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COMBINATIONAL THERAPY OF ROSUVASTATIN CALCIUM AND FENOFIBRATE AS BILAYER TABLET: A POTENTIAL APPROACH TO CONTROL HYPOLIPIDAEMIA

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ABSTRACT: The present investigation studied a novel bilayer tablet having extended release of rosuvastatin calcium (RSTCa) and immediate Release of fenofibrate (FB) in different polymers using wet granulation techniques. Granules were characterized by fourier-transformed Infrared Spectroscopy (FT-IR), differential Scanning Calorimetry (DSC), as well as by content uniformity, *in-vitro* dissolution studies and release kinetics. Selected granular system was subjected to bilayer tablet preparation by direct compression. Compressed tablets were evaluated for drug content, weight variation, friability, hardness, thickness, %assay and *in-vitro* dissolution studies. Prepared tablets were then coated and coated bilayer tablets were evaluated for the same. Functional groups of RSTCa and FB were retained in respective granules, suggesting absence of chemical interaction with any of the excipients used in the preparation of granules. Absence of specific peaks in physical mixtures revealed that FB has been completely converted to molecular form. Among the polymers used to improve drug release, MCC and HPMC K4M showed better control over drug release. Formulated bilayer tablets gave satisfactory results for various physicochemical evaluations and best fitted to Korsemeyer peppas and First-order model rate kinetics. *In - vitro* study showed that optimized bilayer tablet formulation released immediate dose of FB and then sustained release of RSTCa for more than twelve hours. Stability studies conducted for optimized formulation did not show any change in physical properties, drug content, and *in-vitro* drug release. The present study concluded that bilayer tablets of FB and RSTCa can be used as an alternative to the conventional dosage form.

Keywords:

Fenofibrate, Rosuvastatin Calcium, bilayer tablet, coating of bilayer tablet, *in vitro* release studies

INTRODUCTION: Dissolution testing of poorly soluble compounds in immediate-release (IR) solid dosage forms possesses many challenges. These challenges include developing and validating the test method, ensuring that the method is appropriately discriminatory, and addressing the potential for an *in vivo–in vitro* relationship (IVIVR) or correlation (IVIVC).

Satisfying all of these challenges and developing a meaningful dissolution method is a large task, because the extent of release is too low (i.e., one cannot get 100% of the dosage form dissolved) and secondly, the rate of release is too slow (i.e., one cannot get dissolution fast enough for a convenient test [1].

FB is a compound displaying poor aqueous solubility (less than 0.25 mg/ml) across the physiological pH range. FB is a BCS II drug used to decrease elevated plasma concentrations of low density lipoprotein and total cholesterol. Although low bioavailability of the drug is due to its poor solubility in water. Hydroxymethyl hydroxy methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) currently form the mainstay of lipid-regulating therapy, and are the most effective agents for reducing serum cholesterol concentrations and cardiovascular mortality. RSTCa is a new and highly effective inhibitor of HMG-CoA reductase that has completed Phase-III clinical development for the treatment of patients with dyslipidaemia. The absolute bioavailability of RSTCa tablets administered a single dose after a meal was approximately 20% relative to intravenous infusion. The formulation of sustained layer of RSTCa expected to reduce the frequent exposure of dose to upper GIT and thus facilitate in proper distribution to liver rather than skeletal muscles leading to improved patient compliance, maintain therapeutic action.

In current research study, focus was on development of Bilayered oral solid dosage form using anticholesteremic agents that will be used in treatment of hypercholesterolaemia in combination with hypolipidemic agent. When both agents used in combination give better result in mixed dyslipidemias. On the

basis of these considerations, we have proposed a bilayer tablet, in which the one layer is formulated to obtain immediate release of the drug, with the aim of reaching a high serum concentration in a short period of time. The second layer is a controlled release hydrophilic matrix, which is designed to maintain an effective plasma level for a prolonged period of time. The pharmacokinetic advantage relies on the fact that drug release from fast releasing layer leads to a sudden rise in the blood concentration. However, the blood level is maintained at steady state as the drug is released from the sustaining layer.

Drug(s) excipient compatibility screening:

The desired quantities of drug(s) with specified excipient(s) 1:5 were transferred in to an appropriately labelled glass vial. Subsequently, 5 µL of ultra pure water (Milli-Q Academic, Milli-Pore) was added to each vial and mixed using a glass capillary, which was left inside the vial after mixing. Each vial was sealed properly and placed in hot air oven (T26/HAO-L, Technico) at 50°C for 4 weeks. To identify the physical instability, organoleptic parameters of samples such as colour and texture were observed initially and at the end of 1st, 2nd, 3rd and 4 th week. To identify the chemical instability, samples were subjected to FT-IR and DSC [2].

Intrinsic Solubility Studies:

Saturation solubility studies were performed in triplicate by using distilled water, 0.1N HCL, Acetate buffer pH 4.5 and phosphate buffer pH 6.8. Excess of pure drug, were added to 10 mL distilled water 0.1N HCL, Acetate buffer pH 4.5 and phosphate buffer pH 6.8 in a screw-cap tube and shaken in a rotary flask shaker at room temperature (25°C) for 72 hrs. The resultant suspension was treated at 37^{o} C with 100 rpm in incubator shaker. After 24, 48 and 72 hrs samples were withdrawn and filtered through 0.45 µm filter. The filtrate was suitably diluted with distilled water 0.1N HCL, acetate buffer pH 4.5 and phosphate buffer pH 6.8 and analyzed at 290 nm (FB), 248 nm (RSTCa) by UV-visible spectrophotometer [3].

