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Formulation and Development of Colon Specific Etoricoxib CODES™ Tablet: Statistical Optimization and *In Vivo* Roentgenography

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ABSTRACT

The aim of the study was to optimize Etoricoxib (ETX) CODES™ tablet. Core tablets consisting of ETX and lactulose were prepared by wet granulation method and evaluated for pharmacopoeial tests. The core tablets were subsequently coated with optimized levels of (acid soluble layer) Eudragit E100 followed by (barrier layer) HPMC and finally with (enteric layer) Eudragit L100. Concentration of plasticizer was optimized by preparing films, which were evaluated in terms of folding endurance. A full factorial design was employed which comprised of two independent variables: coating level of Eudragit E100 and amount of lactulose, with three different levels and dependent variable was percent drug release at 7th hour i.e. as soon as the formulation reaches to colon. Fermentation study was carried out to decide the inoculation dose of the bacterial culture which was 108mL of *Lactobacillus sporogenes* in 900mL of test medium. The *in vitro* drug release study of nine batches was carried out by change over media method (1.2pH HCl buffer, 5pH and 6.8pH phosphate buffer, with and without addition of microbial culture *Lactobacillus sporogenes*) to select optimized formulation (F2) that was subjected to *in vivo* roentgenography. Dissolution study with addition of microbial culture showed fast drug release (98.929±1.69%) than the dissolution study without addition of culture (94.03±1.72%). Roentgenography study substantiated the intactness of formulation till it reaches to colon. FTIR and DSC revealed absence of any major interaction between drug and formulation excipients. Conclusively, colon targeted ETX CODES™ tablets were prepared using CODES technology and its ability to target colon was validated by roentgenography.

INTRODUCTION

Targeting drug to the specific organ gives certain advantages like low incidences of adverse side effects, delivery of drug to target site and lower conventional dose(1). Delivery of drugs via colon offers numerous therapeutic advantages not only in case of local pathologies such as ulcerative colitis, Crohn's disease, carcinoma and infections but also in case where circadian rhythm is evident e.g. asthma,ulcer,ischemic heart diseases and rheumatoid arthritis.Various approaches are reported for development of colon targeted systems like Colon targeted delivery capsule (CTDC) and CODES^{TM}(2). Rheumatoid arthritis(RA)is an inflammatory autoimmune disease which affects large number of people by causing joint destruction and disability. Two classes of medication are used for treating RA,fast-acting NSAIDS (Nonsteroidal anti-inflammatory drugs) such as Aspirin, Naproxen, Ibuprofen, Celecoxib, Rofecoxib, Etoricoxib and slow-acting DMARDS(Disease modifying anti-rheumatic drugs)such as Adalimumab, Etanercept, Methotrexate and Hydroxychloroquine(3,4).

Etoricoxib (ETX) is an effective nonsteroidal anti-inflammatory agent (5-chloro-6-methyl-3-[4-(methylsulfonyl) phenyl]-2, 3-bipyridine) used for various arthritic conditions. It is a selective inhibitor of cyclooxygenase-2(COX-2) belonging to BCS classII(5).Clinical equivalence for ETX 30mg once daily versus ibuprofen 800mg thrice daily and ETX 60mg once daily versus diclofenac 50mg thrice daily, naproxen 500mg twice daily, and celecoxib 200mg once daily is well established(6).ETX was found to be efficient in extension studies up to 4.5years(7).In patients with rheumatoid arthritis,8weeks treatment with ETX 90mg once daily was significantly effective at improving symptoms such as tender and swollen joint(8).

ETX has not been explored much due to certain dose dependent side effects (9,10). A few ETX formulations like mouth dissolving tablets (11) and oral disintegrating tablets are available in market. Few attempts have been made to increase the solubility of ETX by preparing solid dispersions and cyclodextrin inclusion complexes (12-14).The present study aims for the development of chronotherapeutic delivery of ETX for colon region in the form of oral tablet dosage form. ETX when given in the conventional tablet form fails to produce the desired drug release due to rapid systemic absorption. ETX being an efficient drug for arthritis, if given in the form of colon release tablet will prevent the release of the drug till it reaches the colon and

concentrate maximum drug in colon while avoiding systemic side effects(9). Moreover, in the present work, the proposed ETX formulation is intended to be released when there is a localized pH fall, in which it is freely soluble so there is no need to enhance solubility. Also the dose dependent side effects of the ETX can be effectively reduced by achieving colon targeted delivery with reduced dose through new formulation approaches. Thus ETX is a very good candidate for colon delivery system as luminal delivery can focus the maximum drug concentration in colon while avoiding significant systemic exposure.CTDC approach is reported to target ETX to colon with succinic acid as an efficient carrier (15).

To the best of information, although ETX is a good candidate for colon delivery, there is lack of study of ETX in CODES^{TM} form. CODES^{TM}(16)is a novel technology which utilizes mechanism of both pH dependent and microbial triggered system (Figure 1) (17).Briefly, it is hypothesized that the system consists of drug, lactulose and other excipients in tablet core which is first coated with acid soluble polymer Eudragit E100,followed by a barrier layer of HPMCand final enteric coating of Eudragit L100 which releases the drug at targeted site i.e. colon.Enteric coating protects the system in acidic environment of stomach and degrades in small intestine whereas acid soluble polymer protects the formulation in small intestine. Barrier layer of HPMC prevents possible interaction between cationic Eudragit E100 and anionic Eudragit L100 coatings (18). When system reaches colon, lactulose is converted into lactate and other short-chain organic acids via β-galactosidase by colonic microflora(19).These organic acids lower pH around microenvironment of tablet, dissolving acid soluble polymer completely and thus promoting drug release (20) and avoiding the inherent problems associated with pH dependent and microbial triggered system.

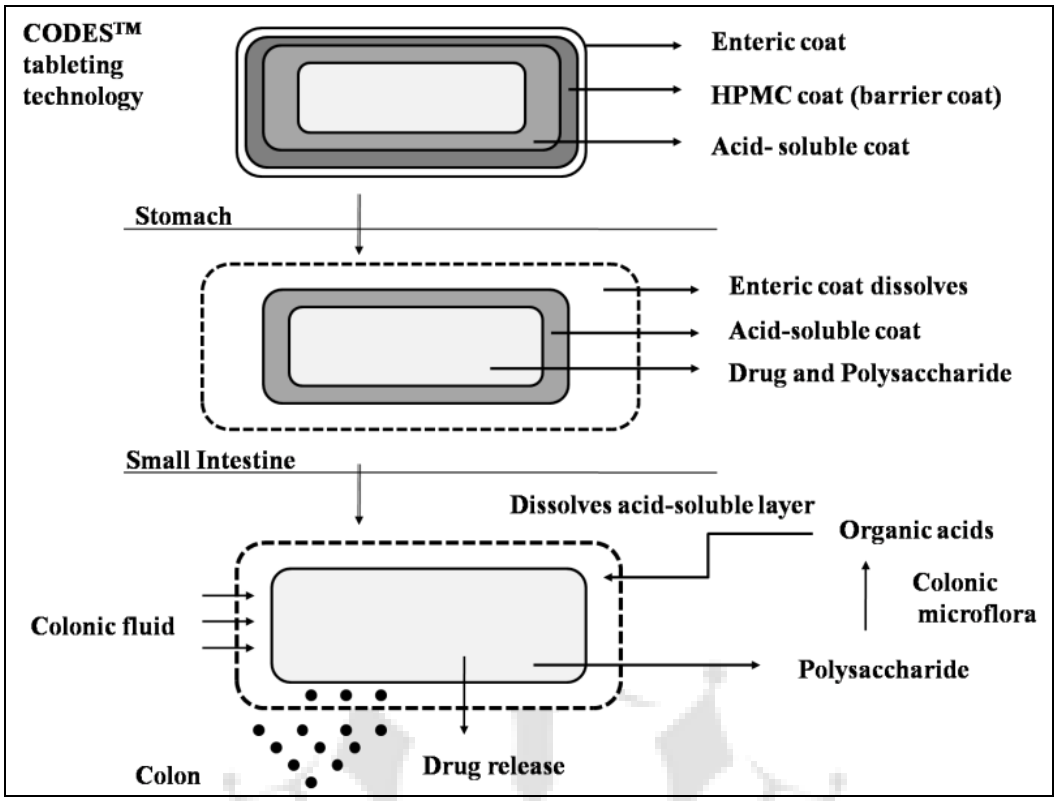

Figure 1: Schematic representation of CODES™ technology.

There are other polysaccharides such as isomalt, pectin, amylose and galactomannan which have been explored as carriers for colon-specific drug delivery. Clinical studies showed that ingesting lactulose resulted in consistent and substantial acidification of proximal colonic contents as a result of fermentation but had little effect on the pH of the distal colonic contents (18). Also, lactulose is used in infant formula, dairy products and as medication for chronic constipation.Moreover,there exists an inverse relationship between colon carcinogenesis and dietary intake of lactulose (21). And in the case of colon carcinoma, adjuvant therapy with NSAIDS is given,as NSAIDS reduce the relative levels of PGE2 production in human colon carcinoma.Thus a combination of colon acidifier (lactulose) and NSAIDS might find their clinical application in the case on colon cancer also. However, in the present study, the selected dose of ETX CODES™ is therapeutically efficient in rheumatoid arthritis.

Thus considering all benefits of CODES™ system and suitability of ETX for this system, present study was aimed (a)To develop , optimize and evaluate colon targeted delivery system using novel combination of CODES™ and ETX, a COX-2 inhibitor for rheumatoid arthritis, which

remains intact throughout GIT and releases the drug as soon as the formulation reaches ascending colon i.e.at about 7th h.(b)To carry out fermentation studies using different bacterial cultures to determine their lactulose-metabolizing capacity.(c)To perform i*n vivo* roentgenographic study in rabbits for determining coating integrity of formulation.

