International Journal of Drug Delivery 3 (2011) 597-618

http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Formulation and evaluation of bilayered tablets of montelukast and levocetrizine dihydrochloride Using natural and synthetic polymers

Moiz Md, Prathima Srinivas M*, Sadanandam M

*Corresponding author:

Prathima Srinivas M

Department of Pharmaceutics, Sri Venkateshwara College of Pharmacy and Research center, Affiliated to Osmania University, Madhapur, Hitech City, Hyderabad-500081, Andhra Pradesh, India.

Abstract

The objective of present work was to formulate and evaluate bilayered tablets of Levocetrzine and Montelukast for treating allergic rhinitis effectively. Anti-allergic medicines (eg, some antihistamines) can cause adverse events such as somnolence and sedation. The Combining Montelukast with Levocetirizine gives additional benefits in comparison with either drug alone and could be considered for patients whose quality of life is impaired by persistent allergic rhinitis. Montelukast sodium is alkaline stable (bioavailability 64%), most of drug being absorbed from the intestine while Levocetirizine Dihydrochloride is acid stable. When tablets of the combination of these are prepared, they tend to become unstable during the shelf life of the formulation. Hence it is recommended to prepare a bilayer tablet, by formulating Montelukast in sustained release layer and Levocetrizine as immediate release layer as it improves and increases the stability by reducing the acid base interactions of both the drugs in combination there by increasing the bioavailability. Taking this into account different formulations were prepared by wet granulation method using natural Tamarind Seed Polysaccharide and synthetic HPMCK100,K15M and K4M release rate controlling hydrophilic polymers. The formulations were evaluated for hardness, weight variation, friability, swelling index and drug content uniformity. The in vitro release of drug from the formulations was studied in pH 1.2 acidic buffer and pH 7.4 phosphate buffer, and it was found that the prepared tablets were able to sustain the release of the drug upto 12hours. The release of Montelukast and Levocetrizine of both layers from the tablets was found to be diffusion controlled and the release mechanism was non-Fickian based on the n value of Korsmeyer-peppas plot. The FTIR studies were performed on three optimized formulations (F4, F12, F16) and the plain drug controls(Levocetrizine,Montelukast).From the observed peaks it is evident that the polymers used and the drugs were found to be mutually compatible chemically. The Pharmacokinetic Studies were performed in two groups of male wistar rats. One group was administered with the optimized formulation containing tamarind Seed Polysaccharide(F12) while Plain Montelukast oral suspension acted as control in the second group.The results indicate that the formulation optimised with 1:4(drug:TSP) was able to sustain the release of montelukast upto 12hours.Insrease in Tmax and $AUC_{(0-t)}$ also were also observed in the studies indicating efficient sustained action and improved bioavailability of the drug. The formulated bilayered tablets using natural polymers provided immediate release of Levocetrizine and sustained release of Montelukast and therefore hold promise as an alternative dosage form in the treatment of allergic rhinitis and bronchial asthma.

Keywords: Tamarind Seed Polysacharide, Hydroxypropyl methyl cellulose, Bilayered tablets.

Introduction

The preferred treatment option in the treatment of chronic diseases i.e hypertension, diabetes and allergic rhinitis is combination therapy in the form of bilayer tablets. The advantages include minimization of side effects, anda reduction of dose-related risk. Using low dosage of two different agents minimizes the clinical and metabolic effects that may occur with higher doses of individual component s of the combined tablet [1-4].

Bilayered tablets

Bilayered tablets are tablets containing subunits that may be either the same (homogeneous) or different (heterogeneous). Bilayer tablets are prepared with one layer of drug for immediate release and second layer designed to release the drug as a second dose or for extended release. These tablets are suitable for sequential release of two drugs in combination, separate two incompatible substances and when the release profiles of the two drugs are different from one another[4]. Levocetirizine dihydrochloride is a selective, long acting peripheral H_1 receptor antagonist. Allergic rhinitis is a symptomatic disorder of the nose induced by inflammation mediated by immunoglobulin E (IgE) in the membrane lining the nose after allergen exposure. Formulating Levocetirizine into an immediate release layer dosage of bilayer tablets would provide fast relief .

Montelukast is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma attacks and to relieve symptoms of seasonal allergies. Because of poor bioavailability of Montelukast sodium by oral route(mainly absorbed by intestine)and it shorter half life of ($t_{1/2}$=2to5hours), there is a need to increase its bioavailability by formulating as a sustained release layer in the bilayer tablets to prevent frequent administration.

In formulation of immediate release layer of Levocetizine super disintegrants like Sodium starch glycolate and Croscarmellose were used. The sustained release layer of Montelukast was formulated using hydrophilic polymers (Tamarind seed polysaccharide, HPMC K100M,K15M and K4M).

The aim of the present study was to design and evaluate bilayer tablets of Montelukast and Levocetrizine for the treatment of allergic rhinitis. The immediate release layer was fabricated to release levocetrizine within 30min into the stomach and the second layer for the sequential release of Montelukast in small intestinal for sustained action .Swelling studies and *Invivo* studies were performed for sustained release layer containing Tamarind seed polysaccharide. The results indicated that the formulated bilayered tablets using natural polymers provided immediate release of Levocetrizine and sustained release of Montelukast.

Materials and methods

Materials:

Levocetrizine dihydrochloride was a kind gift from Symed labs, Hyderabad, India, Montelukast sodium was a kind gift sample from Matrix laboratories, Hyderabad, India. Hydroxy propyl methyl cellulose was purchased from Colocron Asia Pvt limited India. Poly Vinyl Pyrollidine (PVP)K90D was a kind gift sample from Dr.Reddy's Laboratories,Hyderabad. All the solvents used were of HPLC grade purchased from Merck Chemicals,India.

Methods:

Isolation of Tamarind seed polysaccharide(TSP):

Tamarind seed polysaccharide (TSP) was isolated by the method reported by Rao et al [5]. To 20g of tamarind kernel powder, 200ml of cold distilled water was added and slurry was prepared. The slurry was poured into 800ml of boiling distilled water. The solution was boiled for 20 minutes under Stirring condition in a water bath. The resulting thin clear solution was kept overnight so that most of the proteins and fibers settled out. The solution was then centrifuged at 5000 rpm for 20 minutes. The supernatant was separated and poured into twice the volume of absolute ethanol by continuous stirring. The product was filtered through muslin cloth and was pressed between felt. The precipitate was washed with absolute ethanol, iso-propanol and methanol and then dried at 50-60ºC in hot air oven for 24 hours. The dried material was ground and sieved to obtain granules of different particle size range and stored in a desiccator until further use.

Preparation of bilayer tablets

Immediate release layer:

Levocetrizine tablets were prepared by wet granulation method. Levocetrizine, super disintegrants (Sodium starch glycolate, croscarmellose) and lactose were weighed as in Table**1** and mixed thoroughly. Starch Paste (5%) was used as a Binder. The wet mass was passed through sieve no 12. The obtained granules were dried at 40ºC for 30min in tray drier. The dried granules were screened through sieve no 22 and later using sieve no.44 Magnesium stearate(1%) and talc(q.s) were used for lubrication.

Sustained release layer:

Montelukast sodium tablets were also prepared by wet granulation method. Specified quantity of drug, polymer (Tamarind Seed Polysaccharide, HPMC Grades K100M, K15M, and K4M.) and Microcrystalline celluose were weighed as in Table 2 and mixed thoroughly. PVP (1%) was used as binder. Granules were obtained by passing the sluggy mass through sieve no 12. The prepared granules were dried at 40ºC in a tray drier for 1 hour. The dried granules were screened through sieve no 22. Magnesium stearate(1%) and Talc(q.s) were finally added and mixed by triangular mixing. These lubricated granules were compressed into tablets weighing about 250 mg containing 75 mg of Levocetrizine and 175mg Montelukast in a Rotary tablet compression machine (12 station, RIMEK, India) using 9 mm circular, flat punches plain on both sides .The compressed tablets were stored in HDPE containers for stability studies.

Evaluation of both immediate release and sustained release granules:

Flow properties [6-8]

Bulk Density (D_b): It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed (25 grams) powder (passed through standard sieve # 20) into a 100ml

measuring cylinder and initial weight will be noted. This initial volume is called bulk volume. From this the bulk density was calculated according to the formula mentioned below. It is expressed in g/ml and is given by

Bulk Density (g/ml) = Mass of the powder/Bulk Volume

Tapped Density (D_t): It is the ratio of total mass of the powder to the tapped volume of the powder. Volume was measured by tapping the powder for 750 times and the volume was noted, if the difference between these two volumes is less than 2%. If it is more than 2%, tapping was continued for 1250 times and tapped volume was noted. Tapping was continued until the difference between successive volumes is less than 2 % (in a bulk density apparatus). It is expressed in g/ml and is given by

Tapped density (g/ml) = Mass of the powder/Tapped volume

Angle of Repose (θ): The friction forces in a loose powder can be measured by the angle of repose (θ). It is an indicative of the flow properties of the powder. It is defined as maximum angle possible between the surface of the pile of powder and the horizontal plane. The powder mixture was allowed to flow through the funnel fixed to a stand at definite height (h). The angle of repose was calculated by measuring the height and radius of the heap of powder formed.