Intrinsic Dissolution Studies:

Pure drug 160 mg FB were subjected to in vitro dissolution studies using USP dissolution test apparatus II at 37±0.5ºC using 900 ml each water, 0.1N HCl and phosphate buffer ph 6.8 with or without 2% sodium lauryl sulfate in dissolution media. The rotation speed of the paddle was adjusted to 50 rpm. Samples were collected at 5, 10, 15, 20, 30 and 60 minutes and passed through a 0.45µm filter and analyzed by direct UV spectrophotometry at 290 nm. The cumulative percent drug release was calculated and plotted. This method is in house only for idea how the API performs their activity in different pH medias [4].

Granulation of FB Granules:

Table 1 showed composition of granule formulation. Trial 1 was manufactured by sifting FB, lactose, dibasic calcium phosphate, sodium starch glycolate, croscarmellose sodium, sodium lauryl sulphate mix, colloidal silicon dioxide through sieve # 40 and mixed. Magnesium stearate was mixed in final blend for 10 minutes. Other Trials were manufactured by sifting FB, lactose, maize starch, starch glycolate, croscarmellose sodium and sodium lauryl sulphate through sieve#40 and mixed for 15 min. Mixture was granulated with the blend of polysorbate 80, polyvinyl pyrrolidone K30 (PVP K30) in isopropyl alcohal. Prepared granules were dried in an oven at 50^{o}C till LOD of granules comes between (1-2) %. Granules were passed through sieve#20. Cab- O- Sil and magnesium stearate, each at 0.5%w/w, were sequentially mixed with the granules [5].

Granulation of RSTCA

Table 2 showed composition of granule formulation. Trial 1 was manufactured by sifting RSTCa, lactose, hydroxy propylmethyl cellulose K-4M, pregelatinized starch 1500, tribasic calcium phosphate, polyvinyl pyrrolidone k30, talcum, colloidal silicon dioxide, magnesium stearate through sieve #40 and colour sunset Yellow, butylated hydroxytoluene through sieve #100 and mixed. Magnesium stearate was mixed in final blend for 10 minutes. Other trials were manufactured by sifting RSTCa, lactose, hydroxy propylmethyl cellulose K-4M, pregelatinized starch 1500, tribasic calcium phosphate, talcum, colloidal silicon dioxide, magnesium stearate through sieve #40 and mixed for 15 min. Mixture was granulated with the blend of butylated hydroxytoluene, polyvinyl pyrrolidone K30 (PVP K30) in isopropyl alcohal. Prepared granules were dried in an oven at 50^{o}C till LOD of granules comes between (1-2) %. Granules were passed through sieve#20. Cab- O- Sil, color sunset yellow and magnesium stearate were sequentially mixed with the granules [6].

Evaluation of Prepared Granules:

Angle of repose:

The angle of repose of granules was determined by the funnel method. The granules were allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation.

$\tan \theta = h/r$ ..... (1)

Where h and r are the height and radius of the powder cone respectively.

Bulk Density:

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined and calculated by using the following formulas.

LBD = weight of the powder / volume of the packing ..... (2)

TBD = weight of the powder / tapped volume of the packing ..... (3)

Hausner's ratio= TBD/ LBD ..... (4)

Compressibility Index

The compressibility index of the granules was determined by Carr's compressibility index.

Carr's index (%) = [TBD-LBD] /TBD X 100 ..... (5)

Preparation and evaluation of tablets:

Tablets were prepared by direct compression technique using FB granules (Trial 1 to Trail 4) and RSTCa granules respectively (Trial 1 to Trial 5). Granules were compressed by using 12 Station tablet compression machine (Punch size 12.7mm Round, standard concave). The blend was subsequently compressed into tablets at the desired strength. Tablets were evaluated for post compression parameters like hardness, friability, drug content uniformity etc [7, 8].

Release kinetics of prepared tablets:

Drug release from 6 tablets of each formulation, in triplicate, was determined using USP apparatus I (Basket Type) where 900 ml of pH 6.8 buffer with

0.75% SLS was used as dissolution media maintained at 37±0.5°C at 100 rpm. A 5.0 ml sample was withdrawn at specific time points over a 30 min. and 12 hrs for FB and RSTCa respectively and equal volume of fresh dissolution medium was used to maintain a constant volume. The aliquot samples were filtered and the drug concentration was determined by ultraviolet (UV) method at 290 nm and 248 nm for FB and RSTCa respectively [9, 10].

Evaluation of release kinetics:

Data obtained from *In vitro* release studies was fitted to various kinetic equations to find out the mechanism of FB release from formulations. The kinetic models used were Zero order, First order, Higuchi, Peppas model and Hixson-crowell. The following plots were made: Q_t Vs t (Zero order kinetic model), log (Q_o-Q_t) Vs t (First order kinetic model), Q_t Vs square root of t (Higuchi model). Where Q_t is the amount of drug released at time t and Q_o is the initial amount of drug present in solid dispersions, $Q = kt^n$ (Peppas model) where Q is the amount of drug release; t is time; k is constant incorporating structural and geometrical characteristic of the release device and n is the release exponent indicative of the mechanism of release. Plots were subjected to regression analysis to find out the regression coefficient and hence the order of release.