MATERIALS AND METHODS

Materials

Etoricoxib was obtained as gift sample from Zim Laboratories, MIDC, Nagpur and Lactulose was obtained as generous gift sample from S.C.M. Fresenius Kabi Company, Austria (GMBH).Eudragit E100 and Eudragit L100 were obtained as gift sample fromEvonik Degussa India Pvt. Ltd. Mumbai. Hydroxypropyl methylcellulose (Methocel E4M) was obtained as gift sample from Colorcon Asia Pvt.Ltd., Goa.All other chemicals used during study were of analytical reagent grade.

Methods

Preformulation studies

Melting point range of drug was determined by using Thiele tube method and solubility of drug was determined in different solvents like methanol, tetrahydrofuran, dimethylsulfoxide, dimethylformamide, chloroform, ethanol, and toluene. Also the solubility of ETX at different pH like 1.2 pH HCl buffer, pH 5 and pH 6.8 phosphate buffer was determined by Ultraviolet spectroscopy (UV) using shake-flask method. Identification and confirmation of drug was carried out by using Fourier transform infrared spectroscopy(FTIR),Differential scanning calorimetry (DSC) & Ultraviolet spectroscopy (UV). FTIR spectrum of pure drug was recorded by FTIR spectrophotometer using KBr disc method (FTIR spectrophotometer, Shimadzu Corporation, Japan). The drug was triturated with KBr powder in a weight ratio of 1:100 and scanned from 4000 to 500 cm^{-1}(22). DSC thermogram for drug was obtained using differential scanning calorimeter (DSC Mettler10 Star system, Mettler-Toledo, Switzerland). Sample weighing 2-3mg was analyzed in standard 40µl aluminum pan, with heating rate of 10°C/min from 30-300°C with continuous nitrogen purging at flow rate of 50mL/min (23). For spectrophotometric determination of $\lambda_{max,}$ drug (ETX) stock solution of concentration

1000µg/mL was prepared which was then further diluted to 10µg/mL concentration. Solution was filtered and analyzed spectrophotometrically to determine λ_{max} and validated on ICH guidelines for parameters as Linearity, Precision, Robustness, Ruggedness, LOD, LOQ and Recovery studies (24). Calibration curves were plotted for ETX in methanol, phosphate buffer (pH6.8), phosphate buffer (pH5), hydrochloric acid buffer (pH1.2) for which series of working standard solutions of concentration ranging from 2–14µg/mL were prepared from stock solutions. Absorbance of these solutions was measured on UV spectrophotometer (V-630, Jasco 2000 series, Japan) at 234nm (25).

Drug excipient compatibility study

Interaction between drug and excipients was studied by FTIR and DSC. FTIR spectra of pure drug, excipients, optimized formulation and physical mixture of tablet composition were recorded by FTIR spectrophotometer using KBr disc method as described above. The spectrum of drug and excipients was compared to that of optimized formulation and physical mixture. DSC thermograms of drug, Eudragit E100, lactulose, physical mixtures (ETX + lactulose+ Microcrystalline cellulose (MCC) PH101, EudragitE100+HPMC+Eudragit L100, magnesium stearate+ talc) and optimized formulation were obtained by differential scanning calorimeter as per the procedure describe above.

Preparation of ETX core tablets

Desired quantity of bulk powder was weighed and granulated using wet granulation technique by employing 2% w/w HPMC in distilled water as binder solution (Table 1). Granules were evaluated for pre-compression properties like angle of repose, bulk density, tap density, Carr's index and Hausners ratio. Granules were then fed manually to pilot press tablet compression machine (Chamunda Pharma Machinery Pvt.Ltd. Ahmedabad). A 250mg tablet was compressed using 8mm concave shaped punches where compression force was kept constant for all formulations (26). Mainly three batches were compressed LF_1, LF2, LF3 containing 20%, 40%, 60% of lactulose.

Table 1: Composition of core tablets of etoricoxib (CODES^{TM})

Batches	Etoricoxib (mg)	Lactulose (mg)	MCC PH 101(mg)	Magnesium stearate (mg)	Talc (mg)	Coating levels (%)
F1	90	50	109.75	0.25	0.25	4
F2	90	100	59.75	0.25	0.25	4
F3	90	150	9.75	0.25	0.25	4
F4	90	50	109.75	0.25	0.25	8
F5	90	100	59.75	0.25	0.25	8
F6	90	150	9.75	0.25	0.25	8
F7	90	50	109.75	0.25	0.25	12
F8	90	100	59.75	0.25	0.25	12
F9	90	150	9.75	0.25	0.25	12

Evaluation of core tablet

Core tablets were evaluated for various parameters namely tablet thickness, diameter, hardness, %friability, drug content, weight variation and *in vitro* disintegration time. A mean of triplicate was used so as to study the variability in the results. Thickness and diameter of tablets were measured using Vernier caliper (Yamayo instruments, Mumbai). Hardness of tablets (n=3) was estimated by Monsanto hardness tester. Friability was estimated using Roche friabilator (Veegoscientific, India). Tablets (n=6) from every batch were selected randomly, weighed and placed in the plastic chamber provided in apparatus. Friabilator was operated for 100 revolutions and tablets were collected, de-dusted and reweighed. Difference in weights was used to estimate percent friability. For drug content determination, tablets were crushed and powder equivalent to 10mg of drug was transferred in 10mL of methanol to give concentration of 1000µg/mL, and filtered. The filtrate was diluted to give a concentration of 4µg/mL and analyzed spectrophotometrically. The weight variation and *in vitro* disintegration test were done in accordance to USP (27).

Formulation of CODES^{TM} ETX tablets

Prepared core tablets were given three layers of polymeric coating using pan coating system i.e. first layer of Eudragit E100 (10% w/w)and 2% of triethyl citrate (TEC) in isopropyl alcohol (IPA), second layer with HPMC E4M(2% (w/w) in distilled water) and third layer of Eudragit L100 (6%(w/w) and 3% of dibutyl phthalate (DBP) in isopropyl alcohol). The concentration of plasticizer was optimized by preparing films using solvent evaporation technique, for which polymeric solution was poured on a glass ring(3.14cm^2) placed on mercury surface and dried at room temperature for 24 hours. Films were evaluated in terms of appearance and folding endurance. The coating solution was sprayed on pre-warmed bed (30^{0}C), and tablets were coated and dried using inlet air temperature (35-40^{0}C) at 25-30rpm. The process of coating was performed till desired weight gain was achieved. At the end of each stage of coating, tablets were cured in coating pan for 15 minutes and dried at 40^{0}C in hot air oven (Spectrum equipments, Hyderabad) for 2h. Percentage weight gain of tablets after coating was assumed to be indicative of coating thickness.Coating of Eudragit E100 was done with respect to increasing weight gain of tablet i.e. 4%,8%,12% per tablet core as per the experimental design levels (Table 1), whereas coating weight gain of 2% and 6% respectively was achieved for HPMC E4M and Eudragit L100 coating polymers(28).

Lactulose release

Lactulose release was determined by measuring change in weight of the tablet. Tablets (n=3) from each batch were selected randomly, weighed and placed in Petri dish containing 15mL of medium. The study was conducted in two different mediums, simulated gastric fluid (SGF) HCl buffer, pH 1.2 for 2h and then 6h in simulated intestinal fluid (SIF) phosphate buffer pH 6.8 (27). At regular intervals, tablets were removed carefully from Petri dish and excess surface water was removed by light blotting with filter paper. Tablets were reweighed and change in weight was recorded.

***In vitro* drug release study**

In vitro drug release from ETX CODES^{TM} tablet was carried out to determine and compare the effect of added microbial culture (*Lactobacillus sporogenes*) [108mL] in dissolution media during dissolution study at colonic pH.First dissolution study of 9 batches in three buffers-

SGF(pH 1.2)for 2h,followed bySIF (pH 6.8)for 4h,and then simulated colonic fluid (SCF) pH 5.0 buffer for 4h was carried out without addition of microbial culture to dissolution media at any stage of process (27). Then dissolution study was repeated in above mentioned SGF, SIF for 2h and 4h respectively followed by addition of microbial culture for further 4h. For the dissolution study carried out by addition of microbial culture, all solutions were degassed for 20 minutes before use and continuous nitrogen purging was provided in vessels using a mini-vap nitrogen evaporator (6-port). Test conditions for dissolution were 900mL medium, stirring speed of 75rpm and temperature 37±0.5°C. Aliquot samples were withdrawn at regular intervals and sink condition was maintained. Samples were analyzed spectrophotometrically at 231nm for SGF, 234nm for SIF and SCF (29,30)

Fermentation studies

Screening of bacterial strains was done to determine their lactulose-metabolizing capacity. It was carried out by inoculating liquid media i.e. lactulose broth (250mL) containing 100mgof lactulose, with different concentration of bacterial culture (with and without lactulose) which had been grown overnight in basal medium. To liquid media was added bromocresol purple (Few drops).It was then incubated at 37°C in anaerobic conditions. Time-course of experiment was over 2h, with pH measurement by microelectrode every hour. Two strains selected were *Lactobacillus sporogenes* and *Enterococcus faecalis* (31).

Experimental design for ETX CODES^{TM} tablets

A full factorial design was selected for optimizing ETX CODES^{TM} tablet. The independent variables were coating levels of Eudragit E100(X_1) and amount of lactulose (X_2), each at three different levels and dependent variable was percent drug release at 7thh (32)i.e. as soon as formulation reaches to ascending colon (Table 2). Total of 9 (F1-F9) formulation batches were prepared and evaluated for percent drug release.

