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Development and characterization of ternary system for solubility enhancement of a second-generation COX-II inhibitor

Girani Santosh*[1], Shidallingapa Zalki[2], Mahantesh Kavatekar[3], Ajay Shahapur[4], Vitthal K. Vijapure[5],
[*1,4]Department of Pharmaceutical Technology V.M.V.V.S College of Pharmacy Hungund, Bagalkot, Karnataka, India.
[2]Department of Pharmaceutics Krupanidhi College of Pharmacy Bangalore, Karnataka, india
[3]Dept. of Pharmaceutics D.S.T.S Mandal College of Pharmacy Solapur, Maharashtra, India.
[5]Department of Pharmaceutics S.J.M College of Pharmacy Chitradurga, Karnataka, India.

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Abstract: Etoricoxib is a highly selective COX-II inhibitor, used to treat pains of different etiologies. Etoricoxib has low aqueous solubility (201µg/ml) and high permeability and therefore classified as BCS class II drug. By formulating these drugs with cyclodextrins as inclusion complexes have shown to increase the bioavailability. Cyclodextrins when used as complexing agents, enhance the solubility of poor water soluble lipophilic drugs. The objective of the present work is to formulate Etoricoxib cyclodextrin complexes by using ternary systems as Citric acid, Tartaric acid and PVP K-30 in order to enhance solubility and evaluate the enhanced solubility by *in-vitro* dissolution.

Key words: NSAIDs; Etoricoxib; cyclodextrins; ternary system; inclusion complex; Kneading method; Solvent evaporation method; Spray drying; Freeze drying; XRD; DSC; FTIR; SEM.

Introduction

Musculoskeletal conditions are often progressive and associated with considerable pain and disability. These conditions place a huge burden on society in terms of lost productivity and the cost of treatment. Rheumatoid arthritis (RA), osteoarthritis (OA), and spinal disorders (including chronic low back pain [LBP]) are among those musculoskeletal conditions with the greatest impact on society. Approximately 14% of all primary care visits are for musculoskeletal pain or dysfunction. Symptomatic OA affects approximately 10% of men and 18% of women over 60 years of age (WHO 2005), while RA affects between 0.3% and 1% of adults worldwide (WHO 2005). Approximately 2.0% of all disability-adjusted life years are lost due to musculoskeletal diseases, including 1.0% due to OA, and 0.3% due to RA.

Dyspeptic upper gastrointestinal symptoms with chronic use of traditional NSAIDs often lead to discontinuation by the patient with consequent inadequate pain control, switching from one NSAID to another, or the addition of a gastroprotective agent to prevent or treat upper gastrointestinal symptoms or clinical. Major gastrointestinal complications, such as perforation, ulcers, and bleeding may require visits to the emergency department, hospitalization, and endoscopic or barium tests[1]. Many studies have indicated that NSAIDs function as inhibitors of isoforms 1 and 2 of the cyclooxygenase enzyme (COX1 and COX2). COX-1, which is constitutively expressed in tissues, stimulates prostaglandin synthesis. The gastric and renal side effects of NSAIDs may thus be explained by their indirect effect on prostaglandin (PG) E2, which has cytoprotective effects in the gastroenteric system, and on PGE2 and PGI2, which are involved in regulating renal blood flow. As a result, efforts have been made to develop selective COX-2 inhibitors (coxibs), such as rofecoxib and celecoxib, and recently the number of COX-2-selective inhibitors has increased with the addition of the second generation coxibs valdecoxib, parecoxib, and etoricoxib[2]. In contrast, selective COX-2 inhibitors have greater affinity for COX-2 than COX-1. Clinical evidence has shown that selective COX-2 inhibitors have comparable efficacy with traditional NSAIDs in the treatment of arthritis and pain, but offer the major advantage of reduced gastrointestinal toxicity, thus providing physicians with an important therapeutic alternative[1].

The differences between etoricoxib or lumbriacoxib and ns-NSAIDs in relation to ulcer complications are apparent early during treatment and remain constant over time during the course of treatment; therefore, benefit of etoricoxib and lumbiracoxib can be seen in patients with high GI ulcer complication risk who require NSAIDs from the beginning of use to throughout the prescription duration[3].

Etoricoxib is regarded as second generation coxibs because of its higher selectivity for cox-2 inhibition than celecoxib and rofecoxib. Etoricoxib, 5-chloro-6-methyl-3 [4-(methyl sulfonyl) phenyl]-2, 3–bypyridine, is a highly selective second. Generation Cyclooxygenage-2 (COX-2) inhibitor administered orally as an analgesic and nonsteroidal anti-inflammatory drug (NSAID)[4]. Coxibs were developed with the anticipation of reducing the serious gastrointestinal complications associated with NSAIDs use in high risk patients. The development of newer coxibs and their use extend our knowledge in understanding the role of COX-1 and COX-2[3].

Solubility is defined in quantitative terms as concentration of a solute in a saturated solution at a certain temperature and a qualitative way it may be defined as the spontaneous interaction of two or more substances to form a homogenous molecular dispersion[5].

Process of solubilisation:
The process of solubilisation involves the breaking of inter-ionic or intermolecular bonds in the solute, the separation of the molecules of the solvent to provide space in the solvent for the solute, interaction between the solvent and the solute molecule or ion[6].

***Corresponding Author:**
Dr. Santosh Girani,
Department of Pharmaceutical Technology
V.M.V.V.S College of Pharmacy Hungund,
Bagalkot, Karnataka, India.

E-mail: santoshgirani@gmail.com

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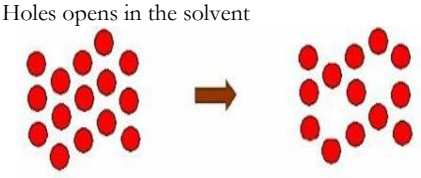
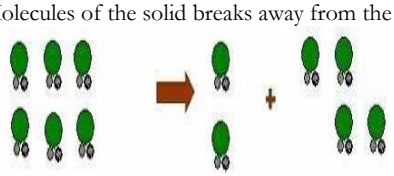
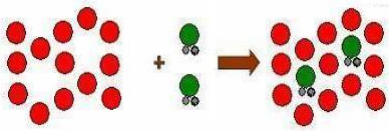
Step 1: Holes opens in the solvent



Step 2: Molecules of the solid breaks away from the bulk



Step 3: The freed solid molecule is integrated into the hole in the solvent



A variety of solubilization techniques have been studied and widely used for enhancement of solubility. Many estimates up to 40% of new chemical entities which are having problem in solubility, discovered by pharmaceutical industry. Today, as per BCS-II, the numbers of drugs are poorly soluble and lipophilic in nature. Aqueous solubility is lesser than 1µg/ml will definitely create a bioavailability problem and thereby affects the efficacy of the drug. There are number of methods through which aqueous solubility of the drug can be increased[7].

Materials and Methods

Standard solution of Etoricoxib:
50mg of pure drug was accurately weighted and transferred into the 50ml volumetric flask and dissolved with 50ml of methanol. The volume was made up using phosphate buffer 6.8 pH. This is standard stock solution (1mg/ml). From the standard stock solution, a series of dilution were made to get 2-20µg/ml solution using phosphate buffer pH 6.8 as dilution medium. The absorbance of these solutions was measured against 6.8pH phosphate buffer as a blank, using UV spectrophotometer at 284nm.

Phase solubility studies:
Phase solubility studies were performed for Etoricoxib complexes in phosphate buffer pH 6.8 containing sequentially increasing concentrations of cyclodextrins, in order to evaluate the role of the unionized species of ETR in improving solubility by CD complexation and to be able to select the most suitable conditions for optimizing drug solubilization.

Method of preparation of tablets by direct compression:
The complexes were selected from the prepared cyclodextrin complexes. The tablets were formulated by employing direct compression method using 12 mm flat punches. It is a process by which tablets are compressed directly from mixtures of drug and excipients without preliminary treatment such as wet/dry granulation.

