IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
June 2017 Vol.:9, Issue:3

Formulation and Characterization of Fixed Dose Combined Trilayer Tablets of Niacin and Rosuvastatin Calcium

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Babita Kumari[1,2]*, H. K. Jain[1], P. Sharma[2], B. Srivastava[2]

1. SCOP, Sudumbare, Pune

2. School of Pharmaceutical Sciences, Jaipur National University, Jagatpura, Jaipur

Submission: 7 June 2017
Accepted: 12 June 2017
Published: 25 June 2017

Keywords: Niacin, Rosuvastatin calcium, Trilayer Tablets, HPMC K100M.

ABSTRACT

The objective of the study was to design fixed-dose combined tri-layer tablets of two lipid lowering drugs of different mechanism of action namely, niacin (NA) and rosuvastatin calcium (RC) to give an immediate release of RC and extended release of NA. The immediate release layer (first layer) comprised of lactose and microcrystalline cellulose as diluents (separately and in combined form) and the extended release layer (second layer) comprised different concentration of HPMC K100M as the release retarding polymers. These two layers were separated by a middle layer barrier consists of some inert diluents. This is more suitable for the delivery of two drugs which have interactions with them. Wet granulation method was employed for formulation of the trilayer tablets. Various pre-formulation studies of NA and RC, prior to granulation, like flow properties evaluation, solubility studies and drug excipient compatibility were performed. The tri-layer tablets were evaluated for weight variation, hardness, friability, drug content, and *in-vitro* drug release using USP dissolution apparatus type I (Basket). More than 85% of RC was released within 45 min. HPMC K100M extended the release of NA from the sustained release layer for 24 hr. After stability tests, drug contents were found within the range. The release of NA was found to follow a mixed pattern of Korsmeyer-Peppas, Higuchi model and zero order release models and the kinetic studies of the formulations revealed that diffusion is the predominant mechanism of drug release. The stability studies as per ICH guidelines were carried out for the optimized batch for six months and it showed acceptable results.

INTRODUCTION

A combination drug is a fixed-dose combination (FDC) that includes two or more active pharmaceutical ingredients (APIs) combined in a single dosage form, which is manufactured and distributed in fixed doses. There are many reasons for the development of two or more drugs incorporated in single dosage form in several diseases like less frequency of dosage administration, synergistic or additive effect, possibility of reducing dose, reduced side effects etc. This concept not only used in case of emergencies but also in common disease like Parkinson, allergic condition etc. This concept was preferred for combination of different APIs in single dose therefore to get relief in single dose and to attain site therapeutic concentration immediately and also maintain same dose over 12hr. FDCs gaining importance globally which are therapeutically justified and are available in critical disease condition e.g. AIDS, Cancer and Pulmonary disease.[1,2,3]

Multilayer tablet technology contains two or more APIs being used in treatment of wide range of chronic condition. It is applicable for broad range of compatible as well as incompatible drug for both immediate and extended release dosage forms. Currently, about two-third of all prescriptions are dispensed as solid dosage forms. Many combinations are available and being used which can provide immediate and modified release of two drugs or dual release rate of same drug in single dosage form by using hydrophilic and hydrophobic polymer matrices. It is therapeutically justified that combination of modified and immediate release matrix found to increase bioavailability. Compaction of different granules in the form of various layer in single tablets are called as multilayer tablets.[4-5] It generally consists of parallel, clear, colored, visually distinct layers two to three or more APIs or APIs along with functional or non-functional placebo layers, sometimes to avoid interaction between different incompatible layers. By using multilayer or trilayer tablet system, it makes possible to design extended release preparations with an immediate release quantity in one layer and an extended release portion in the second, thus maintaining a prolonged blood level. The immediate release portion will disintegrate rapidly after ingestion, thus providing the initial dose of medication for immediate onset of action whereas the matrix layer remains intact during most of the time of its passage through the intestine, while dissolving slowly from its exposed faces in this passage, which helps to maintain the blood level that initially reached. Similarly, one drug can be administered for immediate release and another drug can be for sustained release.[5,6,7]

Niacin (NA) and Rosuvastatin calcium (RC) is available separately as tablet dosage form. Rosuvastatin is available in 5 mg, 10 mg, 20 mg and 40 mg strengths while Niacin is available in 500 mg, 750 mg and 1000 mg strengths. Rosuvastatin has a daily dose of 40 mg while Niacin has a daily dose of 500-2000 mg/day. Both the drugs although are anti-hyperlipidemic agent having different mechanism of action.[4-7]

Niacin is reported to inhibit hepatocyte diacylglycerolacyltransferase-2, a key enzyme for triglycerides (TG) synthesis. This results in accelerated intracellular hepatic apo B degradation which causes significant reduction in secretion of VLDL and LDL particles. Niacin also retards the hepatic catabolism of apo A-I which increases the HDL half-life and concentrations of lipoprotein A-I HDL subfraction, which increases the reverse cholesterol transport. Rosuvastatin is a selective and competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol.[5,6,7,8]

It has been reported that Niacin has shown positive results with other statins like Simvastatin in many clinical trials. So, there is a need to develop a FDC dosage form of collagen turnover increasing drug Rosuvastatin with Niacin, a lipid profile modifying drug. Rosuvastatin has a plasma half-life of 19 hours. Therefore, it can be incorporated in proposed FDC as immediate release component which will suffice the targeted profile for 24 hours. Niacin is water soluble drug with elimination half-life of 20 to 45 minutes, so this needs to be incorporated in same FDC as an extended release part. The combined therapy may have a synergistic effect on the total lipid profile and, in addition, the dose and adverse effect (flushing, palpitation etc.) of niacin can be reduced. Therefore, there is a fair chance to study the new combination fixed dose formulation of Rosuvastatin as immediate release part and Niacin as extended release part.[9-10]

The purpose of this study was to formulate tri-layer tablets of Rosuvastatin calcium as an immediate release layer and Niacin as an extended release layer. Both the layers were separated by a middle layer barrier of inert diluents so as to avoid any unfavorable interaction or incompatibility of two drugs. Both drugs having different mechanism of action were combined to achieve the same goal. The combination of these two drugs shows a significant reduction in low density lipoprotein cholesterol with favorable changes in high-density lipoprotein cholesterol, lipoprotein and triglycerides. Compared to other types of formulation of NA, extended release form has fewer side effects. The selected drug NA is a water soluble

drug and hence judicious selection of release retarding excipients is necessary to achieve a constant in vivo input rate of the drug. In this study, HPMC K100M was used as polymeric carrier for NA to investigate the drug release behavior for 24 hr.