Table 2: Experimental design: factors and responses

Factors (independent variables)	Levels used			Responses (dependent variables)
	-1	0	1	
X_1(Eudragit E 100 coating)	4%	8%	12 %	Y_1= %DR at the end of 7th hr
X_2(Lactulose loading)	20 %	40 %	60 %	

Statistical analysis

Effect of two independent variables, coating levels of Eudragit E100(X_1) and amount of lactulose (X_2) on response (Y) was observed by employing Design-Expert Software (Version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN). The regression equation for response was calculated using following equation-

Response: $\mathbf{Y=b_0+b_1X_1+b_2X_2+b_3X_1^2+b_4X_2^2+b_5X_1X_2}$

Responses in above equation Y are quantitative effect of formulation components or independent variables X_1 and X_2; b is coefficient of term X. The main effects (X_1 and X_2) show the average result of changing factors one at a time from its low to high value. The interaction terms(X1, X2) represent response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) investigate the non-linearity parameter. Contour plots and 3D-graphs were generated using output files by Design-Expert Software. Significance of these parameters on variables was assessed by analysis of variance (ANOVA) (34).

***In vivo* roentgenography study**

The roentgenography study was accomplished in accordance with institutional ethical and regulatory principles. The investigations were performed with approval by the Institutional Animal Ethics Committee, Department of Pharmaceutical sciences, R.T.M.N.U., India (IAEC/UDPS/2014/29).The study involved the use of three rabbits, weighing 2–2.5 kg.The study was conducted in two steps, first, barium meal study was carried out to trace gastrointestinal tract

of animal and then the actual study was performed. All rabbits under test were fasted overnight with free access to water. Barium meal study was conducted by administering 15-20mL of standard barium sulphate suspension (50% w/v) (35) and x-ray images were captured periodically until barium meal reached colon. For actual study, optimized CODES^{TM}tablets (6mm) were prepared by replacing ETX with radio-opaque compound barium sulphate and coating steps were accomplished. After overnight fasting, tablets were administered to three rabbits with 10-15mL of water taking care that animal does not chew tablet. X-ray images of rabbits in lateral position were captured using Siemens X-ray machine (Heliophos D X-ray generator, Nagpur Veterinary College, India) at 80mAs and 42kV voltages, to trace movement and coating integrity of CODES^{TM} tablet in gastrointestinal tract(36-37).

Stability studies

Stability studies were performed to check effect of environmental or storage conditions. Optimized batch was kept for accelerated stability condition at 40^0C±2^0C temperature and 75±5% relative humidity for a period 3 months. The samples were withdrawn at end of 0, 30, 60 and 90 days' interval and evaluated for physical appearance, hardness, friability, weight variation, drug content, and *in vitro* drug release.

RESULTS

Preformulation studies

The melting point range of ETX by Thiele tube method was found to be 127-128°C. ETX formed a clear solution in methanol, tetrahydrofuran, dimethyl sulfoxide, dimethyl formamide and chloroform, isopropyl acetate, ethanol, toluene. ETX was found to be insoluble in water. The solubility of ETX was found to be 14.06 mg/mLin 1.2 pH HCl buffer, 0.21 mg/mL in pH 5 phosphate buffer, and 0.11 mg/mL in pH 6.8 phosphate buffer. The FTIR spectra of ETX (Figure 2) showed characteristic peaks at 3057.17cm^{-1} for (C-H) stretching, 1678.07 cm^{-1} for (C=C) stretching, 1083.99cm^{-1} for (S=O) bond, and 1598.99cm^{-1} for (C=N) bond. However, DSC thermogram of ETX (Figure 3) shows a sharp endothermic peak at 134.55. UV spectrophotometric analysis of ETX solution in methanol gave λ_{max} at 234nm. Similarly, wavelength of maximum absorption (λ_{max}) for solution of ETX prepared in 6.8pH phosphate

buffer and 5pH phosphate buffer was found to be 234nm, while in 1.2pH hydrochloric acid buffer it was found to be 231nm.

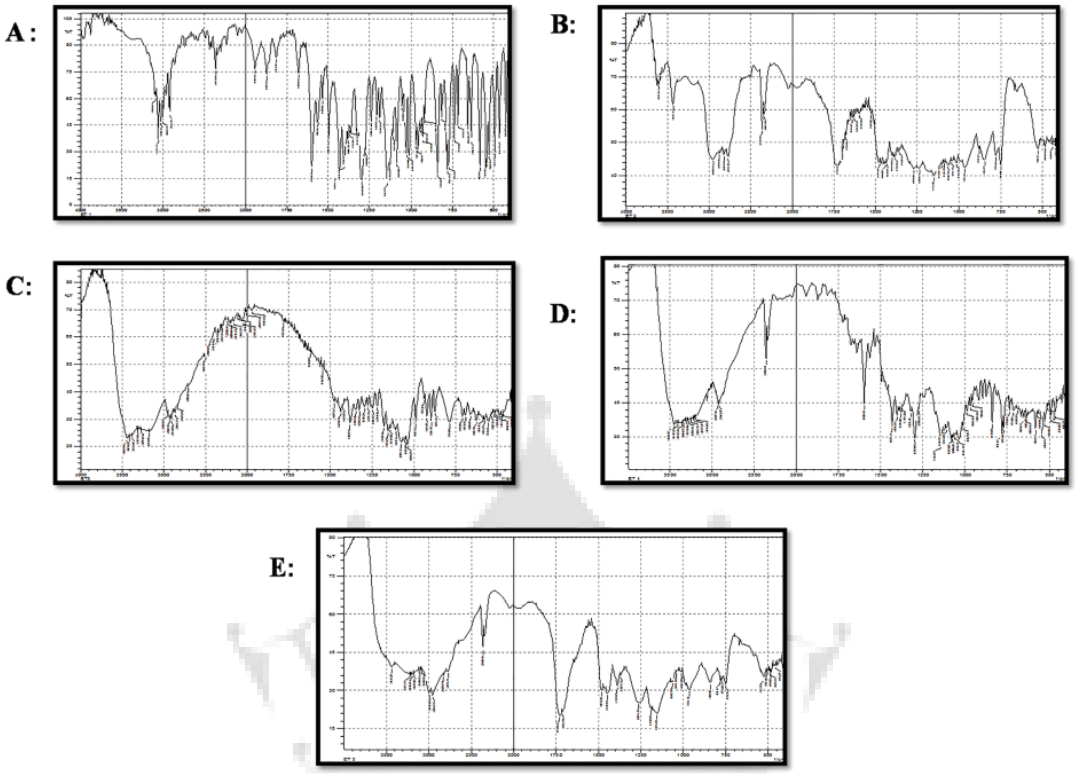

Figure 2: FTIR spectrums of (A) ETX, (B) Eudragit E 100, (C) Lactulose, (D) Physical mixture, (E) Optimized formulation.

The standard calibration curve of ETX in methanol was found in the range y = 0.075x – 0.005 with R^2 as 0.999. The %RSD values for intra-day and inter-day validation parameters was found to be 0.4382 and 0.4710. While the % RSD values for robustness and ruggedness was found to be 0.8276 and 0.8207. The percent recovery for18, 20 and 22 µg/mL solutions were found to be 98.2037, 100.9800, 103.9123% respectively. The Limit of Detection (LOD) for 2 µg /mL and 10 µg/mL was found to be 0.0262 and 0.0219 respectively. While the limit of quantification (LOQ) for 2 µg /mL and 10 µg /mL was found to be 0.0794 and 0.0663 respectively.

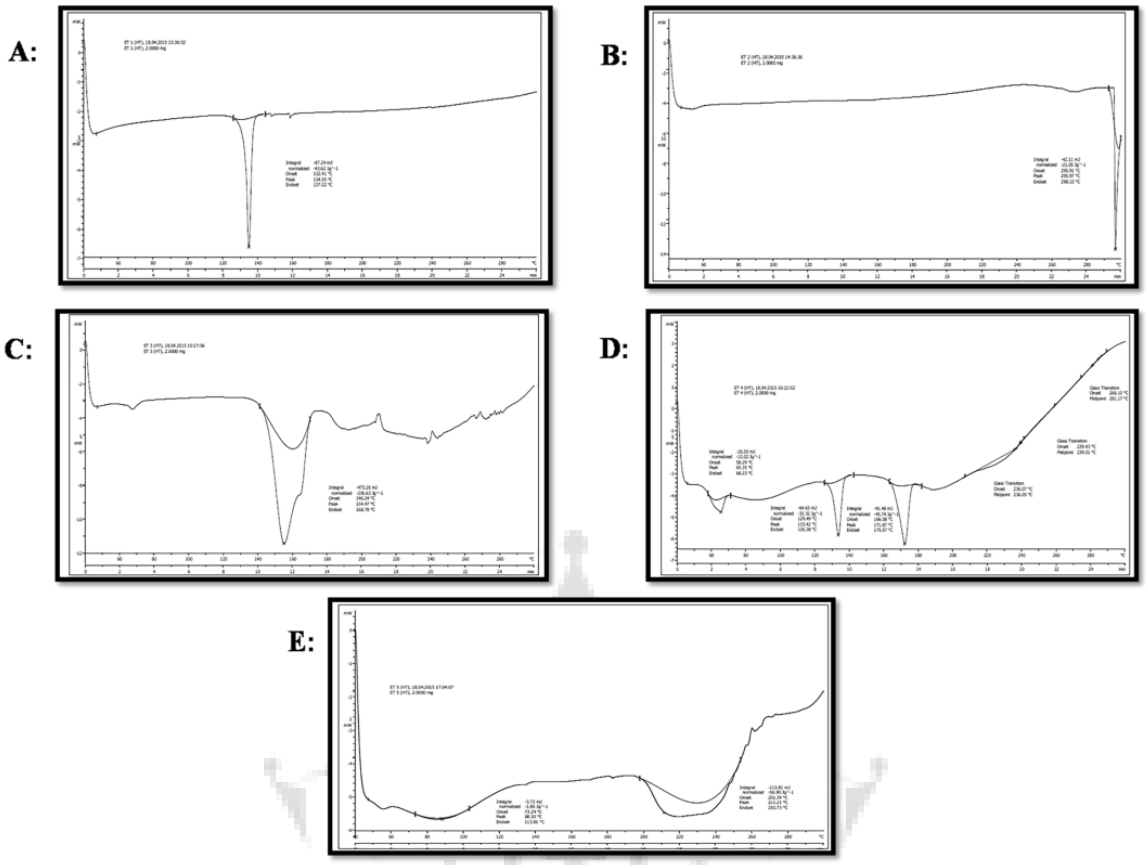

Figure 3: DSC thermograms of(A) ETX , (B) Eudragit E 100, (C) Lactulose, (D) Physical mixture, (E) Optimized formulation.

