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Formulation development and *in vitro* characterization of triple layer tablet containing amlodipine besylate, rosuvastatin calcium and hydrochlorothiazide

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Abstract: Triple layered tablet having various excipients and a new combination of APIs i.e. amlodipine besylate, rosuvastatin calcium and hydrochlorothiazide was prepared through wet granulation. The concentration of disintegrant and diluent was kept different in formulations of all APIs. At compression stage, nine different formulations from H1 to H9 having different combinations were prepared. Layers T1, T2 and T3 of all the three APIs had disintegrant concentration of 3%, 5% and 7 % respectively. *In vitro* analysis of granules was made by determining angle of repose, loss on drying, bulk density, tapped density, hausner ratio. Results of all these parameters were quite similar in all layers, which showed that change in disintegrant concentration does not affect the flow ability of granules to much extent. After compression, tablets were further subjected to weight variation, hardness, thickness, friability, disintegration, dissolution studies and FTIR. *In vitro* drug release data of all formulations were studied which showed that all the formulations exhibited zero order release. Results indicated that H8 had the best results in terms of physicochemical properties, assay and dissolution studies. The external morphology of formulations were further analyzed using scanning electron microscopy and differential scanning calorimetry. Triple layered tablet was successfully developed and characterized.

Keywords: Amlodipine besylate, rosuvastatin calcium and hydrochlorothiazide, FTIR, scanning electron microscopy and differential scanning calorimetry.

INTRODUCTION

The different systems or mechanisms that have been studied for introducing various drugs, prodrugs or pharmaceutical ingredients in the systemic circulatory system are immensely and diversely modified for the smart release of drug. Despite being all of them available, the most common and conventional route or dosage form till date is the oral route. It is just because of patient friendly and needle free administration of drug by this route, but also due to the fact that most of the routinely used drugs are very well absorbed when administered orally. The most common thought, while formulating any drug or drug product, irrespective of its mechanism of action and dosage form design, is that it must be in accordance with the normal physiology of the gastrointestinal tract. Hence, study of various parameters of drug products like pharmacokinetics, pharmacodynamics and design of dosage form must be correlated well with the gastrointestinal characteristics before going ahead in the research and formulation process. Advanced technologies can help us getting most of the results from the same drug of same dose as compared to that when the same drug was used without any detailed modifications in its delivery system.

The most common and widely used procedure of making layered tablets is by compressing the two or more types of granular layers together, which gives them a sandwich like look with the edges of each layer exposed till they got smoothed. Layered tablets can be of different types, described as bi, tri or multilayered depending upon the type of drug substances combined and purpose of their use (Nirmal *et al*. 2008)

An ideal triple layered tablet that can provide maximum and optimal commercial and research related output should not have any kind of microbial contamination, cracks, discoloration, pigmentation, unwanted features, and broken edges. Production procedures, packaging, shipping and dispensing are the steps during which there can be a sufficient pressure and press on the tablet, so there must be sufficient mechanical strength in the tablet to bear all these shocks along with stable physical and chemical nature to go through all the processes to produce the desired effect in a predictable and reproducible manner. Like every other formulation or dosage system or as a part of manufacturing process, there can be a few

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challenges of varying potential that can be raised during formulating a triple layered tablet, like cross contamination, yield production, delamination, degradation and cost effectiveness (Islam *et al*. 2018).

Amlodipine is basically an antagonist of dihydropyridine calcium (slow-channel blocker and/or calcium ion antagonist), which involve in inhibiting the invasion of calcium ion across the transmembrane of cardiac and vascular smooth muscle (Ahsan and Khan 2017).

Rosuvastatin calcium is a selective and competitive inhibitor of reductase HMG-CoA which is the rate determine step that changes 3-hydroxy-3- methylglutaryl coenzyme A to mevalonate i.e., the precursor of cholesterol. The *in vivo* studies in animals have shown a high intake of drug into the selective action in liver which is the cholesterol lowering target. These studies also revealed its effect in two ways (Bajaj and Giwa 2020).

Hydrochlorothiazide belongs to the class of thiazide diuretic which affects the distal renal tubular mechanism of electrolyte reabsorption. The diuretic effectiveness of all diuretics is approximately equal at the dose of maximal therapeutic. It enhances the excretion of chloride and sodium and inhibits the re-absorption of water in the nephron. Natriuresis may be accompanied by some loss of potassium and bicarbonate (Quan *et al*. 2006)

MATERIALS AND METHODS

Materials

Amlodipine besylate, rosuvastatin calcium and hydrochlorothiazide, Avicel PH 101, Avecil PH 102, Croscarmellose sodium, crospovidone, aerosol 200, dicalcium phosphate, lactose anhydrous, magnesium stearate, iron oxide red and lake green were obtained from Highnoon Laboratories, Lahore, Pakistan as all the other solvents and chemicals were of analytical grade.