MATERIALS AND METHODS:

MATERIALS:

Rosuvastatin calcium and niacin were received as a gift sample from Lupin Ltd., Pune, India. Microcrystalline cellulose (MCC; Avicel PH 101), MCC (Avicel PH 112), Lactose monohydrate and Lactose anhydrous were purchased from Gufic Biosciences, India. Polyvinylpyrrolidone (PVP K-90), PVP K30, Hydroxypropyl methylcellulose (HPMC K100M) were purchased from Colorcon India, Goa. Colloidal silicon dioxide and Croscarmellose sodium were purchased from Ashland, Mumbai, India. Other chemicals and solvents used were of analytical grade.

METHODS:

Pre-formulation Studies

Physical evaluation

The physical parameters like angle of repose, bulk and tap densities, Carr's index and Hausner's ratio were determined for RC and NA.

Angle of repose

The angle of repose was determined by the funnel method. The determination of angle of repose by this method is referred to as static angle of repose. Powder is poured onto the center of the dish from the funnel that can be raised vertically until the maximum cone height (h) is obtained.[10]

The angle of repose can be calculated by the given formula,

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

Where 'h' is height of pile and 'r' is radius of pile.

Bulk density

Bulk density of RC and NA were determined by USP bulk density apparatus (Electrolab). It was measured by pouring the weighed quantity of polymers into a 250 mL measuring cylinder, and the volume was noted. It is expressed in gm/cc and is given by

$$D_b = M/V$$

Where, M is the mass of powder and V is the bulk volume of the powder.[10]

Tapped density

The tapped density was measured using USP bulk density apparatus (Electrolab) by tapping the polymers of fixed mass for 100 and then 500 tapped until it reached a constant volume. It is expressed in gm/cc and is given by

$$D_T = M/V_T$$

Where, M is the mass of powder, VT is the tapped volume of the powder.

Carr's index

Based on the apparent bulk density and the tapped density, the percentage compressibility of the bulk drug was determined by using the following formula.

$$Compressibility\ index = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} \times 100$$

Hausner's ratio

It was calculated on the basis of bulk and tapped density data and given by

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density}$$

Compatibility study of NA and RC with excipients

The physical mixtures consisting of RC and NA with various excipients, separately, in 1:1 ratio were taken in a glass vial and kept in stability chamber (Newtronic Walk-in Humidity chamber, India) at 40°C/75% RH for 1 month in open and closed condition. The samples were withdrawn after 4 weeks and checked for color change and assay. Finally, the combination of mixtures without any color changes and acceptable assay were selected for tablet formulation.[12-13]

Solubility studies of NA and RC

Saturation solubility for both niacin and rosuvastatin calcium was determined in water and at physiological pH ranges; 0.1 N HCl (pH 1.2), Acetate Buffer pH 4.5, Phosphate buffer pH 6.8 and Phosphate buffer pH 7.4.

Saturated solution of the respective drugs was prepared in the above stated media separately in 25 mL volumetric flasks in triplicate. Excess drug was added as a precaution so that undissolved drugs remains in the media at the end of the experiment, and saturation solubility can be determined correctly.

These flasks were sealed using parafilm to avoid any possible entry of mechanical shaker's bath water (used to maintain temperature) in the experimental flask. The experimental flasks were suitably clamped and were exposed in submerged mechanical shaker at 37˚C for 2 hours, shaking at 15 Hz. The sampling was done at the end of 24 hr. The samples were withdrawn from each flask separately in pre-identified test tubes. These samples were immediately filtered through 0.45 µm filter. 1mL of these samples were further suitably diluted with respective drugs mobile phase system for HPLC. The content of the drug in each sample was determined against freshly prepared standard samples.[14-15]

Preparation of granules and tablets of NA and RC (separately for optimization)

Preparation of sustained release NA granules

Table 1: Different composition for NA granules

Ingredients	Formulation code Quantity (mg/tablet)					
	N1	**N2**	**N3**	**N4**	**N5**	**N6**
Niacin	1000	1000	1000	1000	1000	1000
HPMC K 100M	100	130	150	140	120	110
MCC PH112	70	40	-	-	-	-
Pre-gelatinized starch 1500	-	-	-	-	55	65
PVP K90	55	55	75	85	50	50
Talc	15	15	-	-	-	-
Magnesium stearate	10	10	-	-	-	-
Stearic acid			25	25	25	25
IPA and Methylene chloride	-	-	Qs	Qs	Qs	Qs
Purified water	Qs	Qs	-	-	-	-
Total weight (mg)	1250	1250	1250	1250	1250	1250

Matrix tablets were prepared by aqueous and non-aqueous granulation method. All materials as shown in Table 1, except polyvinyl pyrrolidone (PVP) and lubricants, were sifted through 40 # sieve and mixed thoroughly in a poly-bag for 15 min and then granulated using PVP as a binder solution. The granules prepared from aqueous granulation were dried at 50 °C for 1 hr in a fluidized bed drier. The non-aqueous granules were air dried till the loss on drying (LOD) value becomes below 2%. The dried granules were then passed through 20 # sieve. To this, the lubricants were added and mixed for 2 min. The tablets of N1 to N6 were compressed using 20 mm diameter flat circular punch in a single station compression machine (Cadmach, India).

Preparation of immediate release RC granules

The RC lactose monohydrate, MCC and croscarmellose sodium as shown in Table 2 passed through # 40 sieve and were mixed well in a polybag for 10 min. To this mixture, purified water was added and granulated. The wet granules were dried at 50 °C for 5 hr in a tray drier. The dried granules were then passed through 20 # sieve and to this lubricant, which were previously passed through # 40 sieve, were added and mixed well for 2 min.

Table 2: Different composition for RC granules

Ingredients	Formulation code Quantity (mg/tablet)			
	RC1	**RC2**	**RC3**	**RC4**
Rosuvastatin calcium	20	20	20	20
Lactose monohydrate	70	80	90	40
MCC (Avicel pH 101)	30	25	20	55
Croscarmellose sodium	15	10	10	20
Aerosil 200	10	10	5	10
Magnesium stearate	5	5	5	5
Total weight (mg)	150	150	150	150

Preparation of middle layer barrier

The Lactose anhydrous and MCC as shown in Table 3 passed through # 40 sieve and were mixed well in a poly-bag for 10 min. To this mixture, PVP K30 was added and granulated. The wet granules were dried at 50 °C for 1 hr in a tray drier. The dried granules were then passed through 20 # sieve and to this magnesium stearate, which were previously passed through # 60 sieve, were added and mixed well for 2 min.

Table 3: Composition for middle layer granules.

Ingredients	Quantity (mg)
Lactose anhydrous	45
MCC (Avicel pH 101)	40
PVP K30	10
Magnesium stearate	5
Water	Qs

Evaluation of prepared granules

The physical parameters like angle of repose, bulk and tapped densities, Carr's index and Hausner's ratio were determined for above prepared granules of different batches of NA and RC.

