Research Article

[Chandel et al., 2(8): Aug., 2011]

ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Co-crystalization of aceclofenac and paracetamol and their characterization

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Abstract

The increase in the aqueous solubility and physicochemical property of insoluble and slightly soluble drugs is of major concern in pharmaceutical formulations. It is commonly recognized in the pharmaceutical industry that on average more than 40% of newly discovered drug candidates are poorly water-soluble. Poor "drug like" properties of lead compounds led to ineffective absorption from the site of administration, which has been designated as an important part of the high clinical failure due to poor pharmacokinetics. In the present work, aceclofenac, a non steroidal anti-inflammatory agent of phenyl acetic acid group, which possesses remarkable antiinflammatory, analgesic and antipyretic properties is selected as a model drug which is BCS class II drug (highly permeable and low soluble). Aceclofenac exhibits slight solubility in water and as a consequence it exhibits low bioavailability after oral administration.

Key-Words: Co-crystallization, Solubility, Drug, Characterization

Introduction

Co-crystals incorporate pharmaceutically acceptable guest molecules into a crystal lattice along with the API. Co-crystals have regained attention as attractive alternate solid forms for drug development. Physiochemical properties of pharmaceuticals can be improved by obtaining co-crystals using co-crystallization.[1] Co-crystallization with pharmaceutically acceptable (GRAS) compounds does not affect pharmacological activity of API but can improve physical properties, such as solubility, hygroscopicity, compaction behavior.[2] Cocrystals with the same active pharmaceutical ingredient will have strikingly different pharmaceutical properties (melting point, solubility, dissolution, bioavailability, moisture uptake, chemical stability, etc.) depending on the nature of the second component. Paracetamol Paracetamol is is a non-steroidal anti-inflammatory drug It is commonly used for the relief of headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies having very slightly soluble in cold water.

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The ultimate objective of any research done in the field of pharmaceuticals is to serve the society's needs by developing a formulation that is highly efficient and most effective. For the research to be successful, the work to be done should be logically and properly based upon the literature surveyed. The literature review on 'Co crystal' reveals that most of the research work has been done to elucidate the mechanism of co crystallization. Also, some research work has been carried out in this field to develop the aqueous solubility of few poorly water-soluble drugs using 'co crystallization' technique. Co-crystals having advantages like stable crystalline form (as compared to amorphous solids), no need to make or break covalent bonds, theoretical capability of all types of API molecules.[3-4]

The purpose of this investigation is to explore the possibility of employing "Co crystallization" in developing an Aqueous soluble co crystal'. This technique may provide the synergistic enhancement in aqueous solubility of poorly water-soluble drugs. The conventional form like tablet or capsule of the Aceclofenac and Paracetamol faces some problem such as low aqueous solubility and bioavailability to overcome these problem there is a need to alter the property of the drug by modifying the form of drug so co-crystalting Aceclofenac and Paracetamol combination for good result.

The co crystal of Aceclofanec and paracetamol is may be reduce problem like low aqueous solubility as well as bioavailability. The study further opens the chances of preparing co crystal of other drug having poor solubility. This work open a new era of more stable products of poorly water soluble drugs in cheaper cost in the market. In brief the major objective of the project is to develop such a formulation which can provide immediate onset of diuretic action by oral route, avoid the side effects of drug, increase the oral bioavailability of aceclofenac and paracetamol and prolong the duration of nsaids action thus improving the patient compliance.

Material and Methods

Identification test of drug

A-Aceclofenac

Test Dissolve 50.0 mg in methanol R and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 50.0 ml with methanol R. Examined between 220 nm and 370 nm (2.2.25), the solution shows an absorption maximum at 275 nm. The specific absorbance at the maximum is 320 to 350 nm.

Test Dissolve about 10 mg in 10 ml of alcohol *R*. To 1 ml of the solution, add 0.2 ml of a mixture, prepared immediately before use, of equal volumes of a 6 g/l solution of potassium ferricyanide *R* and a 9 g/l solution of ferric chloride *R*. Allow to stand protected from light for 5 min. Add 3 ml of a 10.0 g/l solution of hydrochloric acid *R*. Allow to stand protected from light for 15 min. A blue color develops and a precipitate is formed.