Etoricoxib- hpβ cd- citric acid freeze dried complex (310 mg), disintegrant and excipients were blended using mortar and pestle. Before blending, the drug and 5% disintegrant were sieved through mesh 80#. The blended mixture is evaluated for bulk density, compressibility and angle of repose. The blended mixture was mixed with 1% Magnesium stearate, and 1% talc as lubricants. The mixture was then compressed by Rimek, 10 stationary tablet compression machine using 9.45mm flat punch. The hardness was adjusted to 4 to 5 kg/cm^{2}.[8] Formulation of conventional tablet by using freeze dried (Etoricoxib- HP β cd-citric acid 1:1:2M) method cyclodextrin complexes

Table 1: Composition development of F1 batch

Ingredients	F1 (mg/tablet)
ETR- HP β cd- citric acid complexes	310
Croscarmellose sodium	5
Microcrystalline cellulose	183
Magnesium stearate	1
Talc	1
Total weight of tablet	**500**

Procedure for evaluation of powder parameters:
a) Bulk density:
It is the ratio of total mass of powder and the bulk volume of powder. It was measured by pouring the weighed powder into graduated measuring cylinder and the volume was recorded. It is expressed in gm/ml.

$$Bulk\ density = \frac{M}{V_b}$$

Where M is mass of powder, V_b is the bulk volume of powder.
Method: Accurately weighed quantities of the blend mixture were carefully poured in to the graduated cylinder through a funnel and the bulk volume was recorded with and without tapping. The untapped and tapped bulk densities were calculated from the following formula, weight of blend mixture/ untapped volume and weight/ tapped volume, respectively.

b) Carr's index (I) / percentage compressibility:
An important measure that can be obtained from bulk density is the determination of percentage compressibility or Carr's index, which is defined

$$I = \frac{D_t - D_b}{D_b} X100$$

Without tapping. The untapped and tapped bulk densities were calculated from the following formula, weight of blend mixture/ untapped volume and weight/ tapped volume, respectively. Where, D_t is the tapped density of the powder, D_b is the bulk density of the powder. In theory, a blended mixture having an 'I' value of less than 20% is defined to have more free-flowing property and good compressibility.

Table 2: Experimental Considerations for the compressibility Index and Hausner's Ratio:

Compressibility Index (%)	Flow Character	Hausner Ratio
10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very Poor	1.46-1.59
>38	Very, very poor	>1.60

c) Angle of repose:
Angle of repose indicates the frictional forces existing between the particles. It is defined as the maximum angle possible between the surface of the pile of powder and the horizontal plane.

$\tan\theta = h/r$

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

Where θ is angle of repose h is heap of powder in cm's and is radius. Value of θ is rarely less than 20^{o} and values up to 40^{o} indicates good flow potential.

Method: A funnel was fixed at a particular height 'h' cm on a burette stand. A graph paper was placed below the funnel on the table. The given blend mixture whose angle of repose is to be determined was made to pass through the funnel, until it forms a pile. Circumference of the circle is drawn with a pencil without disturbing the pile. The radius of the pile of powder was noted as 'r' cm. Angle of repose θ of the granules, calculated from the above formula.

Procedure for evaluation of tablets:

Evaluation of tablets:

The tablets were compressed using 12mm diameter, round, flat, punches on a CIP tablet compression machine.

Pre-compression parameters:

Following properties were evaluated as Pre-compression parameters

- Bulk density
- Tapped density
- Compressibility index

Compression parameters:

Following parameters were evaluated during compression

- Average weight
- Hardness test
- Friability test
- Disintegration test

Post compression parameters:

Following parameters were evaluated as post compression parameters

- Hardness
- Friability
- Thickness
- Weight variation
- Disintegration
- Assay
- Content uniformity

Pre-compression parameters:

The inter-particulate interaction influences the bulk properties of the drug powder and interferes with powder flow. Comparison of the bulk and tapped density can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow. Tapped density was achieved by tapping a measuring cylinder which contains a powder sample and the change in the volume is noted.

Method: Bulk and tapped density determination were carried according to USP 'Bulk density' and 'Tapped density' method-1 respectively. 50 gm of active ingredient weighed and was taken in 250ml graduated cylinder for bulk density. For tapped density the cylinder was tapped 100 times.

Bulk density, tapped density and Carr's index were calculated by the following formula.

$$Bulkdensity(D_b) = \frac{\text{Mass of the powder(M)}}{\text{Bulk volume}(V_b)}$$

$$Tappeddensity(D_t) = \frac{\text{Mass of the powder(M)}}{\text{Tapped volume}(V_t)}$$

The Carr's index is the measure of propensity of the powder to be compressed. It is calculated using the formula given below

$$Carrsindex(CI) = \frac{D_t - D_b}{D_t} X100$$

Post compression parameters:

1. Hardness: The hardness of the tablets is an official test for the tablets as per IP and was determined for all the formulations using Monsanto type hardness tester,

2. Friability: It is not an official method but required for the shipment of the product. It is carried out by using Friabilator. The 20 tablets were weighed initially and transferred into a Friabilator and allowed to rotate 100 rotations. After the completion, tablets were weighed. The % friability was calculated using the formula,

$$F = \frac{W_{Initial} - W_{Final}}{W_{Initial}} X100$$

3. Weight variation as per IP: The weight variation of the tablets was carried out using 10 tablets by taking average weight of 10 tablets.

4. Thickness: The thicknesses of tablets were measured using digital Vernier calipers.

5. Disintegration time: The disintegration time of the tablet was determined using USP disintegration test apparatus. The limit for disintegration is not more than 3 mins at 37^{0}C.

Procedure: Six tablets were placed individually in each tube of disintegration test apparatus in which water bath temperature was maintained at 37^{0}C ± 0.5^{0}C and disc were placed on each tablet. The disintegration time of each tablet was noted.

Drug content:

Ten tablets of each formulation were weighed and powdered. A quantity of powder equivalent to 60 mg of Etoricoxib was taken. The amount of drug present in a 60mg equivalent amount of powder was determined; by dissolving the powder mixture in 60 ml of 6.8 pH phosphate buffer and from that 1 ml was taken in to 100ml volumetric flask and diluted with phosphate buffer. UV absorbance was measured at 284.0 nm and drug concentration was determined from the standard graph.

***In vitro* dissolution studies**

In-vitro release studies were carried out for the formulations in dissolution test apparatus USP type II.[46] The release of drug from the formulated tablet was compared with that of marketed tablet.

Instrument: Lab India Disso 2000
Apparatus: USP Type II paddle.
Temperature: 37 ± 0.5^o C.
RPM: 100
Dissolution medium: phosphate buffer 6.8 pH.
Volume of medium: 900 ml
Sampling intervals: 5, 10, 15, 30, 45 and 60 min.
Sample volume: 5ml withdrawn and replaced with 5 ml of 6.8pH phosphate buffer.

A sample of 5ml withdrawn and filtered through 0.45µm whattman filter and 1ml of the filtrate was made up to 10ml with phosphate buffer 6.8 pH in 10ml volumetric flask. Suitable dilutions were further made when required. The absorbance of the sample was read at 284 nm against phosphate buffer as blank. The dissolution studies were also performed with marketed product in 6.8pH phosphate buffer for 30 min.

Results

Table 3: Melting point of pure drug by Thiel's tube method

Reported	Observed		
	Trial 1	Trial 2	Trial 3
134^{o}C– 135^{o}C	136^{o}C	135^{o}C	135^{o}C

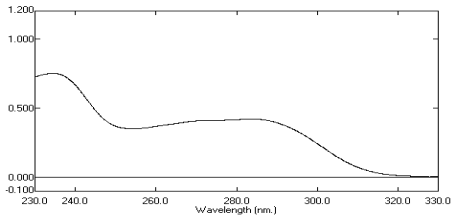
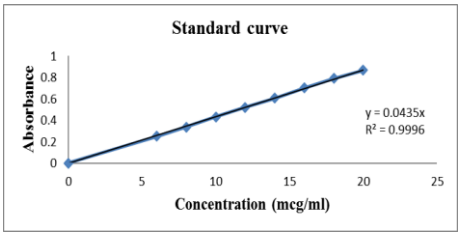


Figure 1: UV Spectrum of Etoricoxib in phosphate buffer pH 6.8.