Preparation and evaluation of tablets

Tablets were prepared by direct compression technique using NA granules (Batches N1 to N6) and RC granules (Batches RC1 to RC4) respectively. Granules were compressed by using single station tablet compression machine (Punch size 12.7 mm 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.

In-vitro dissolution studies of prepared tablets

The dissolution study of NA and RC tablets were carried out using USP type I (basket) method at a stirring speed of 100 rpm at 37±0.5 °C in 900 mL of phosphate buffer pH 6.8. The dissolution samples (5 mL) were collected at an interval of 0.5, 1, 2, 4, 6, 8, 12 and 24 hr with replacement of equal volume of dissolution media and were filtered through a membrane filter. The filtrate was collected and the drug release at different time intervals was measured by HPLC. The inertsil ODS column using a mixture of 0.05 M monobasic sodium phosphate solution (pH 3.0) and methanol containing 1.5 mM sodium 1-octanesulfonate as the mobile phase at 35 °C were employed and the samples were analyzed at 260 nm.[16-17]

Evaluation of release kinetics

Data obtained from *in-vitro* release studies were fitted to various kinetic equations to find out the mechanism of NA 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 = ktn (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.[15-17]

Preparation of trilayer tablets

Optimized formulations of sustained release NA (Batch N6) and immediate release RC (RC4) were selected and final tri-layer tablets were prepared according to the formula shown in Table 4.

Table 4: Composition of trilayer tablets of NA and RC

Layer	Ingredients	Quantity (mg/tablet)
Extended release upper NA layer	Niacin	1000
	HPMC K 100M	110
	Pre-gelatinized starch 1500	65
	PVP K90	50
	Stearic acid	25
	IPA and Methylene chloride	Qs
Middle barrier layer	MCC (Avicel pH 101)	40
	Lactose anhydrous	45
	PVP K30	10
	Magnesium stearate	5
Immediate release bottom RC layer	Rosuvastatin calcium	20
	Lactose monohydrate	40
	MCC (Avicel pH 101)	55
	Croscarmellose sodium	20
	Aerosil 200	10
	Magnesium stearate	5
	Total weight (mg)	1500

Initially, the volume of die cavity was adjusted in equivalence to the weight of tri-layer matrix tablets (1500 mg). Then, the pre-weighed amount of powder equivalent to bottom layer (150 mg) of RC was taken and placed in the die cavity and slightly compressed for uniform spreading using 19.8 x 8.7 mm round shape punch in single station tablet

compression machine (Cadmach, India). The upper punch was lifted up and 100 mg of the powdered granulated mixture containing middle layer formulation was placed over the bottom layer in the die cavity and again slightly compressed.

The remaining volume of the die cavity was filled with pre-weighed amount of powder equivalent to top layer and compressed with the full force of compression on rotary tablet press to obtain trilayer tablets.

Evaluation of trilayer tablet

Post-compression evaluation

Post compression parameters of trilayer tablets were evaluated for weight variation, hardness, friability, thickness, disintegration and in vitro dissolution studies.

Swelling and erosion studies

The swelling behavior of dosage form can be measured by studying its dimensional changes, weight gain or water uptake ability. The water uptake study of the dosage form was conducted by using Type II USP dissolution apparatus in 900 mL of distilled water which was maintained at 37±0.5 $^{\circ}$C and rotated at 50 rpm. At selected intervals (1 to 24 hr), the tablet was withdrawn and blotted with an absorbent tissue to remove any excess dissolution medium on the surface and weighed. Percentage swelling of the tablet was expressed as percentage water uptake (%WU) calculated from following equation:[16-18]

$$\text{Degree of swelling (\% water uptake)} = \frac{(Wt - Wo)}{Wo} 100$$

Where, W_o is the initial weight of the dry tablet, and W_t is the weight of the wet, swollen tablet.

Matrix erosion was determined after completion of swelling studies, on the same tablets used for the swelling determinations. After weighing, the hydrated matrices were dried in an oven at 100 °C for 24hrs and the remaining dry weight Wr, was determined. Matrix erosion was calculated according to the formula:[16-18]

$$\text{Erosion (\% mass loss)} = \frac{(Wo - Wr)}{Wo} 100$$

In-vitro drug release study

The release characteristics of RC was studied using dissolution apparatus USP type I basket method (TDT-O8L, Electrolab, India) with a stirring speed of 100 rpm at 37 ± 0.5 °C in 900 mL of 0.5 % SDS in phosphate buffer, pH 6.8 for 1 hr. The dissolution samples (5 mL) were collected at an interval of 5, 10, 20, 30, 45 and 60 min with replacement of equal volume of dissolution media and were filtered through a 0.45 micron filter. The concentration of RC released at various time intervals were analysed at 248 nm by HPLC. The test was performed in a Luna C18 column using a mobile phase containing acetonitrile: ammonium acetate buffer of pH 4: tetrahydrofuran at a flow rate of 1.0 mL/min.

The dissolution study of NA was carried out using USP type I (basket) method at a stirring speed of 100 rpm at 37±0.5 °C in 900 mL of phosphate buffer pH 6.8. The dissolution samples (5 mL) were collected at an interval of 1, 2, 4, 6, 8, 12 and 24 hr with replacement of equal volume of dissolution media and were filtered through a membrane filter. The filtrate was collected and the drug release at different time intervals was measured by HPLC. The inertsil ODS column using a mixture of 0.05 M monobasic sodium phosphate solution (pH 3) and methanol containing 1.5 mM sodium 1-octanesulfonate as the mobile phase at 35 °C were employed and the samples were analyzed at 260 nm. [15-17]

The release studies of both the drugs were conducted in triplicate (6 tablets in each set) and the mean values were plotted versus time.

Accelerated stability study of trilayer tablet

Stability is defined as the ability of a particular drug or dosage form in a specific container to remain within its physical, chemical, therapeutic, and toxicological specifications.

Drug decomposition or degradation occurs during storage, because of chemical alteration of the active ingredients or due to product instability, lowering the concentration of the drug in the dosage form. The optimized formulations were stored in aluminum capped clear glass vials and were subjected to a storage condition of 40°C±2 °C/ 75%±5 % RH for 6 months in humidity chamber. The samples were withdrawn and evaluated for hardness, friability, disintegration time, drug content and in vitro dissolution study.