Formulation of triple layered tablets

03 different types of formulations were designed for each API in these formulations. The quantity of API's was constant whereas excipients concentration was different. Amlodipine and hydrochlorothiazide layers were prepared through wet granulation whereas rosuvastatin calcium layer was prepared by dry granulation technique.

Preparation of layer containing the amlodipine besylate

In a container, purified water was taken to which kollidon 30 was added. Kollidon was dissolved in water to get a clear appearance with the help of propeller mixer. Sieving of amlodipine besylate, croscarmellose sodium (Ac-di-sol)/sodium starch glycolate, mannitol and colloidal silicon dioxide (Aerosil 200) was done via 40 sized mesh screen. Microcrystalline cellulose (Avicel PH 101) was divided into two equal portions. Geometric mixing was done to blend the one portion of microcrystalline cellulose and iron oxide red. The mixture was sieved with 100 sized mesh screen. The left microcrystalline cellulose was mixed with the blend of step C and sieving was done through 40 sized mesh screen. In the H mixer, the sieved material of Step B and D were mixed for about 10 minutes. Binding solution prepared in step A was added in H mixer and the mixing continued for 10 min. With the help of the 10 sized mesh screen, the wet mass obtained from Step f was sieved. At a temperature of 50-0°C, the granules were dried in tablet tray dryer for 90 minutes, the moisture was checked via IR moisture analyzer and process was stopped when about 2% moisture content was achieved. The dried material was then transferred to Double cone mixer and mixed for 05min. Finally magnesium stearate was transferred to double cone mixer and mixing was performed for 3 min.

The same procedure was adopted for preparation of hydrochlorothiazide layer. The API hydrochlorithiazide was used inspite of amlodipine besylate.

Preparation of rosuvastatin calcium layer

Lactose spray dried and rosuvastatin calcium were sieved through 60 mesh screen separately. Rosuvastatin calcium was added to lactose via geometrical mixing. Mixture was transferred to double cone mixer. Sieving of microcrystalline cellulose (Avicel PH 102) and crospovidone was done through 40 sized mesh screen and mixed. In the double cone mixer di calcium phosphate and the sieved material of Step B and C were added and mixed for about 20 minutes. Finally magnesium stearate was transferred to double cone mixer and mixing was performed for 3 min.

The prepared granules were divided into three batches for being compressed into a triple layer tablet. Each batch was compressed into 700 mg round plain tablets, using ZPW125 triple layer tablet press which was a machine specially designed for compression of triple layer tablets. The prepared tablets were stored in airtight, amber colored glass bottles till further evaluation. Before compression, pre-formulation tests were performed on each layer of tablets.

Formulation composition for amlodipine besylate

T1, T2 and T3 formulations were prepared. Amount of Amlodipine besylate was kept constant in each layer, whereas the amount of disintegrant was added in percentages of 3%, 5% and 7% in T1, T2 and T3 respectively. The percentage of diluents was decreased with respect to disintegrant. Details of all formulations have been given in table 1.

Formulation composition for rosuvastatin calcium

T1, T2 and T3 formulations were prepared. Amount of API i.e., Rosuvastatin calcium was kept constant in each

layer whereas the amount of disintegrant was added in percentages of 3%, 5% and 7% in T1, T2 and T3 respectively. The percentage of diluents was decreased with respect to disintegrant. Details of all formulations have been given in table 2.

Formulation composition for hydrochlorothiazide

T1, T2 and T3 formulations were prepared. Amount of API i.e., Hydrochlorothiazide was kept constant in each layer whereas the amount of disintegrant was added in percentages of 3%, 5% and 7% in T1, T2 and T3 respectively. The percentage of diluents was decreased with respect to disintegrant. Details of all formulations have been given in table 3.

Angle of repose

The angle of repose helps to determine the flow properties of the granules and ranges between 0-90°. Accurate quantity of granules was weighed and poured into a funnel, so that the granules could move freely the on the paper surface forming a conical shape. Angle of repose was measured and results were recorded (Al-Hashemi and Al-Amoudi 2018).

Loss on drying

Loss on drying of mixed blend was determined using the Sartorius moisture analyzer. The formula used was:

Loss on Drying = Initial Weight- Final weight x 100

Bulk density

It is the type of density which is without tapping. This type of density is calculated with help of a graduated cylinder. The granules were weighed and put in measuring cylinder. The volume occupied by granules was noted from the mark of the cylinder. (van den Ban and Goodwin 2017)

Bulk density was measured via using the below given formula. The unit of bulk density is g/cm$^{3.}$

$$\text{Bulk density} = \frac{\text{Weight of powder taken}}{\text{Volume of powder in cylinder}}$$

Tapped density

The weighed quantity of granules was filled in the measuring cylinder and the cylinder was mechanically tapped. Unit of Tapped density is g/cm^3. It is denoted by 'p'. Formula of tapped density is as follows (Inácio *et al*. 2020):

$$\text{Tapped bulk density (TBD)} = \frac{\text{Weight of powder taken}}{\text{Tapped volume of powder in cylinder}}$$

Carr's Compressibility index

The compressibility index of the granules was determined by Carr's compressibility index. (%) Carr's Index was calculated by using the following formula:

$$\text{Carr's Index (\%)} \frac{\text{TBD - LBD}}{\text{TBD}} \times 100$$

Where LBD is loose bulk density and TBD is Tapped bulk density.