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

Where, **θ** - angle of repose, h- height in cms, r -radius in cms

Carr's index (or) % compressibility: It indicates powder flow properties. It is expressed in percentage and is given by

Carr's Index (%) = [(Tapped density – Bulk Density) / Tapped Density] x 100

Hausner ratio: Hausner ratio is an indirect index of ease of powder flow. It is calculated by the following formula.
Hausner's Ratio = Tapped density / Bulk Density

Evaluation of bilayer tablets of Montelukast and Levocetrizine dihydrochloride[9]:

Hardness: The hardness of the tablet was measured by Monsanto hardness tester. The lower plunger was placed in contact with the tablet and a zero reading was taken. The plunger was then forced against a spring by tuning a threaded bolt until the tablet fractured. As the spring was compressed a pointer rides along a gauge in the barrel to indicate the force. The hardness was measured in terms of kg/cm^2.

Weight variation: Formulated matrix tablets were tested for weight uniformity, 20 tablets were weighed collectively and individually. From the collective weight, average weight was calculated. The percent weight variation was calculated by using the following formula.

$$\% \text{ WeightVariation} = \frac{\text{Average Weight} - \text{Individual Weight}}{\text{Average Weight}}$$

Friability: The Roche friability test apparatus was used to determine the friability of the tablets. Twenty pre-weighed tablets were placed in the apparatus and operated for 100 revolutions and then the tablets were reweighed. The percentage friability was calculated according to the following formula.

$$\text{Friability} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Swelling characteristics (water uptake study)[10]

The swelling properties of HPMC matrices containing drug were determined by placing the tablet matrices in the dissolution test apparatus, in 900 ml of 7.4PBS at 37 ± 0.5 0C. The tablets were removed periodically from dissolution medium. After draining free from water by blotting paper, these were measured for weight gain.Swelling characteristics were expressed in terms of percentagewater uptake (WU %) show relationship between swelling index and time.28-30

$$\text{WU \%} = \frac{\text{Weight of swollen tablet} - \text{Initial weight of the tablet}}{\text{Initial weight of the tablet}} \times 100$$

Drug content of Levocetrizine layer :

Ten tablets of each formulation were weighed and powdered. A quantity of powder equivalent to 5 mg of Levocetirizine dihydrochloride was taken into 50 ml volumetric flask. The amount of drug present in a 5 mg equivalent amount of powder was determined by dissolving the powder mixture in 10ml of methanol and UV absorbance was measured at 231nm.

Drug content of Montelukast layer:

Ten tablets of each formulation were weighed and powdered. A quantity of powder equivalent to 10 mg of Montelukast was taken into 50 ml volumetric flask. The amount of drug present in a 10 mg equivalent amount of powder was dissolved in 0.5% SLS solution. The volume of this solution(2ml) was taken and diluted

with water up to 10ml with methanol . UV absorbance was measured at 344 nm.

In vitro drug release studies

The release of drug from different batches of prepared tablets was studied by using USP dissolution apparatus type II [11]. Buffer was used as dissolution medium pH 1.2 for 2 h and phosphate buffer of pH 7.4for 12 h. The medium volume was maintained at 900ml, the temperature was maintained at 37°C ± 0.5°C and the stirring rate was 50 RPM. Samples were withdrawn at regular time intervals and the same volume was replaced with fresh dissolution medium. The samples were measured by UV Spectrophotometer at 231 nm for immediate release layer Levocetrizine and Montelukast at 344nm for sustained release layer against a blank.

Predicting Mechanism of drug release

Various models were tested for explaining the kinetics of drug release.
To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data was fitted in to zero order, first order, Higuchi and Korsmeyer-Peppas release model, to study the drug release from the dosage form

Zero order release rate kinetics:-

To study the zero—order release kinetics the release data are fitted to the following equation.

$$F=K_o .t$$

Where 'F' is the drug release, 'K' is the release rate constant and't' is the release time. The plot of %drug release versus time is linear.

First order release kinetics:

The release rate data are fitted to the following equation.

$$Log (100-F)= kt$$

A plot of log % drug release versus time is linear.

Higuchi release model:

To study the Higuchi release kinetics, the release rate data were fitted to the following equation,

$$F= k t^{1/2}$$

Where 'k' is the Higuchi constant
In Higuchi model, a plot of % release versus square root of time is linear.

Korsemeyer and Peppas release model:

The release rate data were fitted into the following equation,

$$M_t / M_\infty = K.t^n$$

Where M_t / M_∞ is the fraction of drug released,
'K' is the release constant,
't ' is the release time.
'n' is diffusion exponent, if n is equal to 0.89 the release is zero order, if n=0.45 the release is best explained by Fickian diffusion, and if 0.45<n<0.89 then the release through anomalous diffusion or non-fickian diffusion (Swellable and Cylindrical matrix).
In this model a plot of log (M_t / M_∞) versus log(time) is linear.
The drug release data of optimized bilayer tablets were fitted to zero order, first order, Higuchi and Korsmeyer-Peppas model to study the kinetics of drug release.

In vivo Evaluation [12,13]:

Male rats weighing 150-250grams shall be selected. Rats shall be divided into 2groups (n=2).The animals shall be housed individually at (25ºC, 12 h light and dark cycle). The rats shall be fasted overnight and allowed free accesses to water only. The optimized formulation were administered to one group and marketed formulation were administered to other group orally by using gastric intubation method . Samples of blood shall be withdrawn at predetermined time interval from reteroorbital plexus. The Institutional Animal Ethical Committee approved the protocol for this study bearing no SVCP/IAEC/2011/08.

Sample preparation:

Samples of blood were withdrawn at predetermined time intervals from retro orbital plexus. The collected blood was centrifuged at 2000 rpm for 10 minutes to obtain the plasma. To 200µl of plasma sample, 750µl of acetonitrile was added. The mixture was vortex-mixed on a vortex mixer for five minutes and then centrifuged at 13000 rpm for 5.0min .After centrifugation, the supernatant was transferred to glass tubes with caps .Analysis was carried out by injecting 20 µl of this sample into the HPLC system.

HPLC Assay

The samples analyzed using HPLC with Reverse Phase stainless steel C18(2) column250x4.60mm,5µm dimensions The mobile phase was prepared using Acetonitrile: potassium dihydrogen phosphate (0.05 M (70:30v/v) adjusted to pH 3.5 ± 0.1 with Ortho Phosphoric acid. Flow rate was maintained at 2.0 ml/min. The samples were analyzed at 344 nm.

Results & discussion

Characterization of Levocetrizine and Montelukast immediate bilayered tablets.

Pre-compression parameters

The Levocetrizine and Montelukast granules were prepared by wet granulation method. The prepared granules of different batches were evaluated for their granule size, angle of repose, bulk density, tapped density, compressibility index and Hausner's

ratio, as shown in Table 3 and 4. The granules have an average size in the range of 0.448 ± 0.11 to 0.683 ± 0.15 mm, which indicates narrow size distribution. The bulk densities of the granules were found to be in the range of 0.400 to 0.48 gm/ml. The angle of repose varied from 27.51 ± 0.18 to 31.37 ± 0.18. The low values of angle of repose indicate the free flowing nature of the granules. The tapped densities ranged 0.444 to 0.545 gm/ml and the Carr's indexes were in the range of 9.05 to 13.53. Hausner's ratio was found in the range of 1.02 ± 0.04 to 1.15 ± 0.09 and the values showed the low interparticle friction between the granules.

Post-compression parameters

The bilayer tablets of Montelukast and Levocetrizine were prepared by wet granulation method. The prepared tablets were evaluated for their weight variation, hardness, friability and drug content uniformity, and the results are presented in Table 5and 6. The values were found to be within the prescribed limits varied between 0.09 ± 0.024 to 1.4 ± 0.02 %. Hardness was in the range of 4.5 ± 0.14 to 7.7 ± 0.35 kg/cm2. Friability was found to be less than 1% in all the batches which indicates the ability of the prepared tablets to withstand shock during the time of transportation and handling. Drug content was uniform within the prepared batches and ranged between 72.87 ± 0.34 to 96.84 ± 0.16%.

Swelling index:

The swelling index was calculated with respect to time. With increasing time, the swelling index was increased. This is because of the weight gain by tablets proportionally with increased rate of hydration. After 3hours a gradual decrease in the swelling index was seen due to dissolution of outermost gelled layer of tablet into dissolution medium. A direct relationship was observed between swelling index and Tamarind Seed Polysaccharide concentration as seen in Fig 1 and 2. From the Fig 1 it is clear that the matrices underwent swelling in the dissolution media almost immediately. From the results it can be seen that the release profile of the drug is influenced by this swelling. A constant release is observed here due to the increase in diffusional path length caused by swelling, which is compensated by continuous erosion of the matrix.

Cumulative percentage release profiles of LEVO layer of various formulations using SSG and CCS:

The quantitative formula of each of the formulations using different superdisintegrants is given in Table 1.The drug release data of various formulations using different superdisintegrants is graphically represented in Fig 3 and Fig 4. The percentage drug released for the formulations F_1, F_2 and F_3 was found to be more than 90% after 30 minutes. With the increase in the concentration of SSG from 1.5mg to 3.75mg a gradual increase in the drug release is observed.However with F4(3.75mg) ,more than 95% drug release was achieved in 25minutes

The comparative *in vitro* release profile is depicted in Fig 4. The percentage drug released for the formulations F5 and F6 were found to be more than 90% after 30 minutes. With the increase in the concentration of CCS from 1.5 mg to 3.75mg a gradual increase in the drug release is observed. However with F7 (3mg) and F8 (3.75mg) more than 95% drug release was achieved in 25 minutes .