Similarity and dissimilarity factors

The similarity factor (f_2 factor) was used to compare dissolution profiles before and after the stability studies. The *in-vitro* release profiles of the formulations before the stability studies

were considered as reference and the profiles after the stability studies were considered as test. A model independent approach was used to estimate the dissimilarity factor (f_1) and similarity factor (f_2) to compare the dissolution profile of optimized formulation.[14-16]

The dissimilarity factor (f_1) calculates the percent difference between the reference and test curve at each time point and is a measurement of the relative error between two curves. The similarity factor (f_2) is given by the following equation:

$$f_1 = \{[\Sigma_{t=1}^{n} | R_t - T_t |] / [\Sigma_{t=1}^{n} R_t]\} \cdot 100$$

$$f_2 = 50 \cdot \log \{[1 + (1/n) \Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \cdot 100\}$$

Where n is the number of pull points, R_t is the reference batch profile at time point t and T_t is the test batch profile at the same time t. The FDA suggested that two dissolution profiles were declared similar if f_2 value between 50- 100 and f_1 was 0-15.

RESULTS AND DISCUSSION:

Pre-formulation studies

Physical evaluation

The various physical parameters like angle of repose, bulk density, tapped density Carr's index and Hausner's ratio were determined for pure RC and NA and reported in Table 5.

Table 5: Physical evaluation of NA and RC.

Drug	Angle of repose (θ)	Bulk density (gm/cc)	Tapped density (gm/cc)	Carr's index	Hausner's ratio
NA	30±1.5	0.492±0.02	0.630±0.01	22.23±0.12	1.30±0.01
RC	28±2.0	0.370±0.01	0.480±0.02	22.92±0.23	1.29±0.03

From the above data, it was confirmed that both the drugs have poor flow properties and compressibility. Good flow of powders/granules is essential in tableting because the compressibility and flow properties of the drugs are likely to influence the compression process in the preparation of trilayer tablets. In view of this, the formulations were prepared by wet granulation technique to improve the flow as well as compressibility.

Solubility studies of NA and RC

Aqueous solubility NA and RC as a function of pH in various media are shown in below Table 6 and Table 7 respectively.

Table 6: Solubility of NA in various media

Media	Immediate solubility (mg/mL)	Saturation solubility (mg/mL) after 24 hrs
Purified water	16.01	16.92
0.1N HCl	30.21	30.34
Acetate buffer pH 4.5	17.65	17.82
Phosphate buffer pH 6.8	20.63	21.23

Table 7: Solubility of RC in various media

Media	Immediate solubility (mg/mL)	Saturation solubility (mg/mL) after 24 hrs
Purified water	4.68	4.95
0.1N HCl	0.16	0.17
Acetate buffer pH 4.5	0.64	0.70
Sodium citrate buffer pH 6.6	21.24	22.52
Phosphate buffer pH 6.8	11.85	12.34
Phosphate buffer pH 7.4	12.21	12.94

NA showed highest solubility in 0.1 N HCl media followed by phosphate buffer pH 6.8 at both immediate and saturation solubilities at highest strengths. While solubility of RC increases as pH increases. It shows lowest solubility in 0.1 N HCl and maximum solubility was observed in sodium citrate buffer pH 6.6 at both immediate and saturation solubilities at highest strengths.

From the solubility data, it has been observed that both the drugs also showed good solubility in phosphate buffer pH 6.8. So, based on this studies phosphate buffer pH 6.8 media was finalized as dissolution media for trilayer tablets.

Compatibility studies of NA and RC with excipients

Excipient-drug substance (NA and RC) compatibility was assessed through HPLC analysis of binary mixtures of excipient and drug substance at a 1:1 ratio in the solid state (untreated and water treated). Samples were stored at 40 °C/75 % RH in both open and closed containers for 1 month. Common excipients functioning as filler, disintegrant, binder, glidant and lubricant were evaluated in the excipient compatibility study. Table8 and Table 9 summarizes the results.

Table 8: Compatibility study of NA with various excipients at 40 °C/ 75%RH for 1 month.

NA + Excipients (1:1)	% Assay				Colour change
	Open Untreated	Open Water treated	Closed Untreated	Closed Water treated	
NA + HPMC	98.9	97.6	98.9	97.6	ND
NA + MCC	97.2	98.5	97.2	98.5	ND
NA + PVP	99.4	99.4	99.4	99.4	ND
NA + Talc	100.2	99.5	100.2	99.5	ND
NA + Mg stearate	99.9	99.9	99.9	99.9	ND
NA + Stearic acid	96.4	96.4	96.4	96.4	ND
NA + N (All excipient)	97.3	97.1	97.3	97.1	ND
NA + N - HPMC	99.4	98.4	99.4	98.4	ND
NA + N – MCC	99.8	99.2	99.8	99.2	ND
NA + N – PVP	96.4	96.1	96.4	96.1	ND
NA + N – Talc	99.6	99.0	99.6	99.0	ND
NA + N – Mg stearate	100.2	100.2	100.2	100.2	ND
NA + N – stearic acid	101.7	99.8	101.7	99.8	ND

Table 9: Compatibility study of RC with various excipients at 40 °C/ 75%RH for 1 month.

RC + Excipients (1:1)	% Assay				Colour change
	Open Untreated	Open Water treated	Closed Untreated	Closed Water treated	
RC + Lactose	98.9	97.6	98.9	97.6	ND
RC + MCC 101	97.2	98.5	97.2	98.5	ND
RC + Cross. Na	99.4	99.4	99.4	99.4	ND
RC + Aerosil 200	100.2	99.5	100.2	99.5	ND
RC + Mg stearate	99.9	99.9	99.9	99.9	ND
NA + N (All excipient)	97.3	97.1	97.3	97.1	ND
NA + N - Lactose	99.4	98.4	99.4	98.4	ND
NA + N – MCC 101	99.8	99.2	99.8	99.2	ND
NA + N – Cross. Na	96.4	96.1	96.4	96.1	ND
NA + N – Mg stearate	100.2	100.2	100.2	100.2	ND

Loss in assay or detection of degradants indicative of an incompatibility was not observed for the selected excipients. No loss in assay was observed in any of these mixtures at 40 °C/75% RH. There is no incompatibility with the selected excipients. However, assurance of compatibility was provided by long-term stability data for optimized formulation batches.

Preparation and evaluation of NA granules

For each designed formulation (N1-N6), granules of drug and excipients were prepared by wet granulation method and evaluated for their pre-compression properties as shown in Table 10. Bulk density was found to be between 0.37±0.04 to 0.63±0.01 g/mL and tapped density

between 0.40±0.01 to 0.72±0.03 g/mL for all the batches. Carr's Index was calculated and was found to be between 7.50±0.03% to 13.33±0.04%. Angle of repose was found to be in the range of 18.21±0.03 to 23.1±0.02. Hausner's ratio was found to be between 1.08±0.02 to 1.15±0.05. All granules from various batches (N1-N6), exhibited good flow properties and compressibility after wet granulation, were then compressed into tablets.

