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NOVEL FORMULATION AND ANALYTICAL EVALUATION OF BI-LAYERED TABLETS OF ENALAPRIL MALEATE, HYDROCHLOROTHIAZIDE AND NIFEDIPINE

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Keywords:

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ABSTRACT: The present research is intended for the formulation development, optimization, and *in-vitro* evaluation of bi-layer tablets containing Enalapril maleate, hydrochlorothiazide in the immediate release layer and Nifedipine in the sustained release layer based on GEMINEX technology. The impact of formulation variables such as combination of polymers and coating solution on the drug release was evaluated from the developed formulation. The prepared formulation blend was assessed for compressibility characteristics. The homogeneity of each API in a blend was tested by determining the %assay of individual drugs from different layers. Both the immediate and controlled release granules were formulated into bilayer tablets by the direct compression method. SEM analysis of bi-layered tablets was also performed. The mean drug content for all formulations was found to be within specified limits. The *In-vitro* dissolution studies can be performed at USP type II dissolution apparatus for coated tablets. The release of Enalapril maleate and hydrochlorothiazide from the immediate-release layer was found to be 99.2±0.8% and 99.5±1.1, and Nifedipine from the sustained release layer was found to be 100.7%±0.5%, respectively. Hence the bilayer tablets of Enalapril maleate Hydrochlorothiazide and Nifedipine were used to improve patient compliance towards the effective management of hypertension.

INTRODUCTION: Hypertension is an unusual high arterial blood pressure. Hypertension is a major risk factor for various cardiovascular diseases (CVDs) – uncontrolled hypertension increases the relative risk from two to four times for coronary disease, stroke, heart failure, peripheral arterial disease, renal insufficiency, atrial fibrillation and dementia/cognitive impairment. Poorly controlled hypertensive patients have an increased risk for cardiovascular complications [1].

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As per the world health organization (WHO), normal adult blood pressure is described as a systolic BP of 120 mm Hg and diastolic BP of 80 mm Hg. when a systolic BP is ≥140 mmHg or a diastolic BP is ≥90 mm Hg, generally measured as high blood pressure [2]. One of the global non-communicable diseases (NCDs) targets adopted by the World Health Assembly in 2013 is to lower the prevalence of raised blood pressure by 25% by 2025 compared with its 2010 level [3].

In contrast, Secondary or Resistant hypertension occurs earlier than 30 or later than 55 years of age. Its systolic BP > 180mm Hg or diastolic BP >120 mm Hg. Older age, Black race, obesity, diabetes mellitus, obstructive sleep apnea, enlargement of the heart chambers, and chronic kidney disease are the common risk factors for Resistant hypertension [4].

Therefore, there is a necessity for the progress of appropriate medication to treat or manage hypertension. The objective of any drug delivery system is to provide a therapeutic quantity of drug to the proper site in the body to attain and maintain desired drug concentration. Nowadays, various developed and developing countries move towards combination therapy for the cure of various diseases and disorders requiring long-term therapy, such as hypertension, diabetes and cardiovascular diseases. Combination products are two or more active drug substances in a single dosage form. They perform significantly in clinical treatment because of their improved and wider therapeutic synergism and lower side effects [5]. Clinically combination therapy in hypertension involves two or more drugs with different mechanisms of action in a low dose combination at a fixed dose is a suitable choice for the initial treatment of hypertension [6-8].

Four main classes of medications are practiced in combination therapy for managing hypertension: thiazide diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors (ACEIs), and angiotensin receptor blockers (ARBs) [9]. The use of dual combination therapy is preferred in high-risk patients for immediate BP response, better short- and long-term BP control, continued/improved patient adherence [10, 11]. Most dual combinations of antihypertensive agents include ARBs/ACEIs with either calcium channel blockers (CCBs) or thiazide diuretics [12-16]. Triple-combination therapies are required to achieve BP control in approximately one-fourth to one-third of patients and is efficacious for moderate to severe hypertension, with substantial additional BP reduction over dual regimens [17].

Generally, three classes of drugs in combination for managing hypertension comprise ACEIs, CCBs and thiazide diuretics [18-20]. One of the crucial challenges for combined therapy is the management of the release behavior of each drug independently. On the origin of these deliberations, a new oral drug delivery device is an antihypertensive bi-layer tablet. Bilayer tablet is a new era for the successful development of controlled release formulations along with various features to provide a way of successful drug delivery system [21]. Therapeutic strategies based on oral delivery of bilayer (multilayer) tablets are gaining more acceptance among brand and generic products due to various factors, including advanced delivery strategies, patient compliance, and combination therapy. Successful manufacturing of these ever more complex systems has to overcome various challenges, from formulation design to tablet press monitoring and control [22, 23]. Bi-layered tablets are suitable for the chronological release of two or more active pharmaceutical ingredients (API) in a combined form and incorporation of chemically incompatible active pharmaceutical ingredients by physical separation in the single-dose unit. Bilayer tablet is a single dosage form that promotes patient compliance and reduces the dose frequency [24, 25].

This technology has enabled the development of sustained-release dosage forms with predetermined release profiles of APIs, in which an immediate-release layer consists of super disintegrates, allowing sufficient drug concentration in the plasma to produce an immediate therapeutic effect. Sustained-release layer has various polymers that release the drug sustainably and maintain the plasma drug concentration for an extended period to continue the impact. Diabetes, antihypertensive, antihistamines, analgesics, antipyretics and antiallergenic agents are mainly suitable for this drug delivery [26-28]. Various approaches are used in the designing of bi-layered tablets [29]. Different techniques are available for the manufacturing of bilayer tablets. GEMINEX technology delivers one or more drugs with different release rates in a single dosage form. It is helpful for both industries as well as patients.