Flow Rate

In this test, a metallic funnel was used which had a narrow orifice at its bottom. Properly weighed quantity of granules was passed through the metallic funnel. Time for the flow of granules through orifice was measured with the help of stopwatch.

The formula of flow is given below:

$$\text{Flaw rate} = \frac{\text{Mass}}{\text{Time}}$$

Hausner Ratio

This test is related to the flow properties of granules. Its formula is:

$$\text{Hausner ratio} = \frac{\rho^{\text{t}}}{\rho^{\text{d}}}$$

Where t is tapped density and d represents bulk density. Powders having Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25). Hausner's ratio greater than 1.25 indicates poor flow of material (Bowker and Stahl 2008).

Average weight

Weight variation test of all formulations (H1 to H9) was conducted. The test was conducted on 20 tablets of each formulation. For this purpose, a petri dish and analytical balance (Sartorius) were used. Test was conducted by placing petri dish on balance and tare button was pressed. Gross weight of tablets was calculated and the average weight of each Tablet was obtained by dividing it with 20.

Tablet hardness and thickness

The tablets which fall under the required weight were taken. 10 Tablets of each formulation were checked with the help of Erweka hardness and thickness tester.

Friability

10 tablets with the required weight of formulation H1 to H9 were weighed (W1) separately. Those 10 tablets were individually placed in friability tester apparatus (Erweka). The friabilator was run for 4 mins at 25 rpm. Tablets were taken out and then de-dusted via blower and re-weighed (W2). The weight loss was calculated by following formula.

$$\text{Percentage weight loss F (\%)} = \frac{W_1 - W_2}{W_2} \times 100$$

Disintegration test

06 tablets with the required weight of formulation H1 to H9 were taken and subjected to in-vitro disintegration test for tablets. The apparatus was filled with RO water up to the marked given on the tub of apparatus and temperature was maintained at 37.0 °C which could be checked on the display of the apparatus. The requirement of test is that all the 6 tablets should disintegrate completely within 15 min.

Table 1: Different compositions of amlodipine besylate tablet formulations

Ingredients	T1	T2	T3
Amlodipine besylate	6.944	6.944	6.944
Avicel PH 101	113.998	109.998	99.998
Mannitol	58.558	58.558	58.558
Kollidon 30	10.000	10.000	10.000
Colloidal silica	2.000	2.000	2.000
Ac-di-sol	6.000	10.000	14.000
Yellow color iron oxide	0.500	0.500	0.500
Magnesium Stearate	2.000	2.000	2.000
Total	200.00	200.00	200.00

Table 2: Different compositions of rosuvastatin calcium tablet formulations

Ingredients	T1	T2	T3
Rosuvastatin Calcium	5.120	5.120	5.120
Lactose Spray dried	13.900	13.900	13.900
Dicalcium Phosphate	9.300	9.300	9.300
Avicel PH 102	108.830	111.830	114.830
Crospovidone	4.500	7.500	10.500
Magnesium Stearate	2.350	2.350	2.350
Total	150.000	150.000	150.000

Table 3: Different compositions of hydrochlorothiazide tablet formulations

Ingredients	T1	T2	T3
Hydrochlorothiazide	12.500	12.500	12.500
Avicel PH 101	94.000	90.000	86.000
Mannitol	73.000	73.000	73.000
Kollidon 30	10.000	10.000	10.000
Colloidal silica	2.000	2.000	2.000
Ac-di-sol	6.000	10.000	14.000
Green lake BBA 2072	0.500	0.500	0.500
Magnesium Stearate	2.000	2.000	2.000
Total	200.00	200.00	200.00

Table 4: Pharmacokinetics models of amlodipine besylate on nine tablet formulations**.**

Amlodipine Besylate					
Tab Sample	Zero order	First order	Higuchi	Korsmeyer-Peppas	Hixon-Crowell
H1	0.9674	0.9631	0.9313	0.943	0.9315
H2	0.9723	0.9604	0.9350	0.949	0.9300
H3	0.9910	0.9918	0.9698	0.833	0.9866
H4	0.9945	0.9821	0.9520	0.976	0.9738
H5	0.9970	0.9889	0.9606	0.937	0.9843
H6	0.9996	0.9822	0.9513	1.029	0.9725
H7	0.9970	0.9933	0.9705	0.863	0.9927
H8	0.9986	0.9816	0.9609	0.942	0.9752
H9	0.9988	0.9746	0.9579	0.965	0.9635

Table 5: Pharmacokinetics models of rosuvastatin calcium on nine tablet formulations