Infrared spectroscopy

Spectroscopic analysis

10 mg of aceclofenac was weighed accurately and transferred to a 100 ml volumetric flask. To this 10 ml methanol was added to dissolve and the volume was made up to 100 ml with distil water so as to obtain stock solution of 100 µg/ml. From this stock solution, dilution of 20µg/ml was made with distilled water, and the sample was scanned between 200 nm to 400 nm on a double beam UV/Visible spectrophotometer (Shimadzu 1100). The UV spectrum of aceclofenac is shown in fig

Melting point

The melting point of aceclofenac was determined using open capillary method. Aceclofenac was packed into capillary. The average of three values was taken as the melting point of drug.[5]

Solubility study

The solubility of aceclofenac in distilled water at the wavelength of 274 nm Excess amount of aceclofenac in 10 ml water matrices were added in conical flasks in triplicate These conical flasks were kept in a mechanical water shaker bath at the temperature for 72 h to reach equilibrium. After 72 h, solutions were filtered and diluted up to 100 ml with distilled water and subjected for quantification of aceclofenac by UV spectrophotometric method at the wavelength of 273 nm.

B-Paracetamol

Test Dissolve 50 mg drug in sufficient methanol to produce 100 ml. 10 ml of this solution add 0.5 ml of 0.1hydrochloric acid and diluted up to 100 ml with methanol Protect the resulting solution from bright light and immediately absorbance at about 249 nm absorbance at 249 nm about 0.44.

Test Boil 0.1 gram of drug in 1 ml of hydrochloric acid for 5 minute add 10 ml of water and cool no precipitate is produce. Add 0.05 ml of 0.0167 M potassium dichromate a violate colour developed which does not turn red.

Infra red spectroscopy

Spectroscopic analysis

Accurately weighed quantity of paraetamol (10 mg) was dissolved in methanol 100 ml volumetric flask and volume was made up to 100 ml with methanol. Aliquot of the above solution was taken and diluted to get paraetamol concentration 10 Cg/ml. The resulting dilution was scanned between 200- 400 nm on Shimadzu-1100 UV spectrophotometer against distilled water blank. The spectrum is shown in figure

Melting point determination

The melting points of paracetamol were determined using open capillary method. The capillary filled with drug powder was placed in melting point apparatus. and heated it when drug is melt the melting point of drug powder was noted.

Solubility study

The solubility of paracetamol in distilled water at the wavelength of 248 nm Excess amount of aceclofenac in 10 ml water matrices were added in conical flasks in triplicate These conical flasks were kept in a mechanical water shaker bath at the temperature for 72 h to reach equilibrium. After 72 h, solutions were filtered and diluted up to 100 ml with distilled water and subjected for quantification of aceclofenac by UV spectrophotometric method at the wavelength of 248 nm.

Standard curve of Aceclofenac and Paracetamol

Determination of λmax

100 mg Aceclofenac and 100 mg Paracetamol was dissolved separately in 100 ml in methanol to obtain a 1000mcg/mL solution. This solution was subjected to scanning between 200 – 400 nm and absorption maximum was determined.

Standard Stock Solution

A stock solution containing 1 mg/mL of pure drug was prepared by dissolving 100 mg of Aceclofenac and 100 mg of Paracetamol in 100 mL methanol in a volumetric flask.

Working standard solution

10 mL of the stock solution was further diluted to 100 mL with methanol to obtain a working standard solutioncontaining 100mcg/mL.

Linearity and Calibration

0.1 to 1 ml from working standard solution diluted up to 10 ml with methano to obtain the concentration range of 1 – 10 mcg/mL. A calibration curve for aceclofenac and paracetamol was obtained by measuring the absorbance at the λmax of 273 nm and 248 nm. Statistical parameters like the slope, intercept, coefficient of correlation, standard deviation, Relative standard deviation, and error were determined.

Method of preparation

Co crystal of Aceclofenac and paracetamol is prepared by four following method.

Solution co-crystallization[4]

In solution co-crystallization, aceclofenac and paracetamol have similar solubility, For developing co crystal of Aceclofenac and Paracetamol an appropriate ratio of Aceclofenac and Paracetamol is selected i.e. 1:5.and 2:5 the solvent used here methanol due to the solubility of drug. Firstly all apparatus arrange than take a beaker in which solvent incorporate in to the beaker with continuously heating 50-60 ˚C and stirring. Both drug added continuously in small amount when saturated solution obtained to stop incorporation of drug and after a uniform solution the solution put on a dish for drying at room temperature. after drying co-crystal was found and collected.

