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Development of Novel Combination Drug Treatment for Arthritis Using Chronopharmacological Approach.

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ABSTRACT

Concomitant therapy of anti inflammatory agent and disease modifying drug in pulsatile pattern proves the best treatment for pain relief and in remission of disease. In the current work, combination therapy of aceclofenac and leflunomide was studies for rheumatoid arthritis. Tab in tab formulation was developed with inner leflunomide and outer aceclofenac fraction. Outer fraction provides instant relief from symptomatic pain and inner part provides disease remission. pH sensitive polymers were investigated for coating and coating parameters were optimized. Considering the pH solubility of Eudragit S 100 and Eudragit L 100 in different ratio, final composition was derived. With final composition, desired lag phase observed in the drug release study. Further, X-ray studies and Pharmacokinetic study with final formulation also proves the efficacy of the final formulation w.r.t. desired lag phase and drug plasma concentration in-vivo. Final formulation found stable in 3 months stability study w.r.t. physical and chemical parameters. Optimized novel pulse release combination therapy developed to provide the relief to arthritis patient.

Keywords: Arthritis, chronobiological, pH sensitive polymer, pulsatile, combination therapy.

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INTRODUCTION

Arthritis (from Greek arthro-, joint + -itis, inflammation; plural: arthritides) is a group of conditions involving damage to the joints of the body. There are over 100 different forms of arthritis. The most common forms are osteoarthritis (OA) (degenerative joint disease), rheumatoid arthritis (RA) (joint inflammation of, synovial proliferation and destruction of articular cartilage) and psoriatic arthritis (inflammatory arthritis)[1]. Chronobiological patterns have been observed with arthritis pain. People with osteoarthritis, the most common form of the disease, tend to have less pain in the morning and more at night. But for people with rheumatoid arthritis, the pain usually peaks in the morning and decreases as the day wears on. Many animal studies showing that joint inflammation in rats fluctuates over a 24-hour period support these observations by both patients and physicians. Symptoms of rheumatoid arthritis (RA) frequently show diurnal variation, with exacerbations in the morning[2]. This variation in disease expression is accompanied by daily oscillations in circulating concentrations of disease-mediating cytokines[3]. In particular, IL-6 shows robust oscillations, and fluctuations in serum IL-6 levels correlate with changes in disease symptoms[4]. We consider how this information can be utilised, not only to modify existing treatment regimens, but to develop new therapeutic strategies to treat RA.

Rheumatoid arthritis shows diurnal variation in disease symptoms and markers. Joint stiffness and pain are more pronounced in the early morning[2], and this correlates with the early morning rise in plasma IL-6 levels[4]. The circadian hormone melatonin (which is considered to exacerbate the inflammatory response) is released only during the night, and circulating levels peak in the mid-night. The anti-inflammatory glucocorticoid - cortisol - is also under circadian control, peaking in the early morning. For the treatment of RA, NSAIDs are used first; they afford symptomatic relief (pain, swelling, morning stiffness, immobility) but do not arrest the disease process. The disease modifying drugs (DMDs) are added if deformity and bony changes progress rapidly. Early introduction of these drugs is now a days favored, and combination of these drugs with NSAIDs is better way to treat the arthritis patient. Sometimes, Corticosteroids are also employed as a adjuvant to NSAIDs or with DMDs. A circadian rhythm is a 24-hour cycle in the biochemical, physiological or behavioral processes of living entities. For example, many body functions and diseases follow a circadian rhythm. So, by timing administration of a programmed release device, therapeutic plasma concentration can be obtained at an optimal time to counter the diurnal nature of certain diseases, such as angina, hypertension, asthmatic attacks and stiffness of arthritic patients during early morning hours, and heart attacks at night etc[5]. Combination therapy

including one NSAID and one Disease Modifying Drug (DMD) is the best way to treat arthritis patient. DMD suppresses the arthritic process and bring about a remission in disease condition. Further dose reduction can also be accomplished by multi drug pulse system in a single dosage form, particularly in case of steroids. In this research article, combination therapy including one NSAID (Aceclofenac) and one DMD (Leflunomide) were studied for arthritis treatment using pulsatile release. pH sensitive polymer systems were used for coating to provide the pulsatile effect. Different ratios of Eudragit L-100 & S-100 were studied to provide the desire the lag phase *in-vivo*.

MATERIALS AND METHOD

Both the drugs (Aceclofenac and Leflunomide) were received from Suvik Pharma as gift samples. Coating polymers, Eudragit® S 100 & Eudragit® L 100, were received as a gift sample from Evonik. All other materials and reagents used were of pharmaceutical and analytical grade.