Table 4: Calibration curve of Etoricoxib in phosphate buffer pH 6.8.

Concentration in µg/ml	Absorbance
6	0.252
8	0.339
10	0.434
12	0.521
14	0.606
16	0.702
18	0.252
20	0.339

Figure 2: Standard curve of Etoricoxib in phosphate buffer pH 6.8 at 284nm**.**



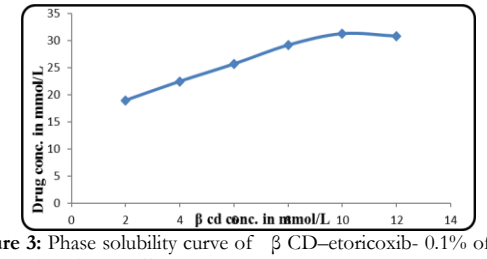


Figure 3: Phase solubility curve of β CD–etoricoxib- 0.1% of citric acid in phosphate buffer 6.8pH.

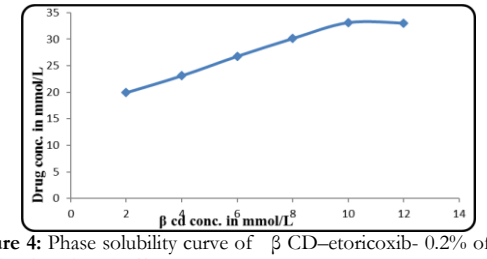


Figure 4: Phase solubility curve of β CD–etoricoxib- 0.2% of citric acid in phosphate buffer 6.8pH.

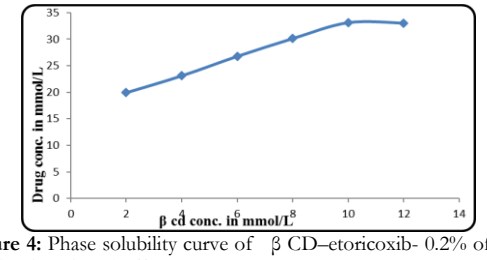


Figure 4: Phase solubility curve of β CD–etoricoxib- 0.2% of citric acid in phosphate buffer 6.8pH.

Table 5: Phase solubility studies of Etoricoxib: β Cyclodextrin: Citric acid (0.1%w/v) complexes.

Concentration of β-cyclodextrin in mmol/L with 0.1%w/v citric acid	Concentration of Etoricoxib (mmol/L)
2	18.94
4	22.45
6	25.65
8	29.13
10	31.23
12	30.76

Table 6: Phase solubility studies of Etoricoxib: β Cyclodextrin: Citric acid (0.2%w/v) complexes.

Concentration of β-cyclodextrin in mmol/L with 0.2% citric acid	Concentration of Etoricoxib (mmol/L)
2	19.94
4	23.12
6	26.78
8	30.12
10	33.12
12	33.02

Table 7: Phase solubility studies of Etoricoxib: β- cyclodextrin: 0.1%w/v tartaric acid complexes.

Concentration of β-cyclodextrin in mmol/L with 0.1% tartaric acid	Concentration of Etoricoxib (mmol/L)
2	18.56
4	21.56
6	23.89
8	26.76
10	29.31
12	28.78

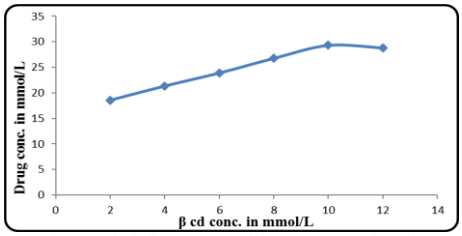


Figure 5: Phase solubility curve of β CD –etoricoxib-: 0.1%w/v tartaric acid in phosphate buffer 6.8pH.

Table 8: Phase solubility studies of Etoricoxib: β- cyclodextrin: 0.2%w/v tartaric acid complexes.

Concentration of β-cyclodextrin in mmol/L with 0.2% Tartaric acid	concentration of Etoricoxib (mmol/L)
2	19.21
4	22.34
6	24.76
8	27.56
10	30.12
12	29.98

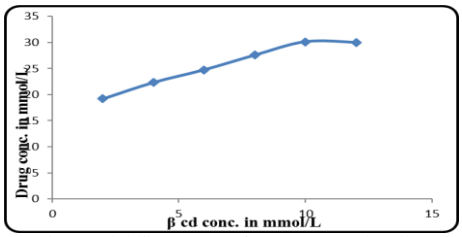


Figure 6: Phase solubility curve of β CD –etoricoxib- : 0.2%w/v tartaric acid in phosphate buffer 6.8pH.

Table 9: Phase solubility studies of Etoricoxib: HP β cd: Citric acid(0.1%w/v) complexes.

Concentration of HP β-cd in mmol/L with 0.1%w/v citric acid	Concentration of Etoricoxib (mmol/L)
2	22.34
4	26.34
6	29.98
8	34.23
10	37.75
12	36.98

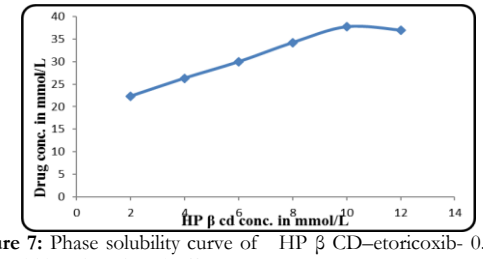


Figure 7: Phase solubility curve of HP β CD–etoricoxib- 0.1% of citric acid in phosphate buffer 6.8pH.

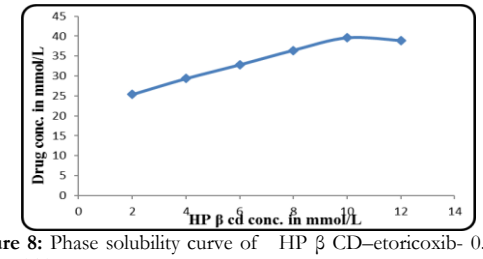


Figure 8: Phase solubility curve of HP β CD–etoricoxib- 0.2% of citric acid in

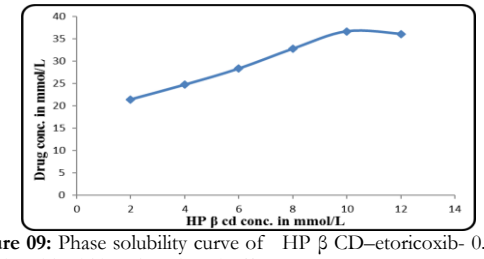


Figure 09: Phase solubility curve of HP β CD–etoricoxib- 0.1% of tartaric acid acid in phosphate buffer 6.8pH.

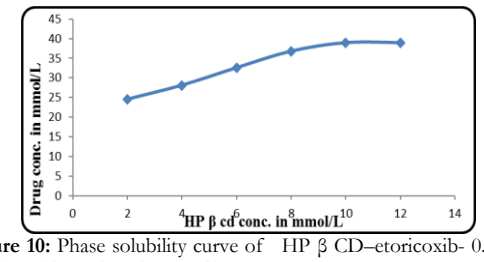


Figure 10: Phase solubility curve of HP β CD–etoricoxib- 0.2% of tartaric acid in phosphate buffer 6.8pH.

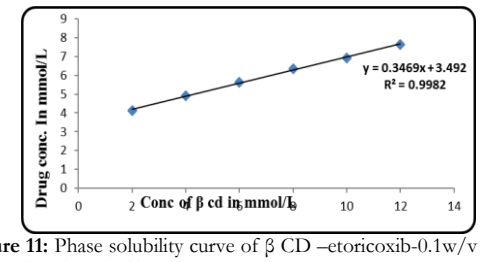


Figure 11: Phase solubility curve of β CD –etoricoxib-0.1w/v pvp k-30 in phosphate buffer 6.8pH.