Preparation and evaluation of bilayer tablets:

Optimized formulations of sustained release RSTCa (Trial 5) and FB immediate release (Trial 4) were selected and final Bilayer tablets were prepared according to the following formula (Table 4.6). Final Bilayer tablets were compressed as one layer only for RSTCa and second layer for FB using 19.8 x 8.7 mm round shape punch in 27 station tablet compression machine (Cadmach, India) (Table 3). In this, RSTCa granules were introduced first into the die cavity and a slight pre compression was made so that the layer was uniformly distributed, after that FB granules were added and a final compression was made [17, 18]. Post compression parameters of bilayer tablets were evaluated for weight variation, hardness, friability, thickness, disintegration and in vitro dissolution studies. Further evaluation was done by assay method as follows:

Standard preparation:

50 mg of RSTCa was weighed and dissolved in 100 ml of methanol. 2 ml of this solution was pippetted out and mixed with 50 ml of buffer: acetnitrile solution. 160 mg of FB was weighed and dissolved in 100 ml of methanol. 5 ml of this solution was pippetted out and mixed with RSTCa sample and sonicated the solution for 5 minutes.

Test preparation:

20 tablets were taken and crushed. Accurately weighed 560 mg powder and dissolved in 100 ml of methanol. 5 ml of this solution was mixed with 50 ml of buffer: acetonitrile solution. Solution was sonicated for 5 minutes. Content of RSTCa and FB was determined via HPLC at 215 nm.

*Same procedure is apply for uniformity of dosage unit

Coating of prepared bilayered tablets and its evaluation: The indented bilayer tablets were coated by using a conventional pan-coating process in a pan coater (Shanghai Huanghai Drug Inspection Instrument Co., Ltd, China). Hydroxypropylmethylcellulose E-15 in isopropyl alcohol containing Methylenechloride and propylene glycol was prepared as coating solution (**Table 4**). The temperature of inlet air was 40°C; spray rate was 7 ml/min; pan-rotating rate was 10 rpm. The coated tablets were dried at 40°C for 24 h to remove the residual solvent and then the coating was achieved. Coated tablets were evaluated for disintegration time, assay (%), thickness and diameter [11].

Release kinetics of prepared tablets:

Drug release from white orange color round biconvex film coated bilayered tablet was determined using USP apparatus I (Basket Type) where 900 ml of pH 6.8 buffer with 0.75% SLS was used as dissolution media maintained at 37±0.5°C at 100 rpm. A 5.0 ml sample was withdrawn at specific time points over a 30 min for FB and 16 hrs for RSTCa and equal volume of fresh dissolution medium was used to maintain a constant volume. The aliquot samples were filtered and the drug concentration was determined by ultraviolet (UV) method at 290 nm and 248 nm for FB and RSTCa respectively [12, 13, 20].

Stability studies:

Above all experiment the conclusion made that the formulation of RSTCa & FB tablet of trial 2 has been finalized at all expects i.e., assay, physical parameters of coated tablet and dissolution results. So the sample has been charge for stability in different condition as per climatic zone 4 for specified time as per ICH guideline (**Table 5**) [14, 15, 19].

Note: sample charge for stability in high density polyethylene (HDPE) bottle

RESULTS AND DISCUSSION

Drug excipient compatibility studies:

At the end of four weeks the mixtures were observed for their physical state. The result showed that incompatibility was observed when 0.45% moisture was added in the mixture and in absence of moisture the physical mixtures were compatible with each other. For RSTCa and FB, the IR stretching band was still visible in physical mixtures with excipients suggesting that there was no interaction between drug and polymer in physical mixtures.

Spectral analysis:

The IR spectrum (**Fig.1**) showed percentage transmission (%T) versus wave number of RSTCa with characteristic peaks of aromatic N-H stretching and C=O stretching at 3316 cm^{-1} and 1600 cm^{-1} respectively. From the figure it was observed that functional group of RSTCa was retained in granules, suggesting absence of chemical interaction with any of the excipients used in the preparation of granules. Pure FB has four characteristic peaks at, 2997 cm^{-1}, 1746 cm^{-1}, 1658 cm^{-1} and 1597 cm^{-1} for O–H stretching vibration, C-H vibration and ester stretching vibration and lactone carbonyl functional group respectively. The FTIR spectrum of prepared granules has four characteristic peaks at 2990cm^{-1}, 1740cm^{-1}, 1660cm^{-1}, and at 1600cm^{-1}. The FTIR spectrum of pure FB and granules were almost similar because of the same functional groups. It indicates that there was no interaction between FB and excipients used in the formulation of granules depicted on **Fig.2**.

TABLE 1: COMPOSITIN OF FB TABLET

S. no	Composition	Trial 1	Trial 2	Trial 3	Trial 4
1.	FB (mg) (micronized)	162.43	162.43	162.43	162.43
2.	Lactose (mg)	81.68	81.68	81.68	81.68
3.	Microcrystalline cellulose (mg)	-------	-------	29.40	58.80
4.	Mannitol (mg)	-------	-------	29.40	-------

5.	Maize starch (mg)	--------	1.25	-------	-------
6.	Dibasic calcium phosphate (Anhydrous) (mg)	71.50	-------	-------	-------
7.	Sodium starch glycolate (mg)	7.70	7.70	7.70	7.70
8.	Croscarmellose sodium (mg)	15.60	15.60	15.60	15.60
9.	Sodium lauryl sulphate (mg)	3.84	3.84	3.84	3.84
10.	Plysorbate 80 (mg)	--------	5.00	5.00	5.00
11.	Polyvinyl pyrrolidone k-30 (mg)	--------	5.25	7.70	7.70
12.	Isopropylalcohal *				
13.	Colloidal silicon dioxide (mg)	2.00	2.00	2.00	2.00
14.	Magnesium stearate (mg)	5.25	5.25	5.25	5.25
Average weight (mg)		350.00	350.00	350.00	350.00