With this assistance, the therapeutic effects of drugs can be increased significantly also beneficial in lowering side effects [30-32]. In the present study, Hydrochlorothiazide, Enalapril maleate, and Nifedipine are selected in triple combination for the formulation of bi-layer tablets to treat mild to moderate hypertension. Enalapril maleate is a peptide molecule chemically described as (S)-1[N-[1-(ethoxy carbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt. It is sparingly soluble in alcohol, freely soluble in dimethyl formamide, slightly soluble in water, and semi-polar organic solvents. It is practically insoluble in non-polar organic solvents [33].

Hydrochlorothiazide is 6-chloro-1, 1-dioxo-3,4-dihydro - 2H - 1λ6, 2, 4-benzothiadiazine-7-sulfonamide. It is soluble in acetone, slightly soluble in water, sparingly soluble in alcohol, freely soluble in a solution of alkali hydroxide and insoluble in ether [34, 35]. Chemically Nifedipine is a Dimethyl 2, 6-dimethyl-4-(2-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate and is odorless, yellow crystals or powder, soluble in acetone, sparingly soluble in absolute ethanol and practically insoluble in water [36]. In the proposed bilayer tablet, Hydrochlorothiazide and Enalapril maleate exist as an Immediate-release layer for rapid medicine release to attain a high serum concentration in a short period and Nifedipine as a sustained-release layer for extended-release to maintain an efficient blood level for a prolonged period time and avoid repeated administration. The research is challenging to formulate a fixed-dose combination of Hydrochlorothiazide, Enalapril maleate and Nifedipine Bi layered tablet with sufficient stability. Hence, there is a necessity for building up a simple, stable fixed dose composition of these drugs. However, it is complicated to achieve, especially for once-daily dosage forms, partly because the environment for drug diffusion and absorption varies along with the gastrointestinal (GI) tract. Based on these considerations, we have proposed a bi-layer tablet [37-39].

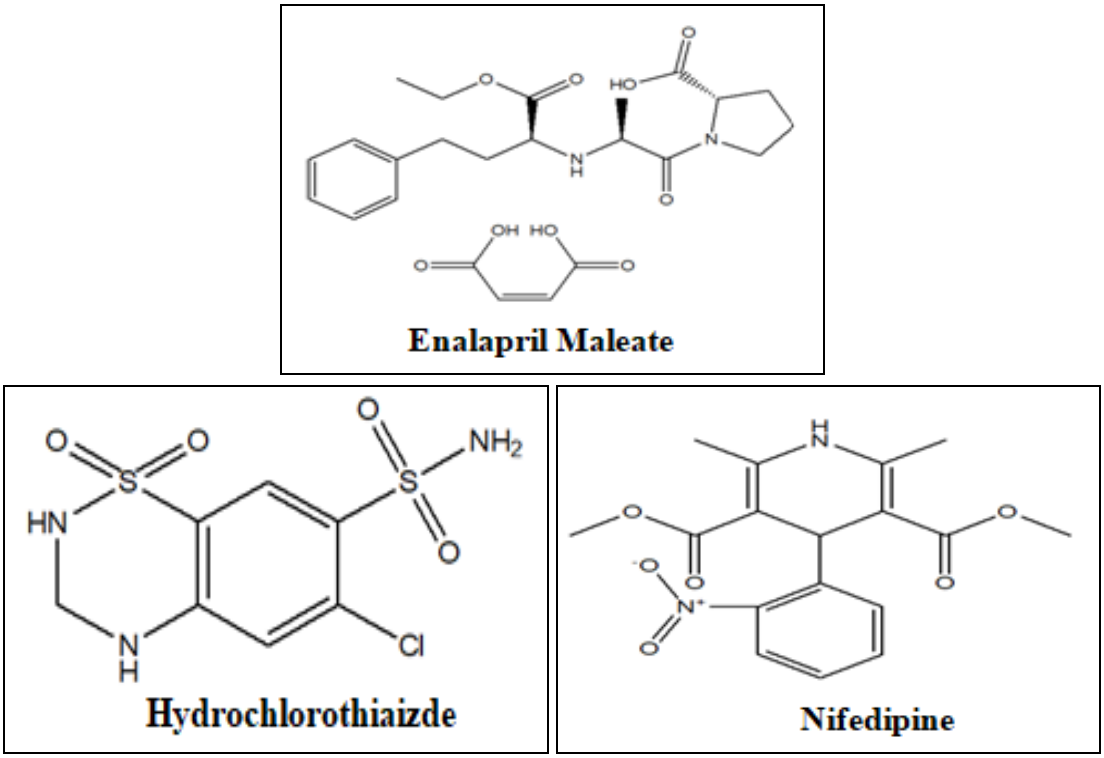

FIG. 1: CHEMICAL STRUCTURE OF APIS OF BI-LAYERED TABLETS

MATERIALS AND METHODS:

Chemicals and Reagents: Nifedipine (Unique Chemicals, Gujarat), Enalapril maleate were procured from Sri Krishna Pharmaceuticals Ltd, Hyderabad, Hydrochlorothiazide (HTZ) was as a gift sample from Amoli Organics Ltd, Mumbai. Corn starch was procured from S.A. Pharma chem Pvt Ltd, Mumbai, MCC (Avicel PH 101); Crospovidone; PVP K-30, magnesium stearate, croscarmellose sodium, DCP, HPMC E-15 & 5, Carbomer 974p, Polyethylene Glycol 3350, SSG and Ethyl cellulose, Xanthan gum, Red ferric oxide and Zinc oxide were donated by SM Pharmaceuticals, Malaysia. Chemicals such as formic acid, Hydrochloric acid, Sodium Hydroxide, Dipotassium hydrogen phosphate, Dipotassium hydrogen phosphate, Dipotassium hydrogen phosphate and Sodium Lauryl sulfate used were of analytical grade and solvents Methanol, Acetonitrile, Water was of HPLC grade obtained from Merck. The reference materials used in analytical testing were procured from the European Pharmacopoeia.