Cumulative percentage release profiles of MONT layer of various formulations using TSP and HPMC grades:

The quantitative formulae of various preparations using different polymers in different concentrations is depicted in Table 2.The release rate of Montelukast was found to be effected by the concentration of the polymer used in the preparation of tablets.A decrease in release rate was observed by increase in TSP concentration. The release rate for optimized formulation (F12) showed release up to 12hours. The influence of polymer concentration on drug release, formulations (F_{14}-F_{28}) using different concentrations of HPMC K100M, HPMC K15M and HPMC K4M is shown in Fig 6 and Fig 7. At 1:3 drug to polymer ratio, HPMC K100 showed release up to 12hours whereas in case of K15M, 1:4 ratio was optimized. In case of K4M the sustained action could be seen only at 5parts of the polymer as seen in Fig 8. With the increase in viscosity of HPMC grades, a retarding effect on the drug release was observed.

Release kinetics

The in vitro release data obtained from Formulations F1-F8 using different concentrations of SSG and CCS was fitted to kinetic models such as zero order, first order, Higuchi and Korsmeyer-Peppas models [14] to explore the release mechanism and the values are given in Table 7 and 8. The cumulative percentage drug released was found to be in the order of F_4>F_3>F_2>F_1 in case of SSG formulations. The values of release exponents 'n' for these formulations were found to be 0.514, 0.531,0.463 and 0.471 respectively indicating the release is governed by non-Fickian anomalous transport. The values of release exponents 'n' for CCS formulations F_5, F_6, F_7 and F_8 were found to be 0.578, 0.631, 0.584 and 0.544 respectively indicating the release is governed by non-Fickian anomalous transport.

The in vitro release data of optimized formulations F12, F16, F22 and F28 using TSP and HPMC grades was fitted to kinetic models such as zero order, first order, Higuchi and Korsmeyer-Peppas models [13] to explore the release mechanism and the values are given in Table 9. The data obtained from the release kinetics fitted with Higuchi model indicated that the release of drug from the tablets was found to depend on the square root of time. Further, it is important to note that a linear relationship was obtained for a plot of release profile verses time, and the regression coefficient was very close to zero (R^2 = 0.995, 0.987, 0.979 and 0.981)for these formulations respectively. The n values obtained from the Korsmeyer-Peppas model showed that the release mechanism was non-Fickian [15].

Accelerated Stability studies:

The data of Stability studies of batch F12 is given in Tables 11 and 12.The studies indicate no change in physical appearance of tablets over a period of three months in accelerated conditions(40°C/75 % RH). The results of stability study after three months, cumulative percentage drug release of batch F12 shows 95.89% for LEVO layer and 98.61% for the MONT layer in 3months.

Drug - Excipient compatibility studies:

The FTIR studies of pure drugs LEVO and MONT ,the natural polymer TSP and optimized formulations (F12) were carried out to detect any major interfering incompatibility between the drugs and the polymer . Few FTIR stectra are given in Fig 9, 10, 11 and the data is given in
Tables 13.
The absorption peaks of tamarind seed polysaccharide were found at 1036 cm-1 (C-O-C, ether group absorbance), 1637 and 1655 cm-1 (C=O, aldehyde absorption), 2924 cm-1 (C-H stretching), 3356 cm-1 (primary OH), 3385 cm-1 (secondary OH), which indicate that isolated product was polysaccharide. There is no change in shift of major peaks of drug (C=O str, C=H str , OH-str) observed.

In vivo studies:

In vivo studies were carried out to evaluate the pharmacokinetic behavior of the optimized F12 formulation. The sample chromatograms of the studies are given in Fig 15 - 17.The retention time of Montelukast is 5.5.

Quantification and Linearity:

The plasma standard curves were prepared over the range of 50-250µg/ml. The calibration curves of MONT were typically described by the regression analysis equation Y=169.6x+0.997.
Plasma concentration and pharmacokinetic parameters after oral administration of formulated sustained release layer bilayer tablet and plain drug are summarized in Table 14 and Figure 15. From the data, the control preparations have shown immediate release upto 6 hours.Significantly higher plasma concentrations were seen after 90min of dose administration, but were found to decline rapidly. It is important to note that the formulated Sustained release bilayer tablets (Code F12) showed significantly higher C_{max} (74.7µg) compared to control formulations.

These tablets also maintained constant plasma concentrations up to 12 hours. The improvement in the blood levels indicate that the bilayered tablets made from TSP are therapeutically more effective in the treatment of bronchial asthma. A four-fold increase in AUC was observed in case of bilayered tablets when compared to the plain drug data. The increase in AUC indicates improvement in the bioavailability due to the bilayered tablets. It is interesting to note that the T_{max} has increased from 3 hrs(control) to 6 hrs in case of our F12 formulation indicating slow and constant release of the drug contributing to sustained action.Overall the preliminary pharmacokinetic data suggest that the F12 formulation is a suitable canditate in providing sustained drug levels of MONT upto 12hours.

Conclusion

The prepared bilayered tablets of Montelukast and Levocetrizine DiHydrochloride using sodium starch glycolate and Croscarmellose sodium showed immediate release of Levocetrizine layer in 25min (F4 and F8). The second layer formulated using Tamarind seed polysaccharide(F 12) showed sustained release up to 12 hours compared to the formulations (F16, F22, F28) using HPMC Grades (K100M, K15M, K4M). From the stability studies Bilayered tablets with tamarind seed polysaccharide were found to be stable. The formulated Sustained release bilayered tablets (F12) showed significantly higher C_{max} (74.7µg) compared to control formulations. The plasma concentrations remained constant up to 12 hours.The AUC of F12 was found to show a four fold increase compared to the control indicating improvement in the bioavailability. The tmax of the formulated F12 tablets was found to be doubled indicating slower and sustained release of the formulated tablets.Hence from our investigations we can conclude that the bilayered tablets of LEVO and MONT prepared using TSP are a promising alternative in the effective treatment of patients suffering from Allergic rhinitis and bronchial asthma.

Acknowledgements

The authors are thankful to matrix laboratories and Symed labs, Hyderabad for their kind gift samples.

References

[1]. Rathod RT, Misra D, FDC of Montelukast with levocetirizine: focus on bilayer technology, J Indian Med Assoc. 2009 ;107(10):734.

[2]. Rathod RT, Misra D; FDC of Montelukast with levocetirizine : focus on bilayer technology Journal of the Indian Medical Association (JIMA). 2009; 107 (8); 562-4.

[3]. Jan Vogeleer ,Challenges in Developing A Bilayer Tablet , Powder technology division,Niro Pharma systems.

[4]. Patel Mehul,Ganesh Nanjan Sockan, kavitha, Tamizh Mani. Challenges in the formulation of bilayered tablets. International Journal of Pharma Research and Development, 2010; 2:23-35

[5]. R. L. Whistler ,Industrial Gums, Ed. (Academic Press, New York, 1973;2nd ed:369-411.

[6]. Indian pharmacopoeia, The Controller of publications, New Delhi, 1996;4th edition:vol II, 469.

[7]. Sameer H Lakade, "Formulation development and evaluation of mouth dissolving tablets of Ondansetron hydrochloride", Asian Journal of pharmaceutics. 200; I:150-153.

[8]. Michael E Aulton, Pharmaceutics, The Design and manufacture of medicines, Harcourt Publishers Limited, London. 2007; 3:175-177.

[9]. USP 27/NF 22, Asian edition, General test procedures, U.S. Pharmacopoeial convention, Rockville MD. 2004:1204.

[10]. Al-Taani BM, Tashtoush BM. Effect of microenvironment pH of swellable and erodable buffered matrices on the release characteristics of diclofenac sodium. AAPS Pharm Sci Tech. 2003;4:E43.

[11]. Glicksman M. Tamarind seed gum in food hydrocolloids. Florida CRC Press.1986; 3: 191-202.

[12]. Ibrahim A. Alsarra. Development of a stability-indicating HPLC method for the determination of montelukast in tablets and human plasma and its applications to pharmacokinetic and stability studies. Saudi Pharmaceutical Journal Saudi Pharmaceutical Journal. 2004; 12: 22-33.

[13]. Pattana Sripalakit, Bungon Kongthong, Aurasorn Saraphanchotiwitthaya. A simple bioanalytical assay for determination of montelukast in human plasma: Application to a pharmacokinetic study, Journal of Chromatography. 2008; 869: 38–44

[14]. Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose. Advances Drug Delivery. Revised. 2001;48:139-157.

[15]. Korsmeyer RW, Gurny R, Docler E, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int J Pharm.1983;15:25-35.