The calibration curve of ETX in 6.8pH phosphate buffer was in the range y = 0.076x+0.054 with R^2value 0.994. The calibration curve of ETX in pH5 phosphate buffer was found in the range y = 0.129x– 0.007 with R^2value of 0.997. While the calibration curve of ETX in pH1.2 hydrochloric acid buffer was in the range y = 0.113x+0.043 with R^2value of 0.997.

Drug excipient compatibility study

FTIR spectra of Eudargit E100 showed characteristic vibration at wave number 2819.93cm^{-1} due to dimethylamino bond. While spectrum of lactulose showed peak at 3442cm^{-1} for (OH) stretching and at 1155cm^{-1} due to the presence of (C=O) group in lactulose structure. The spectra of physical mixture and ETX was compared and no major deviation was noted(Figure 2).While DSC thermograms of ETX, physical mixture and optimized formulation when compared did not show any major shifting of endothermic peak of drug and lactulose(drug peak shifted from

134.55° to 133.42° and lactulose peak shifted from 154.47° to 171.87°).However,there was a presence a new peak in thermogram D and the drug showed diffused peak in thermogram E(Figure 3).

Evaluation of ETX core tablet

The pre-compression properties of granules and post-compression properties of core ETX tablets were evaluated as per pharmacopoeial test standards. Angle of repose ranged between 20°.05 to 22°.58 while Carr's index values and Hausners ratio values ranged between 1.96 to 7.27 and 1.02 to 1.07 respectively. Tablet thickness ranged 6.1 to 6.6 mm and the diameter nearly varied in range 8.02 to 8.08 mm. The average weight of tablets ranged from 250.7 to 252.7 mg while hardness of tablets ranged from 3.4 to 3.8 kg/cm^2.A friability of less than 1% was obtained which ranged between 0.116 to 0.165 %.Drug content values varied between 93.9 to 94.9 %. Variation in *in vitro* disintegration time from 13.51 to 15.25 min was found to be inversely related to MCC content. Higher *in vitro* disintegration time was displayed by formulation LF_3 (15.25±0.87) containing lowest amount of MCC and lower disintegration time was exhibited by formulation LF_1 (13.51±0.24) containing highest level of MCC.

Evaluation of CODES^{TM} ETX formulation

The core ETX tablet was successfully coated to formulate CODES^{TM} ETX tablet by applying three polymer coatings which consisted of acid soluble Eudragit E100, barrier coating of HPMC, and enteric coating of Eudragit L100 respectively. The plasticizer content was optimized in different solutions which were evaluated in terms of folding endurance (Table3).Brittle films and low numbers of folds were obtained when TEC and DBP were casted using acetone and ethanol/water mixture(9:1). Highest numbers of folds were obtained by 2% concentration of TEC in IPA which showed 189 ± 1.03 folds and 3% concentration of DBP in IPA showed 178 ± 3.01folds.

Table 3: Optimization of plasticizer content in polymer solution.

Plasticizer I	**Concentration(% w/w)**	**2%**	**4%**	**6%**
Triethyl citrate	**Solvents**	**Folding endurance (no. of folds)**		
	Isopropyl alcohol(IPA)	**189 ±1.03**	185 ± 3.68	182 ± 2.08
	Ethanol/water mixture [9:1]	Brittle	34 ± 2.21	57 ± 4.8
	Acetone	40 ± 1.15	68 ± 2.1	112 ± 3.48
Plasticizer II	**Concentration(% w/w)**	**1%**	**2%**	**3%**
Di-butyl phthalate	**Solvents**	**Folding endurance (no. of folds)**		
	Isopropyl alcohol(IPA)	93 ± 2.93	154 ± 3.42	**178 ± 3.01**
	Ethanol/water mixture [9:1]	Brittle	Brittle	Brittle
	Acetone	Brittle	23 ± 3.34	59 ± 4.51

*The data is presented as mean value ± S.D. (n = 3)

Lactulose release

The change in weight of the tablet for each batch per hour is given (Table4). The % change in weight of the tablet in the initial 2h (SGF pH 1.2) was insignificant and found in the range 1.2± 1.05% to 8.8 ± 3.05 %. But when the medium was changed to SIF (pH 6.8), % change in weight of the tablet started increasing and was found in the range 8 ± 1.12 % to 60.4± 3.62%.

Table 4: Lactulose release per hour.

Batch code	Change in weight per hour(%)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
F1 (20:4)	1.2 ±1.05	2 ±2.08	8 ±1.12	19.2 ±2.11	31.6 ±4.06	41.2 ±2.72	48.4 ±2.56
F2 (40:4)	1.6 ±1.08	3.2 ±2.03	6.8 ±1.04	16 ±3.29	30.4 ±3.54	40.4 ±2.08	14.4 ±2.73
F3 (60:4)	3.2 ±1.02	9.6 ±2.01	22 ±2.56	35.2 ±3.38	49.6 ±2.45	29.2 ±2.12	12.2 ±2.92
F4 (20:8)	1.6 ±1.09	8.8 ±3.05	18.8 ±3.71	28 ±2.62	34 ±1.36	40.8 ±1.16	52.8 ±2.06
F5 (40:8)	2 ±2.06	8.8 ±3.08	17.2 ±1.48	22 ±3.12	36 ±2.27	46 ±2.20	20.4 ±3.21
F6 (60:8)	3.2 ±1.05	10 ±3.06	23.2 ±2.62	38 ±1.24	51.2 ±3.18	30.8 ±2.24	14 ±3.88
F7 (20:12)	1.6 ±1.04	4.4 ±2.34	18 ±3.16	31.6 ±2.32	39.2 ±4.71	47.6 ±2.28	60.4 ±3.62
F8 (40:12)	2 ±1.07	6.4 ±2.72	12 ±2.12	18.4 ±2.54	33.6 ±2.64	43.2 ±2.32	18 ±3.45
F9 (60:12)	2.4 ±2.03	6.8 ±2.41	21.6 ±1.69	35.6 ±4.48	49.2 ±3.52	28.8 ±3.36	11.2 ±3.22

*The data is presented as mean value ± S.D. (n = 3)

***In vitro* drug release study**

The percent average drug release from ETX CODES^{TM} tablet in SGF and SIF with and without addition of bacterial culture *Lactobacillus sporogenes*is given in Table 5. Drug release study showed that there was no change in dimensions of tablet in first 2h.In SIF the tablet was found to be a little swollen while in SCF complete solubilization of acid soluble Eudragit E100 coating started where size of tablet was found to be decreased up to end of the study. The drug release

values without addition of microbial culture ranged from (56.412± 1.51% to 94.03 ± 1.72%) and maximum percent drug release (94.03 ± 1.72%) was shown by F8 batch containing highest coating of Eudragit E100 (12%w/w) and lactulose (40%w/w), followed by formulation F5 (92.714 ± 1.88%) containing medium coating of Eudragit E100 (8%w/w) with lactulose (40%w/w) and thereafter of batch F2 (98.847± 1.55%) containing low coating of Eudragit E100 (4%w/w) with lactulose (40 %w/w). The drug release values with addition of microbial culture ranged from (63.671 ± 1.62% to 98.929 ± 1.69%) and maximum percent drug release (98.929 ± 1.69%) was exhibited by batch F8 containing highest coating of Eudragit E100 (12%w/w) and lactulose (40%w/w), followed by formulation F5 (98.734 ± 1.81%) containing medium coating of Eudragit E100 (8%w/w) with lactulose (40%w/w) and thereafter of batch F2 (98.328 ± 1.39%) containing low coating of Eudragit E100 (4%w/w) with lactulose (40%w/w). The drug release study with addition of microbial culture *Lactobacillus sporogenes* showed higher drug release (98.929 ± 1.69%) than without addition of microbial culture (94.03 ± 1.72%).

Table 5: Percentaveragedrug release from ETX CODES™ tablet with and without addition of bacterial culture *(Lactobacillus sporogenes).**

Batches	Drug release (without addition of culture)	Drug release with addition of culture
F1(20:4)	89.489 ±1.81	94.637 ±1.28
F2(40:4)	92.847 ±1.55	98.328 ±1.39
F3(60:4)	90.869 ±1.33	97.614 ±1.35
F4(20:8)	90.599 ±1.36	96.212 ±1.59
F5(40:8)	92.714 ±1.88	98.734 ±1.81
F6(60:8)	91.929 ±1.47	97.241 ±1.02
F7(20:12)	91.05±1.82	96.515 ±1.83
F8(40:12)	94.03±1.72	98.929 ±1.69
F9(60:12)	91.565 ±1.12	96.515 ±1.64

*The data is presented as mean value ± S.D. (n = 3)

Lactulose fermentation studies

Enterococcus faecalis showed reduction in pH6.8 to pH5 after 4h, so study was further carried out with *Lactobacillus sporogenes* which showed reduction in pH within an hour. The change in color of bromocresol purple indicator from purple to yellow was observed. The change in pH is given in Table 6 showing inverse relationship between concentration of culture and pH. Thus volume of bacterial inoculum required in 900mL of test media was found to be 108mL in order to obtain 5pH.