Slurry conversion[4]

For preparing co crystal from slurry conversion weighted amount of aceclofenac and paracetamol incorporated into methanol.100 ml methanol was added to the and the resulting suspension was stirred at room temperature for one days. After one days, the solvent was decanted and the solid material was dried at room temperature.

Liquid-assisted grinding[4]

Involves co-grinding of Aceclofenac and Paracetamol. Weighted quantity of aceclofenac and paracetamol with the addition of a minor quantity of solvent methanol grinded, when uniform past obtained past was dried and co crystal collected.

Crystallization from the melt[3]

For developing co crystal by melting method weighted quantity of both drug in two test tube is introduced into a heated stand (liquid bath) in close proximity to a high accuracy thermometer. The temperature in the heating stand is maintain at melting point of both drug fixed rate until the sample in the tube transitions into the liquid state. While both drug drug melted stop heating and both drug solution mixed with each other. And after drying at room temperature co crystal collected.

Formulation code	Drug Ratio (Aceclofenac: Paracetamol)	Method of Preparation
F1	1:5	Solution co crystallization
F2	2:5	Solution co crystallization
F3	1:5	Slurry conversion
F4	2:5	Slurry conversion
F5	1:5	Liquid-assisted grinding
F6	2:5	Liquid-assisted grinding
F7	1:5	crystallization from the melt
F8	2:5	crystallization from the melt

Characterization of co-crystal

Drug content analysis

Preparations 120 mg equivalent to 100 mg Paracetamol and 20 mg of aceclofenac was weighed accurately and transferred to 100 ml volumetric flask and dissolved in methanol. The volume was made up with methanol up to the mark. After suitable dilution,(20 mcg/ml aceclofenac and 100 mcg/ml paracetamol for F1,F3,F5,F7 and 40 mcg/ml aceclofenac and 100 mcg/ml paracetamol for F2,F4,F6,F8) the absorbance of the above solution was measured at 273 nm and 243 nm using appropriate blank solution. The drug content of aceclofenac and paracetamol was calculated using calibration curve.[6]

The concentration of drug in present sample calculating using formula.

$$Concentration = \frac{\text{Absorbance}}{\text{Slop}}$$

The drug content calculated using formula.

$$Drug\ Content = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

Physical appearance of co crystal

The physical appearance of co crystal are given in table

Percent practical yield

Percentage practical yield were calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production. Inclusion complex were collected and

weighed to determine practical yield (PY) from the following equation.

PY (%) = Practical Mass (SD) / Theoretical Mass (Drug + Carrier)] ×100

Solubility study

Solubility measurement by UV absorption Kinetic solubility in high-throughput assays can be also quantitated by UV plate readers. The excess amount of co crystal was added In 10 ml water. Fresh distilled water was used as medium and flasks were protected from light, sealed and were placed in a shaker for 72 h. After equilibrium was reached the sample of flask was filtered through Millipore filter (0.45 µm). The filtered solution was then diluted up to 100 ml with water and concentration of aceclofenac and paracetamol was analyzed by U.V spectrophotometer (Shimadzu Double Beam Spectrophotometer 1601) at 273 nm and 248 nm. The drug concentration in the filtrate is quantitated by UV absorption, and solubility is calculated using a calibration curve[7]

The solubility of drug is the concentration of drug present in the solution. The concentration of drug calculated by following formula

$$Concentration = \frac{\text{Absorbance}}{\text{Slop}}$$

Determination of pH

pH of formulation determined by dispersing 0.5gm of co crystal in 50ml of water. Checked using digital pH meter at constant temperature prior to this, the pH meter was calibrated using buffer solution of pH 4.0 and 9.2, and then electrode was washed with demineralised water. The electrode was then directly dipped in to gel formulation and constant reading as noted.

Melting Point

The melting point of a solid is the temperature at which the vapor pressure of the solid and the liquid are equal. The melting point determination by DSC method

Dissolution study of co-crystal

In vitro dissolution testing offers a convenient and inexpensive means of predicting absorption and bioavailability of formulations of the same drug.

The release profile of co crystal of Aceclofenac and Paracelamol drug predicts how a delivery system might function and gives valuable insight into its in vivo behavior. In vitro release profile for co crystal as well as pure drug were performed using USP XXII type 2 dissolution apparatus. Take 120 mg of co crystal Sample equivalent to 20 mg of Aceclofenac and 100 mg of Paracetamol was added to 900 ml phosphate buffer pH 7.4 at 37± 0.5ºC and stirred at 50 rpm. Aliquot of 5ml was withdrawn at time intervals of 5,10, 20, 30, 40, 50 and 60 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 λmax 273 nm and 248 nm[8].