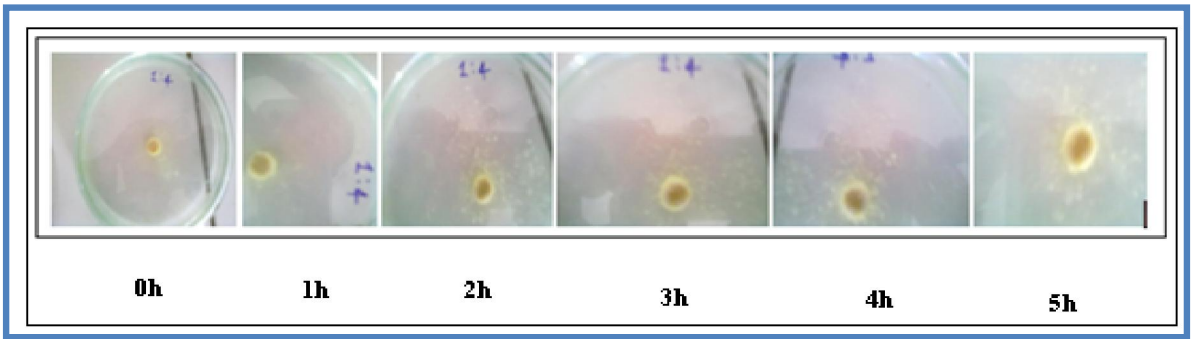

Fig.1: Swelling index of optimized formulation as a function of time (F12).

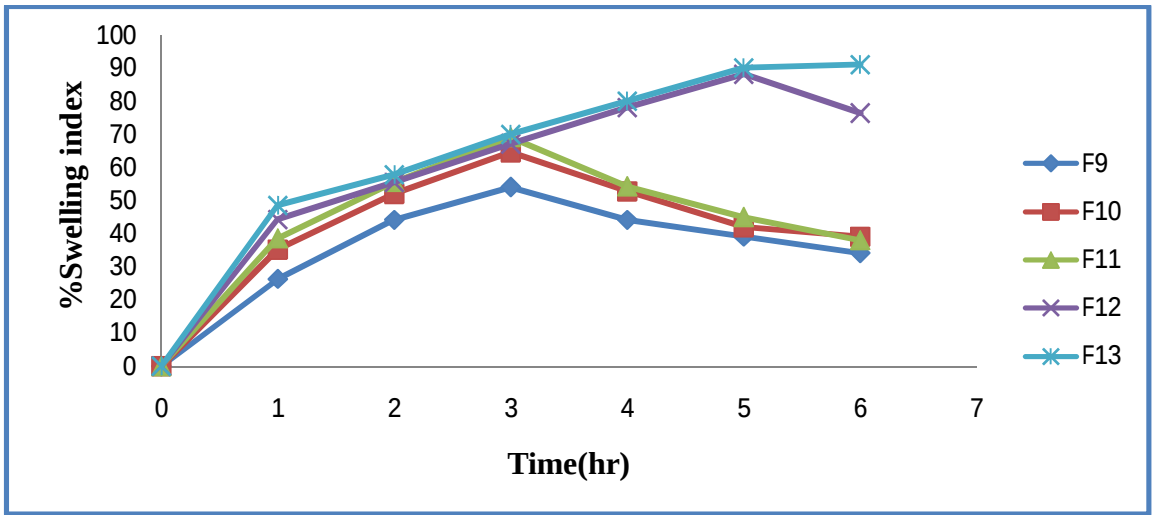

Fig 2: Comparative profile of swelling index with that of Tamarind Seed Polysaccharide (F9 to F13).

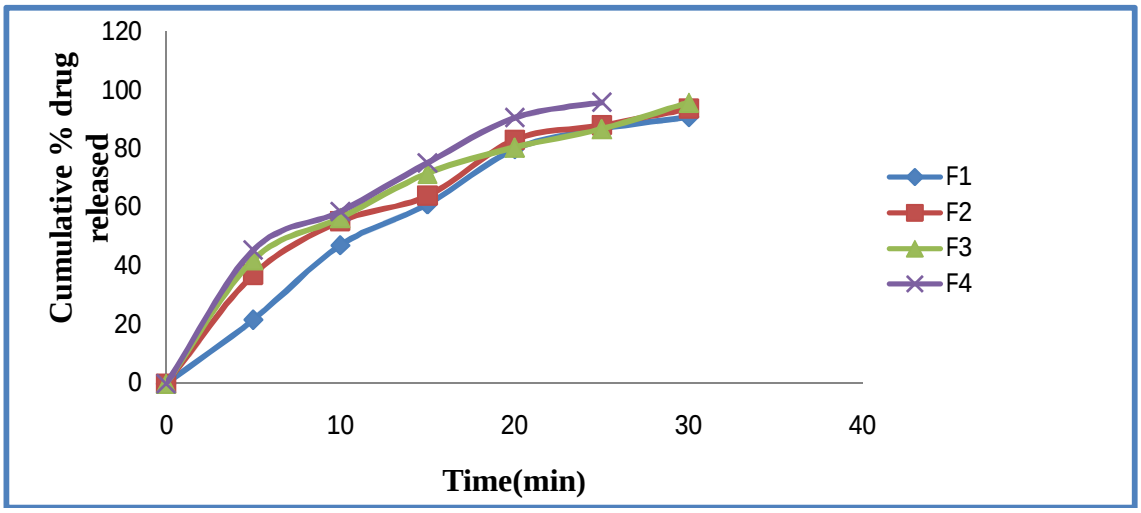

Fig 3: In vitro release profiles of immediate release layer for bilayer tablets of Levocetrizine DiHydrochloride formulated with SSG (F1-F4)

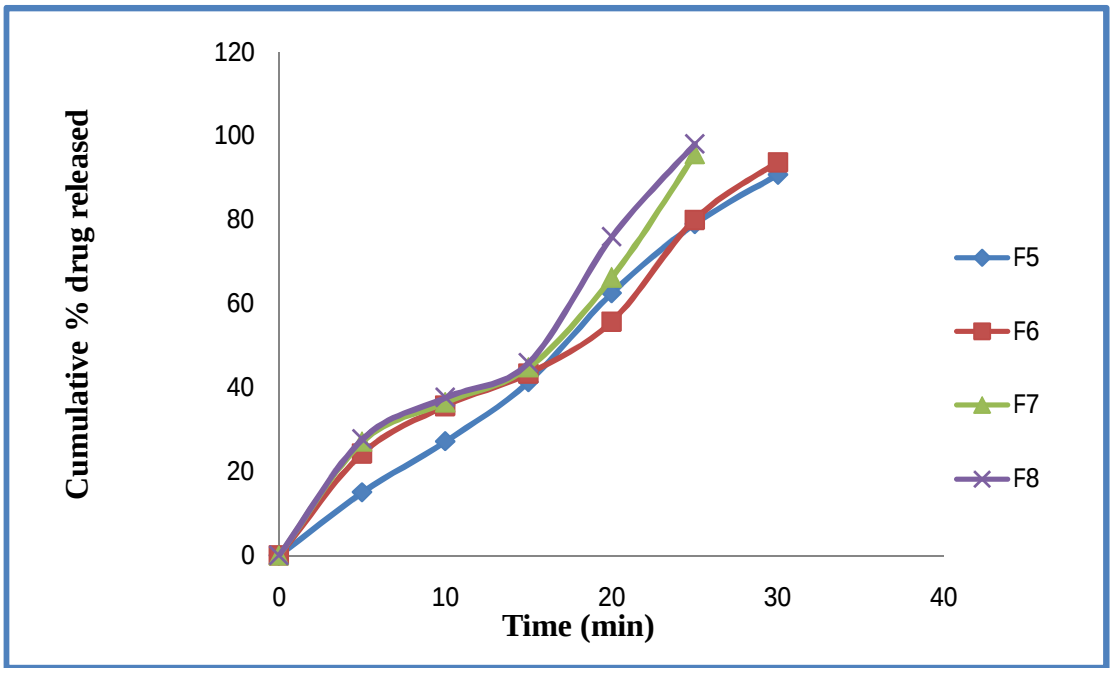

Fig 4. In vitro release profile of immediate release layer for bilayer tablets of Levocetrizine DiHydrochloride formulated with Croscarmellose sodium(F5-F8)

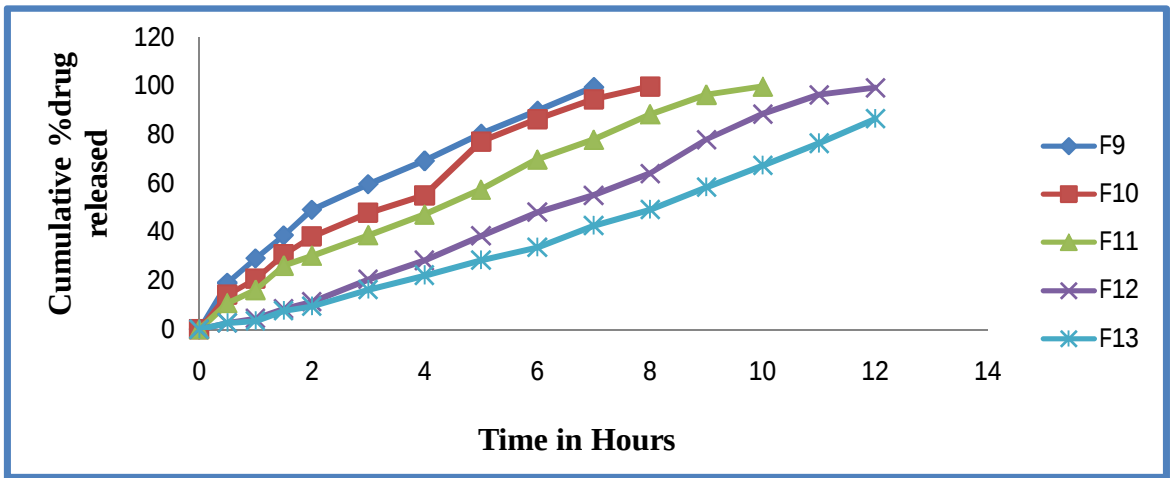

Fig. 5.Invitro release profile of sustained release layer of formulation with TSP (F9 to F13)