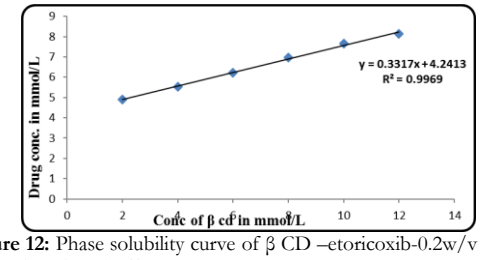


Figure 12: Phase solubility curve of β CD –etoricoxib-0.2w/v pvp k-30 in phosphate buffer 6.8pH

Table 10: Phase solubility studies of Etoricoxib: HP β cd: Citric acid(0.2%w/v) complexes.

Concentration of HP β-cd in mmol/L with 0.2%w/v citric acid	Concentration of Etoricoxib (mmol/L)
2	25.35
4	29.34
6	32.79
8	36.43
10	39.56
12	38.86

Table 11: Phase solubility studies of Etoricoxib: HP β cd: Tartaric acid acid (0.1%w/v) complexes.

Concentration of HP β-cd in mmol/L with 0.1%w/v tartaric acid	Concentration of Etoricoxib (mmol/L)
2	21.43
4	24.73
6	28.34
8	32.79
10	36.71
12	36.12

Table 12: Phase solubility studies of Etoricoxib: HP β cd: Tartaric acid acid(0.2%w/v) complexes.

Concentration of HP β-cd in mmol/L with 0.2%w/v tartaric acid	Concentration of Etoricoxib in mmol/L
2	24.56
4	28.12
6	32.56
8	36.76
10	38.9
12	38.89

Table 13: Phase solubility studies of Etoricoxib: β- cyclodextrin: 0.1w/v pvp k-30 complexes.

Concentration of β-cyclodextrin in mmol/L with 0.1%w/v pvp k-30	Concentration of Etoricoxib (mmol/L)
2	4.12
4	4.91
6	5.61
8	6.34
10	6.91
12	7.63

Table 14: Phase solubility studies of Etoricoxib: β- cyclodextrin: 0.2w/v pvp k-30 complexes.

Concentration of β-cyclodextrin in mmol/L with 0.2%w/v pvp k-30	concentration of Etoricoxib (mmol/L)
2	4.89
4	5.54
6	6.22
8	6.96
10	7.65
12	8.12

Table 15: Phase solubility curve of HP β cd –etoricoxib-0.1w/v pvp k-30 in phosphate buffer 6.8pH

Concentration of HP β-cyclodextrin in mmol/L with 0.2%w/v pvp k-30	concentration of Etoricoxib (mmol/L)
2	6.12
4	7.45
6	9.11
8	10.64
10	12.13
12	13.76

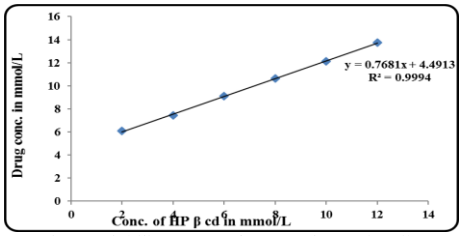


Figure 13: Phase solubility curve of HP β cd –etoricoxib-0.1w/v pvp k-30 in phosphate buffer 6.8pH

Table 16: Phase solubility curve of HP β cd –etoricoxib-0.2w/v pvp k-30 in phosphate buffer 6.8pH

Concentration of HP β-cyclodextrin in mmol/L with 0.2%w/v pvp k-30	concentration of Etoricoxib (mmol/L)
2	8.12
4	9.54
6	10.89
8	12.64
10	14.32
12	15.56

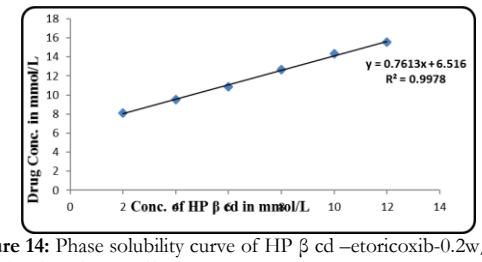


Figure 14: Phase solubility curve of HP β cd –etoricoxib-0.2w/v pvp k-30 in phosphate buffer 6.8pH

Table 17: Stability constant of various cyclodextrins and type of phase diagram.

Drug	Cyclodextrin	Hydroxy Acids	$K_{1:1}$	Phase Diagram
Etoricoxib	β- cyclodextrin	Citric acid	--	A_N Type
Etoricoxib	HP β- cyclodextrin	Tartaric acid	--	A_N Type
Etoricoxib	β- cyclodextrin	PVP K-30	57.56	A_L Type
Etoricoxib	HP β- cyclodextrin	PVP K-30	360.21	A_L Type

$$\text{Where } K_{1:1} = \frac{\text{slope}}{S_o\,(1\text{-slope})}$$

S_o is the intrinsic solubility of Etoricoxib in absence of cyclodextrin and slope is obtained from the phase solubility diagram

Table 18: Dissolution study of Etoricoxib in phosphate buffer pH 6.8.

Time	Percentage drug release*± SD
0	0
5	8.37±0.21
10	13.35±0.16
20	18.67±0.61
30	24.55±0.45
45	29.45±0.76
60	35.65±0.24

*= average cumulative drug release of triplicate samples (n=3).

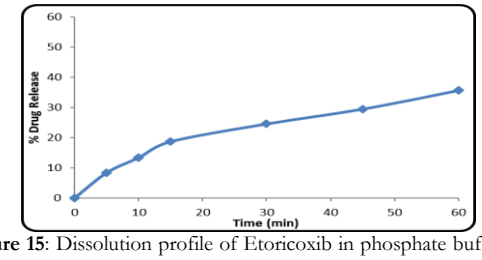


Figure 15: Dissolution profile of Etoricoxib in phosphate buffer pH 6.8

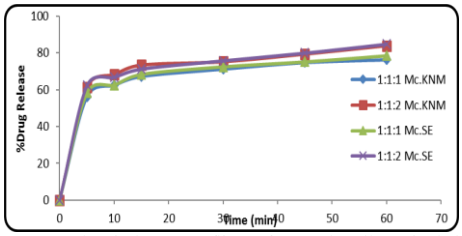


Figure 16: Dissolution profile of Etoricoxib-βcd-Citric acid complexes in phosphate buffer pH 6.8.

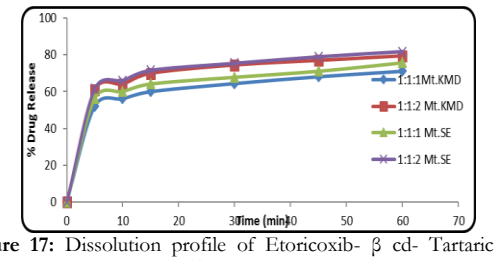


Figure 17: Dissolution profile of Etoricoxib- β cd- Tartaric acid complexes in phosphate buffer pH 6.8

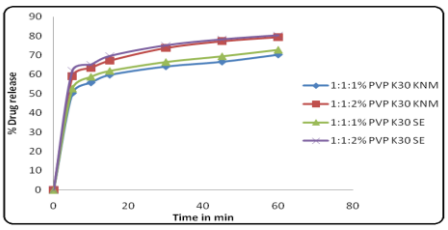


Figure 18: Dissolution studies of Etoricoxib- β cd PVP K30 complexes in phosphate buffer pH 6.8.

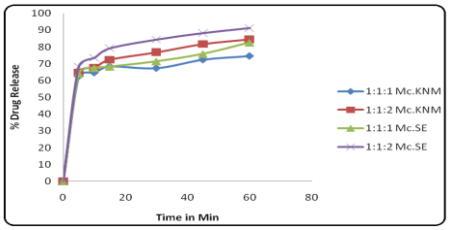


Figure 19: Dissolution studies of Etoricoxib-HP β cd- Citric acid complexes in phosphate buffer pH 6.8.