Rosuvastatin Calcium					
Tab Sample	Kinetic Models				
	Zero order	First order	Higuchi	Korsmeyer-Peppas	Hixon-Crowell
H1	0.9978	0.9858	0.9652	0.906	0.9933
H2	0.9986	0.9756	0.9637	0.923	0.9873
H3	0.9932	0.9954	0.9683	0.861	0.9976
H4	0.9977	0.9938	0.9644	0.915	0.9976
H5	0.9903	0.9982	0.9753	0.803	0.9985
H6	0.9985	0.9869	0.9678	0.896	0.9946
H7	0.9981	0.9862	0.9466	1.056	0.9915
H8	0.9978	0.9818	0.9674	0.893	0.9918
H9	0.9923	0.9884	0.9690	0.852	0.9949

Table 6: Pharmacokinetics models of hydrochlorothiazide on nine tablet formulations

Hydrochlorothiazide					
Tab Sample	Kinetic Models				
	Zero order	First order	Higuchi	Korsmeyer-Peppas	Hixon-Crowell
H1	0.9986	0.9806	0.9566	0.974	0.9886
H2	0.9964	0.9803	0.9648	0.903	0.9907
H3	0.9976	0.9946	0.9750	0.744	0.9927
H4	0.9974	0.9891	0.9590	0.949	0.9931
H5	0.9967	0.9872	0.9581	0.950	0.9917
H6	0.9919	0.9963	0.9795	0.784	0.9990
H7	0.9937	0.9917	0.9692	0.850	0.9940
H8	0.9970	0.9702	0.9426	1.083	0.9808
H9	0.9998	0.9810	0.9573	0.982	0.9903

Drug dissolution

For in vitro release of drug, studies were conducted in accordance with "Dissolution procedure" monograph for a time period over 30 min. Dissolution apparatus 2 (Paddle dissolution system) was used (DT6, Erweka, Germany). 900 ml dissolution medium (Phosphate Buffer of 6.8 pH) was used which was maintained thermostatically at 37°C. The buffer was prepared by dissolving about 6.805g of monobasic potassium phosphate and 0.896 g of sodium hydroxide in purified water to produce 1000 ml. The final pH was adjusted to pH 6.8 by adding 0.2N sodium hydroxide (USP 41-NF 36). All the 9 formulations were tested. Drug release was calculated after 30 min. Agilent Caraway UV visible spectrophotometer was used to analyze the release and dissolution of all three active ingredients. The drug release of these tablets was tested in 0.1N HCl solution.

Quantitative assay

Sample Preparation

Accurately weighed 700mg tablet was put in to the volumetric flask and volume make up to 100 ml was done with methanol. Stirring was applied continuously for about 15 minutes. The solution was filtered using 0.45 micron sized membrane filter. The drug content was checked by UV-Vis spectrophotometer and outcome was evaluated.

Standard preparation

10mg, 20mg and 30mg of Amlodipine Besylate, Rosuvastatin Calcium and Hydrochlorothiazide were accurately weighed and were added into 100ml volumetric flask. 100ml methanol was added and the contents were mixed well by using magnetic stirrer. The solution was filtered using 0.45 micron sized membrane filter. The drug content was checked by UV-Vis spectrophotometer and outcome was evaluated.

FTIR

Polymer-polymer and drug-polymer interactions were studied on FTIR spectrophotometer. Sample (2% w/w) was added to potassium bromide and mixed. By using mortar with agitation, the mixture of potassium bromide and sample was grounded to fine powder. This mixture was compressed at hydraulic press at a pressure of 10000 psi to form KBr discs. Each KBr disc was scanned at FTIR apparatus and characteristic peaks were recorded. (Halith *et al*., 2011)

Scanning electron microscopy

All the three layers of final tablet were observed through scanning electron microscopy. For the external morphology studies, the layers were visualized using scanning electron microscope (ZEISS) operated at 15Kv to check external morphology and to determine the particle size of granules in every layer. Coating of platinum/palladium alloy was applied on the samples under vacuum. By using double tape, the coated samples were mounted on the metallic stub. The shape and surface characteristic and particle size of the granules was observed under electron micro analyzer and photographs were taken (Bhandwalkar, Bhandwalkar *et al*. 2015)

Differential scanning calorimetry

Differential scanning calorimeter (DSC7, Perkin-Elmer) was used. The equipment was calibrated using indium and zinc. Samples were heated at 5ºC/min in aluminum pans under nitrogen atmosphere. The onset of the melting points and enthalpies of fusion were calculated by the software (Pyris, Perkin-Elmer). The cell had a nitrogen purge flowing at approximately 20 cm3/min. The cell and sample were held isothermally at -79ºC for 30 min to purge the headspace and sample with nitrogen before heating (Pachori *et al*., 2010; Halith *et al*., 2011)