Table 6: Lactulose fermentation studies*

Bacterial inoculums(mL) {*Lactobacillus sporogenes*}	**Change of pH in lactulose broth (initial pH 6.8)**	
	First hour	**Second hour**
10mL	6.34± 0.02	6.28± 0.01
20mL	6.21± 0.01	6.08± 0.01
30mL	5.09± 0.02	4.85± 0.01
40mL	4.25± 0.02	2.22± 0.01
50mL	4.04± 0.02	3.93± 0.02

*The data is presented as mean value ± S.D. (n = 3)

Statistical analysis

Statistical analysis was carried out after determining the percent drug release of all nine formulation batches by Design-Expert Software (Version 8.0.7.1, Stat-EaseInc., Minneapolis, MN).The effect on percent drug release(Y) was observed to be significant by ANOVA and the polynomial equation was found as follows:

$$\mathbf{Y= 97.72+0.48\ X_1+0.80\ X_2 - 2.31\ X_1^2 - 0.12\ X_2^2\ -0.21\ X_1X_2}$$

Where X_1& X_2 are independent variables, 97.72 is arithmetic mean response and 0.48, 0.80, 2.31, 0.12, 0.21 are estimated coefficient of factors X_1 & X_2 which represent the average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows the

response changes when two factors are simultaneously changed. The polynomial terms(X_1^2 and X_2^2) are included to investigate non-linearity. The positive sign for coefficient of X_1& X_2 indicates that as lactulose concentration and Eudragit E100 coating level is increased, the percent drug release (Y_1) also increases.3D figures (Figure 4) showed linear ascending pattern for drug release with increasing lactulose and Eudragit E100 coating levels. However, negative sign indicates increase in lactulose loading and Eudragit E100 coating delayed and decreased the drug release.

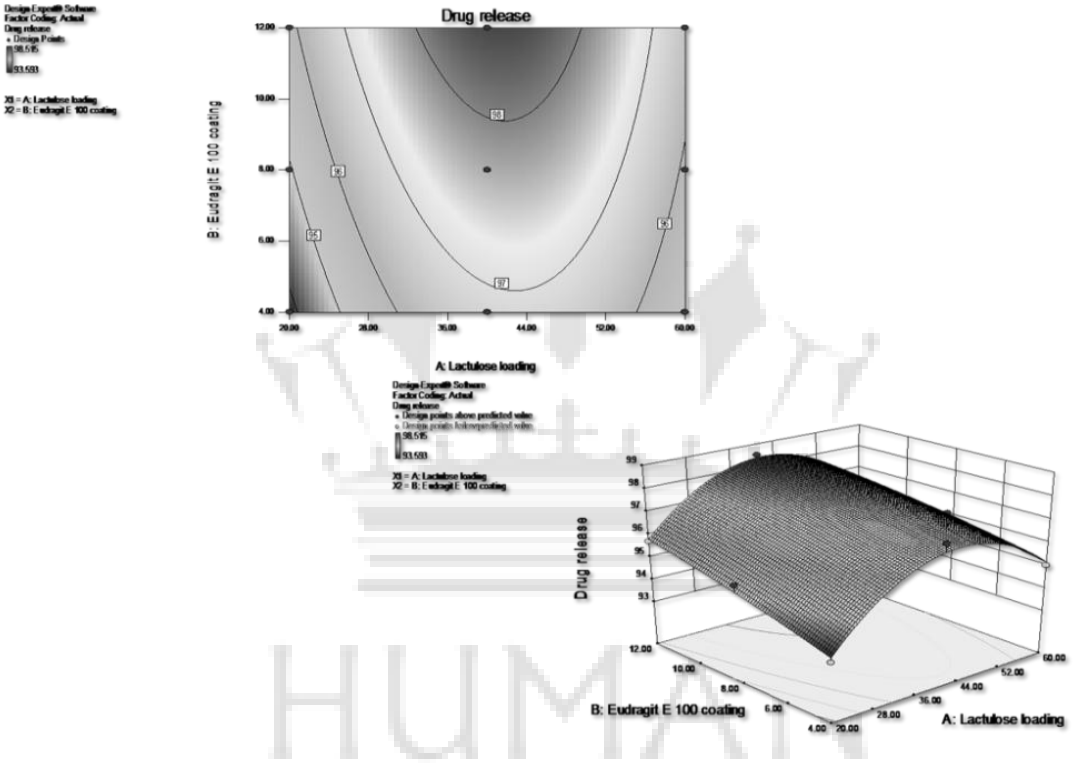

Figure 4: Contour plot & Response surface 3D plots showing effect of independent variables on the dependent variables viz lactulose loading and eudragit E 100 coating on drug release by design expert on CODES^{TM} formulations.

***In vivo* roentgenography study**

The roentgenography results are given in Table7 and Figure 5(a-b). Barium meal movement ascertained gastrointestinal tract of animal. The study was then carried out with radio-opaque optimized CODES^{TM} tablet. The actual position and coating integrity of CODES^{TM} tablet was

determined by comparing it with X-ray images of barium meal study. The x ray graphs showed that the tablet remained intact in stomach [Figure 5 (a)]. Also, no major change in size and shape of tablet (Figure 5b–d) proved that tablet remained intact in small intestine; while Figure 5(e) clearly depicts decrease in tablet size.

Table 7: *In vivo* roentgenography data of CODES^{TM} based tablets in rabbits*

Parameter	Time (h)
Mean gastric retention time	3.15 ±0.38
Mean intestinal transit time	4.05 ±0.66
Mean time of apparent reduction in size of tablet	8.10 ±0.80

*The data is presented as mean value ± S.D. (n = 3)

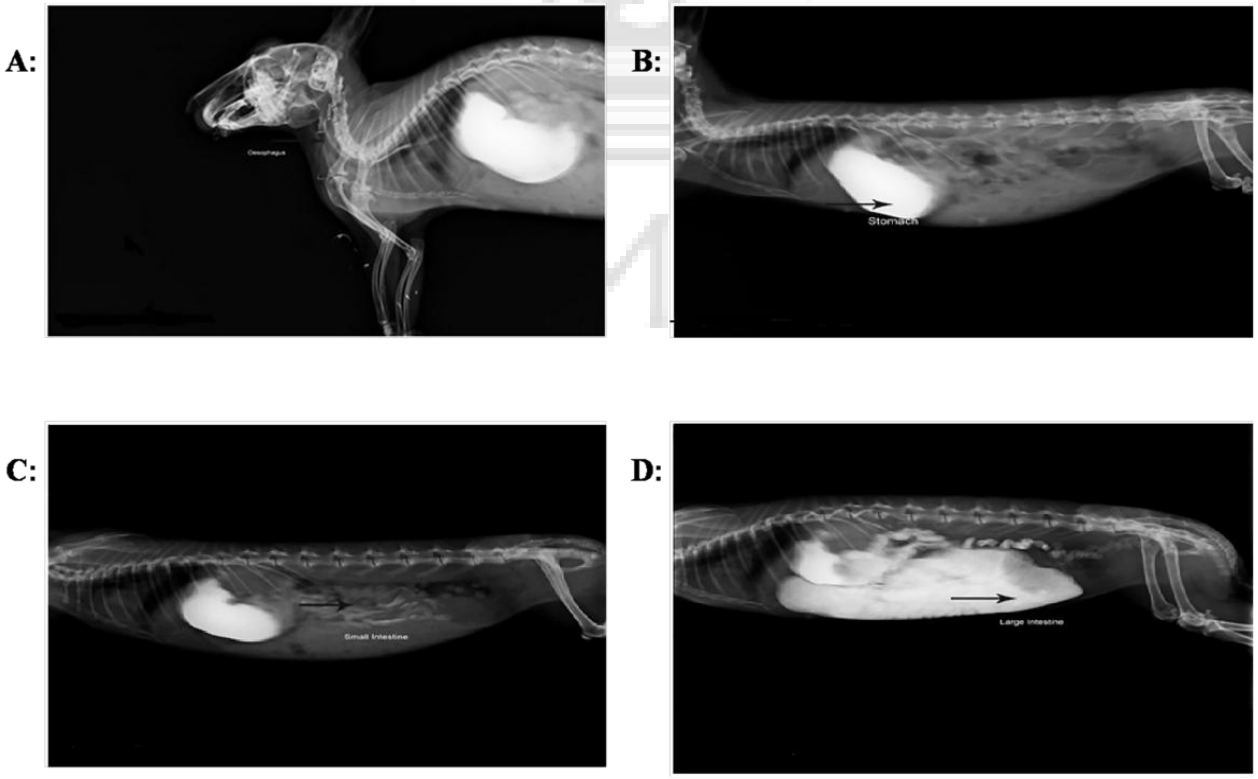

Figure 5(a): Barium meal movement through GI tract of rabbit.