Preformulation study (PFS)

Preformulation study was carried out for LEF, ACE, LEF and ACE (1:1), and each drug with proposed excipients i.e. lactose, starch, poly ethylene glycol, magnesium stearate, Aerosil, sodium starch glycolate, micro crystalline cellulose, croscarmellose sodium, povidone, ethylcellulose, Eudragit, titanium dioxide, poly ethylene glycol, and talc. The study was carried out at 40^0 C/75% RH in open vials for a period of 1 month. Samples were evaluated for physical appearance and drug content. The IR spectra were obtained using KBr disk method using an FTIR spectrometer (FTIRPERKINELMERFT-I Insf. USA) and spectra of drug alone and physical mixture of drug and excipients were compared.

Preparation of inner tablets

Leflunomide, lactose and starch were sifted through 40 mesh sieve and dry mixed. Powder blend was granulated with starch paste (8% w/w). The wet mass was dried and sieved through 18 mesh sieve. Dried granules were mixed with colloidal silicon dioxide and Sodium starch glycolate, and then lubricated with magnesium stearate. Blend was compressed in to tablet (average weight – 90 mg) to a hardness value of 50 N with rotary tablets compression machine (6.35 mm round FFBE punches). Core tablet was characterized for IPQC parameters (like thickness, hardness, friability, weight variation, disintegration time and drug content etc.) and other parameters. Composition of Leflunomide was shown in Table 1. The *in vitro* drug release study of tablets was carried out with following conditions. In vitro drug release study was compared with available market formulation. (Market Formulation: Lefra 20® Mfg By: TPL, India). Dissolution was carried out in 7.8

phosphate buffer using paddle apparatus (volume 900 ml and rpm 100)

Table-1: Inner tablet formulation of Leflunomide

Inner tablet of Leflunomide (B.No.- A1)				
Process	Ingredients	Category	mg/tab	% w/w
Dry mix	Leflunomide	API	20	22.22
	Lactose	Diluent	44.66	49.62
	Starch	Diluent/Disintegrant	12	13.33
Binding	Starch	Binder	4	4.44
	Water#	Binder vehicle	30	-
Blending	Aerosil	Glidant	1.67	1.86
	SSG	Disintegrant	6	6.67
Lubrication	Magnesium stearate	Lubricant	1.67	1.86
	Total wt (inner tablet)		90	100

Preparation tab in tab for combination drug therapy (TIT)

Aceclofenac, micro crystalline cellulose and Povidone were sifted through 40 mesh, dry mixed and granulated with water. Wet mass was dried, sieved through 18 mesh. Sized granules were mixed with croscarmellose sodium and lubricated with magnesium stearate

Table 1.a: Outer fraction of Aceclofenac Part

Outer part of Aceclofenac fraction			**mg/tab**
Dry mix	Aceclofenac	API	100
	Microcrystalline cellulose	Diluent	84
	Povidone	Binder	5
Binder solution	Water#	Binder vehicle	q.s.
Blending	Croscarmellose Sodium	Disintegrant	8
Lubrication	Magnesium stearate	Lubricant	3
Total wt (Outer fraction)			200 mg

Final tab in tab was compressed with 9.50 mm round concave punches at 110 N hardness using rotary tablets compression machine at an average weight of 290 mg. Overall tab in tab was characterized for IPQC and chemical parameters like thickness, hardness friability, weight variation, disintegration time and drug content etc. Composition of TIT formulation was shown in Table 2.

Table 2: Tab in Tab formulation for Combination Therapy

Compression Coated Tablet (Tab In Tab)			
Inner tablet of Leflunomide			B.No.LA/001
Process	Ingredients	Category	mg/tab
Dry mix	Leflunomide	API	20
	Lactose	Diluent	44.66
	Starch	Diluent/Disintegrant	12
Binding	Starch	Binder	4

	Water#	Binder vehicle	q.s.
Blending	Aerosil	Glidant	1.67
	Sodium starch glycolate	Disintegrant	6
Lubrication	Magnesium stearate	Lubricant	1.67
Compression coat of Aceclofenac			
Dry mix	Aceclofenac	API	100
	Microcrystalline cellulose	Diluent	84
	Povidone	Binder	5
Binder solution	Water#	Binder vehicle	q.s.
Blending	Croscarmellose Sodium	Disintegrant	8
Lubrication	Magnesium stearate	Lubricant	3
Total wt (Compression coated Tab)			290

Dissolution was carried out in 7.2 phosphate buffer using paddle apparatus for TIT formulation. (volume: 900 ml and rpm: 100)

Coating composition optimization

Qualitative composition selected for pH sensitive coating is given in Table 3. Ratio of two pH sensitive polymers was optimized by film solubility method in different media. Due to the variability of in vivo pH in GI tract, only single polymer cannot serve the purpose. Eudragit S 100 dissolves at pH 7.0 and Eudragit L 100 starts dissolving at pH 6.0. Coating with only Eudragit L 100 may release the drug to early and cannot provide the sufficient lag phase. Coating with only Eudragit S 100 may result in failure in drug release and tablet may come out in stool as such due to lower in vivo pH in some patient. Different ratios of S 100 and L 100 were studied for pH dependent solubility. Enteric films were casted with different ratios of Eudragit L & S and films were checked for the solubility in different pH media media with respect to time to select the proper ratio of Eudragit S 100: Eudargit L 100.