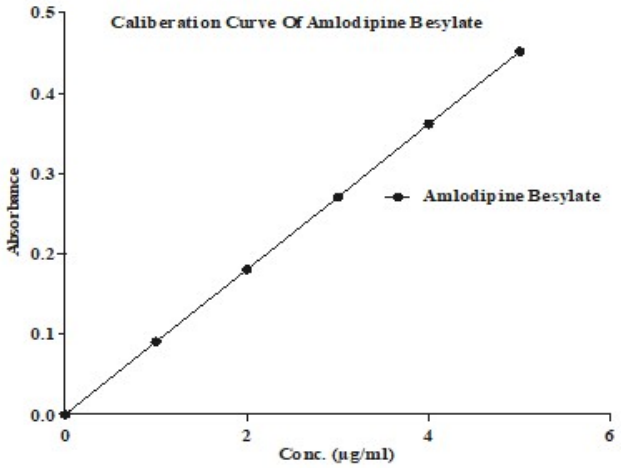

Fig. 1: Calibration curve of amlodipine besylates

RESULTS

Pre-formulation studies

Calibration Curve

Standard curve of API of each drug was determined to estimate the concentration of drug by using UV visible spectrophotometer.

Amlodipine besylate

The absorbance of 237nm was used for Amlodipine Besylate. The UV spectrophotometer was set at the specific wavelength 200-400 nm ranges and maximum absorbance was taken. Then absorbance of all solutions was measured on UV spectrometer.

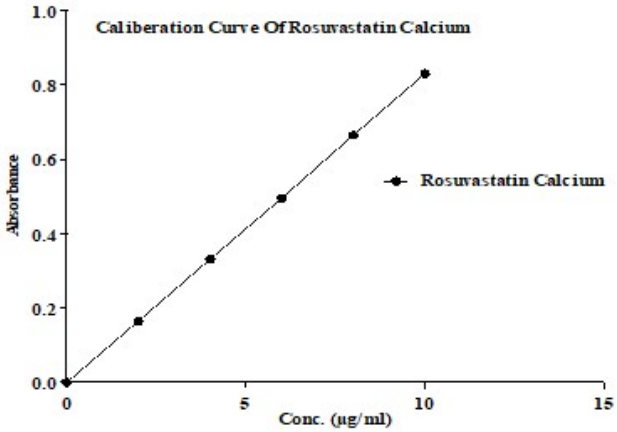

Fig. 2: Calibration curve of rosuvastatin calcium.

Rosuvastatin calcium

The absorbance of rosuvastatin calcium was observed at wavelength 244nm. The UV spectrophotometer was set at the specific wavelength. The UV spectrophotometer was set at the specific wavelength 200-400nm ranges and maximum absorbance was taken. Then absorbance of all solutions was measured on UV spectrometer.

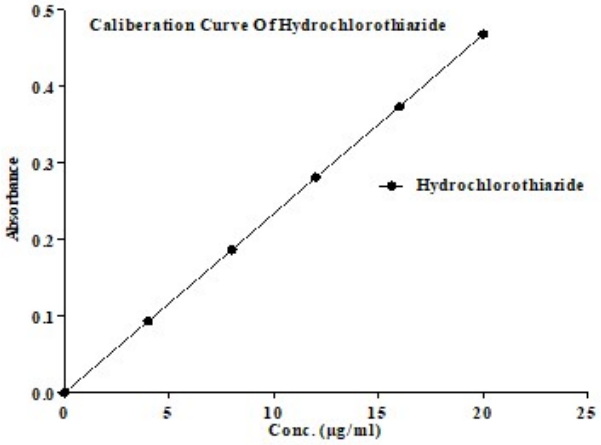

Fig. 3: Calibration curve of hydrochlorothiazide.

Hydrochlorothiazide

The absorbance of 244nm was used for Hydrochlorothiazide. The UV spectrophotometer was set at the specific wavelength 200-400nm ranges and maximum absorbance was taken. Then absorbance of all solutions was measured on UV spectrometer.

Angle of repose

Angle of Repose of final blend of T1, T2 and T3 of Amlodipine Besylate were 31.367º, 29.300º and 29.467º, Rosuvastatin Calcium layers were 32.33º, 30.46º and 30.46º and T1, T2 and T3 of hydrochlorothiazide were

33.73º, 31.200º 32.033º respectively. All showing good flow properties.

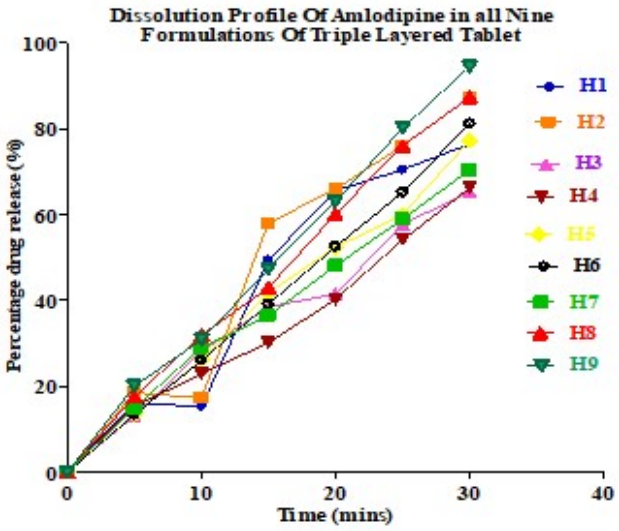

Fig. 4: Dissolution profile of amlodipine.