X-Ray Powder Diffraction

Powder diffraction is a scientific technique using X-ray, neutron, or electron diffraction on powder or microcrystalline samples for structural characterization of materials Structure determination of organic solids from powder The powder x-ray diffraction (XRD) was performed by X'pert Pro with Spinner PW3064 using Nifiltered, CuKα radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4o/min over a range of 5oC to 40^0C[9]. The diffraction spectra of Aceclofenac and Parecetamol show numerous distinct peaks indicating that both are present in a highly crystalline state. The XRD pattern of co crystal of aceclofenac and paracetamol exhibits all the characteristic diffraction peaks of aceclofenac and and paracetamol.

Differential scanning calorimetry

Differential scanning calorimetry was performed by using DSC-60. The instrument comprised of calorimeter (DSC 60), flow controller (FCL 60), Thermal analyzer (TA 60) and operating software TA 60 from (Shimadzu Corporation, Japan.) The samples were placed in aluminum pans and were crimped, followed by heating under nitrogen flow (30 ml/min) at a scanning rate of 5oC/min from 25oC to 200oC. Aluminum pan containing same quantity of indium was used as reference. The heat flow as a function of temperature was measured for both the drug and drug-excipient.

Results and Conclusion

The aim of the present research study was to explore the possibility of employing co crystallization technique in the two drug i.e. aceclofenac and paracetamol and characterization of co crystal. Co crystallization is a novel, safe and effective way to enhance physicochemical property and also solubility of poorly aqueous soluble drugs. Immediate dissolution of practically insoluble drug i.e. aceclofenac and paracetamol indicates it's great potential to solublize the drug in biological fluids and thus appreciable enhancement in bioavailability and onset of action can be expected. Thus the concept of co crystallization is an emerging field which can serve as a milestone for solubility enhancement and therefore deserves an urgent attention of scientific community to asses it's efficiency and applicability. Solubility studies co crystallization showed that the enhancement in solubility of aceclofenac and paracetamol. Therefore

ISSN: 0976-7126

tremendous enhancement in solubility of drug in co crystal form is attributed due to co crystallization phenomenon.

Dissolution studies reveal that there is marked increase in the dissolution rate of aceclofenac and paracetamol co crystal. as compared to pure aceclofenac and paracetamol itself. The DSC thermogram of pure aceclofenac showed a sharp endotherm at 153.49°C corresponding to its melting point/transition temperature and pure paracetamol showed a sharp endotherm at 169-58°C. The co crystal of aceclofenac and paracetamol show one sharp characteristic endothermic peak at 160.46±0.82.pH of co crystal is pH 5 Melting point of Aceclofenac is 152º C melting point of Parecetamol is 170º C and melting point of co crystal is 16oº C. The melting point of co crystal is different from the melting point of pure drug.

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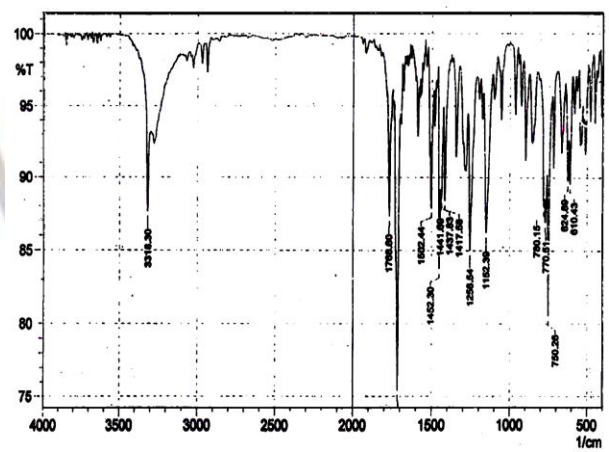

Fig. 1 IR Spectrum of aceclofenac

Table 1: Interpretations of infrared spectrum bands of aceclofenac sample

Wave No. (cm^{-1})	Interpretations
1096.00	C-Cl stretching
1152.39	C-O-C stretching
1168.60	C=O stretching of ketones
1502.60	C=C stretching of aromatic ring
1716.53	C=O stretching of COOH group
3296.30	O-H stretching of COOH group
3318.30	N-H (secondary amine) stretching