Table 3: Composition of pH sensitive coating system

Ingredients	**Category**	**Qty/unit (mg/tab)**
Methacrylic acid Co-polymer (Eudragit S-100)	Enteric coating polymer	Ratio to be optimized
Methacrylic acid Co-polymer (Eudragit L-100)	Enteric coating polymer	
Talc	Anti-tacking agent	2.1
Triehtyl citrate	Plasticizer	1.95
Titanium dioxide	Opacifying agent	1.95
Isopropyl alcohol@ (IPA)	Vehicle	q.s.

@ does not remain in final product, will be lost during processing.

Coating of tablet in tablet

Isopropyl alcohol was selected as a coating solvent. Required quantity of Eudragit S 100 and

Eudargit L 100 were dispersed in the IPA by stirring. Mixture of talc and titanium dioxide were homogenized and milled in part of solvent and then added to above dispersion. Coating solution was stirred properly under stirrer. At last triethyl citrate was added and mixed properly under stirring. Coating of tab in tab was carried out using Neocota® and coating parameters are given in Table 4.

Table 4: Coating Parameter for Eudragit Coating

Solid Content of coating solution	6-7 % w/w
Inlet air temperature	45 °± 5° c
Exhaust temperature	32 °± 5° c
Spray rate	30-40 gm/min
Pan RPM	0.5 to 4 RPM during coating processing
Atomization pressure	2.00 kg/cm² ± 0.5
Drying of coated tab	20 min at inlet 40°c in coating pan at low RPM

Dissolution study and method for coated tablets

Dissolution of coated tablets were carried out in different media in the sequence given below

0.1 N HCl for 2hrs, followed by 4.5 acetate for 1 hr, followed by 6.0 phosphate buffer 1 hr and than in 7.2 phosphate buffer (considering the transit of tablet through GIT and pH of different part of GIT) Dissolution was carried out for 6 units and their mean results were reported. A first order UV derivative spectrum was selected for the quantitative estimation using methanol as a common solvent. From the overlain spectra of both drugs, wavelength selected for quantification was 259.0 nm for aceclofenac and 249 nm for leflunomide.

X-ray and pharmacokinetic studies (Project no.: KB/13/457 – Ethics approval)

X-ray imaging technique was used to monitor tablets throughout the GI system. The inclusion of radioopaque material into the solid dosage form enables it to be visualized by the use of X-rays. By incorporating barium sulphate into the pharmaceutical dosage forms, it is possible to follow the movement, location and integrity of the dosage form after oral administration by placing the subject under a fluoroscope and taking a series of X-rays at various time points. Three healthy male human volunteers, between 22 and 30 years of age and 50–70 kg body weight, were participated in X-ray imaging studies. They were non-alcoholics, non-smokers and have not taken any drugs. The purpose of the study was fully explained to the volunteers and the volunteers had given their written consent. Dummy tablets coated optimized coating formula studied for in-vivo lag time in human through X-ray study[6,7]. Each subject ingested barium sulphate containing optimized coated formulation (placebo) orally with 200 mL water, and the tablets were visualized

using X-rays at different time interval. Final optimized formulation was studied for in-vivo lag time and for pharmacokinetic study.

Pharmacokinetic studies in rabbit (Project no.: KB/13/457 – Ethics approval)

Final coated tablets were studied for in-vivo plasma concentration in rabbits [8, 9].

The study was carried out after obtaining approval from the Institutional Animal Ethical Committee, KBIPER, Gandhinagar. (Project no.: KB/13/457).

Rabbits weighing between 1.5 to 3 kg of either sex will be used. In the study, overnight fasted rabbits with free access to water will be used. Zero hour blood sample (blank) will be collected. Single dose of combination drug is administered to the animal. Blood samples (1.0 ml) will be collected from marginal ear vein of animals into centrifuge tubes. The same method will be followed in all cases at an interval specified as per following Table-5. Plasma samples collected from the rabbits will be analyzed using HPLC method. Sampling planning for kinetic study in rabbits

Table 5: Sampling interval (in hours) for plasma collection for *in-vivo* study

No	Treatment	Hours													
1	Group	0	0.50	1.00	2.00	3.00	5.00	6.00	7.00	8.00	9.00	10.00	11.00	12.00	16.00	20.00	24.00

The blood collected was mixed with the EDTA properly and centrifuged at 5000 rpm for 25 min for separation of plasma. The separated plasma was stored at -20^{o}C until drug analysis was carried out using high performance liquid chromatographic (HPLC) method.