Loss on drying

Moisture content of Amlodipine Besylate layer and Hydrochlorothiazide layer was checked on IR moisture analyzer. The moisture content of T1, T2, and T3 of Amlodipine Besylate layer was 1.543%, 1.580% and 1.630% respectively. Moisture content of T1, T2 and T3 of Hydrochlorothiazide layer was 1.640%, 1.530% and 1.587% respectively. The acceptance criteria for this test less than 2.0%.

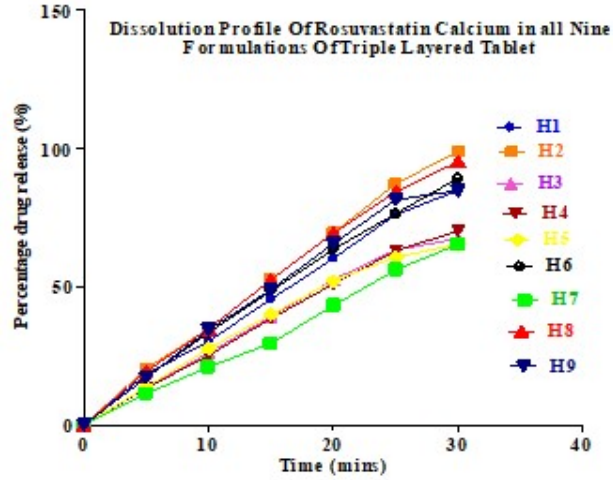

Fig. 5: Dissolution profile of rosuvastatin calcium

Bulk density

Bulk density of T1, T2 and T3 of Amlodipine Besylate layer as 0.490g/ml, 0.467g/ml and 0.530g/ml respectively. Results of bulk density of T1, T2 and T3 Rosuvastatin calcium layer were 0.457 g/ml, 0.567 g/ml and 0.547 g/ml respectively. Bulk density values of T1, T2 and T3 of Hydrochlorothiazide layer were 0.513 g/ml, 0.553 g/ml and 0.510 g/ml respectively. Acceptance criteria for this test are 0.40-0.70 g/ml.

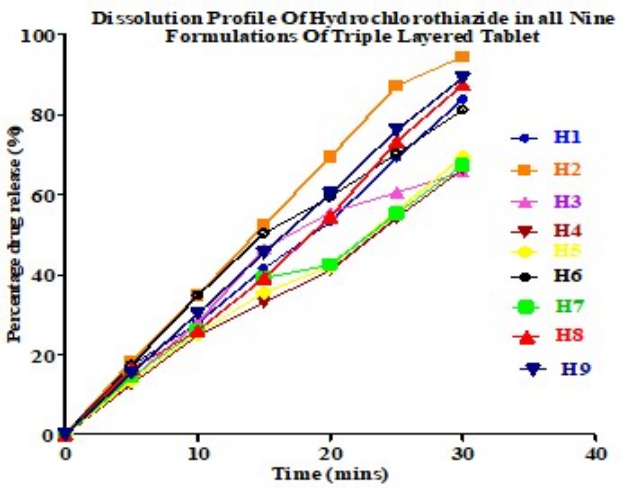

Fig. 6: Dissolution profile of hydrochlorothiazide

Tapped density

Tapped density values of T1, T2 and T3 of Amlodipine Besylate were 0.513g/ml, 0.510g/ml and 0.510g/ml, T1, T2 and T3 of Rosuvastatin calcium were 0.497g/ml, 0.597g/ml and 0.587g/ml and T1, T2 and T3 Hydrochlorothiazide were 0.567g/ml, 0.587g/ml and 0.587g/ml respectively. All fall within the acceptance limit of 0.50-0.80gml.

Compressibility index

Compressibility Index values of T1, T2 and T3 of Amlodipine Besylate layer were 1.153, 1.180 and 1.180, T1, T2 and T3 of Rosuvastatin calcium were 1.167, 1.187 and 1.157 respectively. Acceptance criteria for CI of all the samples was 1.10-1.34.

Hausner Ratio

Hausner Ratio for T1, T2, and T3 of Amlodipine Besylate was 1.153, 1.180 and 1.180 respectively, which indicates good flow properties as the values fall in the range of 1.12-1.18. Hausner Ratio for T1, T2, and T3 of Rosuvastatin Calcium were 1.177, 1.177 and 1.187. This also represents good flow properties of Final blend of Rosuvastatin Calcium layers. Hausner Ratio of T1, T2, and T3 of Hydrochlorothiazide were 1.167, 1.187 and 1.157 which shows good flow ability of all the three layers of hydrochlorothiazide as well.