Material Sifting: Assemble the vibratory sifter (Make: Shree Bhagwati Pharma Machinery, Model:

SBVS-48) by placing a discharge chute on top of the body of the required sieve. Then keep the position frame and fit the gasket and tight the clamps. Tie the polybag (inside the container) properly to the outlet of the sifter.

Load the materials in chronological order of Corn Starch, MCC, Crospovidone, SSG, Magnesium stearate, HPMC K-15 & E-5, and Croscarmellose sodium, Ethylcellulose and Nifedipine, Enalapril maleate, Hydrochlorothiazide through # 40 sieve

Granulation: Above sifted materials were loaded into a rapid Mixer Granulator and mixed the contents for 10 minutes at agitator slow speed and chopper off. Binder solution was prepared by dissolving Corn starch; subsequently, PVP K-30 insufficient quantity of purified water with agitator/impeller dead & chopper off.

Scraped the contents from the sidewalls of a bowl, added the additional amount of distilled water and continued mixing with the agitator and chopper at slow speed till granular mass was obtained.

Drying: The wet mass was discharged into the Fluid bed dryer bowl using tipper assembly, air-dried for 5 min at an inlet air temperature of 50-60°C with intermittent raking.

The drying was stopped when the product temperature reached 41°C - 46°C. Final loss on drying was determined using an Infrared moisture analyzer at 105°C and was less than 1.5 % w/w**.**

Milling, Sizing, Sifting: Dried granules were sifted through a Vibratory sifter using a 25mesh stainless steel sieve. Large granules were milled using Comminuting mill through 1.5 mm screen at slow speed, knives forward and passed through 25mesh. Now large granules were milled at medium speed, blades forward.

This process was continued till all granules were passed into 25mesh. Oversized material was milled using Co-mill fitted with a 1.5 mm stainless steel screen at a slow speed and sifted through 25mesh. The granules were dried. Crospovidone, Colloidal Silicon dioxide and Magnesium stearate were sifted through # 30mesh.

The dried granules Crospovidone and Colloidal Silicon dioxide were loaded into the Octagonal blender and mixed for 10 min at a slow speed of 5rpm. Magnesium stearate was also added to a blender and all the contents were lubricated for 5 min. The prepared granules were subjected to analysis of pre-compression parameters before formulating them into tablets. Six formulations of Enalapril maleate and Hydrochlorothiazide immediate release granules were prepared.

Blending and Lubrication: Zinc oxide (extra granular) and Colloidal Silicon dioxide (extra granular) were sifted through # 30mesh. The dried granules and sifted materials were loaded in the Octagonal blender and mixed for 10 minutes at 5rpm.

The prepared granules were subjected for evaluation of pre compression parameters before formulating them into tablets. Similarly, six formulations of Nifedipine Controlled release granules were prepared. The composition for all six formulations of both immediate and control release layers were given in **Tables 1** and **2.**

TABLE 1: FORMULATION TRIALS FOR IMMEDIATE RELEASE LAYER

Ingredients	F1	F2	F3	F4	F5	F6
Enalapril maleate	10	10	10	10	10	10
Hydrochloro-thiazide	25	25	25	25	25	25
Corn starch	86.5	86.5	86.5	86.5	86.5	86.5
MCC (Avicel PH 101)	15	20	10	-	-	-
Crospovidone	-	-	-	5	10	15
PVP K 30	15	15	15	15	15	15
Sodium starch glycolate	3	3	3	3	3	3
Purified water for granulation(in ml)	q.s	q.s	q.s	q.s	q.s	q.s
Lubrication stage:						
Magnesium stearate	3	3	3	3	3	3
Croscarmellose sodium	2	2	2	2	2	2
Dibasic calcium phosphate	3	3	3	3	3	3
Target weight (mg)	150	150	150	150	150	150

TABLE 2: FORMULATION TRIALS FOR THE CONTROL RELEASE LAYER

Ingredients	F1	F2	F3	F4	F5	F6
Nifedipine	30	30	30	30	30	30
HPMC K-15	10	12	15	16	13	14
Carbomer 974p	16	22	24	18	19	20
HPMC E-5	6	10	8	-	-	-
Ethyl cellulose	68	56	53	51	43	46
PVP K 30	0	0	0	15	25	20
Croscarmellose sodium	10	10	10	10	10	10
Xanthan Gum	3	3	3	3	3	3
Water (in ml)	1	1	1	1	1	1
Lubrication stage:						
Zinc oxide	3	3	3	3	3	3
Colloidal silicon dioxide	2	2	2	2	2	2
Target weight (mg)	150	150	150	150	150	150

Evaluation of Pre-Compression Parameters: The prepared powder granules and blend were evaluated for flow properties such as Angle of Repose, Bulk Density, Tapped density, and compressibilities such as Carr's Index and Hausner's ratio.