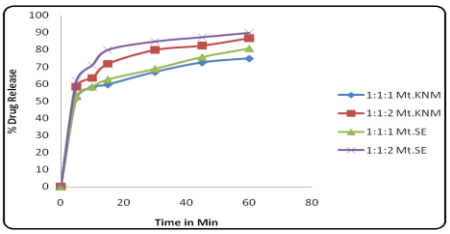


Figure 20: Dissolution studies of Etoricoxib-HP β cd- Tartaric acid complexes in phosphate buffer pH 6.8.

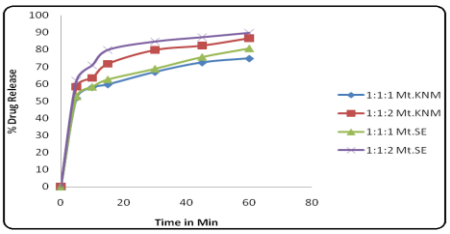


Figure 20: Dissolution studies of Etoricoxib-HP β cd- Tartaric acid complexes in phosphate buffer pH 6.8.

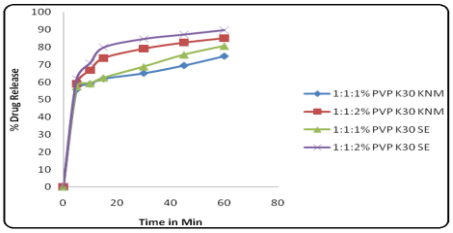


Figure 21: Dissolution studies of Etoricoxib-HP β cd- PVP K30 complexes in phosphate buffer pH 6.8.

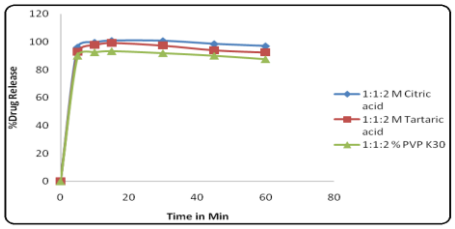


Figure 22: Dissolution studies of Etoricoxib-Hp β cd/citric acid/tartaric acid/pvpk30 by Freeze drying method

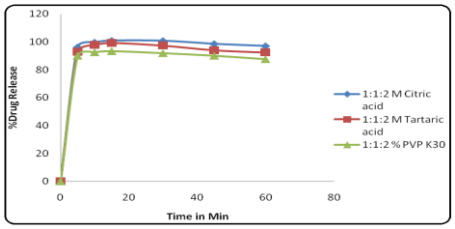


Figure 22: Dissolution studies of Etoricoxib-Hp β cd/citric acid/tartaric acid/pvpk30 by Freeze drying method

Table 26: Loose bulk density, Tapped bulk density and Carr's compressibility index

Formulations	Loose bulk density (g/ml)	Tapped bulk density (g/ml)	% Compressibility	Hausner's ratio
F1	0.449	0.492	8.739	1.09

Table 27: Evaluation parameters of prepared Etoricoxib Tablets

Formulation	Hardness (Kg) (n=6)	Disintegration (sec)	Thickness (mm) (n=6)	Weight variation (mg) (n=20)	Friability (%)	Drug content (%)
F1	4.4±0.68	560	3.30±0.019	619±2.1	0.59	99.4±0.12

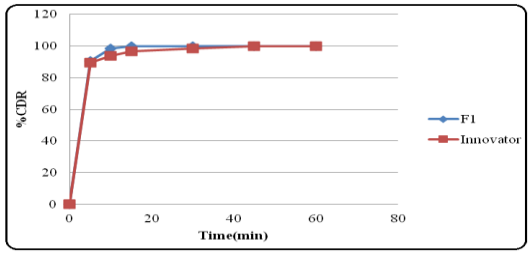


Figure 23: Comparison profiles of optimized formulation with innovator.

Table 29: Comparison of release profile

Formulation trials	F2 value	F1 value	Influence of dissolution profile in comparison to innovators product as per FDA standards
F1	78.89	1.83	Similar

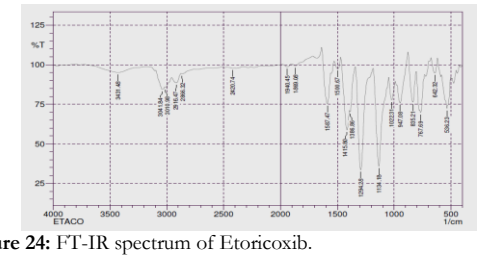


Figure 24: FT-IR spectrum of Etoricoxib.

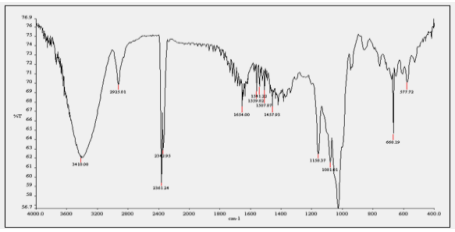


Figure 25: FT-IR spectrum of β CD.

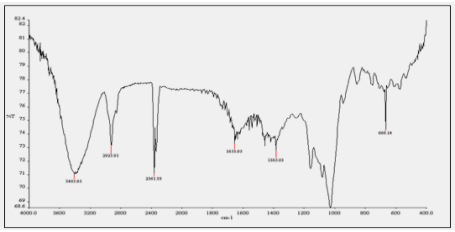


Figure 26: FT-IR spectrum of HP β CD.

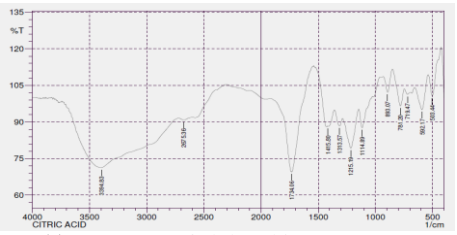


Figure 27: FT-IR spectrum of Citric Acid

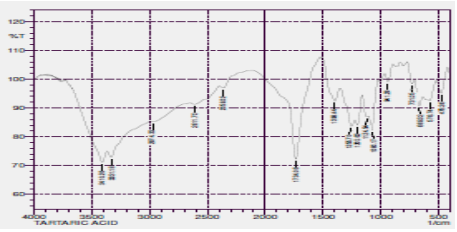


Figure 28: FT-IR spectrum of Tartaric acid

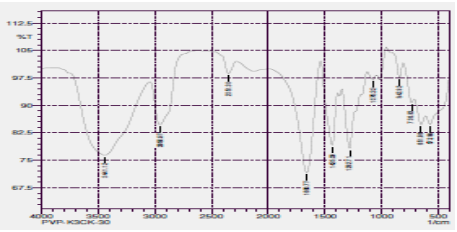


Figure 29: FT-IR spectrum of PVP K30

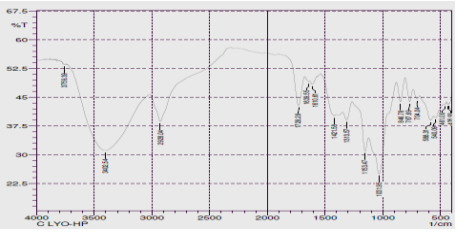


Figure 30: FT-IR spectrum Etoricoxib- HP β cd- Citric acid freeze dried formulation

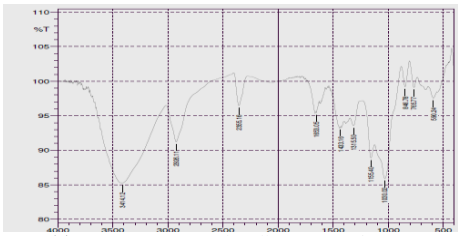


Figure 31: FT-IR spectrum Etoricoxib- HP β cd- PVP K30 freeze dried formulation

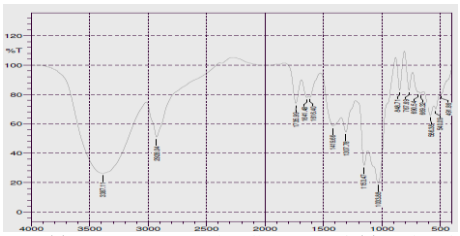


Figure 32: FT-IR spectrum Etoricoxib- HP β cd- Tartaric acid freeze dried formulation