Weight variation

Weight variation Test was performed after compression. 09 different tablet combinations from H1 to H9 were made. Tablet weight was set at 550mg. Acceptance limit was 512-590mg. Average weight of 10 tablets of all samples from H1 to H9 was in limit.

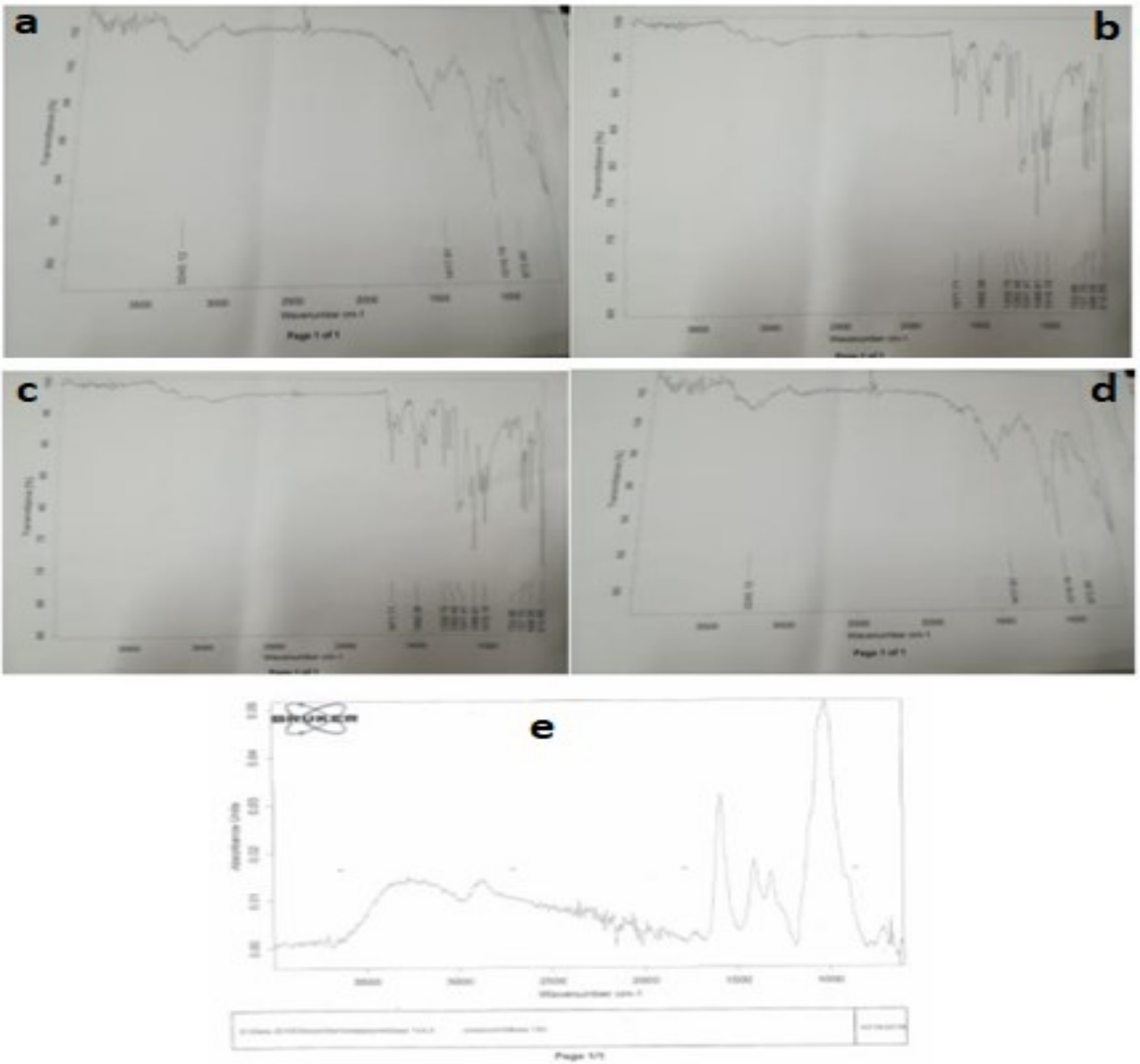

Fig. 7: FTIR of (a) Triple Layer Tablet, (b) Amlodipine Besylate, (c) Rosuvastatin Calcium, (d) Hydrochlorothiazide, (e) Croscarmellose sodium