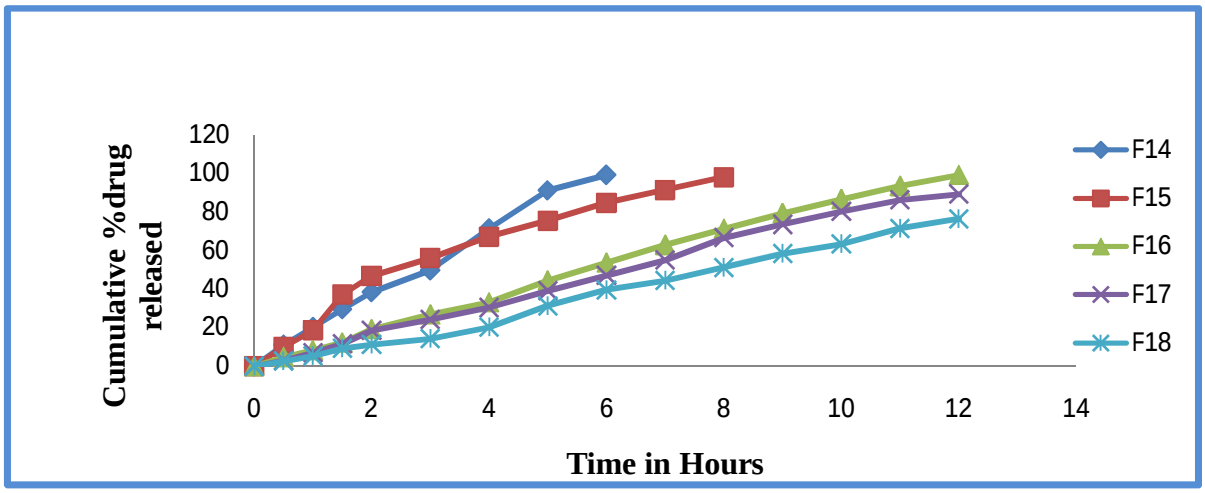

Fig.6.Invitro release profile of sustained release layer of formulation withHPMCK100M (F14 to F18)

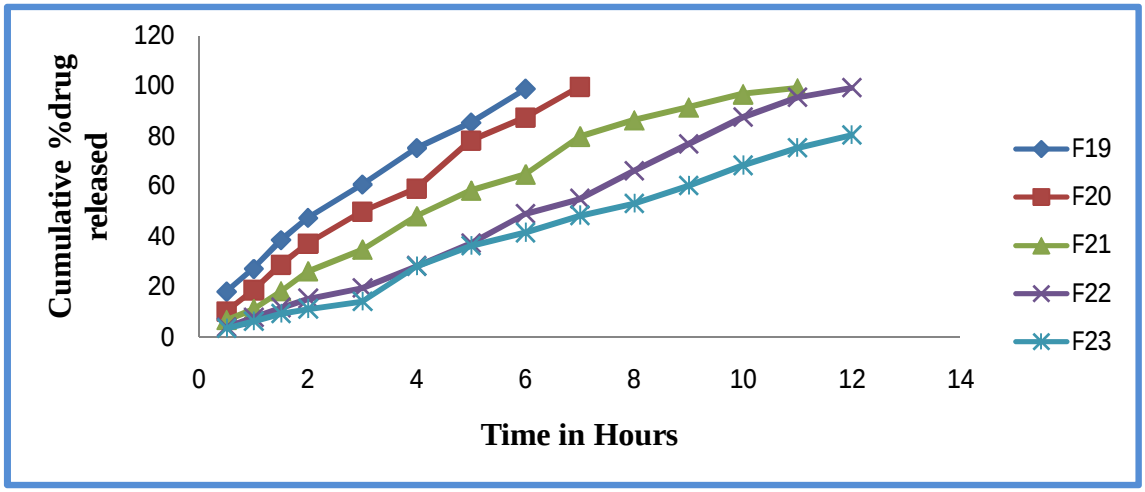

Fig.7.Invitro release profile of sustained release layer of formulation with HPMCK15M (F19 to F23)

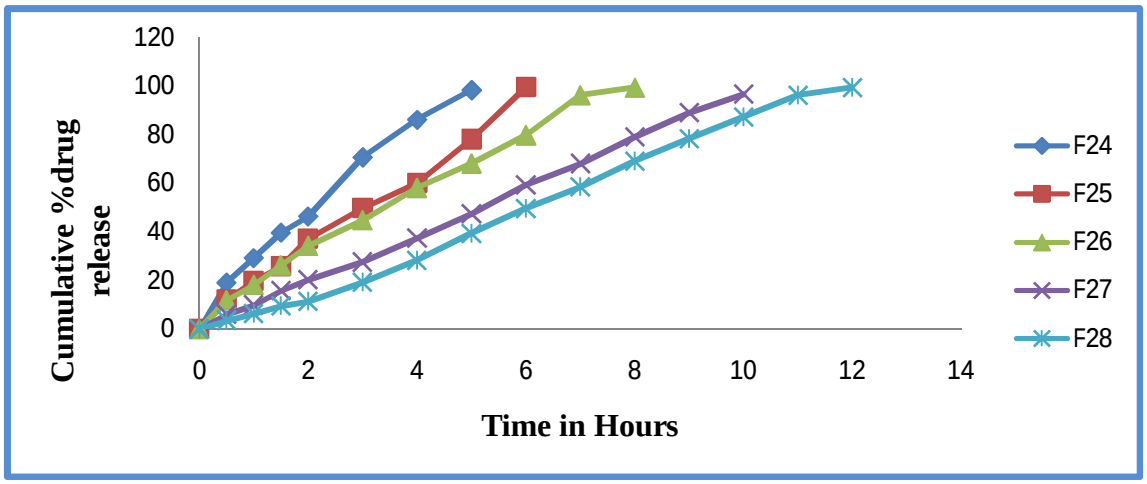

Fig.8.Invitro release profile of sustained release layer of formulation with HPMCK4M (F24 to F28)