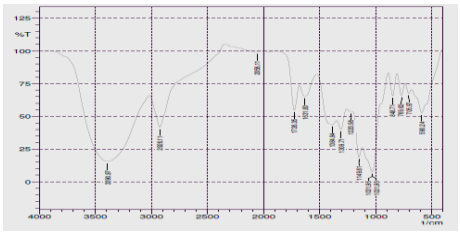


Figure 33: FT-IR spectrum Etoricoxib- HP β cd- Citric acid spray dried formulation

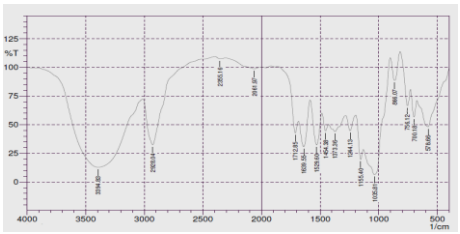


Figure 34: FT-IR spectrum Etoricoxib- HP β cd- Tartaric acid spray dried formulation

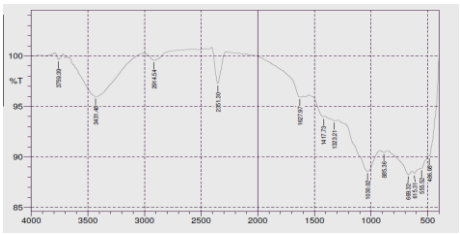


Figure 35: FT-IR spectrum Etoricoxib- HP β cd- PVP K 30 spray dried formulation

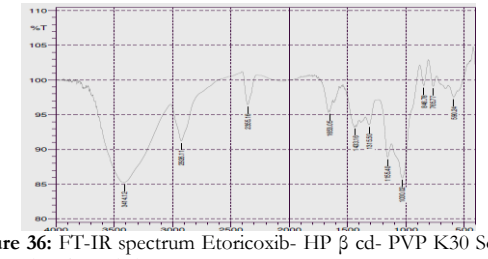


Figure 36: FT-IR spectrum Etoricoxib- HP β cd- PVP K30 Solvent evaporation formulation

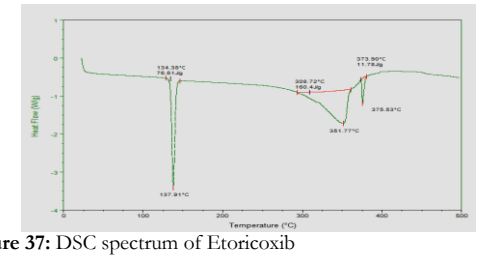


Figure 37: DSC spectrum of Etoricoxib

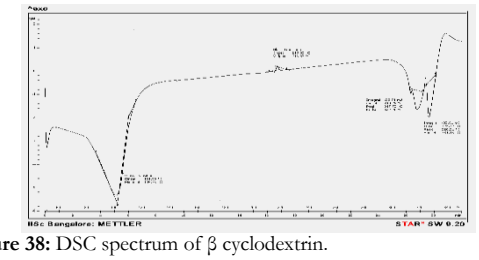


Figure 38: DSC spectrum of β cyclodextrin.

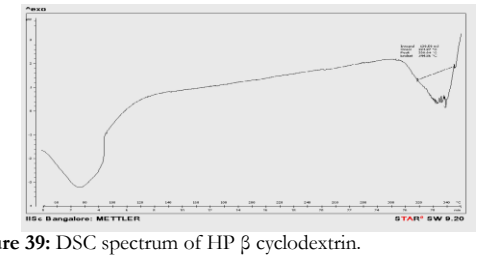


Figure 39: DSC spectrum of HP β cyclodextrin.

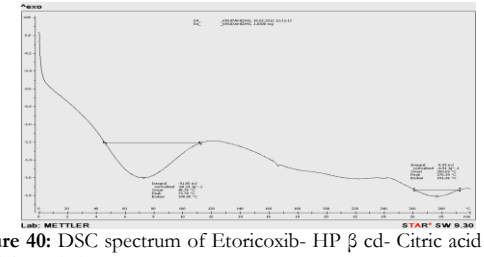


Figure 40: DSC spectrum of Etoricoxib- HP β cd- Citric acid freeze dried formulation

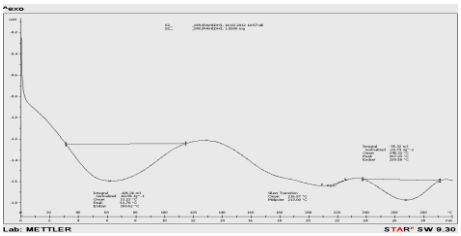


Figure 41: DSC spectrum of Etoricoxib- HP β cd- Citric acid kneading method formulation

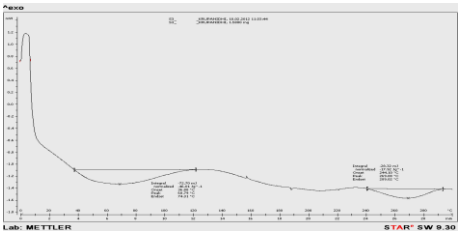


Figure 42: DSC spectrum of Etoricoxib- HP β cd- Citric acid Solvent evaporation formulation

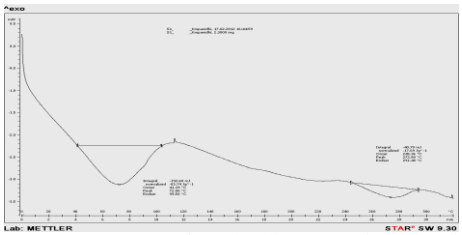


Figure 43: DSC spectrum of Etoricoxib- HP β cd- Citric acid Spray driedformulation

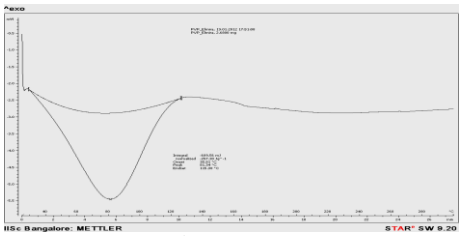


Figure 44: DSC spectrum of PVP K30

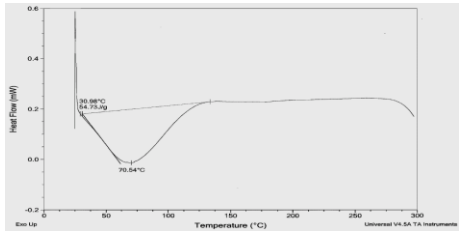


Figure 45: DSC spectrum of MCCX-Ray Diffraction study

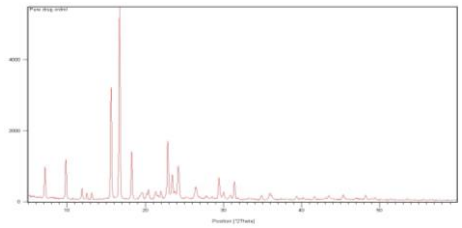


Figure 46: XRD of Etoricoxib

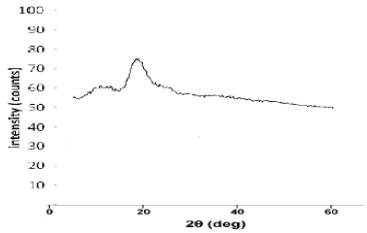


Figure 47: XRD of β cyclodextrin.

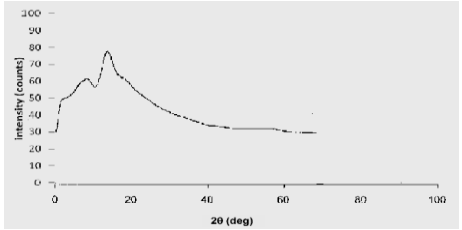


Figure 48: XRD of HP β cyclodextrin.