*Not to be found in the final stage

TABLE 2: COMPOSITION OF RSTCa TABLET

S. no	Composition	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
1.	RSTCa (mg)	21.90	21.90	21.90	21.90	21.90
2.	Lactose (mg)	53.18	44.35	33.01	34.18	24.18
3.	Microcrystalline cellulose101 Avicel (mg)	------	------	25.35	14.18	14.18
4.	Hydroxy propylmethyl cellulose (mg) K-4M	30.00	53.00	70.00	80.00	90.00
5.	Pregelatinized Starch 1500 (mg)	49.18	33.01	------	------	------
6.	Tribasic calcium phosphate (mg)	30.00	30.00	30.00	30.00	30.00
7.	Colour sunset yellow (Lake) (mg)	0.7	0.35	0.35	0.35	0.35
8.	Polyvinyl pyrrolidone K30 (mg)	4.00	6.00	8.00	8.00	8.00
9.	Butylated hydroxytoluene (mg)	0.04	0.04	0.04	0.04	0.04
10.	Isopropyl alcohal*	------				
11.	Talcum (mg)	4.00	4.00	4.00	4.00	4.00
12.	Colloidal silicon dioxide (mg)	4.00	4.00	4.00	4.00	4.00
13.	Colour sunset yellow (Lake) (mg)	------	0.35	0.35	0.35	0.35
14.	Magnesium stearate (mg)	3.00	3.00	3.00	3.00	3.00
Average Weight (mg)		**200.00**	**200.00**	**200.00**	**200.00**	**200.00**

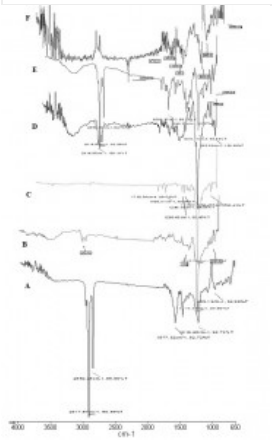

FIG.1: FTIR SPECTROSCOPY OF (A) RSTCa, (B) RSTCa- Acdisol, (C) RSTCa- Aerosil, (D) RSTCa- HPMC K15M, (E) RSTCa- Butyl Hydroxyl Toluene, (F) RSTCa- HPMC K4M

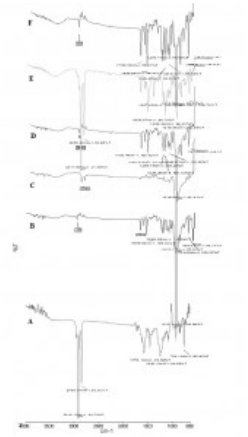

FIG.2: FTIR SPECTROSCOPY OF (A) FB, (B) FB- Acdisol, (C) FB- Aerosil, (D) FB- HPMC K15M, (E) FB- Butyl hydroxyl toluene, (F) FB- HPMC K4M

The possible interactions between a drug entity and excipients were determined by differential scanning calorimetry (DSC). **Fig. 3** showed the thermal behavior of the pure components as well as of granules. The RSTCa peaks appeared clear, demonstrating a sharp characteristic endothermic peak at 125.38°C corresponding to its melting temperature (Tm); such a sharp endothermic peak showed that the RSTCa used was in a pure crystalline state. On the other hand, granules showed that the characteristic peaks of RSTCa had disappeared; this agrees that the drug was molecularly dispersed within the excipients. That was accompanied by the formation of a new endothermic peak at 93.5°C indicating the melting of drug and excipients [14].

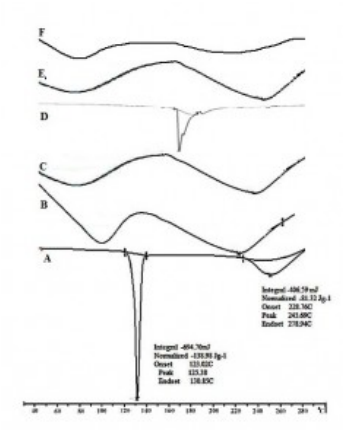

TABLE 3: PREPARATION OF BILAYER TABLETS

S. no	Composition	Grade	Trial 1	Trial 2
FB Part Composition			**Mg/tablet**	**Mg/tablet**
1	FB (micronized)	BP	162.43	162.43
2	Lactose	IP	81.68	81.68
3	Microcrystalline cellulose	USP	58.80	58.80
4	Sodium starch glycolate	IP	7.70	7.70
5	Croscarmellose sodium	IP	15.60	15.60
6	Sodium lauryl sulphate	IP	3.84	3.84
7	Plysorbate 80	IP	5.00	5.00
8	Povidone K-30	IP	7.70	7.70
9	Isopropylalcohal	BP	0.2 ml	0.2 ml
10	Colloidal silicon dioxide	IP	2.00	2.00
11	Magnesium stearate	IP	5.25	5.25
	Average weight (mg)		350.00	350.00
RSTCa Part Composition				
1	RSTCa	IP	21.90	21.90
2	Lactose	IP	24.18	14.18
3	Microcrystalline cellulose 101 Avicel	USP	14.18	14.18
4	Hypromellose K-4M	USP	90.00	100.00
5	Tribasic calcium phosphate	USP	30.00	30.00
6	Colour sunset yellow (Lake)	IH	0.35	0.35
7	Povidone K30	USP	8.00	8.00
8	Butylated hydroxytoluene	IP	0.04	0.04