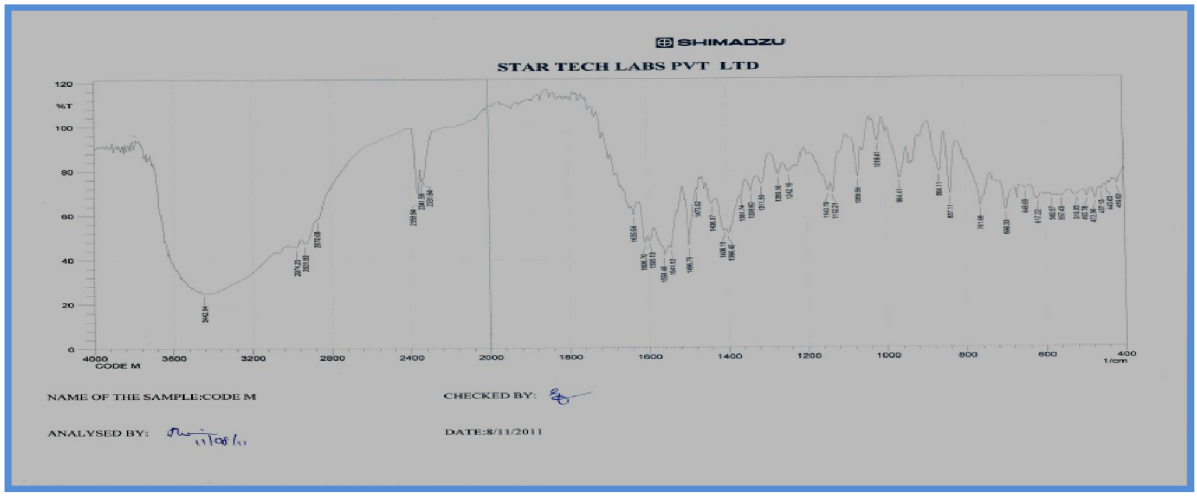

Fig.9: FTIR spectrum of pure drug montelukast

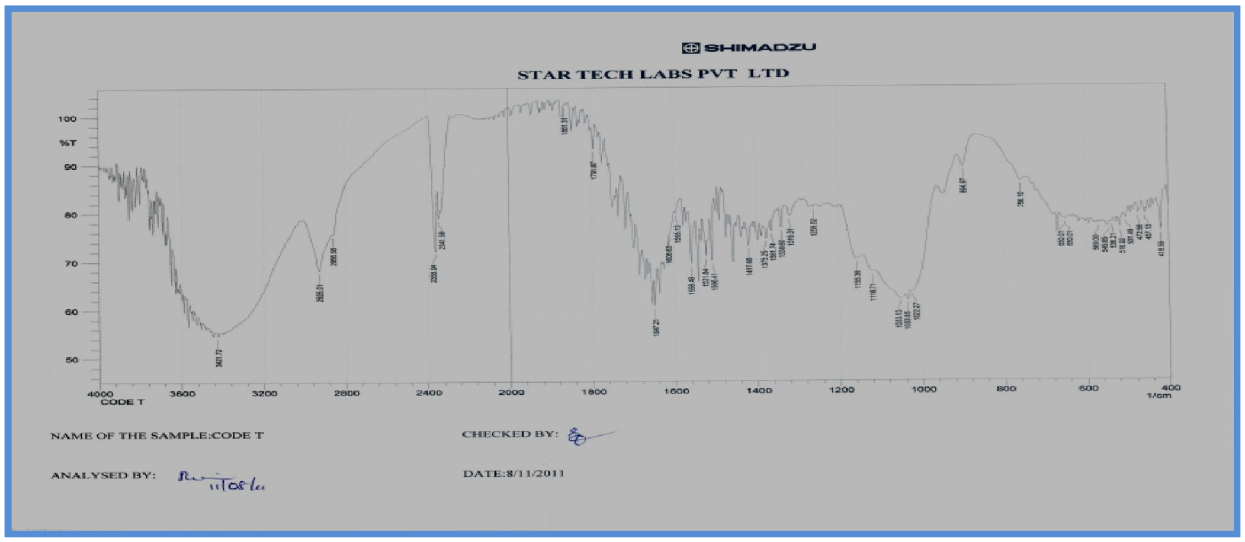

Fig.10: FTIR spectrum of tamarind seed polysacharide

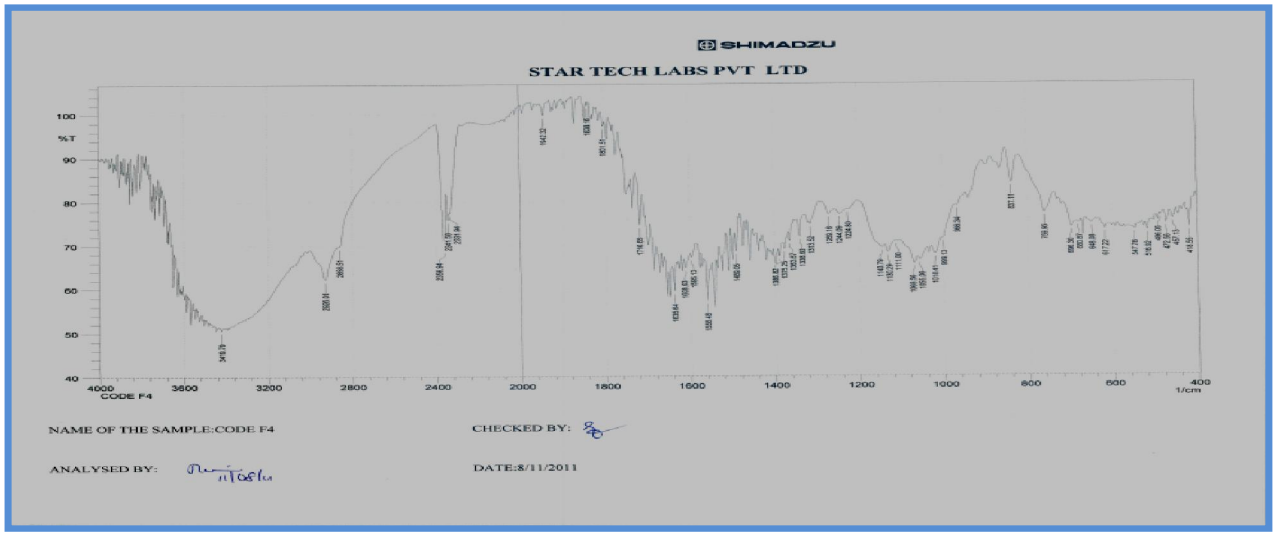

Fig.11: FTIR spectrum of optimised formulation (F12)