Table 2: Interpretations of infrared spectrum bands of Paracetamol

Drug	Absorbance	Concentration
Aceclofenac	0.375	15 mcg/ml
Paracetamol	6.45	83.77 mcg/ml

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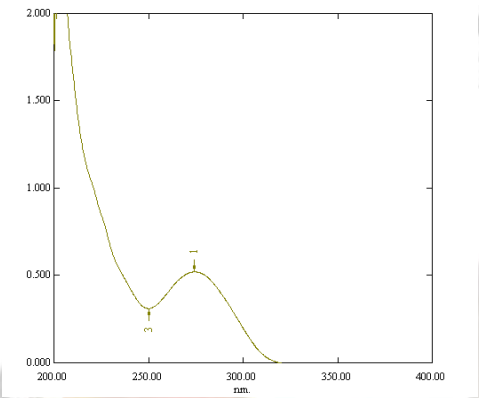

Fig. 2: UV spectrum of aceclofenac in methanol

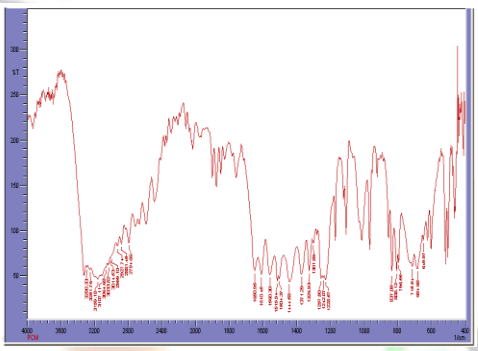

Fig. 3 Infra red spectrum of paracetamol

Wave No.(cm^{-1})	Interpretations
3290	OH stretching
1242	C_6H_5OH stretching
1690	X-$COCH_3$ stretching
2927.7	Aliphatic CH_3
790	C_6H_5-R stretching
1262	Aromatic amine stretching

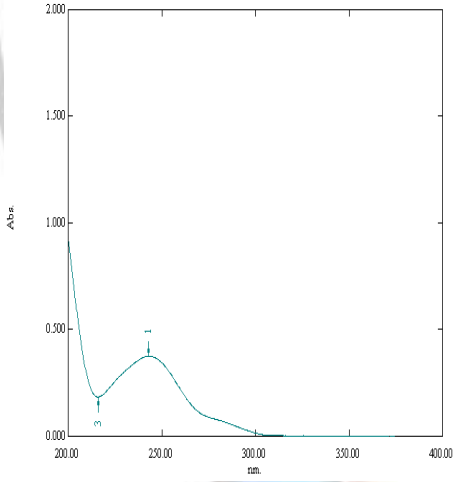

Fig. 4: UV spectrum of paracetamol

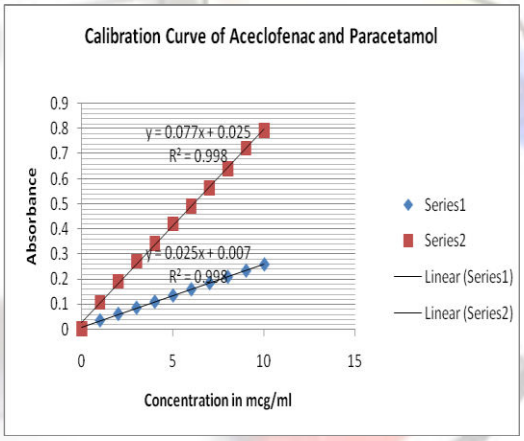

Fig. 5: Calibration Curve
Serial 1 is Aceclofenac, Serial 2 is Paracetamol

ISSN: 0976-7126

Table 3: Calibration Curve of Drug

Concentration	Absorbance at 273 nm	Absorbance at 248 nm
1	0.035	0.106
2	0.061	0.19
3	0.086	0.271
4	0.11	0.342
5	0.135	0.42
6	0.160	0.49
7	0.185	0.562
8	0.210	0.640
9	0.235	0.721
10	0.260	0.793

Table 4: Optical characteristics and precision for Aceclofenac

Absorption maxima	273 nm
Beer's law limit	0 – 20 mcg/mL
Coefficient of Correlation	0.998
Regression equation	Y = 0.025 X + 0.007
Slope	0.025
y intercept	0.007