Bioanalysis of Aceclofenac and Leflunomide in rabbit plasma

Analytical method for plasma concentration measurement of Aceclofenac[8]

Aceclofenac in plasma was determined by the reported validated HPLC method. Analysis was performed on a C18 RP-HPLC column. The mobile phase consisted of acetonitrile : phosphate buffer (40:60). The mobile phase was delivered at the flow rate of 1 ml/min. Detection was performed at 282 nm. Injection volume was 20 µl. The concentration of unknown plasma samples was calculated from the calibration curve plotted between peak area ratios of Aceclofenac to IS against corresponding Aceclofenac concentrations. Analytical method for plasma concentration measurement of Leflunomide[9]. Reported Reverse-phase high-performance liquid chromatography (HPLC) methods have been used for concentration measurement. The mobile phase consisted of 10 mmol/L potassium dihydrogen phosphate and 100 mmol/L potassium chloride in aqueous 25% acetonitrile, acidified to pH 3 with *o*-phosphoric acid (50:50). A mobile phase flow rate of 0.7 mL/min was used, corresponding to a column pressure of about 65 bar (6,500 kPa). Peaks were

detected at an absorbance wavelength of 280 nm. The concentration of unknown plasma samples was calculated from the calibration curve plotted between peak area ratios of Leflunomide to IS against corresponding Leflunomide concentrations.

Stability study

The tablets were charged for the accelerated stability studies as per ICH guidelines (40±2^{o}C and 75±5% RH) for a period of 3 months in stability chambers (Thermolab, Bombay). They were placed in flint vials and hermetically sealed with rubber plugs and aluminum caps. The samples were taken out at the end of 3 months and evaluated for the drug content, in vitro drug release and appearance.

RESULTS AND DISCUSSION

Preformulation study suggested no significant difference in 1month study in both chemical analysis and FTIR Spectra. (Figure 1)

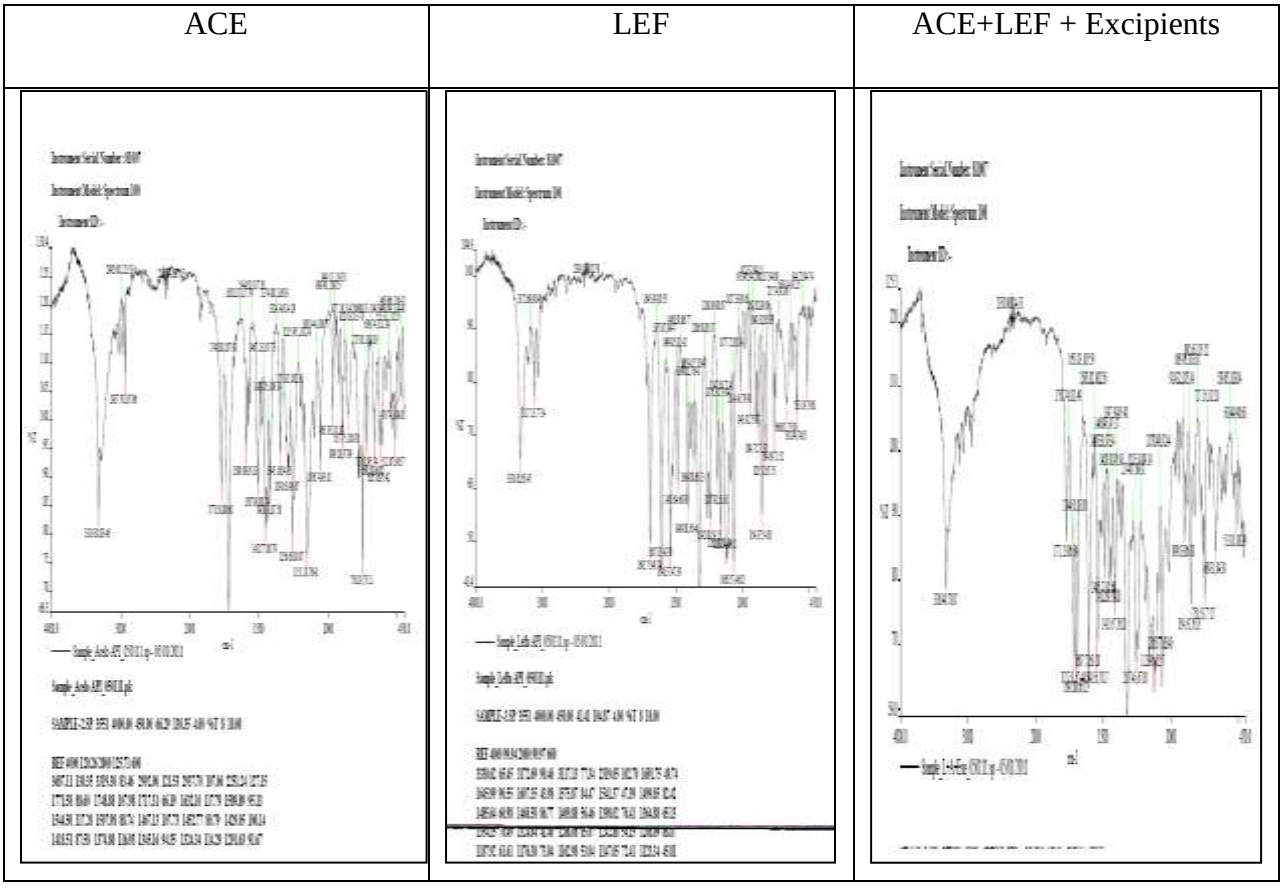


Figure 1: FTIR Spectra for drug alone and in combination with Excipients in PFS Study