9	Isopropyl alcohal	BP	0.08 ml	0.09 ml
10	Talcum	IP	4.00	4.00
11	Colloidal silicon dioxide	IP	4.00	4.00
12	Colour sunset yellow (Lake)	IH	0.35	0.35
13	Magnesium stearate	IP	3.00	3.00
Average Weight (mg)			200.00	200.00

This disappearance of drug peaks upon melting was in agreement with McCauley and Brittain who declared that the complete suppression of all drug thermal features undoubtedly indicates the formation of an amorphous solid solution. In addition, Mura et al. found that the total disappearance of the drug melting peak indicates that drug amorphization had taken place. FB peak was clearly seen in its DSC thermogram (**Fig. 4**) indicating a sharp characteristic peak at temperature range 79-82°C corresponding to its melting temperature (T*m*). This showed that FB used was in pure form. Prepared granules showed characteristic endothermic peaks of FB. This behavior is also observed in case of mixture of all these components. These two results indicated that there is no incompatibility between drug and excipients.

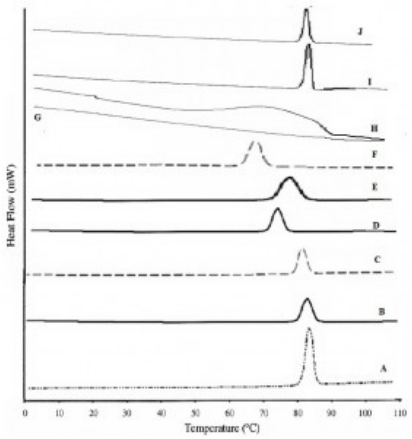

FIG.4: DSC SPECTRA OF (A) FB, (B) FB- Acdisol, (C) FB- Aerosil, (D) FB- HPMC K15M, (E) FB- Butyl hydroxyl toluene, (F) FB- HPMC K4M, (G) FB- Lactose, (H) FB- Magnesium stearate, (I) FB- MCC PH101, (J) MCC

TABLE 4: FILM COATING SOLUTION COMPOSITION

S. No	Ingredients	Quantity (mg/tablet)
1	Hydroxy propylmethylcellulose E-15	8.35
2	Propyleneglycol	1.65
3	Isopropyl alcohal	0.076
4	Methylenechloride	0.114
Average weight (mg)		10.00

Intrinsic Dissolution Studies:

Dissolution testing of poorly soluble compounds in immediate-release (IR) solid dosage forms possesses many challenges. These challenges include developing and validating the test method, ensuring that the method is appropriately discriminatory, and addressing the potential for an in vivo–in vitro relationship (IVIVR) or correlation (IVIVC). Satisfying all of these challenges and developing a meaningful dissolution method is a large task, because the extent of release is too low (i.e., one cannot get 100% of the dosage form dissolved) and secondly, the rate of release is too slow (i.e., one cannot get dissolution fast enough for a convenient test). FB is a compound displaying poor aqueous solubility (less than 0.25 mg/ml) across the physiological pH range. FB is a BCS II drug used to decrease elevated plasma concentrations of low density lipoprotein and total cholesterol. Although low bioavailability of the drug is due to its poor solubility in water.

The intrinsic dissolution showed the rate of dissolution of a pure FB (**Table 5**). The intrinsic dissolution studies were performed with USP – type l dissolution apparatus using 0.75% SLS as a dissolution media. The Percentage release of FB from the intrinsic dissolution was found to be increased (**Fig.5**). **Fig. 5** showed the enhanced solubility (total solubility divided by aqueous phosphate buffer solubility) of FB in different concentrations of SLS plotted as function of surfactant concentration. Enhanced solubility was observed at 0.75% SLS, which is well above the critical micelle concentration (cmc) reported in the literature for pure SLS in water (approximately 0.008M). In pH 6.8, FB exhibits 82.85% drug release at 37^{0}C without SLS. A solution of SLS (0.069 M) increased the solubility of FB 91%, indicating that the incorporation of FB into the micelle was significant.

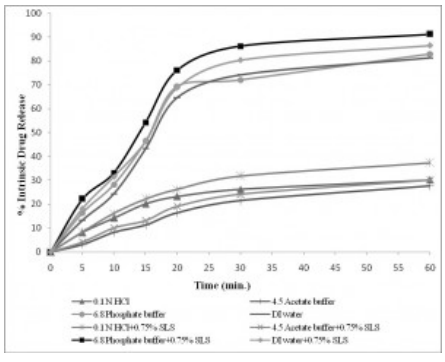

FIG.5: INTRINSIC DISSOLUTION STUDY OF FB IN BIORELEVENT MEDIA WITHOUT SURFACTANT AND WITH SURFACTANT

TABLE 5: TIME PERIOD FOR SAMPLE CHARGE IN STABILITY CHAMBER

Stress condition	Condition	Time period
	60 ^{0}C	**7 days**
	105 ^{0}C	**96 hours**
	Months	
Accelerated condition40 ^{0}C/75% RH	0 3 6	
Intermediate condition30 ^{0}C/65% RH	0 3 6 9 12	
Long time25 ^{0}C/60% RH	3 6 9 12 18 24 36	

***As per ICH guideline**

Evaluation of prepared FB granules:

For each designed formulation, blend of drug and excipients was prepared and evaluated for precompression properties showed in **Table 6**. Bulk density was found to be between 0.27±0.04 to 0.542±0.01gm/cm^3 and tapped density between 0.628±0.01 to 0.780±0.03 gm/cm^3 for all trials. Carr's Index was calculated and was found to be between 20.40±0.03% to 65.8±0.04%. Angle of repose was found to be in the range of 33.66±0.03 to 39.8±0.02. Hausner ratio was found below 1.25±0.02 to 2.8±0.05. All the formulation shows the fair to good flow properties for direct compression and hence tablets were prepared by using direct compression technology.

