ezogabine with Benzoic acid E



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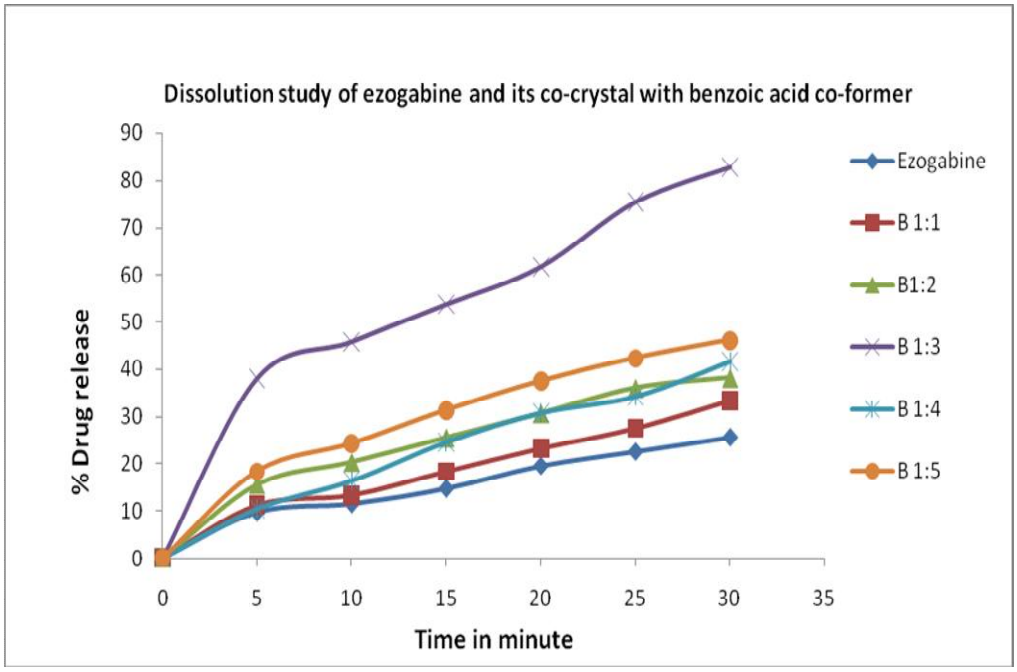

B

Figure 6. *In vitro* dissolution study of Ezogabine co-crystals with *p*-Amino benzoic acid A and Benzoic acid B