Table 5: Optical characteristics and precision for Paracetamol

Absorption maxima	248 nm
Beer's law limit	0 – 20 mcg/mL
Coefficient of Correlation	0.998
Regression equation	Y = 0.077 X + 0.025
Slope	0.077
y intercept	0.025

Table 6: Drug content analysis

Formulation	Absorbance		Concentration		Total drug Content	
	At 273 nm	At 248 nm	Aceclofenac	Paracetamol	Aceclofenac	Paracetamol
F1	0.480	7.480	19.60	97.14	98%	97.14%
F2	0.961	7.481	39.20	97.16	98%	97.16%
F3	0.460	7.47	19.40	97.01	97%	97.01%
F4	0.923	7.471	38.82	97.03	97%	97.03%
F5	0.486	7.55	19.44	98.05	97%	97.05%
F6	0.972	7.53	38.84	97.79	97%	97.79%
F7	0.476	7.39	19.04	95.97	95%	95.97%
F8	0.950	7.41	38.00	96.23	95%	96.23%

ISSN: 0976-7126

Table 7: Physical Appearance

Formulation	Color	Form
F1	White	Crystaline
F2	White	Crystaline
F3	White	Crystaline
F4	White	Crystaline
F5	White	Crystaline
F6	White	Crystaline
F7	White	Crystaline
F8	White	Crystaline

Table 8: Percent practical yield

Formulation	% yield
F1	90.3 %
F2	90.5%
F3	89.32%
F4	89.30%
F5	99.90%
F6	99.90%
F7	86.5%
F8	86.4%

Table 9: Determination of pH and Melting point

Formulation	pH	Melting point
F1	5	160
F2	5.1	158
F3	5.2	161
F4	5	157
F5	4.97	163
F6	5.1	160
F7	5	162
F8	5.12	159

Table 10: Dissolution profile of Aceclofenac and Paracetamol

Time in (Min)	Aceclofenac			Paracetamol		
	Absorbance At 273 nm	Concentration In 900 ml	% Drug Release	Absorbance At 248 nm	Concentration In 900 ml	% Drug Release
0	0	0	0	0	0	0
5	0.0261	1.04	4.72	0.72	9.35	8.50
10	0.0581	2.32	10.56	1.09	14.16	12.87
15	0.0680	2.72	12.37	1.39	18.05	16.41
20	0.077	3.10	14.12	1.80	23.38	21.25
30	0.0972	3.88	17.67	2.80	36.36	33.05
40	0.141	5.64	25.63	3.90	50.65	46.05
50	0.163	6.52	29.63	4.73	61.43	55.85
60	0.184	7.36	33.45	5.87	76.23	69.30

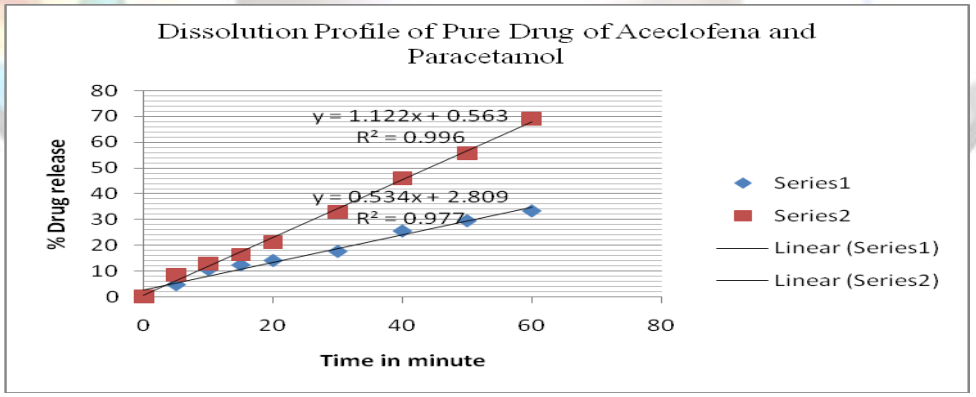

Fig. 6: Dissolution profile

Serial 1 is Release profile of Aceclofenac, Serial 2 is Release profile of Paracetamol