Angle Of Repose (Θ): The angle of repose (θ) of powder was determined using the funnel method. The funnel was fixed to a burette stand at the height of approximately 4.0 cm. A weighed quantity of lubricated blend was poured into the funnel until the top of the powder pile touched the funnel's tip. Once it touched the pile, the height (h) and radius (r) were measured. The angle of repose was expressed in degree and calculated through the following formula:

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

h= Height; r = Radius of the pile

Bulk Density (PB) / Tapped Density (PT): Enough powder was passed tenderly through a sieve with orifices of 1.0 mm. An accurately weighed 100 g of the powder (m) was gently introduced without compacting into a dry 250 mL graduated measuring cylinder with the help of a funnel. Level the powder carefully, and the apparent unsettled volume (V0) to the adjacent graduated unit was noted. The bulk density was calculated in grams per milliliter using the formula m/V0. This process was replicated, and an average of three determinations was considered.

$\rho b = m/V_0$ m is mass of powder and V_0 is the Unsettled apparent volume

The powder was tapped for 500, and 1250 taps on a wooden surface until further volume changes in the powder had not happened. The volume (Vf) was read to the nearby unit as V_{500} and V_{1250}. The difference between 500 and 1250 was less than 2ml. So, we considered V_{1250} as the final tapped volume. This procedure was repeated, and the mean of three measurements was deemed. It was measured in grams per milliliter using the formula m/Vf.

$\rho t = m/Vf$ m is mass of powder and Vf is Final tapped volume

Measurement of Powder Compressibility: Based on the calculated values of bulk and tapped density, the Compressibility index in percentage and Hausner ratio were determined using the following formulas:

Compressibility Index= Tapped density - Bulk density / Tapped density x100

Hausner's ratio = Tapped density / Bulk density

Compression of Bi-Layer Tablets: A blend of lubricated granules of both immediate releases (Enalapril maleate and hydrochlorothiazide) and extended-release (Nifedipine) layers were filled individually into two different hoppers. The tablets were compressed on Tablet Punch Barrel 25.4mm diameter, touter diameter of Die with 38.10 mm on Double Rotary Tablet Press Machine; Model: TPC-27-D equipped with 27 Stations, 10ton operating load, 5ton Pre-compression load with type D tooling, 38.1 mm tablet diameter, 25mm depth of fill, 1-6 upper punch penetration with 5 HP / 3 Phase / AC Main Electric motor, 0.25 HP / 3 Phase / AC feed motor, 1 HP / 3 Phase / AC Hydraulic Power Pack Motor, 6.5 HP / 3 Phase / AC Electrical Supply.

Film Coating: HPMC E-5 was dispersed in a mixture of 2-propanol and methylene dichloride in a SS vessel and stirred for 30 min. Polyethylene Glycol 3350 was also dissolved in the same solvent mixture. The red ferric oxide was passed through mesh #100 and dispersed in the same solvent mixture. Pass the solution through colloidal milling. The core tablets were loaded into the coating pan with a speed of 4-6 rpm, inlet air temperature of 55ºC-65ºC, and outlet air temperature of 40ºC-50ºC. The air pressure of the coating gun was adjusted, and the tablets were coated with the above coating solution.

Container and Closure System: The coated tablets were packed in Alu-Alu Blister using NOACK model Packaging Machine Make: Romaco in a temperature and moisture-controlled area. The 10 x 10s Blisters were packed in a printed box with leaflets. The boxes were then packed into labeled cartons.

Evaluation of Post Compression Parameters: All the prepared Bi-layer tablets were evaluated for physical parameters such as Shape, Thickness, Hardness, Friability, Weight variation, Disintegration time and Drug content as per the standards of an official compendium.

The tablets were inspected under the magnifying lens for shape of tablets. Ten tablets were picked at random from each formulation and measured the thickness and diameter individually using calibrated Vernier calipers. Ten tablets were taken arbitrarily from each formulation and evaluated for hardness by Monsanto hardness tester. The tablet being tested was kept in between moving and the fixed jaw and indicator reading was adjusted to zero. Then the force was slowly increased to the edge of the tablet by 85, operating the screw knob forward till the tablet was broken. The reading was recorded on a scale the denoted the pressure necessary in kg/cm2 to break the tablet. The friability of tablets was accomplished in an Electro lab Model: EF-2W friability tester. Ten tablets were initially weighed (W0) and shifted into a friability. The instrument was then operated at 25 rpm for 4 min or 100 revolutions. Tablets were dusted and again weighed accurately (W). The loss in weight implied friability. The % friability was calculated.

$$\% \text{ friability} = 1- W_0 / W_1 \times 100$$

The weight uniformity of tablets was determined using an Electronic balance. Twenty tablets were picked randomly from each formulation and weighed individually. The average weight was calculated and compared the weight of individual tablets to the average. The deviation if at all in the individual tablet weight from the average was examined. This test depicts that all tablets of a specific formulation should be uniform in weight. The weight variation should be within the pharmacopeial limits. The disintegration time was evaluated using an Electrolab Tablet Disintegration tester Model ED-2L and was calculated in seconds. Water was selected as the disintegration medium. One tablet was kept in each of the six tubes of apparatus. The time taken in seconds to disintegrate the entire tablet without palpable mass in the tube was noted.