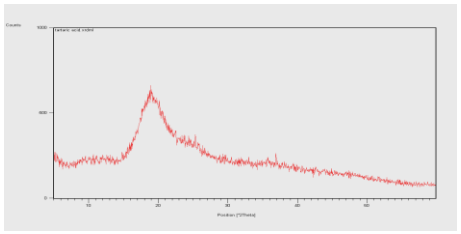


Figure 49: XRD spectrum of Etoricoxib- HP β cd- Citric acid (1:1:2M) freeze dried formulation

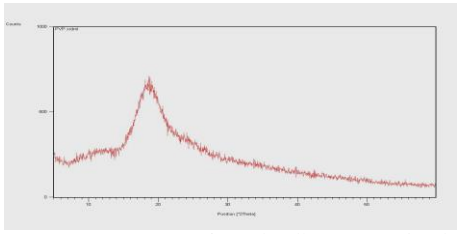


Figure 50: XRD spectrum of Etoricoxib- HP β cd- Citric acid (1:1:2M) kneading method formulation

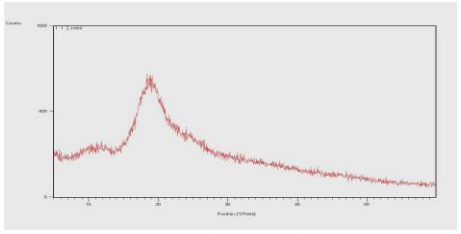


Figure 51: XRD spectrum of Etoricoxib- HP β cd- Citric acid (1:1:2M) solvent evaporation formulation

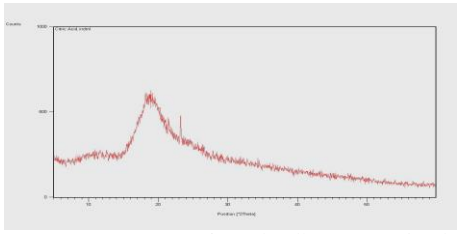


Figure 52: XRD spectrum of Etoricoxib- HP β cd- Citric acid (1:1:2M) spray dried formulation

Scanning electron microscopy study:

Figure 53: SEM of etoricoxib

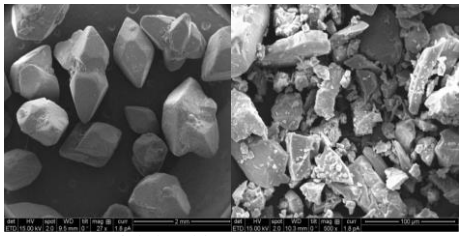


Figure 54: SEM of citric acid

Figure 55: SEM of Etoricoxib- HP β cd- Citric acid (1:1:2M) freeze dried formulation

Table 30: Stability study for Optimized formulation (F1)

Evaluation Parameters	Initial	At 25°C ± 2°C / 60 % RH ± 5%	
		After 1 month	After 2 month
In vitro disintegration Time(s)	560	560.2	560.19
Hardness(kg/cm²)	4.4±0.68	4.3	4.2
Drug content (%)	99.4±0.12	99.50±0.77	97.11±1.07

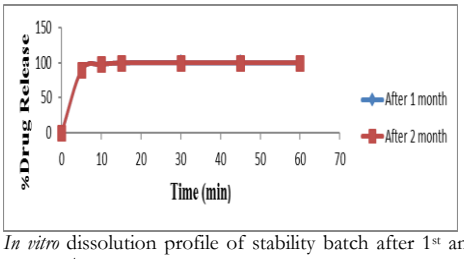


Fig 56: *In vitro* dissolution profile of stability batch after 1st and 2nd months at 25⁰ C/ 60% RH

Discussion

Etoricoxib-Cyclodextrin-Hydroxy Acids/Pvp K-30 Complexes: In the present study inclusion complexes of Etoricoxib were prepared with β cyclodextrin and Hydroxy Propyl β cyclodextrin by kneading, solvent evaporation, Spray drying and freeze drying methods. The complexes of Etoricoxib, cyclodextrin with citric acid/tartaric acid/pvp k-30 were prepared in the ratio of (1:1:1M, 1:1:2M, 1:1:1% and 1:1:2%). The complexes were characterized by Fourier Transform Infrared Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), X-ray Diffraction, and Scanning electron microscopy. The prepared complexes were evaluated by *in vitro* dissolution studies and the results of this evaluation are given in table 19-23.

Phase solubility studies:
The effect of cyclodextrin on aqueous solution of Etoricoxib was evaluated using phase solubility method. Fig. 3-14 show phase diagrams for etoricoxib in presence of two different cyclodextrin in phosphate buffer 6.8 pH. The solubility of Etoricoxib increased linearly as a function of β cd and HP β cd. The phase solubility profiles show that complexation with both cyclodextrins increases the etoricoxib solubility in a linear pattern, displaying A_L type phase diagrams in PVP K-30 and A_N type for hydroxy acids (citric acid, tartaric acid) according to the classification by Higuchi (Higuchi and Connors, 1965). The complexation constants ($K_{1:1}$) of Etoricoxib: cd: pvp k-30 with each cyclodextrin are 57.61 mol^{-1} for β cd, 370.61mol^{-1} for HP β cd using equation. Complexation is generally due to the hydrophobic interaction between poorly water soluble guest molecule, such as Etoricoxib and the polar cavity of cyclodextrin molecule. The hydrophobicity, geometry and cavity size of the guest molecule and the derivative groups of the cyclodextrin are important for complex formation. In the present study, the enhancement of Etoricoxib solubility is highly dependent on the type of cyclodextrin used. Based on the phase solubility diagrams, HP β cd with citric acid (1:1:0.2%w/v) is more effective in solubilising Etoricoxib in aqueous media compare to β CD.

***In vitro* dissolution study:**
In vitro dissolution studies for pure Etoricoxib complexes were carried out in 900 ml of phosphate buffer pH 6.8 using USP Type II dissolution rate test apparatus (Lab India). The dissolution data of etoricoxib- β cd and etoricoxib-HP β cd with citric acid, tartaric acid, pvp k-30 (1:1:1M, 1:1:2M and 1:1:1%, 1:1:2%) sequentially complexes are given in table 19 to 23. Figure 15 to 22 shows the dissolution rate profile of pure Etoricoxib and the inclusion complexes prepared by kneading, solvent evaporation, spray drying and freeze drying method with different ratios (1:1:1M, 1:1:2M, and 1:1:1%, 1:1:2%).

The dissolution rate of Etoricoxib from inclusion complexes was found to be 50.25% to 100.85% in 60 minutes when compared to Active pharmaceutical ingredients form of Etoricoxib in host dissolution which exhibited only 8.37 to 35.65% in 60 minutes. Inclusion complexes of Etoricoxib with β CD, citric acid, tartaric acid(1:1:1M,1:1:2M) and PVP K-30(1:1:1%,1:1:2%) prepared by kneading, solvent evaporation method exhibited release of 83.85%, 84.81%, 79.34%, 81.78% and 79.33%, 80.35% respectively in 60 minutes, using phosphate buffer pH 6.8 as medium. Inclusion complexes of Etoricoxib- HP β CD- tartaric acid prepared by kneading, solvent evaporation, spray drying and freeze drying method exhibited release of 84.75%, 91.21%, 86.76%,89.83%, 89.83%, 85.08%, 91.23%, 91.74%, 83.13%, 96.99%, 92.27% and 87.65 respectively in 60 minutes, using phosphate buffer pH 6.8 as medium.

A marked enhancement in dissolution rates of Etoricoxib from Etoricoxib – HP β cd- Citric acid (1:1:2M) complex prepared by freeze drying method. The higher dissolution rates were observed with inclusion complexes prepared by freeze drying method may be due to better interaction of Etoricoxib-cyclodextrin-citric acid.

Preformulation parameters:
UV scanning of the drug revealed that the drug had a λ_{max} of 284 nm at 6.8 pH phosphate buffer. Also, the IR spectrum was concordant with the reference spectrum of Etoricoxib.