Characteristic peaks for ACE: 965 cm^{-1} (O-H bending out of plane), 3319 cm^{-1} (Secondary amine N-H stretching or O-H stretching), 1508 cm^{-1} (-C=C stretching of aromatic) and Characteristic peaks for LEF: 1691 cm^{-1} (HC=N-O sharp peak of isoxazole ring), 1607 cm^{-1} (C=O of amide) were remained unchanged in FTIR study, indicated no degradation. All physical and chemical

parameters found satisfactory as mentioned in the below Table 6 and 7 for the inner tablets and tab in tab uncoated tablets respectively. Further comparative dissolution of inner tablet of LEF was compared with market formulation and showed no significant different, with f2 value 74.28. (Figure 2). Drug release study was carried out for TIT formulation as shown in the Figure 3. More than 80% drug release observed for both ACE and LEF in TIT formulation in 30minutes and complete drug release observed in 45 minutes.

Table 6: Physical properties of inner tablets (Leflunomide)

Batch	Thickness* (mm)	Hardness* (N)	Friability (%)	Weight Variation (%)	DT*	Drug content (%)
A1	2.28 ±0.078	50.20±0.2	0.12	90.10 ±0.95	3 min 35 s	99.64 ± 0.034

(All values are expressed as Mean± SD; * n=5)

Table-7: Physico-chemical properties of tab in tab uncoated tablets (ACE+LEF)

Batch	Thickness* (mm)	Hardness* (N)	Friability (%)	Weight Variation (%)	DT*	Drug content (%)	
						ACE	LEF
LA/001	3.90±0.11	100.25 ±0.10	0.41	290.71 ±2.11	4 min 10 sec	100.40 ±0.012	99.45 ±0.009

All values are expressed as Mean± SD; * n=5

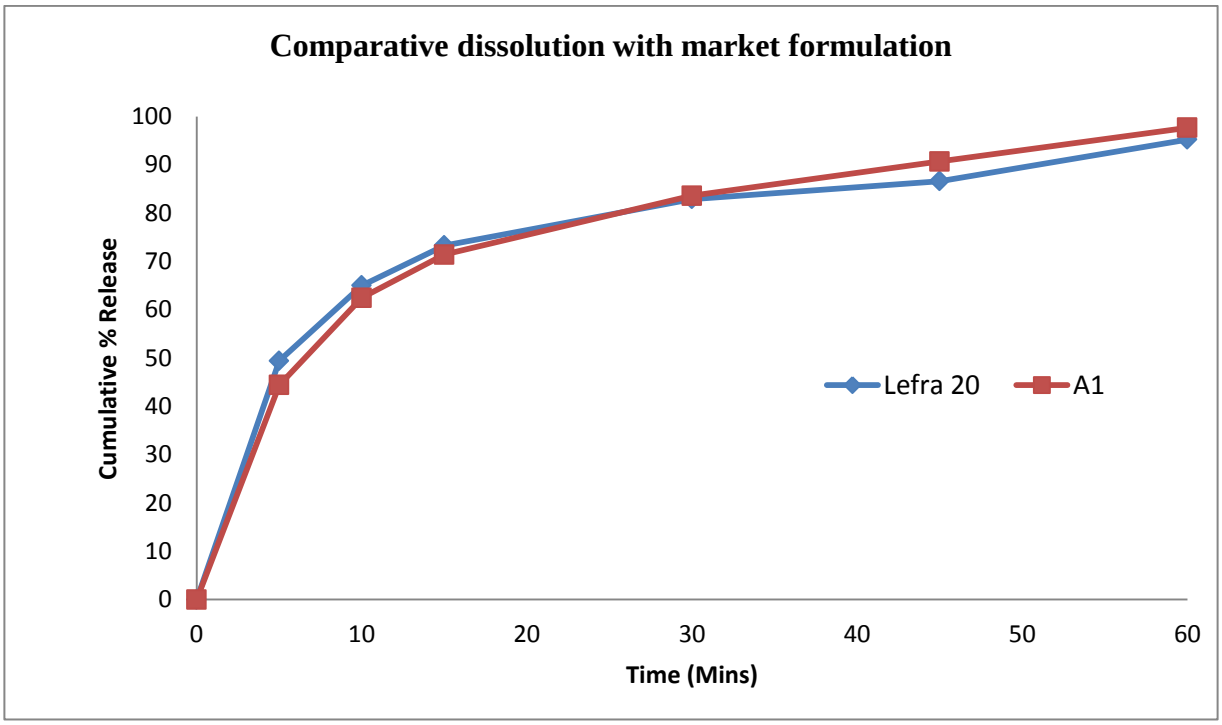


Figure 2: Comparative in vitro drug release with market formulation (*Lefra 20® vs. B.No. A1)

***Lefra 20®, Mfg by: Torrent Pharma. B. No: B13178200

Mfg date: Mar/2013, Expiry: Feb/2016.