TABLE 6: PHYSICAL CHARACTERISTICS OF FB GRANULES

Physical property	Bulk density	Tapped density	Hausner ratio	Carr's index	Results	Angle of repose	Results
Trial 1	0.27±0.04	0.78±0.01	2.8±0.02	65.0±0.03	Very very poor	39.8±0.03	Passable
Trial 2	0.511±0.06	0.642±0.04	1.25±0.06	20.40±0.14	Fair	34.77±0.40	Good
Trial 3	0.498±0.11	0.628±0.20	1.26±0.02	20.70±0.01	Fair	34.68±0.04	Good
Trial 4	0.542±0.01	0.683±0.03	1.26±0.05	20.64±0.04	Fair	33.66±0.02	Good

Evaluation of prepared FB tablets:

The formulated tablets were subjected for the quality control tests such as hardness, friability, diameter, and thickness. Evaluation results of FB tablets were given in **Table 7**. Estimation of drug content in different formulations revealed 75-98% of expected values. The drug content was in good agreement with theoretical drug content. *In vitro* dissolution studies are valuable tools to judge stability and quality of sustain release dosage forms and often used to predict the *in vivo* performance [10].

TABLE 7: EVALUATION PARAMETERS OF PREPARED FB TABLETS

S. no	Physical characteristics	Disintegration (min.)	Hardness (kg/cm^2)	Friability (%)	Diameter (mm)	Thickness (mm)	Assay(%)
1.	Trial 1	0.48	3.5	0.472	12.78	3.1	94.02
2.	Trial 2	1-2	3.0	0.57	12.78	3.0	98.0
3.	Trial 3	4-5	3.0	0.371	12.78	3.0	98.5
4.	Trial 4	4-5	7.0	0.101	12.78	2.9	99.09

The release of FB from prepared formulations was analyzed by plotting the cumulative percent drug release vs. time as shown in **Fig. 6**. The graph showed an initial burst release i.e., over 20% of FB was released within first half an hour of dissolution study. This initial high amount of FB release can be attributed to release of drug from the immediate release layer of the formulation. The initial release of FB was due to MCC and SSG. The initial release of FB with high concentration of MCC in F4 was very high compared to other formulation. This high percent release can be ascribed to burst release of drug and also sustained release of drug after 10 min. The release rate was found to be increasing as the concentration of MCC increase in trial 1 to trial 4. This is due to swelling is more because of higher concentrations of polymer. In trial 4 cumulative percent drug release was about 97.56% in 30 min. Drug release kinetic from the trial 4 exhibit best correlation by Higuchi equation proving that the release is by diffusion mechanism as shown in **Table 5, 10**, Koresmeyer and Peppas equation revealed that F4 formulation have n value 0.493 indicates that they follow Non-fickian diffusion (**Table 8**).

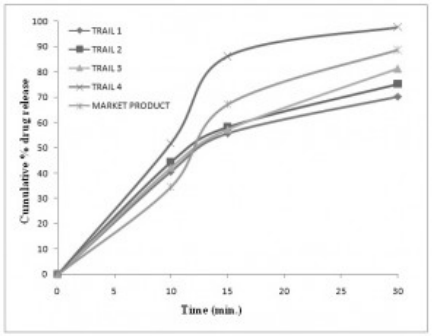

FIG. 6: DISSOLUTION OF TRIALS OF FB IN SELECTED DISSOLUTION MEDIUM pH 6.8 PHOSPHATE BUFFER WITH 0.75% SLS

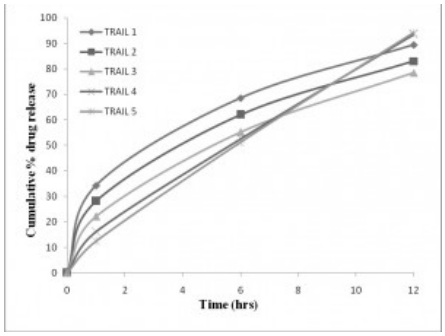

FIG. 7: DISSOLUTION OF TRIALS OF RSTCa IN SELECTED DISSOLUTION MEDIUM pH 6.8 PHOSPHATE BUFFER WITH 0.75% SLS

TABLE 8: MODEL FITTING DATA OF FB

Formulations	Zero order rate model	First order rate model	Higuchi model	Korsemeyer Peppas model	Release exponential in Korsemeyer peppas
Trail 1	0.8318	0.9474	0.9276	0.8283	0.718
Trail 2	0.8873	0.9431	0.9862	0.9519	0.554
Trail 3	0.8337	0.8706	0.9785	0.9655	0.488
Trail 4	0.8662	0.9285	0.9893	0.9899	0.493

Evaluation of RSTCA granules:

For each designed formulation, blend of drug and excipients was prepared and evaluated for precompression properties shown in **Table 10**. Bulk density was found to be between 0.37±0.04 to 0.592±0.01 gm/cm^3 and tapped density between 0.513±0.01 to 0.683±0.03 gm/cm^3 for all trials.