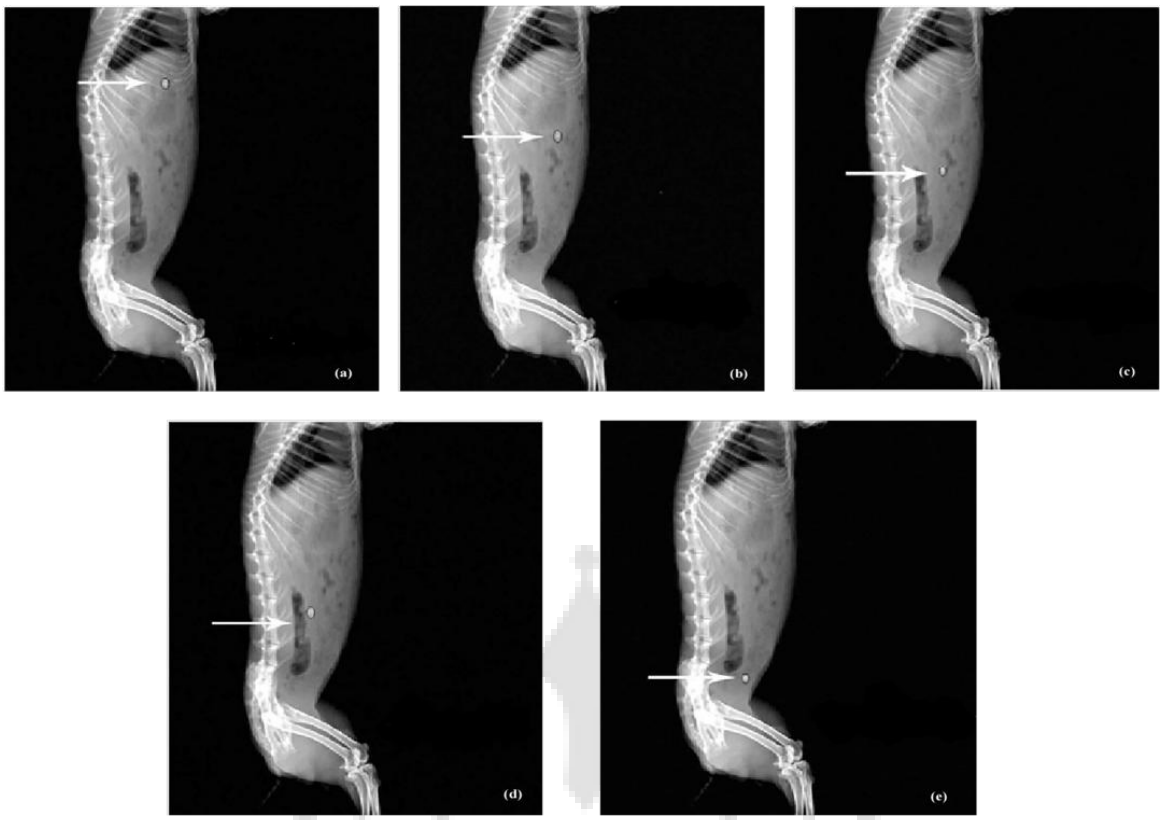

Figure 5(b): Optimized CODES^{TM} tablet along the GI tract of rabbit.

Stability studies

Stability data (Table 8) revealed no change in physical appearance of CODES^{TM} based tablet of ETX at the end of 3 months after storage at $40^0C \pm 2^0C$ and 75±5% relative humidity. The hardness value and drug content value reduced insignificantly from 3.4±0.02 to 3.3±0.05kg/cm^{2}and 94.3±1.36% to 93.9±1.10%, respectively.While friability values and weight variation values deviated slightly from 0.116±0.005% to 0.116±0.008% and 252.7±18.9% to 252.9±18.5%, respectively.The *in vitro* drug release profiles at various time intervals were compared with reference (0 day samples) showed slight change in % drug release from 98.328±1.39% to 97.929±1.08%.

Table 8: Evaluation parameters of stability batch {F2 (20:4)}*

Evaluation parameters	Before stability (0 day)	After 30 days	After 60 days	After 90 days
Hardness (kg/cm^2)	3.4±0.02	3.4±0.06	3.4±0.04	3.3±0.05
Friability (%)	0.116±0.005	0.116±0.004	0.116±0.006	0.116±0.008
Weight variation (%)	252.7±18.9	252.7±18.9	252.9±18.5	252.9±18.5
Drug content (%)	94.3± 1.36	94.3± 1.06	94.2 ± 1.12	93.9± 1.10
% Drug release	98.328 ± 1.39	98.263 ±1.04	98.057±1.09	97.929 ± 1.08

*The data is presented as mean value ± S.D. (n = 3)

DISCUSSION

Preformulation studies

General solubility study indicated that ETX is soluble in nonpolar solvents and insoluble in polar solvents as reported in earlier studies. Also, ETX was freely soluble in pH 1.2 hydrochloric acid buffer,slightly soluble in pH 5 phosphate buffer and pH 6.8 phosphate buffer (29).FTIR study of ETX exhibited the wave numbers which complied with that of standard numbers (22). The DSC thermogram of ETX showed a sharp endothermic peak indicating pure crystalline form of drug as per standard value(23). Wavelength of maximum absorption (λ_{max}) obtained for ETX in different solutions was found to be concordant with the λ_{max} specified in standard reference (24, 25). Calibration curves of ETX obtained in different solutions followed Beer-Lamberts law. The inter-day and intra-day precision study confirms adequate sample stability and method reliability over a period of 24h as the selected concentration lies within linearity range and observed RSD was < 2%. The robustness and ruggedness data shows that % RSD was within limit (less than 2).

Drug and excipient compatibility

The FTIR spectra of physical mixture and tablet excipients along with drug are shown in theFigure 2.When compared with individual spectrum of drug and excipients, it was observed that some peaks of drug were found in FTIR spectra of physical mixture, while some of the characteristic peaks corresponding to the drug were found to overlap with those of the polymer. A shift in(S= O) bond might indicate the possibility of inter-molecular hydrogen bonding via (S=O) group of ETX and (O–H) group of lactulose which may have effect on solubility of drug.In DSC thermogram D (Figure 3), presence of the new peak was may be due to the excipients present.While no major deviation in the endothermic peaks was observed,hence drug and lactulose are considered to be compatible. While DSC thermogram of physical mixture showed diffused peak of drug indicating its conversion to amorphous form and thus also indicated enhancement in drug solubility (23).

Evaluation of ETX core tablet

The precompression properties of powder blend of all three batches (LF_1, LF_2, and LF3) were found within standard limits showing good flow property. Also the post-compressional properties showed values within standard limits.The tablet thickness and diameter values indicated uniform size and regular shape of the tablet thus showing least variation in processing. The range of average weight of tablets showed uniformity in weight variation. Hardness and friability values (less than 1%) indicated good mechanical strength. Least variation in drug content values showed uniform drug distribution. Variation in *in vitro* disintegration time was found to be inversely related to MCC content. Higher *in vitro* disintegration time was shown by formulation containing lowest amount of MCC and lower disintegration time was exhibited by formulation containing highest level of MCC may be due to its disintegrating ability (27).

Evaluation of CODES^{TM} ETX formulation

Brittle films and low number of folds were obtained when TEC and DBP were casted using acetone and ethanol/water mixture (9:1). This might be due to the low solubility of plasticizer in the solvents and also the volatile nature of the solvent.Highest numbers of folds were obtained by 2% concentration of TEC in IPA which gave 189 ± 1.03folds and 3% concentration of DBP in

IPA gave 178 ± 3.01folds,thus a 2% concentration of TEC and 3% concentration of DBP in IPA were used for coating Eudragit E 100 and Eudragit L 100, respectively.

Lactulose release

A plot of % change in weight of tablet versus time (Figure 6) did not show any change in weight of tablets in initial 2 h (SGF pH 1.2) due to enteric coating (Eudragit L100), but when medium was changed to SIF pH6.8, ingression of water in tablet was observed which led to increase in weight of tablets up to a particular time followed by decrease in weight. The decrease in weight of tablet can be attributed to the fact that lactulose got dissolved due to ingress of water in tablet and got diffused through permeable coat of Eudragit E100.This change in weight was used to quantify lactulose release. The tablet containing higher amount of lactulose showed weight loss after 5h (F3-F6-F9); while tablet containing low and medium amount of lactulose showed weight loss after 7h (F2-F5-F8).Also results indicated that lactulose release was inversely regulated by amount of MCC. This may be due to the binding property of MCC (38). Formulation containing highest percentage of MCC showed least weight loss while formulation containing least percentage of MCC showed highest weight loss.

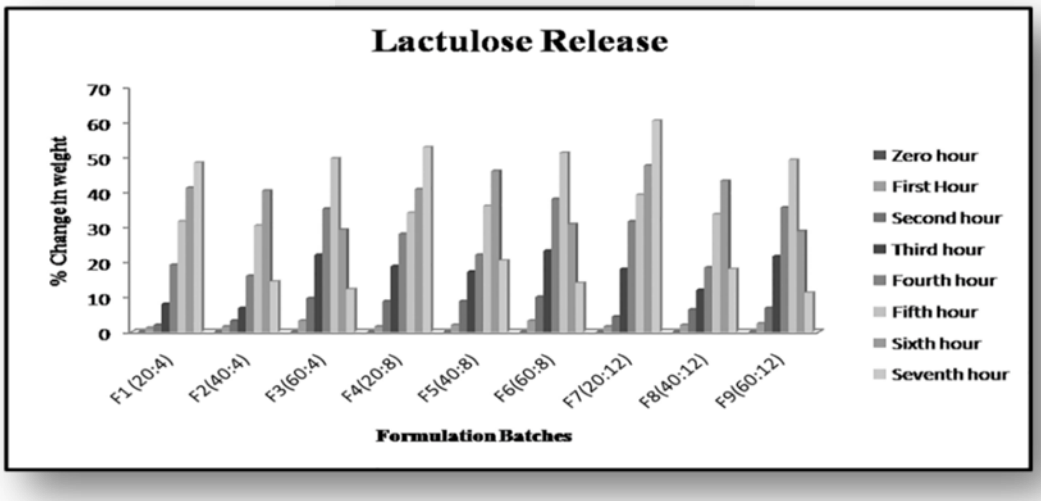

Figure 6: Lactulose release in weight versus time.