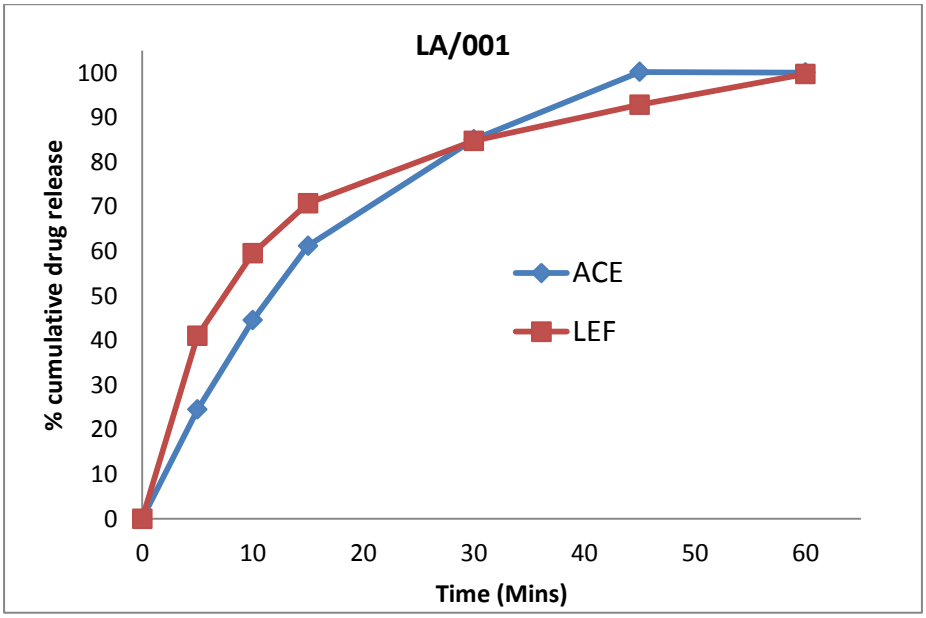


Figure 3: *In vitro* release study for ACE+LEF TIT Formulation

pH solubility (Eudragit S100: Eudragit L100)

Observation of films prepared with different ratio of Eudragit are reported in Table 8. Based on the above observation, 50:50 ratio of S100 & L 100 was selected for coating to provide the desired lag phase and for the successful dosage form delivery.

Table 8: pH Solubility of film casted with different ratios of Eudragit S 100: Eudragit L 100

Observation of Films in Media							
		pH					
Sr No	EudragitS:L ratio	5.5	6	6.3	6.5	6.8	7.2
observation after 2 hrs							
1	L (100%)	Not soluble	Very slightly soluble	More soluble than pH 6	More soluble than pH 6.3	More soluble than pH 6.5	Completely soluble
2	S (100%)	Not soluble	Not soluble	Not soluble	Not soluble	Not soluble	Slightly soluble
3	S: L (50:50)	Not soluble	Not soluble	Not soluble	Not soluble	Slightly soluble	Completely soluble
4	S: L (10:90)	Not soluble	Not soluble	Slightly soluble but less than L (100%) at pH 6.3	More soluble than pH 6.3	More soluble than pH 6.5	Completely soluble
5	S: L (30:70)	Not soluble	Not soluble	Not soluble	Very slightly soluble	Slightly soluble	Completely soluble

Table 10: Physicochemical Evaluation of Eudragit Coated Tab in Tab

Batch	**Thickness* (mm)**	**Weight Variation (%)**	**% weight gain**	**Drug content (%)**	
				ACE	**LEF**
LA/001 (Coated)	4.35±0.12	319.21±1.14	10.07 %	99.21 ±0.047	100.12±0.014

All values are expressed as Mean± SD; * n=5

Optimized coating composition and dissolution profile of novel combination therapy

Based on pH solubility study, following quantitative composition for the pH sensitive coating was derived in Table 9. Physicochemical evaluation of coated tablets was reported in Table 10 and found satisfactory. Further drug release study for coated tablet was reported and shown in Figure 4. Formulation LA/001 (coated) i.e. Tab in Tab of Leflunomide + Aceclofenac coated with optimized pH sensitive coating system provide desired lag phase of around 5 hrs *in vitro*.