Carr's Index was calculated and was found to be between 13.76±0.03% to 27.87±0.04%. Angle of repose was found to be in the range of 32.42^0 ±0.03 to 46.23^0 ±0.02. Hausner ratio was found below 1.13±0.02 to 1.386±0.05 (**Table 9**). All the formulations were subjected for direct compression technology.

TABLE 9: PHYSICAL CHARACTERISTICS OF RSTCa GRANULES

S. no	Physical property	Bulk density	Tapped density	Hausner ratio	Carr's index	Results	Angle of repose	Results
1.	Trial 1	0.37	0.513	1.386	27.87	Poor	46.23	Poor
2.	Trial 2	0.463	0.582	1.257	20.44	Passable	41.15	Passable
3.	Trial 3	0.514	0.619	1.260	16.96	Fair	37.49	Fair
4.	Trial 4	0.589	0.683	1.16	13.76	Good	32.74	Good
5.	Trial 5	0.592	0.672	1.13	11.90	Good	32.42	Good

Evaluation of prepared RSTCA tablets:

The formulated tablets were subjected for the quality control tests such as hardness, friability, diameter, disintegration, thickness. Evaluation results of RSTCa tablets were given in **Table 10**. Estimation of drug content in different formulations revealed 75-98% of expected values. The drug content was in good agreement with theoretical drug content. The results indicated that the rate of drug release was higher for F5 formulation. The rate of drug release decreased by increase in the concentration of HPMC K4M which may be due to the increase in viscosity produced by the gelling of the hydrophilic polymer HPMC K4M.

TABLE 10: EVALUATION PARAMETERS OF PREPARED RSTCa TABLETS

S. no	Physical characteristics	Disintegration (minutes)	Hardness (kg/cm^2)	Friability (%)	Diameter (mm)	Thickness (mm)	Assay(%)
1.	Trial 1	0.48	3.5	0.472	12.78	3.1	97.05
2.	Trial 2	1-2	3.0	0.57	12.78	3.0	98.02
3.	Trial 3	4-5	3.0	0.371	12.78	3.0	99.06
4.	Trial 4	4-5	7.0	0.101	12.78	2.9	99.0
	Trail 5	3-4	6.0	0.401	12.78	3.1	99.21

The Concentration of polymer HPMC K4M was predominant controlling factor. HPMC K4M as the polymer could retard the drug release for 12 h by formation of a viscous gel. When the tablets were exposed to dissolution medium, the solvent penetrates into free spaces between the macromolecular chains of the polymer. After solvation of the polymer chain, the dimensions of the polymer molecule increase due to polymer relaxation by the stress of the penetrated solvent.

This phenomenon is defined as swelling and is characterized by formation of a gel-like network surrounding the tablet. This swelling and hydration property of HPMC causes an immediate formation of a surface barrier around the matrix tablet, which reduces the burst release. Diffusion exponent 'n' value obtained (0.53-0.59) for all formulations indicated that the release mechanism was non fickian or anomalous transport of drug (coupled diffusion/polymer relaxation) (**Table 11**).

TABLE 11: MODEL FITTING DATA OF RSTCA

Formulations	Zero orderratemodel	First orderratemodel	HiguchiModel	KorsemeyerPeppasmodel	Release exponential in KorsemeyerPeppas
Trial 1	0.8935	0.9217	0.9546	0.9045	0.53
Trial 2	0.8543	0.9037	0.9862	0.9013	0.55

Trial 3	0.8363	0.8233	0.9785	0.8985	0.56
Trial 4	0.8435	0.9026	0.9109	0.9100	0.58
Trial 5	0.8126	0.9119	0.9989	0.8998	0.59

This can be explained by the fact that RSTCa is a hydrophilic drug in a hydrophilic polymer matrix. The drug release from hydrophilic matrix is governed sequentially by the following processes:

1. Hydration and swelling of the polymer which results in formation of a gel;

2. Dissolution of drug in hydrated matrix/gel;

3. Diffusion of drug molecule through that hydrated matrix; and finally

4. Surface erosion and/or dissolution of that formed gel-matrix.

5. Diffusion of drug was the main mechanism of drug release from hydrated matrix. The comparison of cumulative percent drug release of all formulations is shown in **Fig. 7**.

Preparation and evaluation of bilayer tablets:

Dissolution data revealed that Trial 4 of FB and Trial 5 of RSTCa displayed desirable drug release for immediate and sustained release of drugs respectively. On the basis of dissolution studies Trial 4 of FB and Trial 5 of RSTCa were selected for bilayer tablet preparation. Percentage purity of FB and RSTCa in bilayer tablets was found to be in desirable range (98.78 and 98.29 respectively). All the tablets were produced under similar conditions to avoid processing variables. Mass of the bilayer tablets was 200 ± 1.20 mg, hardness was 6- 6.5 kg cm^{-2} and thickness was found to be 4.7 mm. The percentage friability of all the formulations was found to be 0.174 and 0.182%. Values of the hardness test and percent friability indicate good handling properties of the prepared bilayer tablets.