In vitro drug release study

The *in vitro* release profiles of ETX CODES^{TM}tablets (F1-F9) in SGF and SIF (With and without addition of microbial culture) are given in Figure 7. Negligible drug release (0.1-0.5%) was found in hydrochloric acid buffer (pH1.2) which can be attributed to the dependent solubility of the Eudragit L100 which dissolves above pH 6. The polymer is insoluble at gastric pH but gets hydrated and ionized as pH increases to 6-7. Hence tablets were intact for initial first two hours which also proved the coating level was optimum in sealing release of ETX in gastric environment. A slight drug release (0.179±2.04% to 6.625±2.02%) was recorded in SIF (phosphate buffer, pH 6.8), as the enteric coating gets dissolved. While the barrier layer HPMC got hydrated and finally got dissolved. The drug release was still restricted in SIF due to Eudragit E100 coating which dissolves at pH 5. While slight drug release was due to the fact that polymer Eudragit E100 coating is swellable and permeable above pH5. Also, ETX release in SIF can say to be inversely regulated by amount of MCC.Higher drug release in SIF was recorded for formulation F3–F6-F9 (6.6±1.49 to 6.3±1.46%) containing low amount of MCC followed by formulation F2–F5-F8 (5.4±1.42 to 5.3±1.71%) and minimum drug release was recorded for formulation F1–F4-F7 (4.09±1.47 to 4.3±1.10%) containing highest amount of MCC.The variation in release may be due to swelling capacity of MCC that increased diffusional path length for traveling of ETX molecules.

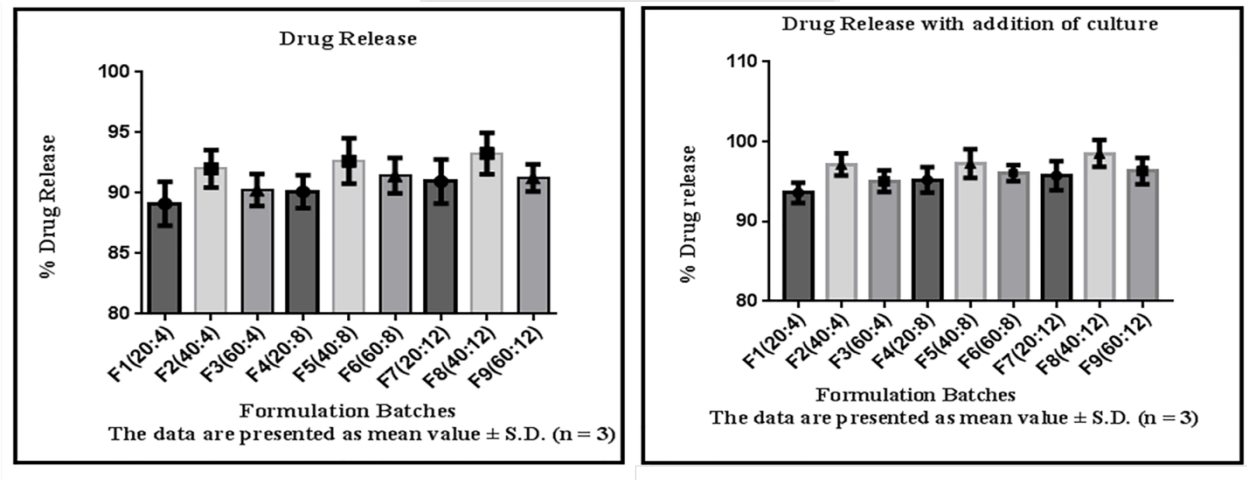

Figure 7: *In vitro* dissolution profiles of CODES™ tablets with and without addition of microbial culture (*Lactobacillus sporogenes*).

Similarly, drug release was determined in SCF (phosphate buffer, pH 6.8 containing microbial culture of *Lactobacillus sporogenes* along with nitrogen purging) to define release behavior of CODES^{TM} formulations in colonic environment. A rapid drug release was exhibited by all formulations which may be due to the action of *Lactobacilli sporogenes* via β-galactosidase on lactulose.Lactulose got converted to lactate via β-galactosidase that reduced the pH surrounding the tablet, adequately to affect dissolution of acid soluble coat.The drug release study with addition of microbial culture *Lactobacillus sporogenes* showed higher drug release values (98.929 ± 1.69%) than drug release study without addition of microbial culture (94.03 ± 1.72%).

Effect of Eudragit E100 coating on drug release: Eudragit E100 is only permeable at pH6.8, and permeability of Eudragit E100 coating will decrease as coating layer thickness increases. Thus increasing the coating level simultaneously increases tablets ability to withstand the time-dependent mechanical erosion of coating layer. That also leads to prolonged lag time of drug release at higher coating levels. It can be seen from Figure 8 that release profile of ETX at different Eudragit E100 coating levels decreases as coating level of Eudragit E100 increases(39).

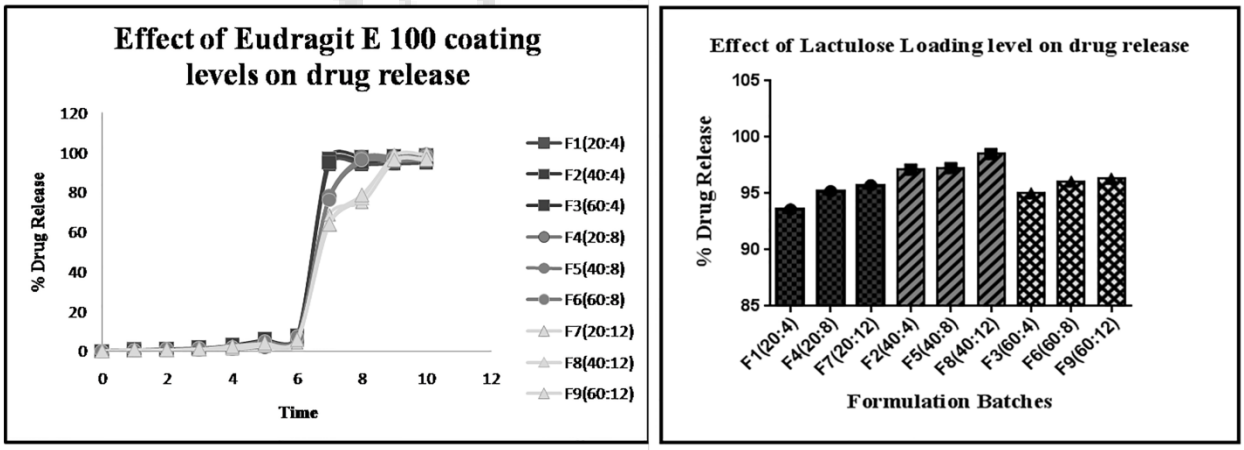

Figure 8: *In vitro* dissolution profiles of CODES™ tablets as a function of Eudragit E coating levels (■ 4%;● 8%; ▲ 12%, coating weight gain) and lactulose loading level(● 20%;■ 40%; ▲ 60% lactulose concentration).

Effect of lactulose loading levels on drug release: There are significant differences among values of percent average release for CODES™ tablets with lactulose loading of 20%,40%,and 60%(Figure 8).This indicates that quantity of organic acid generated from fermentation of lactulose is able to reduce microenvironment pH low enough to trigger drug release in colon.

Thus incorporation of less lactulose in core tablets led to a decrease in lactulose release rate into colon for fermentation, which led to low production of organic acid. This prolonged the accumulation process of organic acids in order to reach threshold pH value for dissolving the Eudragit E100coating, resulting in delay of drug release observed from tablets containing less lactulose (39). Also, a low drug release was observed at high concentration of lactulose might be due to the acidification of whole of the colonic contents.

Fermentation studies

To simulate colonic conditions for *in vitro* drug release studies, simulation of colonic environment was earlier done by addition of caecal content of rat/guinea pig which leads to hindrance in drug analysis due to turbidity of release medium.Furthermore,accurate measurement of drug release was not possible due to partial/complete adsorption of drug on suspended caecal matter.Recently,use of enzyme release media containing pectinase and invertase has been reported by many research groups to precisely assess release behavior of colon targeted formulations.Lactulose,a carbohydrate is utilized by colonic bacteria mediated by secretion of the enzyme β-galactosidase that leads to acidification of colonic content via lactic acid production (40); so in this study in order to perform *in vitro* release studies incorporation of bacterial culture in test media was done for which fermentation studies were conducted separately. Lactulose converts into lactate via β-galactosidase release by bacterial strain that decreases pH surrounding microenvironment of tablet, sufficient to affect dissolution of acid soluble coat so the drug would release as soon as formulation reaches colon i.e. after 6-7thh.Fermentation study was performed in order to decide the inoculation dose of the bacterial culture in the test media.

Validation of experimental design

Upon "trading off" different responses by different batches in percent drug release, following criteria were adopted: 40% lactulose loading in core of tablet was optimum for reduction in microenvironment pH of tablet while 4% coating level of Eudragit E100 gave the drug release in 6-7th hour as soon as the formulation reaches ascending colon. Thus F2 (40:4) was selected as the optimized batch. Validity of generated model was determined by reformulating optimized batch. The predicted value was calculated from reduced polynomial equation generated by software and

reduced by omitting non-significant coefficients using one-way ANOVA which is 96.7981±1.39% with a standard deviation of 0.42 and R^2value of 0.9685.The predicted value was compared with experimental value by calculating percentage error (0.52%) which was found to be quite low, proved validity of generated model in predicting response. The predicted value(96.7981±1.39%)and experimental value(98.458±1.32 %)of response were also compared using t-test at 95% confidence interval at 2 degrees of freedom.No significant difference was recorded between these two values, hence affirming the validity of generated model. Additionally, a total of 39 solutions for nine combinations of numeric factor levels were given by design expert software and all of them with maximum desirability of 1.00.

***In vivo* roentgenography study**

The roentgenography study shows that the tablet remained intact in stomach [Figure 5 (a)] defining *in vivo* efficiency of Eudragit L100 coating in protecting the tablet in gastric environment of stomach. No major change in size and shape of tablet (Figure 5b–d) proved that tablet remained intact in small intestine; confirming that optimized acid soluble coat was able to maintain integrity of tablet in small intestine. The Figure 5(e) clearly depicts decrease in tablet size which indicated that formulated CODES^{TM} system is capable of specific targeting drug to colon without losing its integrity in stomach and small intestine thus in compliance with the hypothesis.