Table 10: Flow properties evaluation of NA granules from various batches (N1-N6)

Batch	Angle of repose (θ)	Bulk density(gm/cc)	Tapped density (gm/cc)	Carr's index	Hausner's ratio
N1	20.5	0.63	0.72	12.50	1.14
N2	19.6	0.54	0.62	12.90	1.14
N3	22.4	0.38	0.43	11.62	1.13
N4	23.1	0.40	0.46	13.04	1.15
N5	18.2	0.37	0.40	7.50	1.08
N6	20.6	0.53	0.61	13.33	1.15

Preparation and evaluation of NA tablets

All granules from various batches (N1-N6), exhibited good flow properties and compressibility after wet granulation, were then compressed into tablets.

The formulated tablets were subjected to the quality control tests such as hardness, friability, average weight and assay. Evaluation results of NA tablets are shown in Table 11. The drug content was in good agreement with theoretical drug content. *In-vitro* dissolution studies are valuable tools to judge quality of sustained release dosage forms and often used to predict the in vivo performance.

Table 11: Physical evaluation of NA tablets

Formulation batches	Average weight (mg)	Hardness (N)	Friability (%)	Assay (%)
N1	1248.21±2.33	115.2±2.18	0.156±0.012	99.6±3.9
N2	1249.32±3.67	113.7±0.11	0.164±0.018	99.9±2.6
N3	1243.84±4.77	118.9±0.21	0.173±0.023	98.4±1.2
N4	1246.71±4.74	116.9±0.11	0.147±0.016	100.2±3.5
N5	1249.57±2.89	118.6±0.49	0.131±0.004	100.6±2.7
N6	1252.75±5.89	101.7±0.19	0.198±0.004	103.6±3.2

In-vitro dissolution studies of NA tablets

In the extended release layer of NA to control its release, high viscous polymer HPMC K100M was incorporated in various proportions. The required release profile of NA was fixed to be not more than 25% at the end of 1 hr, 30-50% at the end of 4 hr, 65-85% at the end of 12 hr and not less than 80% at the end of 24 hr.

In NA1 formulation there was an initial burst release of about 48.9% at 1 hr and at 4 hr all the drug content was released. This might be due to decreased macromolecular association of the matrix components, thus resulting in decreased consistency of the gel layer formed around the tablet core at the initial hours. Thus, the gel layer on the tablet core with reduced viscosity is more susceptible to erosion and diffusion processes, resulting in rapid drug release in the initial hours.

In NA2 formulation also the release at the end of 4 hr was found to be more (67.95%). Tablet when comes in contact with the dissolution medium, HPMC absorbs water, swells and become a hydrated gel. At the same time MCC (Avicel PH 112) having disintegration properties, promoted the disintegration of the tablets. The tablets were therefore easy to erode, resulting in a higher release profile.

In NA3, the purified water for preparing coherent mass was replaced with isopropyl alcohol and methylene chloride (1:1). The non-aqueous granulation may reduce dissolution and transport of dissolved drug across the diffusion path length. The MCC (Avicel PH 112) was not incorporated and the first 4 hr release was brought within the limit (38.62%), but the 12

hr release was only 60.71%. At the end of 24 hr the release was only 76.8%. The lubricants magnesium stearate and talc were replaced with stearic acid because it has the low melting point and hence it can avoid the layer separation when compressed as a multilayer tablets.

In NA4, HPMC K100M was slightly reduced to increase the 12 hr release. The release became less (59.07%) compared to NA3, but there was no much difference between NA3 and NA4 in the release profile at 12 hr. In general, increasing the total polymer content of the tablets decreases the rate of drug release. At higher polymer loading, the viscosity of the gel matrix is increased which results in a decrease in the effective diffusion coefficient of the drug.

The quantity of HPMC K100M was further reduced and disintegrant starch was incorporated in NA5. Even then the release at 24 hr was found to be less (78.30%). Previous investigations have indicated that changing the compression force had effect on the dissolution rate of drugs from HPMC matrices. Hence, in NA6 without altering the polymer and its quantity only the compressional force was reduced and thereby hardness got reduced to 98 N from 118 N. The release at all the hours was found to be satisfactory, that is at the end of 1 hr the release was 20.84%, 12 hr 82.3% and at the end of 24 hr the release was 99.8%.

Diffusion exponent ‘n’ value obtained (0.52-0.59) for all formulations indicated that the release mechanism was non-fickian or anomalous transport of drug (coupled diffusion/polymer relaxation) as shown in Table 12.

Table 12: Model fitting data of NA

Batch	Zero order rate model	First order rate model	Higuchi model	Korsemeyer Peppas model	Release exponential in Korsemeyer Peppas
N1	0.873	0.932	0.954	0.904	0.53
N2	0.844	0.901	0.986	0.901	0.52
N3	0.856	0.823	0.981	0.897	0.56
N4	0.834	0.823	0.978	0.910	0.57
N5	0.843	0.945	0.910	0.892	0.56
N6	0.814	0.911	0.998	0.899	0.59

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

- Hydration and swelling of the polymer which results in formation of a gel
- Dissolution of drug in hydrated matrix/gel
- Diffusion of drug molecule through that hydrated matrix
- Surface erosion and/or dissolution of that formed gel matrix
- Diffusion of drug was the main mechanism of drug release from hydrated matrix

The comparison of cumulative percent drug release of all NA formulations is shown in Figure 1.