Table 9: Quantitative composition of Eudragit coating system for Tab in Tab formulation

Uncoated Tablet weight (tab in tab) - 290 mg		
Coating Formula	Function	mg/tab
Eudragit S-100	Enteric coating polymer	11
Eudragit L-100	Enteric coating polymer	11
Talc	Anti-tacking agent	2.1
PEG 6000	Plasticizer	1.95
TiO2	Opacifying agent	1.95
IPA #	Vehicle	q.s.
Total wt (final coated tablet)		318

does not remain in final product will be lost during process

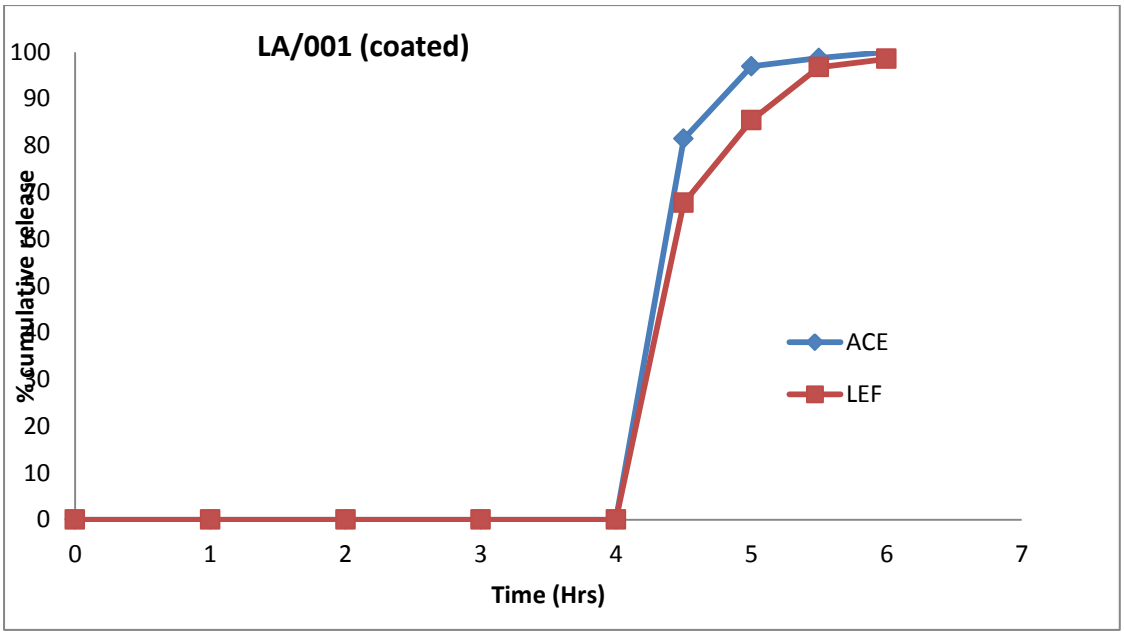


Figure 4: Dissolution profile for combination therapy *in-vitro*

X-ray studies

Dummy tablets were coated with optimized pH sensitive composition and ingested in vivo to study the lag phase. Photographs taken at different intervals were shown on Figure-5. *In vivo* lag phase was observed around 6.5 hrs which was further studied with kinetic study.

Pharmacokinetic study

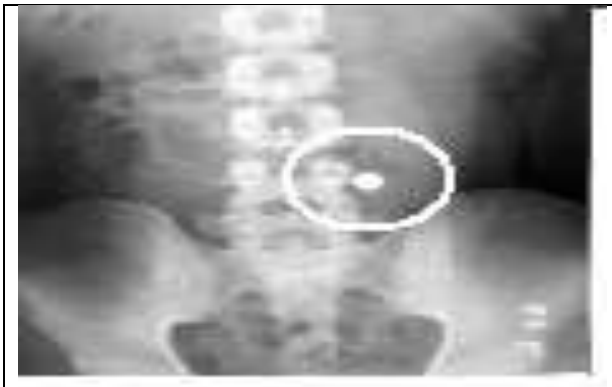
In vivo study was carried out using rabbits. Overnight fasted rabbits were single dosed with optimized coated tablet. Plasma samples were collected and analyzed for mean plasma concentration as shown in Figure-6. Desired *in vivo* plasma profile obtained with pH sensitive novel combination therapy for rheumatoid arthritis with lag phase of around 6-7 hours. Further kinetic parameters (like Cmax, Tmax, AUC, MRT) were calculated and reported in Table 11.

Table 11: Pharmacokinetic parameters derived for Coated Tablet from *In vivo* profile

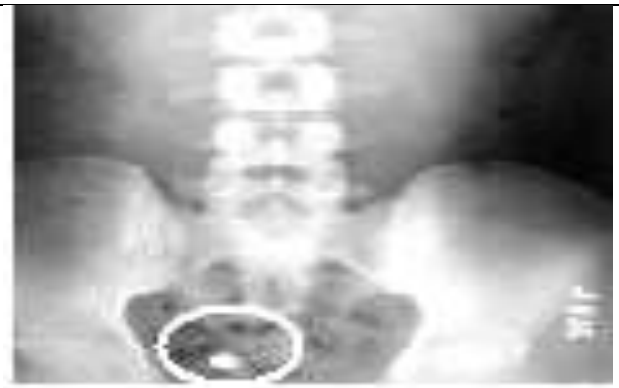
Parameters	For ACE	For LEF
Cmax (µg/ml)	10.5	6.68
Tmax (h)	8	10
AUC o-- t (µg.h/ml)	32.55	33.55
AUC o—infinite (µg.h/ml)	32.7	33.7
MRT (Hrs)	10.2	11.43