Stability studies

The observations show chemical and physical stability of formulation during storage. The insignificant change in the values indicated that the formulation was quite stable.

CONCLUSION

Colon targeted ETX CODES^{TM} tablets were successfully designed, formulated and optimized using experimental design, which remained intact throughout the GI tract and gave release (*in vitro*) as soon as the formulation reached to the colon i.e. in 6-7thh. The dissolution study with addition of microbial culture showed higher drug release values (98.929±1.69%) than the dissolution study without addition of culture (94.03±1.72%). The specificity of formulation to target colon was validated by roentgenographic study. Stability studies revealed absence of

chemical and physical changes over a period of 90 days. Thus ETX can be successfully designed and formulated in CODES^{TM} by providing advantage over conventional dosage form.

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REFERENCES

1) Rubinstein A. Colonic drug delivery. Drug Discov Today Technol 2005; 2(1):33-37.
2) Singh NB. Modified release solid formulations for colon delivery. *Recent Pat Drug* Deliv Formul 2007; 1:53-63.
3) Kaur A, Harikumar SL. Controlled drug delivery approaches for Rheumatoid Arthritis. J Appl Pharm Sci 2012; 2(8):21-32.
4) Kadiyam R, Muzib I. Colon-specific drug delivery of tramadol HCl for chronotherapeutics of arthritis. Int J Pharm Investig 2015; 5(1):43-49.
5) Rodrigues AD, Halpin RA, Geer LA, Cui D, Woolf EJ, Matthews CZ, Gottesdiener KM, Larson PJ, Lasseter KC, Agrawal NGB. Absorption, metabolism and excretion of etoricoxib, a potent & selective cyclooxygenase-2 inhibitor, in healthy male volunteers. Drug Metab Dispos 2003; 31(2):224-232.
6) Brooks P, Kubler P. Review on Etoricoxib for arthritis and pain management. Ther Clin Risk Manag 2006; 2(1):45-57.
7) Croom KC, Siddiqui AAM. Etoricoxib-review of its use in the symptomatic treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and acute gouty arthritis. Drugs 2009; 69 (11):1513-153.
8) Conaghan PG. A review on turbulent decade for NSAIDs: update on current concepts of classification, epidemiology, comparative efficacy, and toxicity. Rheumatol Int 2012; 32:1491-1502.
9) Bansal SS, Joshi A, Bansal KA. New dosage formulations for targeted delivery of cyclooxygenase 2 inhibitors focus on elderly. Drugs Aging 2007; 24(6):441-451.
10)Burkhard H, Bertold R, Brune K. Drug insight: cyclo-oxygenase-2 inhibitors— a critical appraisal nature clinical practice rheumatology. Nat Clin Pract Rheumatol 2007; 3(10):552-560.
11)Thakre A, Bhople A, Jaiswal S, Chandewar A, Ghuge N, Wagh N, Thakre S, Bari M. Formulation and development of oral fast dissolving tablet of etoricoxib. Der Pharmacia Lettre 2012; 4(4):1169-1182.
12)Patra S, Samantaray R, Pattnaik S, Barik BB. Taste masking of etoricoxib by using ion-exchange resin.Pharm Dev Technol 2010; 15(5):511-517.
13)Suhagia BN, Patel HM, Shah SA, Rathod I, Parmar VK. Preparation and characterization of etoricoxib-polyethylene glycol 4000 plus polyvinyl pyrrolidone K30 solid dispersions. Acta Pharm 2006; 56:285-298.

14)DankiL S, Chowdary KPR. A factorial study of enhancement of solubility and dissolution rate of etoricoxib by hydroxypropyl β-cyclodextrin and solutol HS15.IJCP 2012; 8(2).
15)Kumar GBK, Yadav SN, Ahamed MG, Nasir KA. Design and evaluation of a new capsule-type dosage form for colon-targeted delivery of etoricoxib. Int J Pharm 2013; 3(1):147-151.
16)Watanabe S, Kawai H, Katsuma M, Fukui M; Yamanouchi Pharmaceutical company Ltd. Colon specific drug release system. US Patent 6,368,629. 2002 April 9.
17)Rangari N, Puranik P. Review on recent and novel approaches to colon targeted drug delivery systems. Int J *Pharm Pharm* Sci 2015; 3(1):167-186.
18)Yang L, Watanabe S, Li J, Chu JS, Katsuma M, Yokohama S. Effect of colonic lactulose availability on the timing of drug release onset *in vivo* from a unique colon-specific drug delivery system (CODES^{TM}). Pharm Res 2003; 20:429–434.
19)Venema K, Marleen HMC, Ellen G, Pool W, Jos MBM. The effect of lactulose on the composition of the intestinal microbiota and short chain fatty acid production in human volunteers and a computer controlled model of the proximal large intestine. Microbial ecology in health and diseases 2003; 15:94-105.
20)Dehghan MHG, Gupta RM, Shaikh MA, Darwis Y, Rizwan M, Mundada VP. Assessment of isomalt for colon-specific delivery and its comparison with lactulose. AAPS Pharm Sci tech 2012; 14(1):53-59.
21)Challa A, Rao DR, Chawan CB, Shackelford L. Bifidobacterium longum and lactulose suppress azoxymethane induced colonic aberrant crypt foci in rats. Carcinogenesis 1997; 18: 517–521.
22)Sunitha PG, Deattu N, Kumar RR, Rudhra SS, Kalaimathi P, Soundiramani B. FTIR spectroscopic method for qualitative analysis of etoricoxib in tablets. Res. j. pharm. biol. *chem. sci* 2013; 4(3):252.
23)Das A, Nayak AK, Mohanty B, Panda S. Solubility and dissolution enhancement of etoricoxib by solid dispersion technique using sugar carriers. ISRN 2011; 5402.
24)Singh S, Mishra A, Verma A, Ghosh AK, Mishra AK. A simple ultraviolet spectrophotometric method for the determination of etoricoxib in dosage formulations. J
Adv Pharm Technol Res 2014; 3(4):237-240.
25)Thimmaraju MK, Rao V, Hemanth K, Siddartha K. Determination of Etoricoxib in bulk and pharmaceutical dosage forms by UV spectrophotometric method. IJPTR 2012; 4(2):860-865.
26)Li J, Yang L, Ferguson SM, Hudson JT, Watanabe S, Katsuma M, Fix JA. *In vitro* evaluation of dissolution behavior for a colon-specific drug delivery system (CODES™) in multi-pH media using United States Pharmacopeia Apparatus II and III, AAPS PharmSciTech 2002; 3 (4):33.
27)Singh AK, Pathak K. Colon specific CODES^{TM} based piroxicam tablet for colon targeting: statistical optimization, *in vivo* roentgenography and stability assessment.Pharm Dev Technol2013; 20(2):237-245.
28)Omar S, Aldosari B, Refai H, Gohary OA. Colon-specific drug delivery for mebeverine hydrochloride. J Drug Target 2007; 15(10):691–700.
29)Okumu A, Maso DM, Lobenberg R. Computer simulations using Gastro Plus^{TM} to justify a biowaiver for etoricoxib solid oral drug products. Eur. J. Pharm. Biopharm 2009; 72(1):91-98
30)Yang L, Chu JS, Fix JA. Colon-specific drug delivery: new approaches and *in vitro /in vivo* evaluation.Int J Pharm 2002; 235(1-2):1-15.
31)Bingyong M, Dongyao L, Jianxin Z, Xiaoming L, Zhennan G, Yong QC, Zhang H, Chen W. *In vitro* fermentation of lactulose by human gut bacteria. J. Agric. Food Chem 2014; 62:10970−10977.
32)Katsuma M, Watanabe S, Takemura S, Sako K, Sawada T, Masuda Y, Nakamura K, Fukui M, Connor AL, Wilding IR. Scintigraphic evaluation of a novel colon targeted delivery system (CODES^{TM}) in healthy volunteers. J. Pharm. Sci 2004; 93(5):1287-1299.
33)Akhgari A, Garekani HA, Sadeghi F, Azimaie M. Statistical optimization of indomethacin pellets with ph-dependent methacrylic polymers for possible colonic drug delivery. Int. J. Pharm 2005; 305:22-30.
34)Sahu BP, Das MK. Optimization of felodipine nanosuspensions using Full Factorial Design. Int J *Pharm* Technol 2013; 5(2):553-561.
35)Nag A. Stability and flow behavior of barium sulphate suspension and the preparation of stable barium sulphate suspension for medical use. J Sci Ind Res 2005; 64:268-272.

36)Avachat MA, Shinde SA. Feasibility studies of concomitant administration of optimized formulation of probiotic-loaded vancomycin hydrochloride pellets for colon delivery. Drug Dev Ind Pharm 2015; 1-11.
37)Sharma P, Pathak K. Inulin based tablets in capsule device for variable multiple delivery of aceclofenac: optimization and *in vivo* roentgenography. AAPS Pharm Sci Tech 2013; 14:736–747.
38)Bhowmik D, Chiranjib B, Yadav J, Chandira RM, Kumar KPS. Emerging trends of disintegrants used in formulation of solid dosage form. Der Pharm Let 2010; 2(1):495-504.
39)Varshosaz J, Emami J, Tavakoli N, Minaiyam M, Rahmani N, Dorkoosh F, Mahzouni P. Development of novel budesonide pellets based on CODES^{TM} technology: *In vitro/in vivo* evaluation in induced colitis in rats. DARU 2011; 19(2):107-117.
40)Mohapatra SK, Kshirsagar SJ, Bhalekar MR, Shukla GN, Patil AV. Development and evaluation of enzymatically triggered multiparticulate colon targeted drug delivery system. Int J Res *Ayurveda Pharm* 2011; 2(1):211-215.