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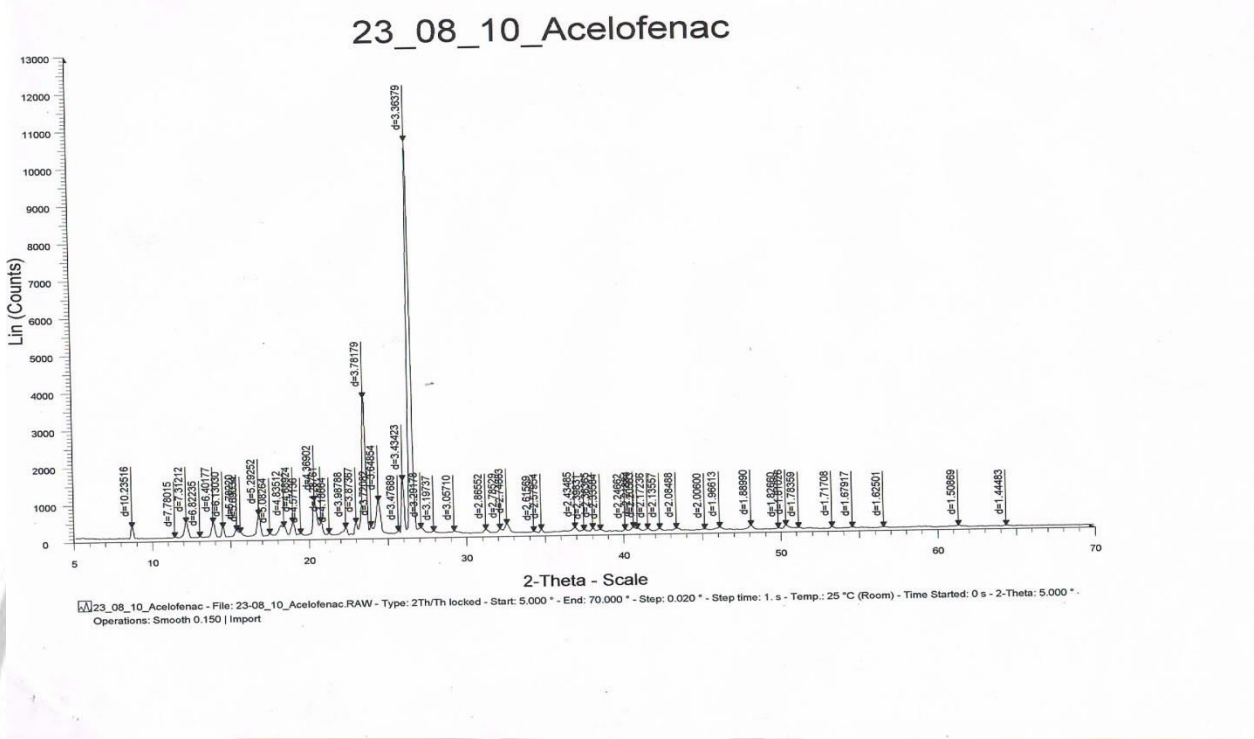

Fig. 7: X-ray Diffraction Patterns of co crystal

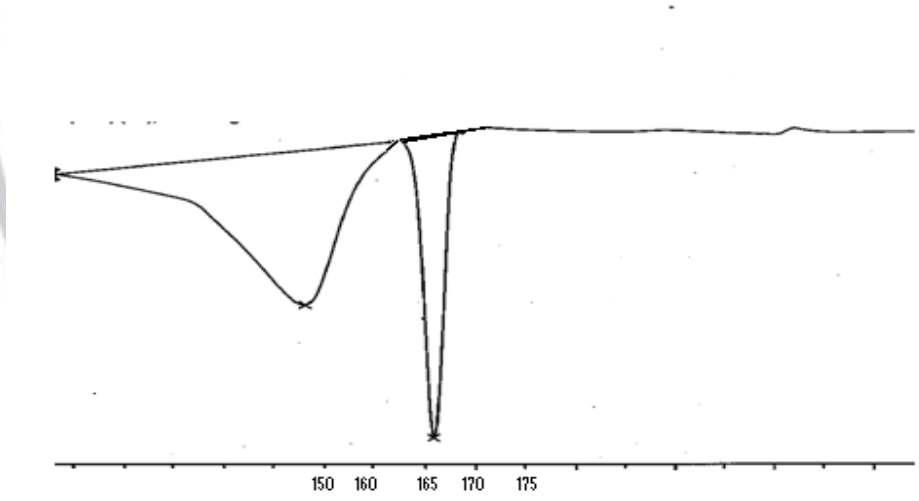

Fig. 8: DSC of co crystal