***In-Vitro* Dissolution Studies:** The dissolution studies were carried out by using an Electro lab Model: EDT-14Lx Dissolution tester. The phosphate buffer was prepared by dissolving 331 g of dibasic sodium phosphate and 38 g of citric acid in water in a 1-L volumetric flask, to this 10 mL of phosphoric acid was added and diluted with water. To 125.0 mL of phosphate buffer, 1 L of 10% sodium lauryl sulfate solution was added, mixed, and diluted to 10 L. pH of 6.8 was adjusted by using Dissolution Media Preparator; (Make: Electrolab, Model: EMP-21). 900ml of media was added for each vessel at a batch temperature of 37°C + 0.5°C Apparatus 2: 50rpm with sinkers. 10ml of the sample was withdrawn at an interval of 5min, 10min, 20min, 30min, 45min and 60min, 4th hour, 8th hour, 16th hour and 24th hour and filtered through a 0.2µm Nylon membrane filter.

Standard Preparation: An accurately weighed 30mg of Nifedipine BPCRS Batch # 2997, 20mg of Enalapril Maleate BPCRS Batch # 2673, and 25mg of Hydrochlorothiazide BPCRS Batch # 3063 examined was dissolved in 20ml methanol and diluted to 100mL with dissolution medium. After 45 min a measured volume of 1.0mL was transferred into to 10mL volumetric flask, diluted to mark with dissolution medium and mixed well, finally filtered through a 0.2µm Nylon membrane

filter. The percentage release of each drug was calculated using the following formula.

For single point

Calculation of % Release

% Release = AT / AS x Ws /100 x 1/ 10 x 900 / 1 x p / LC x 100

For multi-point = % Release + Dilution factor

At	:	Area of analyte peak obtained in the sample solution
As	:	Area of analyte peak obtained in standard solution
Ws	:	Weight of Nifedipine / Enalapril / Hydrochlorothiazide CRS used, in mg
P	:	The potency of Nifedipine / Enalapril / Hydrochlorothiazide CRS used, in %
LC	:	Label Claim

Process Validation Studies: The formulation details and the process parameters were considered as the basis for the development of concurrent process validation. The validation batches were carried out based on the afore said manufacturing process.

The following critical process parameters blend mixing time, drying process concerning time and temperature, lubrication process, the blend hold time studies, punch penetration and compression pressure, and discriminate studies of tablet binder were carried for the study.

RESULTS AND DISCUSSION: The immediate-release layer Enalapril maleate is BCS Class III water-soluble drug; Hydrochlorothiazide is BCS Class II poor water-soluble drug. To enhance the dissolution of hydrochlorothiazide and to increase the solubility of Enalapril maleate, the solubility enhancers PVP-k30 10% and Sodium starch glycolate 2% is added to its theoretical fill weight of 150mg per tablet. Nifedipine is a poor water-soluble BCS Class II drug. To enhance the solubility of Nifedipine, 6.6% of Croscarmellose sodium is added.

The excretion of Nifedipine is about 2 h after being absorbed in the blood stream and it has a synergetic effect on relaxing heart muscles and blood vessels. Control the synergetic effect and prolong the elimination of half-life, extended-release tablets are formulated. The % Release of Nifedipine is extended to 24 h by using HPMC, Carbomer 974p, and Ethylcellulose polymers in a 6 to 12% ratio.

Precompression Parameters:

Precompression Parameters for Immediate Release Layer: The prepared blend of different formulations of Enalapril maleate and Hydrochlorothiazide immediate-release layer were evaluated for their flow properties such as angle of repose, bulk and tapped density, compressibility index and Hausner's Ratio.

The flow properties of different bends of formulations are given in **Table 3.** The angle of repose values for the total formulations blend is in the range of 26.16 to 27.35. The compressibility index ranges from 15.57 to 21.86, and Hausner's ratio is between0.813 and 0.851. The acquired flow property values indicate that all the formulation blends have good and passable flow characteristics and were said to be suitable for compression.

TABLE 3: PRECOMPRESSION PARAMETERS OF IMMEDIATE-RELEASE LAYER BLEND

Formulation	Angle of Repose (θ)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Compressibility Index (%)	Hausner's Ratio	% Lose on drying
F-1	26.22± 0.05	0.543± 0.012	0.668± 0.014	18.71± 0.11	0.813± 0.012	1.54± 0.12
F-2	26.72± 0.09	0.533± 0.011	0.653± 0.015	20.21± 0.17	0.816± .022	1.58± 0.11
F-3	26.88± 0.11	0.538± 0.008	0.645± 0.018	19.46± 0.14	0.834± 0.015	1.38± 0.09
F-4	27.35± .12	0.542± 0.006	0.658± 0.016	18.86± 0.18	0.824± .014	1.43± 0.05
F-5	26.16± 0.05	0.522± 0.009	0.638± 0.012	21.86± 0.19	0.818± 0.018	1.62± 0.09
F-6	26.94± 0.07	0.564± 0.012	0.663± 0.011	15.57± 0.22	0.851± 0.019	1.59± 0.10

Precompression Parameters for Controlled Release Layer: The flow properties for the prepared blend of diverse formulations of the Nifedipine controlled-release layer were also studied. The flow properties of various bends of formulations are given in **Table 4**. The angle of repose values for the blend of entire formulations is found to vary from 29.11to 29.78. The compressibility index is between 5.09 to 7.49 and Hausner's ratio is in the range of 0.814 to 0.854. The flow property values obtained for the various blend of formulations demonstrate that the powder blend has excellent flow characteristics for compression.