Preparation of standard calibration curve of Etoricoxib:
From the standard curve Etoricoxib (Table 4, Figure 1); it was observed that the drug obeys Beer's law in concentration range of 6-20 µg/ml in phosphate buffer 6.8 pH.

Compatibility study:
Physical mixture of drug, cyclodextrin and inclusion complexes was characterized by FTIR, XRD, DSC and SEM analysis for any physical and chemical alteration of the drug characteristics. From the results it was concluded that there was no interference of the functional groups as the principal peaks of the Etoricoxib were found to be unaltered in the spectra of the inclusion complexes. Their respective generated scans are shown in spectra figures 24-55.

Characterization of Etoricoxib-HP β Cyclodextrin-citric acid (1:1:2M) freeze dried blends and tablet properties:
Pre-compression parameters: The Etoricoxib- HP β cd-citric acid complexed blend individually and along with crosscarmellose sodium was evaluated for pre-compression parameters and results are shown in Table 26. The blend was evaluated for bulk density, tapped density, Carr's index and Hausner's ratio. The bulk densities of the blend were found to be 0.449 gm/ml, while the tapped densities of the granules were found to be 0.492 gm/ml. The flow properties of the blend were assessed by determining the Carr's index. The value of the compressibility (8.256-8.379%) signifies excellent flowability. This shows that the blend had smooth flow properties ensuring homogenous filling of the die cavity during the compression (punching) of tablets.

Post compression parameters: The Etoricoxib- HP β cd-citric acid complexed blend individually and along with super

disintegrants were compressed into tablets and tablets were evaluated for their hardness, weight variation, thickness, drug content, friability and results are shown in the Table 27.

The hardness test is one of the control parameter during the manufacturing of tablets. The average hardness of the tablets was found to be4.4±0.68 kg/cm^2. The average weight variation of tablets was found within the limits of 7.5% (USP). Thickness of tablets was found to be of 3.30±0.019**.** Friability value which also affected by the hardness value of tablets should be in the range of 0.5 to 1% limits, which is the usual friability range of tablets. The friability of the prepared tablets was found less than 1% w/w. The drug content in tablets formulation ranged from 99.4% to 100.2%**.** It was found that the physicochemical parameters of the prepared tablets are within standards.

***In vitro* characterization of conventional tablets:**
In vitro drug release studies were carried out in dissolution test apparatus USP Type II with paddle type in 900ml of phosphate buffer 6.8 pH for 60 min. These release studies results are shown in Table and their values are graphically represented in figure 23. As Etoricoxib- HP β cd- citric acid by freeze drying inclusion system in the ratio **(**1:1:2M) gave 100.85 % release in 15 min. Hence in formulation F1 Etoricoxib-Cyclodextrin blend was directly compressed into tablet and the drug release at the end of 60 min was found to be 100±1.37%.

Comparison of release profile
The dissolution release at different time points were taken for the best trial batches and compared with that of the innovator's product. The f2 values, which indicated the extent of similarity between two products, were calculated. FDA prescribed the range of 50-100 for similarity between two dissolution profiles. The release profile of formulation F1 was more similar to innovators release profile shown in table 29 and figure 23. The comparison of release profile for F1 with innovator was done by similarity factor and formulation F1 was optimized with a similarity factor of 78.89 shown in table 29.

FTIR study:
The FTIR spectra of pure Etoricoxib showed characteristic peaks at 1500.670 cm^1 (C=N stretching vibration), 1294.0cm^{-1} (S=O stretching vibration), 1587.47(C=C aromatic benzene) and 767.69 cm^{-1} (C-Cl stretching vibration), 2916.47 (sp3 CH stretching), 3010.48 (sp^2 CH bending) and CN-1134.

The FTIR spectra of citric acid showed (OH- acid) 3394.83, (sp^3 CH stretching), 1734.64 (C=O carbonyl), 1215.19 (C-O). The FTIR spectra of pvp k-30 showed 2956.97(sp^3 CH), 1435.09(pyrole aromatic), 1660.77(ketone), 1282.71 (CN stretching aromatic pyrrole). The FTIR spectra of MCC showed characteristic peaks at 3248.54 cm^{-1} for -OH stretch, 1028.1 for C-O stretch, 2899.11for CH_2 stretch and 111.48, 1165 and 1028 for C_1-O-C_4. FTIR spectrum of Etoricoxib- HP β cd- citric acid/tartaric acid/pvp k-30 freeze dried complex shows characteristic broad peak at 3400.cm^{-1} to 3415.63.cm^{-1}. This shift and broadening of peak indicates interaction between and HP β cd. Intermolecular hydrogen bonding was observed in Etoricoxib cyclodextrin complexes prepared by freeze drying, due to broad absorption at 3402.54 cm^{-1}.

DSC studies:
DSC thermogram of Etoricoxib showed a broad endothermic peak at 134.38 ^{o}C which is corresponding to its melting point. DSC thermogram of β CD showed a broad endothermic peak at 101°c due to loss of water molecule. DSC thermogram of HP β CD showed a broad endothermic peak from 60-80°c. The DSC spectra of the formulation showed a marked difference in melting point and had a pronounced transition temperature indicative of the correctness of formulation formation.

X Ray Diffraction:
In the x- Ray diffractogram of Etoricoxib, sharp peaks at a diffraction angle (2θ) of 4º, 10º, 16 º, 17 º, 18 º, 19 º and 23 º are present and it suggest that the drug is present as crystalline form. The X-ray pattern of formulations prepared with different methods showed 1 sharp peak at a high intensity range of 500 to 1000 cps. The complexed formulation of Etoricoxib showed the sharp peaks with decrease intensity range of 400 – 500 cps. The x – ray scan showed the partial amorphous characteristics. The decreased intensity of peak suggests the partial conversion of crystalline to amorphous form which can assume to responsible for solubility enhancement of the drug.

Scanning Electron Microscopy:
SEM data of Etoricoxib: HP β CD: Citric acid (1:1:2M) prepared by freeze drying method are represented in fig.60. The comparison of surface morphology of API form of etoricoxib is in needle shape form as compared with the complexed formulation it indicates the formation of complexation. The surface morphology shows a marked change in API form (crystalline) to the complexed form (amorphous).

Conclusion

In the present investigation inclusion complexes of Etoricoxib were prepared with β cd, HP β cd. These systems are useful in enhancing aqueous solubility and hence oral bioavailability. Complexes prepared with HP β cd and Citric acid by freeze drying method has shown good release of drug, (100.85% at 15th min).

From the present study following conclusions can be drawn:
Cyclodetxrins like β cd and HP β cd can be used to prepare inclusion complexes of etoricoxib with improved solubility of drug. Phase solubility studies of etoricoxib- β cd- pvp k-30 illustrate the solubility enhancement of β cd. The stability constant $K_{(1:1)}$ of etoricoxib- β cd- pvp k-30 (1:1:2%) complex was found to be 57.56 mol^{-1}. Phase solubility studies of etoricoxib- HP β cd- pvp k-30 illustrate the solubility enhancement of HP β cd. The stability constant $K_{(1:1)}$ of etoricoxib - HP β cd - pvp k-30 (1:1:2%) complex was found to be 360.01mol^{-1}. Phase solubility studies of etoricoxib- β cd- citric acid (1:1:2M) and etoricoxib- HP β cd- citric acid (1:1:2M) illustrate the solubility enhancement of HP β cd phase solubility type was found to be of $A_N$type. Phase solubility studies of etoricoxib- β cd- tartaric acid (1:1:2M) and etoricoxib- HP β cd- tartaric acid (1:1:2M) illustrate the solubility enhancement of HP β cd phase solubility type was found to be of $A_N$type.

Characterization studies like FTIR, DSC, XRD and SEM indicated the correct formulation of complexes of etoricoxib with β cd, HP β cd.

Conventional tablets of etoricoxib was successfully prepared with etoricoxib- HP β cd- Citric acid (1:1:2M) complex using croscarmellose sodium. The formulation F1 was an optimized formulation which has similarity factor of 78.89 with that of marketed product.

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