Dissolution studies of prepared bilayer tablets before coating and after coating:

The immediate release layer of the bilayer tablet containing crosscarmellose sodium swells rapidly upto 4-8 times its original volume on contact with water. So, it performs its disintegrating action by wicking through capillary action and fibrous structure respectively with minimum gelling and liberated FB for immediate action. From bilayer tablets, more than 50% of the RSTCa was released in the first 30 min of the dissolution study. As soon as the bilayer tablet comes in contact with the dissolution media, IR layer disintegrated with initial immediate release of drug within 30 min. with simultaneous imbibition of dissolution medium by the tablet with the formation of gel layer of polymer around the tablet. The controlled release of RSTCa was found to be a function of the polymer concentration. The effect of HPMC K4M on drug release was due to swelling nature of polymer which causes subsequent thicker gel formation with decrease in drug release. So it was concluded from different trials that biphasic release of the FB and RSTCa from bilayer tablets was mainly due to proper proportion of CCS in IR layer and rate retarding polymer in the CR layer respectively (**Fig. 8**) [15, 18, 21].

TABLE 12: STABILITY STUDIES OF OPTIMIZED BILAYER COATED TABLETS

S.No.	Parameters	Stability Studies of TRAIL 2 coated tablets				
		At 60 ^{0}C after 7 days	**At 105 ^{0}C after 96 hrs**	**At accelerated condition after 6 months**	**At intermediate condition after 6 months**	**At long term condition after 6 months**
1.	Physical appearance	Comply in color and physical appearance	Color fade of tablet observed	Comply in color and physical appearance	Comply in color and physical appearance	Comply in color and physical appearance
2.	Thickness (mm)	4.71±0.001	4.72±0.012	4.74±0.031	4.72±0.068	4.74±0.022
3.	Diameter (mm)	12.82±0.059	12.82±0.033	12.83±0.071	12.81±0.050	12.84±0.041
4.	**% Assay**					
	FB	98.74	96.18	98.51	98.61	98.72
	Rosuvastatin	98.49	96.29	98.09	98.34	98.44
5.	**% Drug Release**					
	FB	92.91±0.003	84.12±0.059	91.87±0.041	93.07±0.021	92.27±0.055
	Rosuvastatin	96.17±0.030	87.18±0.031	96.57±0.090	97.17±0.070	96.69±0.030

In vitro drug release studies which are considered the best tool for assessing in vivo drug behavior were carried out and both the percent dissolution and assay were within the acceptable limits as shown in Figure. The figure showed no significant difference between the coated bilayer tablets and uncoated bilayer tablets and all were completely dissolved within 30 minutes (immediate release part). Three different batches of coated bilayer tablets were prepared and tested parameters of the three batches showed no significant differences for each set of these batches, indicating that this manufacturing process is reliable and reproducible. An extended-release of RSTCa was obtained from coated bilayer tablet, demonstrating that the mechanical strength of the viscous-gel layer was strong enough to maintain its integrity and drug release. Coated tablet containing HPMC showed the fast dissolution profile, with complete drug release at 30 min. (**Fig.8**).

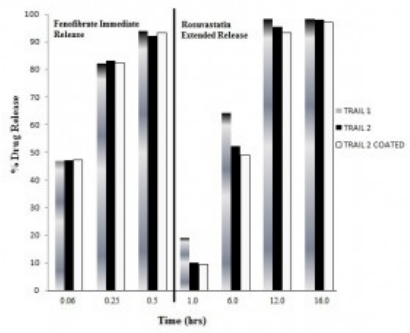

FIG. 8: DISSOLUTION OF TRIALS IN SELECTED DISSOLUTION MEDIUM pH 6.8 PHOSPHATE BUFFER WITH 0.75% SLS OF TRIAL 2 BEFORE COATING AND AFTER COATING.

Stability studies:

Samples stored at 60^0C, accelerated condition, intermediate condition and long term condition revealed no changes in assay, dissolution and physical appearance. While samples stored at 105^0C showed some change in assay, dissolution and physical appearance. But all results were in the range. In stability studies, the increased lag time indicates the possibility of reaction of drug and polymer with moisture during the study period. But, there was very little effect on the dissolution profile of the tablets [16, 17]. Decreased drug release was found from all trials, but drug release complied the official standard of release, since more than 80% of the drug was released. Statistical analysis of dissolution data before and after stability studies was carried out. Student's t-test was used to assess the results. No significant change was observed in percent drug release before and after stability studies for six months.

CONCLUSION: The modest absolute oral bioavailability and high hepatic extraction of RSTCa are consistent with first-pass uptake into the liver after oral dosing. The main aim was to minimize the liver extraction ratio of RSTCa by controlling the release of drug from the dosage form. Produced bilayer tablet proved to give better efficacy by minimizing extraction ratio. Thus from the data obtained, it can be concluded that bilayer tablet dosage form of an antihyperlipidemic drug FB/RSTCa formulated as an approach to modify drug release and thereby minimizing hepatic extraction ratio. Among the polymers used to improve drug release, cellulose polymers MCC and HPMC K4M, showed better control over drug release.

Formulated bilayer tablets gave satisfactory results for various physicochemical evaluations like weight variation, content uniformity and *in vitro* drug release. Further it was concluded that, by the application of optimization technique, optimized formulation can be obtained with IR/SR release of FB and RSTCa respectively. *In vitro* study showed that optimized bilayer tablet formulation released immediate dose of FB and then sustained release of RSTCa for more than twelve hours. Thus the objective of the work of formulating a bilayer tablet dosage form of FB and RSTCa to minimize hepatic extraction has been achieved with success.

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