Stability Study of final formulation

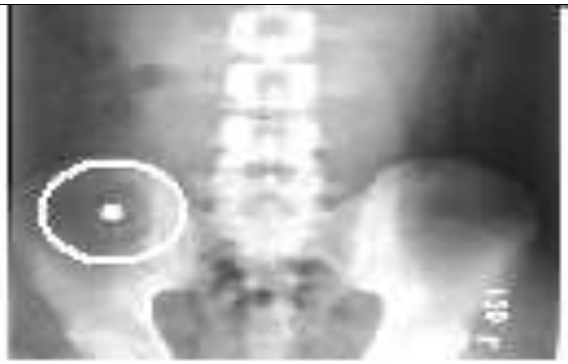
No significant difference observed in any physical and chemical parameters (like assay, dissolution etc.) in 3 months stability study of formulation at 40^0c/75% RH.



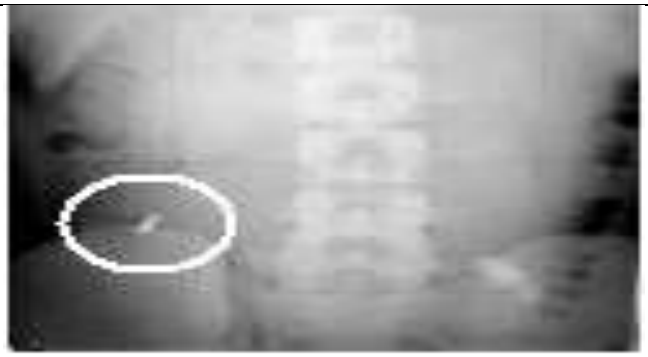
At 1 hr tablet remains intact at upper intestine part



At 3 hrs tablet remains intact in small intestine



At 5 hr tablet remains intact in small intestine part



At around 6.5 hr: At Ileo-cecal junction pH sensitive polymer system dissolves and outer fraction comes out and followed by disintegration of inner tablet

Figure-5: X-ray photographs to study the lag phase

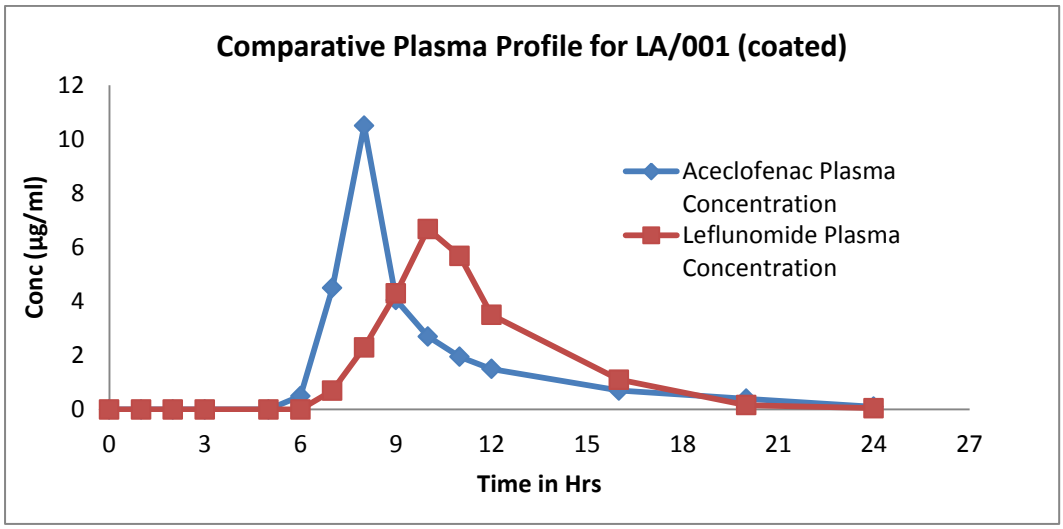


Figure 6: *In vivo* plasma concentration profile for combination therapy (ACE+LEF)

CONCLUSION

Targeted dosage form designed to treat the arthritic patients with combination therapy with pulsatile release system for Aceclofenac+Leflunomide. Optimized novel pulse release combination therapy provide the patient compliance in the following way,

- Chronopharmacological treatment: pain relief in early morning to remove the stiffness
- Pulsatile release: provide the drug release in pulses to maintain the dosage regimen
- Multidrug combination therapy: ease the multidrug administration in single dosage and bring about remission in arthritic condition
- Reducing dosing frequencies and increasing patient compliance
- Single dosage form provide both (i) relief from arthritic pain & (ii) bring about remission from disease condition
- Economical formulation compare to available market formulations.

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