TABLE 4: PRE-COMPRESSION PARAMETERS OF NIFEDIPINE CONTROL RELEASE LAYER BLEND

Formulation	Angle of Repose (θ)	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility Index (%)	Hausner's Ratio	%Lose on drying
F-1	29.15±0.06	0.634±0.014	0.754±0.011	5.09±0.09	0.841±0.011	1.26±0.03
F-2	29.72±0.08	0.631±0.011	0.788±0.012	5.54±0.06	0.801±0.013	1.33±0.04
F-3	29.53±0.02	0.627±0.013	0.765±0.014	6.14±0.08	0.820±0.016	1.16±0.06
F-4	29.11±0.09	0.618±0.009	0.759±0.015	7.49±0.11	0.814±0.011	1.68±0.07
F-5	29.15±0.04	0.622±0.009	0.728±0.011	6.89±0.14	0.854±0.013	1.52±0.09
F-6	29.78±0.03	0.629±0.007	0.745±0.012	5.84±0.10	0.844±0.011	1.43±0.06

Evolution of Blend Uniformity: The blend uniformity is a critical in-process parameter for process validation; this test was carried out to determine the homogeneity of Nifedipine, Enalapril maleate and Hydrochlorothiazide present in optimized formula F-3. The blend samples were collected from different locations of the Octagonal blender and tested for uniformity by Waters Acquity UPLC equipped with a PDA detector.

The uniformity results are graphically illustrated in **Fig. 2**. The % mean assay of three batches blend results are in the range of 97.82%±0.38% to100.28%±0.43% for Nifedipine, 96.82% ±0.17% to 99.76%±0.33%of Enalapril maleate content and 98.08%±0.37% to 99.36%±0.55%for Hydro-chlorothiazide. The results are shown in **Table 5.**

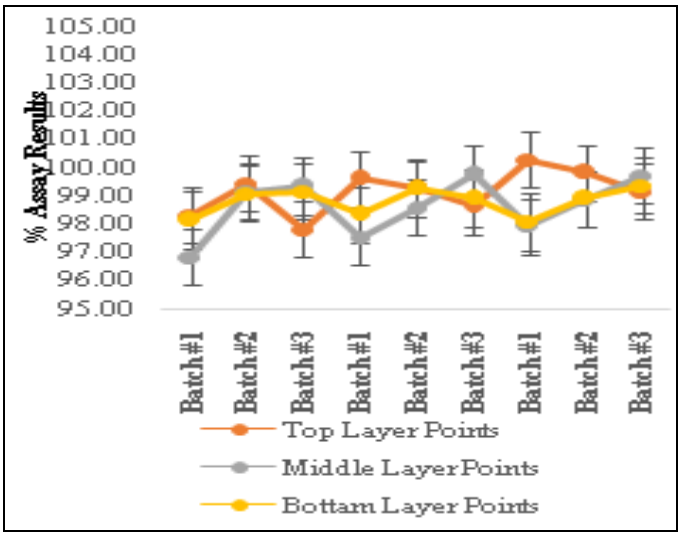

FIG. 2: % ASSAY RESULTS FOR BLEND UNIFORMITY

TABLE 5: BLEND UNIFORMITY ASSAY CONTENT RESULTS

Batch #	Location	% Mean Assay Results		
		NDP	ENL	HTZ
1	Top Layer Points	98.30±0.32	96.82±0.17	98.14±0.44
2		99.44±0.41	99.14±0.31	99.08±0.51
3		97.82±0.38	99.34±0.42	99.16±0.66
1	Middle Layer Points	99.60±0.55	97.52±0.39	98.32±0.23
2		99.24±0.12	98.58±0.32	99.26±0.28
3		98.60±0.41	99.76±0.33	98.92±0.21
1	Bottom Layer Points	100.28±0.43	97.90±0.45	98.08±0.37
2		99.80±0.61	98.86±0.36	98.88±0.61
3		99.16±0.24	99.72±0.28	99.36±0.55

NDP-Nifedipine, ENL-Enalapril Maleate, HTZ-Hydrochlorothiazide

Tablet Compression Results: The lubricated blend of control release and immediate release materials was compressed into tablets. The physical parameters of core tablets are reported in **Table 6.**

TABLE 6: PHYSICAL PARAMETERS OF ENCARDIL NH XR CORE TABLETS

Physical parameters	Batch #1	Batch #2	Batch #3
Weight of 20 Tablets (in g)	6.1896±0.02%	6.1988±0.07%	6.1954±0.05%
Weight variation	309.5±0.3%	309.4±0.7%	308.6±0.5%
Friability	0.09%	0.12%	0.09%
Disintegration Time (mm: sec)	12′:22″±1′:51″	12′:38″±1′:53″	12′:05″±0′:57″
Thickness (mm)	4.93±0.01	4.97±0.03	4.94±0.04
Diameter (mm)	5.07±0.04	5.03±0.01	9.987±0.55
Hardness (kg/cm2)	8.95±0.25	5.05±0.02	8.876±0.77

Evaluation of Post Compression Parameters for Bi-Layer Tablets: The prepared bi-layer tablets of all formulations of Nifedipine, Enalapril maleate, and hydrochlorothiazide were evaluated for post-compression parameters like weight uniformity, thickness, Hardness, Friability, Disintegration time, and drug content. The obtained results for various formulations are shown in **Table 7**. The hardness of tablets of each formulation is between 8.4 to 10.2 kg/cm2 confirms that all formulations have good handling characteristics. The friability is <0.2% indicates that tablets are mechanically stable. The variation of weight in-between tablets from average weight is ≤ 5% of pharmacopeia limits. The measured mean thickness of tablets of each formulation is ranged from 4.91 to 4.95mm with ≤ 0.1% variation from **Table 10**. The disintegration time is found between 8.31″ to 11.03″.