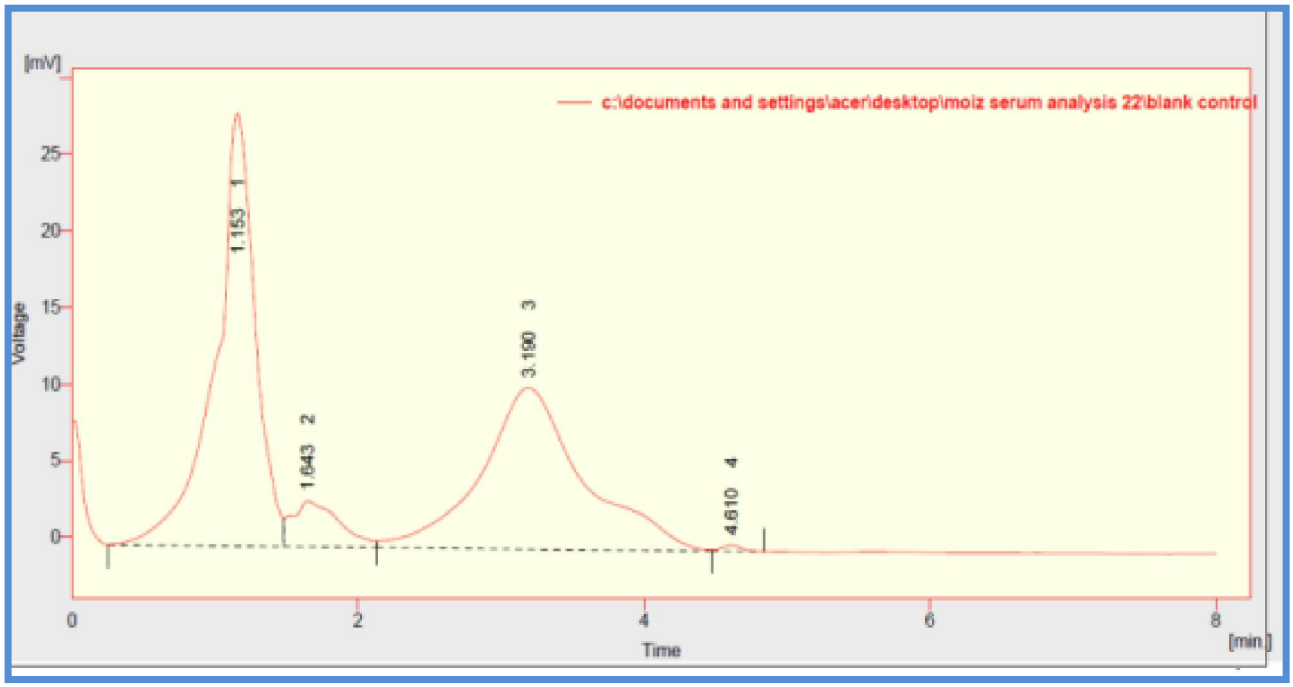

Fig.12.Chromatogram of plain plasma

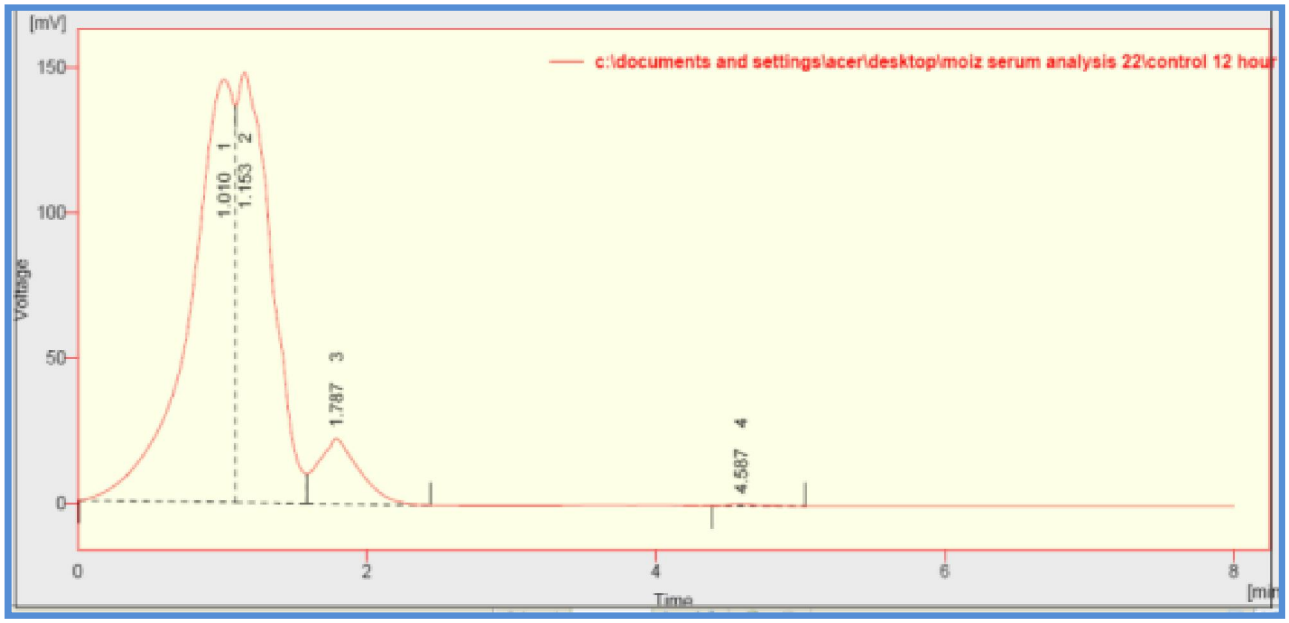

Fig.13.Chromatogram of control sample 12hours after drug administration

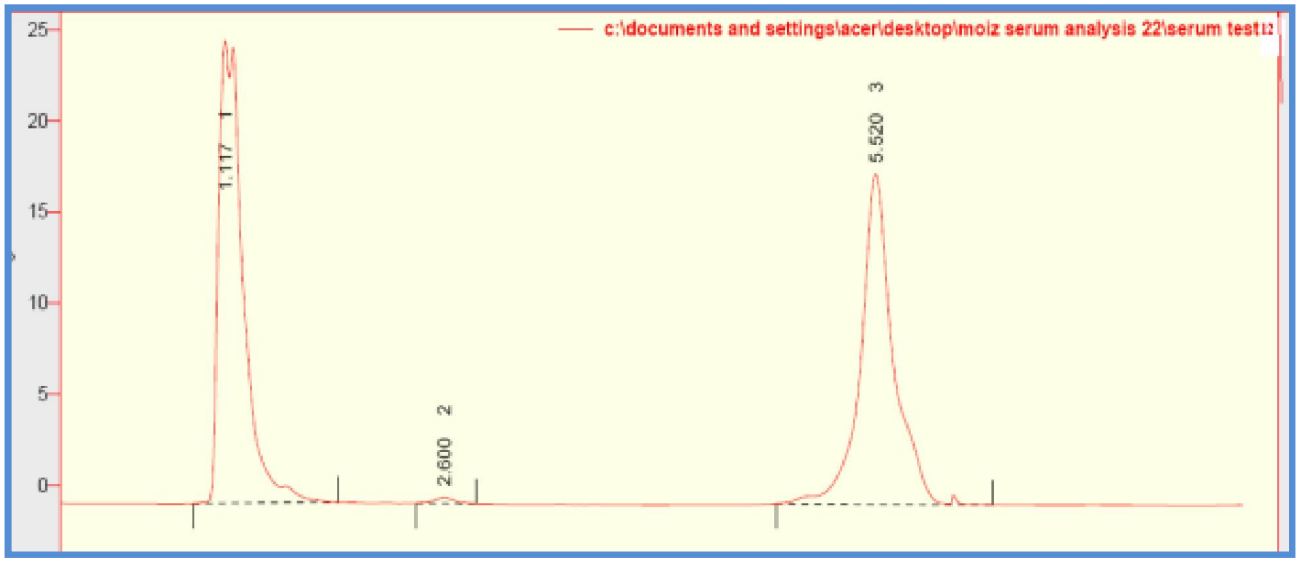

Fig.14.Chromatogram of test sample 12hours after drug administration

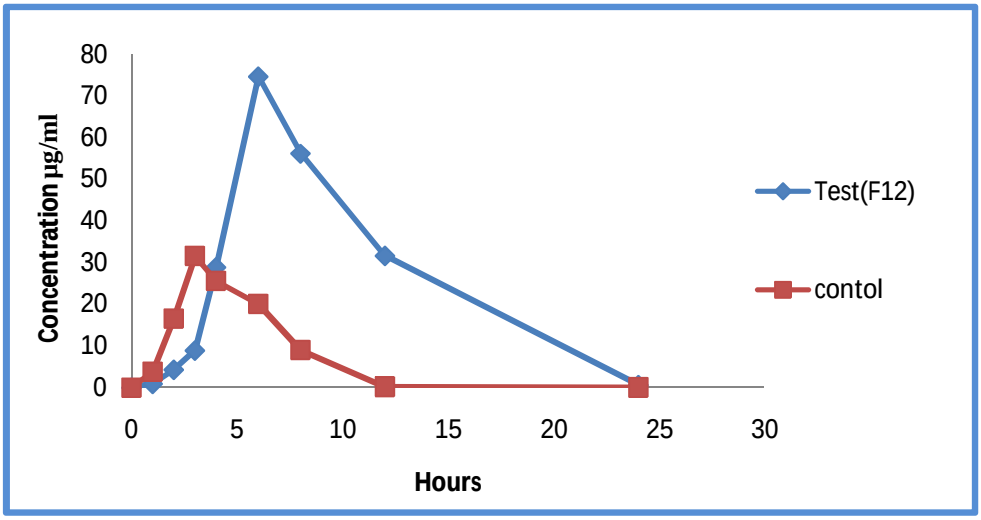

Fig 15. Plasma concentration-time profiles of optimized batch of Sustained Release layer Bilayer tablet (batch F12) and Control plain drug after oral administration.

Table 1. Formulation and optimisation of Levocetrizine immediate release layer.

Batch Code	Levo (mg)	SSG (mg)	CCS (mg)	Lactose (mg)	SP (5%)	MS (mg)	Talc (mg)	Total Weight(mg)
F1	5	1.50	-	67.00	q.s	0.75	0.75	75
F2	5	2.25	-	66.25	q.s	0.75	0.75	75
F3	5	3.00	-	65.50	q.s	0.75	0.75	75
F4	5	3.75	-	64.75	q.s	0.75	0.75	75
F5	5	-	1.50	67.00	q.s	0.75	0.75	75
F6	5	-	2.25	66.25	q.s	0.75	0.75	75
F7	5	-	3.00	65.50	q.s	0.75	0.75	75
F8	5	-	3.75	64.75	q.s	0.75	0.75	75

Levo-Levocetrizine**, SSG-**Sodium starch glycolate**, CCS-Croscarmellose,SP-**starch paste**, MS-**Magnesium Stearate**, mg-**milligrams

Table 2. Formulation and optimisation of Montelukast sustained release layer

Batch code	Mont (mg)	TSP (mg)	HPMC K100 (mg)	HPMC K15 (mg)	HPMC K15 (mg)	MCC (mg)	PVP (1%)	MS (mg)	Talc (mg)	Total weight(mg)
F9	10	10	-	-		146	q.s	6	3	175
F10	10	20	-	-		136	q.s	6	3	175
F11	10	30	-	-		126	q.s	6	3	175
F12	10	40	-	-		116	q.s	6	3	175
F13	10	50	-	-		106	q.s	6	3	175
F14	10	-	10	-		146	q.s	6	3	175
F15	10	-	20	-		136	q.s	6	3	175
F16	10	-	30	-		126	q.s	6	3	175
F17	10	-	40	-		116	q.s	6	3	175
F18	10	-	50	-		106	q.s	6	3	175
F19	10	-	-	10		146	q.s	6	3	175
F20	10	-	-	20		136	q.s	6	3	175
F21	10	-	-	30		126	q.s	6	3	175
F22	10	-	-	40		116	q.s	6	3	175
F23	10	-	-	50		106	q.s	6	3	175
F24	10	-	-	-	10	146	q.s	6	3	175
F25	10	-	-	-	20	136	q.s	6	3	175
F26	10	-	-	-	30	126	q.s	6	3	175
F27	10	-	-	-	40	116	q.s	6	3	175
F28	10	-	-	-	50	106	q.s	6	3	175

Mont-Montelukast, **TSP**- Tamarind seed polysaccharide
HPMC - Hydroxypropyl methylcellulose **(K100M,K-15M,K4M)**;
MCC- Microcrystallinecellulose**(101);PVP-**Polyvinylpyrollidine
MS- Magnesium stearate; **q.s-** quantity sufficient. **Colour -**Tartrazine yellow.

Table 3. Physical evaluation of immediate release layer of Levocetrizine granules

Batch Code	Bulk density (gm/ml)*	Tapped density (gm/ml)*	Carr's Index (%)	Hausner's ratio	Angle of repose (°)*
F-1	0.40 ± 0.06	0.444 ± 0.05	10.01 ± 0.14	1.11 ± 0.06	29.05 ± 0.25
F-2	0.480 ± 0.03	0.545 ± 0.03	11.92 ± 0.12	1.13 ± 0.02	29.74 ± 0.24
F-3	0.440 ± 0.04	0.500 ± 0.02	12.00 ± 0.15	1.13 ± 0.05	27.75 ± 0.15
F-4	0.432 ± 0.05	0.490 ± 0.04	11.83 ± 0.13	1.13 ± 0.03	29.74 ± 0.17
F-5	0.416 ± 0.03	0.476 ± 0.05	12.60 ± 0.09	1.14 ± 0.04	29.05 ±0.26
F-6	0.409 ± 0.04	0.473 ± 0.03	13.53 ± 0.24	1.15 ± 0.09	29.30 ± 0.14
F-7	0.412 ± 0.04	0.460 ± 0.05	10.43 ± 0.20	1.11 ± 0.07	29.60 ± 0.34
F-8	0.420 ± 0.03	0.466 ± 0.06	9.87 ± 0.14	1.10 ± 0.06	28.52 ± 0.14