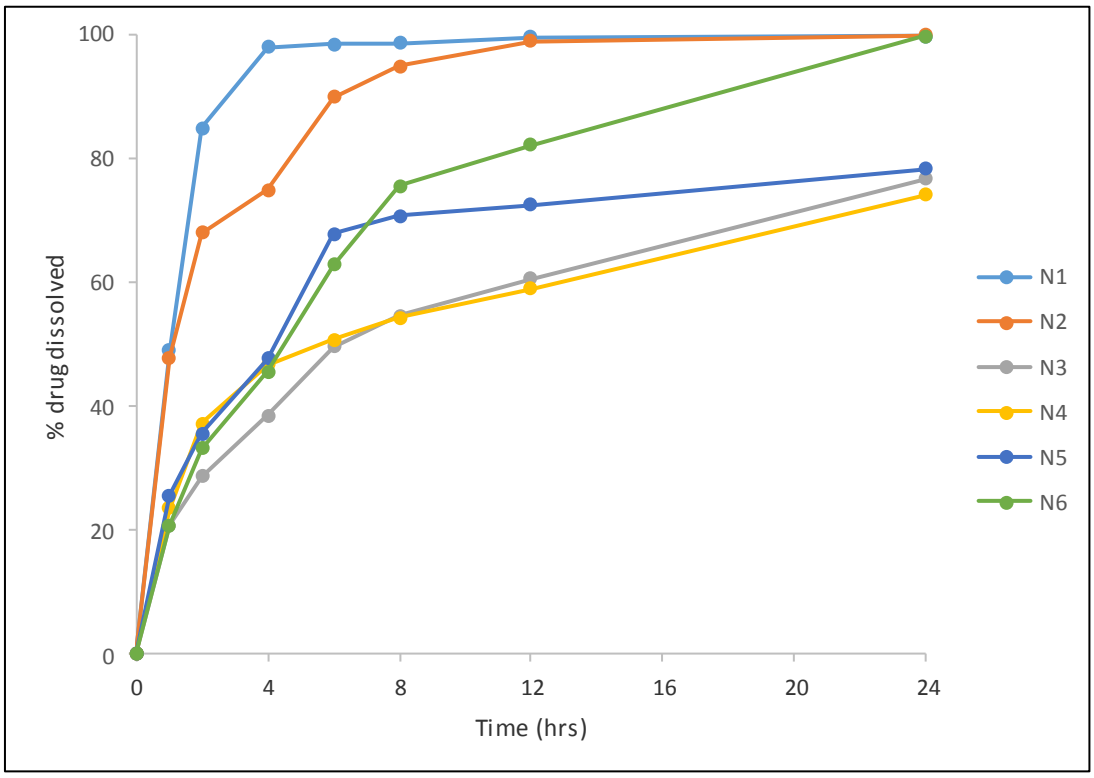

Figure 1: Dissolution of various batches of NA (N1-N6) in selected dissolution medium pH 6.8 phosphate buffer

Preparation and evaluation of RC granules

For each designed formulation (RC1-RC4), granules of drug and excipients were prepared by wet granulation method and evaluated for their pre-compression properties as shown in Table

13. Bulk density was found to be between 0.37±0.04 to 0.52±0.01 g/mL and tapped density between 0.40±0.01 to 0.57±0.03 g/mL for all the batches. Carr's Index was calculated and was found to be between 6.98±0.03% to 9.09±0.04%. Angle of repose was found to be in the range of 19.8±0.03 to 24.5±0.02. Hausner's ratio was found to be between 1.08±0.02 to 1.10±0.05. All granules from various batches (RC1-RC4), exhibited good flow properties and compressibility after wet granulation, were then compressed into tablets.

Table 13: Flow properties evaluation of RC granules from various batches (RC1-RC4)

Batch	Angle of repose (θ)	Bulk density (g/mL)	Tapped density (g/mL)	Carr's index	Hausner's ratio
RC1	24.5	0.40	0.43	6.98	1.08
RC2	20.7	0.37	0.40	7.50	1.08
RC3	19.8	0.52	0.57	8.7	1.10
RC4	23.1	0.50	0.55	9.09	1.10

Preparation and evaluation of RC tablets

All granules from various batches (RC1-RC4), exhibited good flow properties and compressibility after wet granulation, were then compressed into tablets.

The formulated tablets were subjected to the quality control tests such as hardness, friability, average weight and assay. Evaluation results of RC tablets are shown in Table 14. The drug content was in good agreement with theoretical drug content. In vitro dissolution studies are valuable tools to judge quality of immediate release dosage forms and often used to predict the in vivo performance.

Table 14: Physical evaluation of RC Tablets

Formulation batches	Average weight (mg)	Hardness (N)	Friability (%)	Assay (%)
RC1	148.21±2.33	75.2±2.18	0.143±0.012	99.6±3.9
RC2	149.32±3.67	73.7±0.11	0.157±0.018	99.9±2.6
RC3	143.84±4.77	78.9±0.21	0.161±0.023	102.4±1.2
RC4	146.71±4.74	76.9±0.11	0.177±0.016	100.2±3.5

In-vitro dissolution studies of RC tablets

The immediate release layer of RC was prepared as per the composition in Table 2. The MCC (Avicel PH 101) has the disintegration property when it comes in contact with the dissolution medium and hence it facilitates the tablet to erode. Also the super disintegrant croscarmellose sodium facilitates the same. After the disintegration of the tablet, lactose present in the granules takes up high amount of water leading to quicker release of drug. The RC layer (Table 2) was compressed as a single layer tablet before compressing as a trilayer and evaluated the release profile. The required 100% release of drug was achieved at 60 min.

The release of RC from prepared formulations (RC1-RC4) was analyzed by plotting the cumulative percent drug release vs. time as shown in Figure 2. In case of RC1 formulation, the graph showed an initial burst release of 20% of RC within 5 min and at the end of 45 min around 70% of drug was released. This might be due to presence of high concentration of lactose monohydrate which increases hardness as well as total disintegration time of tablets. Similar dissolution behaviour was also observed in case of RC2 and RC3 batches.

In case of RC4, the initial release (~45%) of drug within 5 min was due to high concentration of MCC and croscarmellose sodium promoting rapid disintegration of tablets. The initial release of RC with high concentration of MCC in RC4 was very high compared to other formulation. This high percent release can be ascribed to burst release of drug. In RC4 cumulative percent drug release was about 85.56% in 45 min.

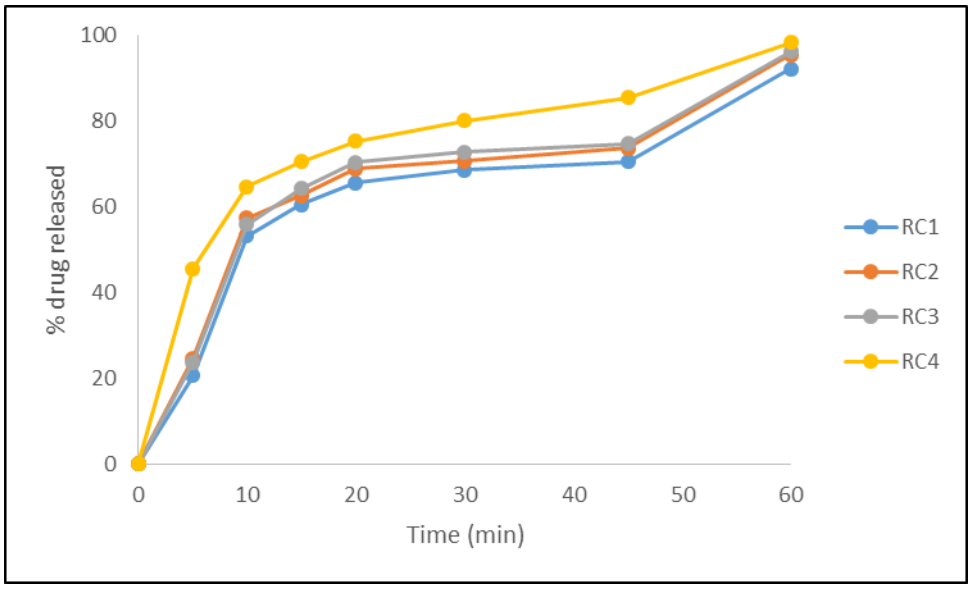

Figure 2: Dissolution of various batches of RC (RC1-RC4) in dissolution medium pH 6.8 phosphate buffer.