TABLE 7: POST COMPRESSION PARAMETERS FOR ENCARDIL NH XR BI-LAYER CORE TABLETS

Formulation	Weight uniformity (mg); N=20	Thickness (mm); N=20	Hardness (Kg/cm2) N=20	Friability (%) N=10	Disintegration Time(Minutes/ seconds); n=12
F-1	311.5 ± 1.22%	4.93±0.05	8.4± 0.7	0.18	8 m31s
F-2	311.3± 0.98%	4.95±0.03	8.6± 0.9	0.15	9m20s
F-3	312.1± 0.84%	4.91±0.04	10.2± 1.2	0.12	10m05s
F-4	311.9± 1.52%	4.94±0.06	9.1± 1.5	0.17	8m47s
F-5	311.8± 2.11%	4.92±0.03	9.3± 1.1	0.13	9m26s
F-6	312.1± 2.43%	4.91±0.07	8.8± 0.6	0.16	11m03s

The estimated mean drug content of each API for all formulations from **Table 8** is as follows. The mean drug content of Nifedipine is found to be 29.89mg+0.09mg (99.63%+0.3%) to 30.18mg +0.07mg (100.60%+0.23%), of Enalapril is found to be 10.11mg ±0.05mg 101.10% + 0.49% to 10.15mg ±0.06mg (101.50%±0.59%), of Hydro-chlorothiazide is found to be 25.02mg ±0.12mg to 25.21mg±0.06mg (100.84%±0.24%). There are no significant changes in the Assay results between the formulations.

TABLE 8: ASSAY RESULTS FOR ENCARDIL NH XR BI-LAYER CORE TABLETS

Formulation	Mean assay results		
	Nifedipine (mg/tab)	Enalapril (mg/tab)	Hydrochlorothiazide (mg/tab)
F-1	29.89±0.09 (99.63%±0.3%)	10.11±0.05 (101.10%±0.49%)	25.21±0.06 (100.84%±0.24%)
F-2	30.12±0.04 (100.40%±0.13%)	10.15±0.06 (101.50%±0.59%)	25.05±0.11 (100.20%±0.44%)
F-3	30.05±0.05 (101.67%±0.13%)	10.04±0.03 (100.40%±0.30%)	25.06±0.13 (100.24%±0.52%)
F-4	30.18±0.07 (100.60%±0.23%)	10.09±0.08 (100.90%±0.79%)	25.13±0.08 (100.52%±0.32%)
F-5	30.09±0.03 (100.30%±0.10%)	10.02±0.04 (100.20%±0.40%)	24.95±0.11 (99.80%±0.44%)
F-6	30.01±0.06 (100.03%±0.20%)	10.07±0.07 (100.70%±0.69%)	25.02±0.12 (100.08%±0.48%)

Hold-Time Studies: The hold-time studies were performed for the Encardil NH XR core tablets, which were packed in black color HDPE bags at 40^{O}C ± 2^{O}C with 75%+5% by using a Stability chamber; Model: TH 400 S/G; Make: Thermolab. The hold-time studies of Description, Disintegration time, Moisture content, Dissolution, and % Assay content at different sampling intervals of initial (T0), 15th Day (T1), 30th Day (T2) and 60th Day (T3) were studied. The hold-time study results are tabulated in **Tables 9, 10,** and **11.**

TABLE 9: HOLD TIME STUDY RESULTS OF DT, MC FOR ENCARDIL NH XR CORE TABLETS

Test	Study period	Batch # 1	Batch # 2	Batch # 3
Disintegration	initial (T_0)	11′:04″±0′:43″	10′:45″±1′:22″	11′:14″±1′:33″
Time (DT) (n=6)	15th Day (T_1)	11′:11″±1′:02″	11′:26″±1′:07″	11′:14″±1′:01″
	30th Day (T_2)	10′:57″±0′:46″	11′:07″±0′:56″	10′:05″±0′:55″
	60th Day (T_3)	11′:46″±1′:05″	11′:06″±1′:15″	11′:03″±0′:95″
Moisture content	initial (T_0)	1.53%	1.66%	1.58%
(MC)	15th Day (T_1)	1.48%	1.63%	1.55%
	30th Day (T_2)	1.45%	1.62%	1.54%
	60th Day (T_3)	1.43%	1.59%	1.52%

TABLE 10: DISSOLUTION STUDIES FOR CORE TABLETS FROM HOLD TIME STUDY

Period	Analyte	Time	% Release
initial (T_0)	NDP	4th hr	12.82% ± 4.5%
		8th hr	34.54%± 3.1%
		24th hr	96.42%± 0.8%
	ENL	45 min	96.8%± 1.0%
	HTZ	45min	99.6%± 0.8%
15th Day (T_1)	NDP	4th hr	12.12%± 3.9%
		8th hr	31.15%± 2.7%
		24th hr	95.56%± 0.8%
	ENL	45 min	97.16%± 0.9%
	HTZ	45min	99.15%± 0.6%
30th Day (T_2)	NDP	4th hr	14.55%± 4.1%
		8th hr	34.88%± 2.7%
		24th hr	95.76%± 0.7%
	ENL	45 min	96.86%± 0.8%
	HTZ	45min	99.23%± 0.9%
60th Day (T_3)	NDP	4th hr	13.85%± 4.4%
		8th hr	36.02%± 3.2%
		24th hr	96.24%± 0.8%
	ENL	45 min	97.32%± 0.8%
	HTZ	45min	99.33%± 0.9%