***(n=3±SD)**

Table 4. Physical evaluation of sustained release Montelukast granules

Batch Code	Bulk density (gm/ml)*	Tapped density (gm/ml)*	Carr's Index (%)	Hausner's ratio	Angle of repose (°)*
F-9	0.421 ± 0.05	0.476 ± 0.04	11.55 ± 0.07	1.13 ± 0.03	29.05 ± 0.26
F-10	0.408 ± 0.03	0.450 ± 0.05	9.30 ± 0.17	1.10 ± 0.08	27.51 ± 0.18
F-11	0.412 ± 0.05	0.454 ± 0.03	9.20 ± 0.23	1.07 ± 0.04	30.76 ± 0.21
F-12	0.410 ± 0.02	0.463 ± 0.03	11.64 ± 0.18	1.12 ± 0.07	30.76 ± 0.36
F-13	0.400 ± 0.02	0.444 ± 0.06	10.01 ± 0.32	1.11 ± 0.03	29.60 ± 0.12
F-14	0.420 ± 0.03	0.474 ± 0.03	11.49 ± 0.12	1.12 ± 0.02	29.05 ± 0.24
F-15	0.411 ± 0.05	0.456 ± 0.02	10.01 ± 0.15	1.10 ± 0.05	28.52 ± 0.15
F-16	0.418 ± 0.05	0.472 ± 0.04	11.44 ± 0.13	1.12 ± 0.03	29.60 ± 0.17
F-17	0.405 ± 0.03	0.450 ± 0.05	9.05 ± 0.09	1.06 ± 0.04	30.71 ± 0.26
F-18	0.411 ± 0.04	0.455 ± 0.03	9.60 ± 0. 24	1.10 ± 0.09	29.05 ± 0.14
F-19	0.430 ± 0.04	0.485 ± 0.05	11.34 ± 0.20	1.12 ± 0.07	29.60 ± 0.34
F-20	0.415 ± 0.03	0.459 ± 0.06	9.50 ± 0.14	1.10 ± 0.06	28.52 ± 0.14
F-21	0.411± 0.05	0.455 ± 0.04	9.60 ± 0.07	1.10 ± 0.03	28.00 ± 0.26

F-22	0.405 ± 0.03	0.457 ± 0.05	11.47 ± 0.17	1.12 ± 0.08	31.37 ± 0.18
F-23	0.400 ± 0.05	0.451 ± 0.03	11.32 ± 0.23	1.12 ± 0.04	30.17 ± 0.21
F-24	0.414 ± 0.02	0.457 ± 0.03	9.40 ± 0.18	1.10 ± 0.07	29.60 ± 0.36
F-25	0.418 ± 0.02	0.472 ± 0.06	11.44 ± 0.32	1.12 ± 0.03	29.60 ± 0.12
F-26	0.424 ± 0.07	0.477 ± 0.04	11.42 ± 0.06	1.13 ± 0.05	29.05 ± 0.07
F27	0.420± 0.03	0.474 ± 0.03	11.49 ± 0.12	1.12 ± 0.02	29.05 ± 0.24
F28	0.410 ± 0.02	0.463 ± 0.03	11.64 ± 0.18	1.12 ± 0.07	30.76 ± 0.36

***(n=3±SD)**

Table 5. Physicochemical evaluations of Immediate release layer (Levocetrizine) Tablets

Batch Code	Parameter			
	Hardness(kg/cm2)*	**Friability (%)****	**Weight variation (%)****	**Drug content (%)*****
F-1	3.8 ± 0.4	0.012 ± 0.03	1.1 ± 0.05	91.23 ± 3.05
F-2	4.4 ± 0.5	0.015 ± 0.02	1.4 ± 0.02	81.5 ± 2.21
F-3	3.7 ± 0.33	0.010 ± 0.02	0.96 ± 0.01	78.2 ± 3.15
F-4	4.7 ± 0.35	0.018 ± 0.03	0.859 ± 0.01	72.87 ± 4.34
F-5	3.3 ± 0.21	0.005 ±0.03	0.09 ± 0.02	96.5 ± 2.42
F-6	4.9 ± 0.15	0.020 ± 0.02	0.65 ± 0.03	96.84 ± 1.16
F-7	4.7 ± 0.42	0.10 ± 0.02	0.43 ± 0.02	94.93 ± 3.09
F-8	4.5 ± 0.17	0.12 ± 0.03	0.19 ± 0.02	92.87 ± 1.48

(n=6±SD)** * (n=20±SD)** ***** (n=3 ±SD)**

Table 6. Physicochemical evaluations of sustained release layer of Montelukast tablets

Batch Code	Parameter			
	Hardness (kg/cm2)*	**Friability (%)****	**Weight variation (%)****	**Drug content (%)**
F-9	4.4 ± 0.16	0.6 ± 0.04	0.29 ± 0.01	83.97 ± 2.26
F-10	4.7 ± 0.24	0.40 ± 0.02	0.14 ± 0.01	91.36 ± 1.35
F-11	3.9 ± 0.25	0.16 ± 0.02	0.56 ± 0.02	86.9 ± 2.42
F-12	4.8 ± 0.34	0.27 ± 0.03	0.98 ± 0.02	94.15 ± 3.13
F-13	4.0 ± 0.18	0.33 ± 0.03	0.132 ± 0.03	92.15 ± 3.18
F-14	5.0 ± 0.29	0.33 ± 0.03	0.234 ± 0.04	86.15 ± 4.38
F-15	6.5 ± 0.24	0.16 ± 0.02	0.287 ± 0.02	90.76 ± 2.27
F-16	7.0 ± 0.18	0.16 ± 0.02	0.290 ± 0.01	86.72 ± 3.36
F-17	5.0 ± 0.13	0.1 ± 0.01	0.76 ± 0.02	94.05 ± 2.15
F-18	6.0 ± 0.25	0.4 ± 0.01	0.56 ± 0.04	92.00 ± 2.5
F-19	5.5 ± 0.24	0.33 ± 0.05	0.54 ± 0.63	86.10 ± 4.04
F-20	5.7 ± 0.13	0.27 ± 0.04	0.75 ± 0.03	89.76 ± 4.08
F-21	6.5 ± 0.12	0.33 ± 0.01	0.76 ± 0.02	85.72 ± 4.04
F-22	4.5 ± 0.14	0.33 ± 0.07	0.86 ± 0.04	94.15 ± 2.06
F-23	5.0 ± 0.25	0.27 ± 0.01	0.56 ± 0.03	90.15 ± 2.07
F-24	5.5 ± 0.12	0.33 ± 0.01	0.05 ±.0.02	86.15 ± 3.02
F-25	6.0 ± 0.24	0.15 ± 0.01	0.3 ± 0.03	91.76 ± 2.05
F-26	5.5 ± 0.05	0.16 ± 0.02	0.12 ± 0.02	86.72 ± 4.05
F-27	6.0 ± 0.35	0.33 ± 0.05	0.54 ± 0.63	86.10 ± 4.04
F-28	6.5 ± 0.44	0.16 ± 0.02	0.290 ± 0.01	86.72 ± 3.36

***(n=6±SD)** **(n=20±SD)** ***(n=3 ±SD)**

Table 7. Swelling index of sustained release layer tablet with that of Tamarind Seed Polysaccharide (F9 T0 F13)

Time	F9	F10	F11	F12	F13
0	0	0	0	0	0
1	26.4	35.29	38.6	44.4	48.6
2	44.3	52.14	55.6	55.6	57.9
3	54.2	64.7	69.2	67.3	70.1
4	44.3	52.94	54.4	78.2	80.2
5	39.2	42.12	45.1	88.3	90.2
6	34.2	39.2	38.1	76.6	91.2

Table 8: In vitro drug release kinetics of immediate release layer for bilayer tablets of Levocetrizine DiHydrochloride formulated with SSG

Formulation	zero order r^2	First order r^2	Higuichi r^2	Korsemeyer-peppas r2	n
F1	0.965	0.984	0.977	0.985	0.514
F2	0.912	0.984	0.994	0996	0.531
F3	0.879	0.958	0.978	0.998	0.463
F4	0.909	0.947	0.988	0.995	0.471

Table 9: In vitro drug release kinetics of immediate release layer for bilayer tablets of Levocetrizine Di Hydrochloride formulated with Croscarmellose sodium

Formulation	zero order r^2	First order r^2	Higuichi r^2	Korsemeyer-peppas r2	n
F5	0.988	0.990	0.965	0.997	0.578
F6	0.960	0.995	0.987	0.995	0.431
F7	0.951	0.994	0.990	0.992	0.584
F8	0.965	0.980	0.984	0.986	0.544

Table 10: Release kinetics of Montelukast layer of optimized formulations.

Formulation	zero order r^2	First order r^2	Higuichi r^2	korsemeyer-peppas r2	n
F12	0.995	0.770	0.896	0.995	0.859
F16	0.987	0.846	0.926	0.987	0.858
F22	0.979	0.846	0.900	0.979	0.850
F28	0.981	0.840	0.906	0.981	0.858

Table 11: Cumulative % drug release of Immediate Release Layer stability study of F4 with sodium starch glycollate.

Time(min)	Cumulative % Drug Released (Initial)	Cumulative % Drug Released (After storage at 40°C after 3M)
5	45.66	44.66
10	58.83	57.99
15	75.43	76.43
20	90.83	89.83
25	96.09	95.89
30	-	-

Table 12: Cumulative % drug release of Sustained Release Layer stability study of F12

Time(hours)	Cumulative % Drug Released (Initial)	Cumulative % Drug Released (After storage at 40°C after 3 month)
0.5	4.01	3.91
1	8.88	7.88
1.5	11.76	11.76
2	15.33	14.33
3	20.54	20.54
4	28.33	28.33
5	38.43	37..43
6	48.22	46.22
7	55.11	55.11
8	64.12	63.12
9	77.98	77.98
10	88.65	88.65
11	96.44	95.44
12	99.33	98.61

TABLE 13: FTIR peak positions (cm^{-1}) of Montelukast

TYPE OF VIBRATION	Frequency(cm-1)
-OH	**3417**
-C-H ar	**2900-3100**
-C=O	**1613**

Table 14.Pharmacokinetic Parameters of Montelukast After Administration of control and Sustained Release Tablets

Formulation Code	AUC (µg/µL)*h	C_{max} (µg)	t_{max} (hours)
F12	0.562	74.7	6
CONTROL	0.122	31.26	3