Preparation and evaluation of trilayer tablets of NA and RC

Dissolution data revealed that N6 batch of NA and RC4 batch of RC displayed desirable drug release for extended and immediate release of drugs respectively. On the basis of dissolution studies, N6 of NA and RC4 of RC were selected for trilayer tablet preparation.

Percentage purity of NA and RC in trilayer 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. Average weight of the tri-layer tablets was 1503 ± 1.20 mg, hardness was 105 ± 5.2 N and thickness was found to be 5.7 mm. The percentage friability of all the formulations was found to be 0.174 to 0.182%. Values of the hardness test and percent friability indicate good handling properties of the prepared trilayer tablets.

Swelling index and % erosion studies

The swelling index was calculated with respect to time and shown in Table 15. With increasing time, the swelling index was increased. This is because of the weight gain by tablets proportionally with increased rate of hydration. In the swelling study, the tablets were found swollen and retained their physical integrity till the end. Table shows that there is increase in both percentage water absorbed and percentage erosion of the tablet. But the percentage water absorbed was more dominant than erosion. The release mechanism was more by diffusion than erosion. The fact was further evident from the Higuchi's plot whose regression value was 0.9874 ± 0.11 compared to regression value of Peppa's plot as 0.7544 ± 0.01. In theory, higher the uptake of water by the polymer, more the amount of drug diffused out from the polymer matrix. Thus the high amount of water uptake by HPMC K100M may lead to considerable swelling of the polymer matrix, allowing drug to diffuse out at a faster rate. For matrix devices, drug is often released by diffusion process such that a receding drug boundary exists within the device. It is also supported by the previous investigation that the slower release rate and greater release duration correlated significantly with greater matrix swelling and negligible erosion.

Table 15: Swelling behavior of trilayer tablets

Time intervals (hr)	% water uptake	% Erosion
1	1.50	0.19
2	1.69	0.39
3	1.77	0.46
4	1.91	0.64
5	2.09	0.86
6	2.72	0.87
8	3.21	0.91
10	3.78	0.94
12	4.01	0.98
24	6.26	0.98

In-vitro drug release study

The immediate release layer of the trilayer tablet containing MCC and 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 RC for immediate action. From trilayer tablets, more than 85% of the RC was released in the first 30 min of the dissolution study. As soon as the trilayer tablet comes in contact with the dissolution media, IR layer disintegrated with initial immediate release of drug (RC) within 45 min followed by disintegration of middle layer rapidly within 1 min followed by simultaneous imbibition of dissolution medium by the tablet with the formation of gel layer of polymer around the tablet. The extended release of NA upto 24 hr was found to be a function of the polymer concentration. The effect of HPMC K100M 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 RC and NA from trilayer tablets was mainly due to proper proportion of MCC and crosscarmellose sodium in immediate release layer and rate retarding polymer (HPMC K100M) in the extended release layer respectively.

Stability studies of tri-layer formulation

The tri-layer formulation was stored in aluminum capped clear glass vials and were subjected to a storage condition of 40 ± 2 °C / 75 ± 5 % RH for 6months in stability chamber. The samples were withdrawn and evaluated for physical appearance, hardness, friability, drug content, related substance and *in-vitro* dissolution study all the formulations as shown in Table 16 and Figure 3. The data showed no significant variation in all the data after completion of stability study.

Table 16: Stability data for trilayer tablets of NA and RC

Parameters	**Time interval (months)**			
	0	**1**	**3**	**6**
Hardness (N)	105 ± 5.2	103 ± 4.7	104 ± 6.8	105 ± 3.2
Friability (%)	0.174 ± 0.02	0.180 ± 0.03	0.230 ± 0.04	0.241 ± 0.02
Average weight (mg)	1503 ± 1.20	1501 ± 2.30	1504 ± 3.65	1502 ± 2.45
% Assay				
NA	98.78 ± 2.24	97.43 ± 1.45	97.67 ± 2.45	96.24 ± 1.78
RT	98.29 ± 1.48	98.12 ± 1.78	98.24 ± 3.68	97.23 ± 2.31
Related substances	ND	ND	ND	ND
% water uptake after 12 hr	4.01 ± 0.04	4.13 ± 0.08	4.09 ± 0.09	4.15 ± 0.09
% erosion after 12 hr	0.98 ± 0.01	0.98± 0.02	0.99 ± 0.02	0.99 ± 0.08