TABLE 11: HOLD TIME STUDY RESULTS OF % ASSAY CONTENT FOR ENCARDIL NH XR CORE TABLETS

Study period	Drug	Batch # 1	Batch # 2	Batch # 3
initial (T_0)	NDP	96.42%± 0.8%	95.55%± 0.9%	96.74%± 0.6%
	ENL	96.8%± 1.0%	97.44%± 0.7%	97.02%± 0.5%
	HTZ	99.6%± 0.8%	99.52%± 0.6%	99.21%± 0.4%
15th Day (T_1)	NDP	95.56%± 0.8%	96.35%± 0.9%	96.88%± 0.4%
	ENL	97.16%± 0.9%	97.89%± 0.4%	97.65%± 0.4%
	HTZ	99.15%± 0.6%	99.28%± 0.7%	99.45%± 0.6%
30th Day (T_2)	NDP	95.76%± 0.7%	96.11%± 0.3%	96.55%± 0.5%
	ENL	96.86%± 0.8%	97.22%± 0.5%	97.69%± 0.7%
	HTZ	99.23%± 0.9%	99.39%± 0.6%	99.31%± 0.6%
60th Day (T_3)	NDP	96.24%± 0.8%	95.97%± 0.6%	96.07%± 0.5%
	ENL	97.32%± 0.8%	97.73%± 0.9%	97.78%± 0.9%
	HTZ	99.33%± 0.9%	99.54%± 0.8%	99.46%± 0.7%

Surface Studies by Using Scanning Electron Microscopy (SEM): The Encardil NH XR tablets granules were analyzed through Scanning Electron Microscopy; Model: JCM-7000; Make: JEOL, SEM microscope software program to investigate the appearance, shape, and size distribution of both immediate-release and extended-release granules. It is found that immediate release granules from **Fig. 3** are spherical, with smooth surfaces aggregated. Consequently, the average sizes of XR granules are round-shaped aggregated particles found in the SEM microscopy and are dispersed in the XR matrix from **Fig. 4.**

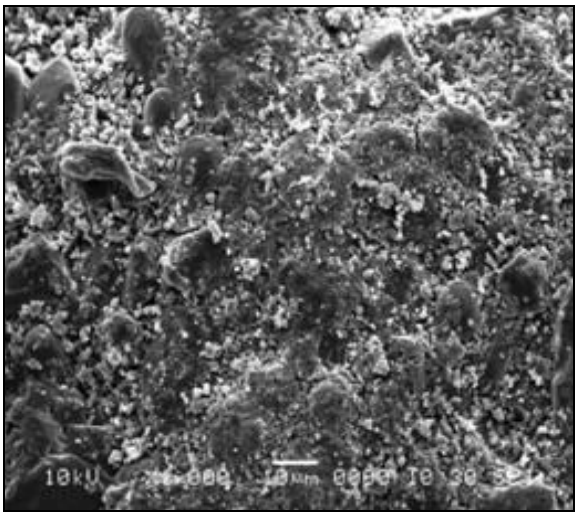

FIG. 3: SEM IMAGE OF IMMEDIATE RELEASE LAYER OF ENCARDIL NH XR TABLETS

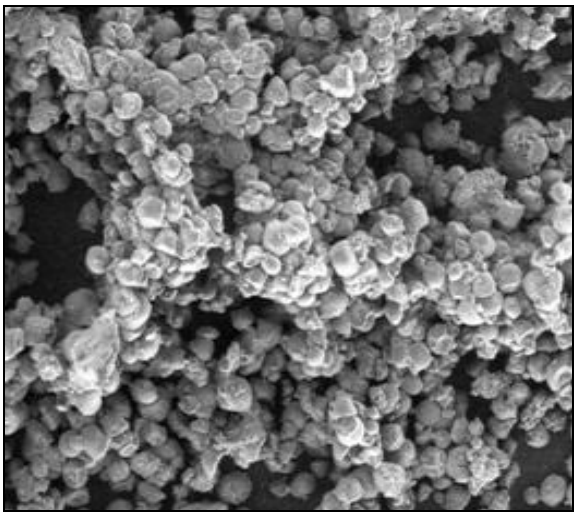

FIG. 4: SEM IMAGE OF CONTROL RELEASE LAYER OF ENCARDIL NH XR TABLETS

CONCLUSION: The direct compression process is desirable for compressing powder blends as bi-layer tablets. The post-compression parameters of all the tablet formulations met I.P specified limits. Based upon the dissolution results and kinetic profile, formulation 3 has been selected as the optimized formulation as the % release of each API has more. The percentage drug content of each API in the optimized formulation has more than 99%, and related substances were within specified limits.

The assay of Enalapril, Hydrochlorothiazide, and Nifedipine bi-layer tablets with %RSD for precision was < 2%. The SEM analysis of bi-layered tablets shows that the IR granules are spherical, and XR granules are round-shaped aggregated particles. Combination therapy provides greater antihypertensive effect than high monotherapy doses, adding several mechanisms of action that block various pathways of increased blood pressure, providing greater protection to target organs than monotherapy and reducing the potential for side effects.

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