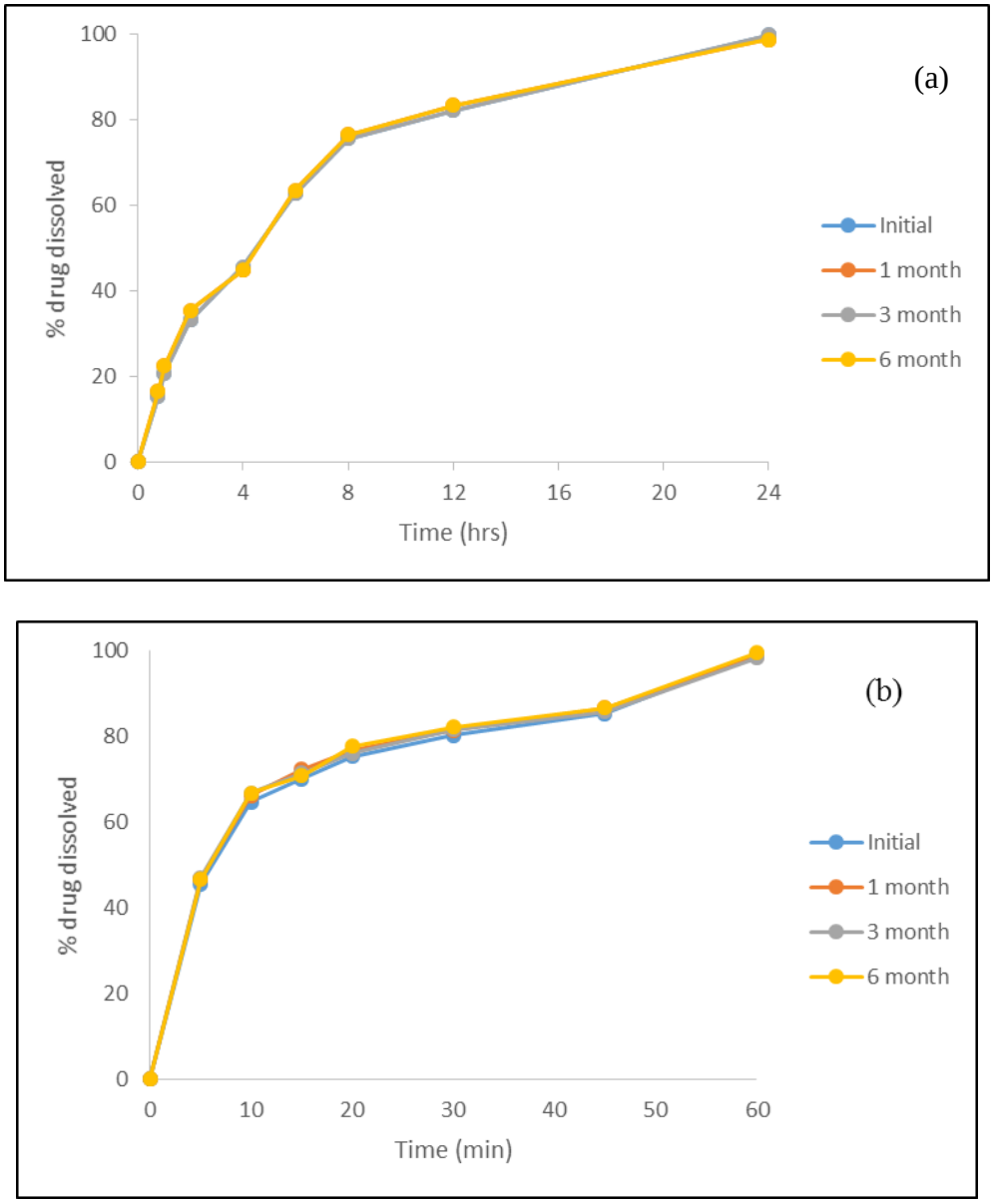

Figure 3: Stability data of in vitro drug release study of trilayer tablet for (a) NA and (b) RC

Similarity factor and dissimilarity factors

All formulations showed (f_2) value between 50 to100 and (f_1) value below 15 indicating similar release profiles of the formulations before and after stability studies.

CONCLUSION:

Combined tri-layer tablet of NA and RC was formulated successfully. HPMC K100M can be used for retarding drug release from matrix tablets. *In-vitro* dissolution studies showed release of RC within 45 min formulated as immediate release layer with the help of polymers or super-disintegrants. Although, RC was formulated as immediate release layer but it was

expected to give sustained effect due to its long half-life (19 hrs). Therefore, using this pharmacokinetic property of RC once a day tri-layer tablet was formulated with NA by extended its release for 24 hr with the help of HPMC K100M. There was no evidence of effect of one drug on the release pattern and compatibility of another. The *in-vitro* release study of all the formulations can sustain the release up to 24 hr. The data obtained from the release kinetics of NA and RC fitted with Higuchi model indicated that the release of drug from the tablets was found to depend on the square root of time showed that the release mechanism was non-fickian diffusion with slower erosion rates. Stability study was conducted for 6 months. Similarity (f_2) and dissimilarity factors (f_1) were calculated. All formulations showed (f_2) value between 50 to 100 and (f_1) value below 15 indicating similar release profiles of the formulations before and after stability studies. There was no significant variation in the physical appearance, hardness, friability and *in-vitro* dissolution profiles for the tri-layer formulation. In conclusion, the objective of formulation development and evaluation of fixed dose combined tri-layer tablet of NA and RC had been achieved.

REFERENCES

1. Yang L, Venkatesh G, Fassihi R. Compactionsimulator study of a novel triple-layer tablet matrix for industrial tableting. Int J Pharm. 1997; 152(1):45-52.
2. Pujara ND, Gokani R, Paun J. Bilayer Tablet – An Emerging Trend. Int J Pharm Res Dev. 2012; 4(4): 102-111.
3. Remya PN, Damodharan N, Kumar CV. Formulation and Evaluation of Bilayered Tablets of Ibuprofen and Methocarbamol. Int J Pharm Tech Res. 2010;2(2): 1250-55.
4. Rathod RT, Misra D. FDC of montelukast with levocetirizine: focus on bilayer technology. J Indian Med Assoc. 2009;107(8): 562-74.
5. Mishra P, Sharma PK, Malviya R. A review on Bi-layer tablets- An emerging trend. J Drug Del Tech 2014; 4(2): 110-4.
6. Nagaraju R, Kaza R. Formulation and evaluation of bilayer sustained release tablet of salbutamol and theophylline. Int J Pharm Sci Nanotech. 2009; 2(3): 638-46.
7. Costa P, Lobo JM. Modeling and comparison of dissolution profiles. EurJ Pharm Sci. 2001; 13:123-133.
8. Fassihi RA, Ritschel WA. Multiple-layer, direct-compression, controlled-release system: in vitro and in vivo evaluation. J Pharm Sci. 1993; 82:750–4.
9. Khule NR, Mahale NB, Chaudhari SR. Formulation development and evaluation of oncea day regioselective dual componentgastroretentive tablet of rosuvastatin calcium and metoprolol succinate. Int J Pharm Res Dev. 2012; 4(07): 69-78.
10. Garg BK, Gnanarajan G, Kothiyal P, Formulation and evaluation of pulsatile drug delivery system of rosuvastatin calcium using different swelling polymers. The Pharm Innovation. 2012; 1(7): 61- 67.
11. Dwivedi J, Mahatma OP. Formulation and evaluation of sustained release formulation of rosuvastatin calcium. Asian J Biochem Pharm Res. 2011; 1(3): 539-548.
12. Krishnarajan D, Senthil KN, Jeyabalan G. Design and evaluation of bioadhesive tablets of rosuvastatin calcium. Int J Bio Pharm Res. 2012; 3(4): 624-630.
13. Amish VP, Mark and M, Viral HS and Umesh U. Formulation and in-vitro evaluation of mucoadhesive bilayered buccal tablets of rosuvastatin calcium. Int J Pharm Sci Res. 2012; 3(8): 2733-2740.
14. Mehrgan H, Mortazavia SA. The release behaviour and kinetic evaluation of diltiazem hydrochloride from various hydrophilic and plastic based matrices. Iran J Pharm Res. 2005; 3: 137-146.

15. Khemariya P, Jain AK, Bhargava M, Singhai SK, Goswami S, Goswami R. Preparation and in-vitro evaluation of sustained-release matrix tablets of diltiazem. Int J Adv Pharm Sci. 2010; 267-273.
16. Gowri R, Narayanan N, Maheswaran A, Vijayalakshmi P, Rekha M. Design development and evaluation of diltiazem hydrochloride sustained release tablet. Int J Pharm Res Dev. 2013; 5(2): 59-65.
17. Brown WV, Bays HE, Hassman DR. Efficacy and safety of rosuvastatin compared with pravastatin and simvastatin in patients with hypercholesterolemia: a randomized, doubleblind, 52-week trial. Am Heart J. 2002;144:1036–1043.
18. Brown GB, Zhao XQ, Chait A. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. N Engl J Med. 2001;345(22):1583–1592.