Scanning electron microscopy

Amlodipine Besylate

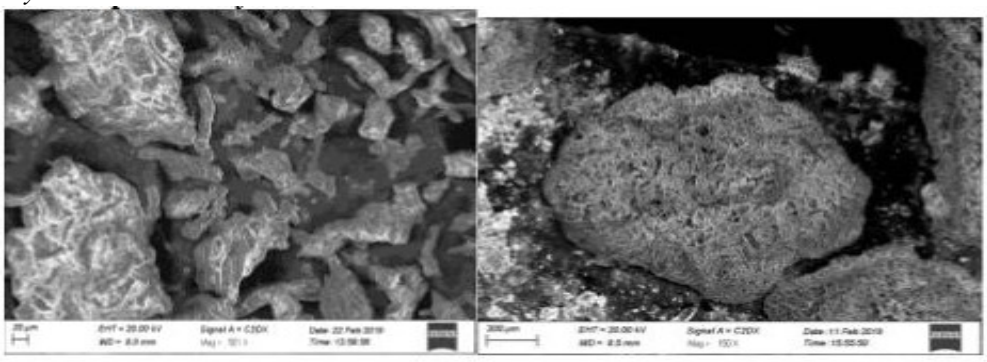

Fig. 8: SEM analysis of amlodipine besylate

Friability

Average results of Friability of H1, H2, H3, H4, H5, H6, H7, H8, H9, were 0.773%, 0.667%, 0.840%, 0.650%, 0.630%, 0.673%, 0.620%, 0.667%, 0.880% respectively. As the passing criteria of Friability test is ≤1%, therefore friability results of all tablet formulations fall within acceptance limit.

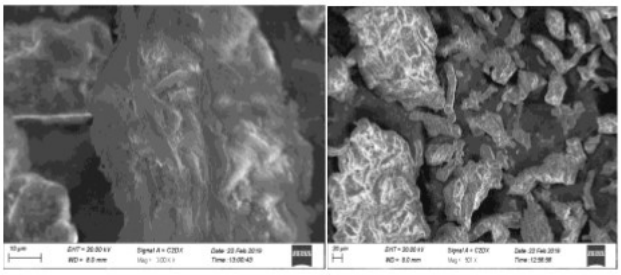

Fig. 9: SEM Analysis of rosuvastatin calcium

Hydrochlorothiazide

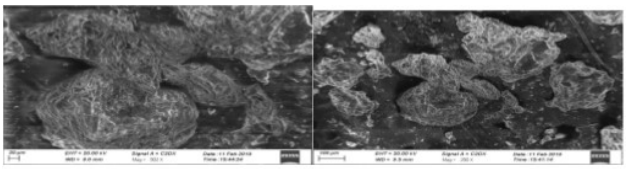

Fig: **10**: SEM Analysis of hydrochlorothiazide

Disintegration test

In nine different combinations of triple layered tablets, disintegrant was present in different concentrations. H3 had the highest percentage of disintegrating agent (7%) and H1 had the lowest amount of disintegrating agent (3 %). Average disintegration time of all tablet formulations H1, H2, H3, H4, H5, H6, H7, H8 and H9 were 15.7, 7.56, 5.3, 14.44, 10.046, 10.4, 11.293, 9.290, 7.297 and 6.237 min respectively.

Dissolution profile of amlodipine besylate

Graph has been plotted between time and percentage release. Dissolution results of amlodipine layers in of all formulations from H1 to H9 were 76.45%, 87.36%, 65.32%, 66.23%, 77.23%, 81.20%, 70.44%, 87.36%, 94.49% respectively. H9 exhibited best dissolution rate.

In vitro release data of amlodipine besylate according to various release kinetic models has been shown in table 4.

Dissolution profile of rosuvastatin calcium

Graph has been plotted between time and percentage release. Dissolution results of Rosuvastatin layer in of all formulations from H1 to H9 were 84.79%, 98.85%, 67.41%, 70.21%, 65.34%, 89.23%, 65.34%, 95.23%, 84.79% respectively.

In vitro release data of rosuvastatin calcium according to various release kinetic models has been mentioned in table 5.

Dissolution profile of hydrochlorothiazide

Graph has been plotted between time and percentage release. Dissolution results of hydrochlorothiazide in of all formulations from H1 to H9 were 83.84%, 94.49%, 65.64%, 66.45%, 69.50%, 81.20%, 67.41%, 87.49%, 89.23% respectively.

In vitro release data of hydrochlorothiazide according to various release kinetic models has been shown in table 6.

FTIR

FTIR analysis of triple layered tablet and all the three active ingredients of formulation were performed. Individual FTIR curves of APIs was different from that of the triple layered tablet. In FTIR scan of triple layered tablet, peaks were found at 14781.4, 1019.19 and 873.90. FTIR scan of individual APIs do not have peaks at these wavenumbers.

DISCUSSION

The calibration curves of all the three APIs shows that with an increase in concentration an increase in absorbance values was found which exhibits linear relationship. Not much variation is found in the values of angle of repose in all the layers which indicate that change in amount of disintegrating agent does not affect angle of repose.

Similarly, the results of Bulk density, tapped density, Carr's index, Hausner's ratio, were also found within acceptable range which indicated that change in disintegrant concentration has no remarkable effect on these tests. This is in accordance with the literature in which no profound effect on preformulation studies was observed by change in disintegrant concentration (Bhargavi *et al*. 2013).

The results of the loss on drying are falling within the acceptance range. Therefore, it can be assumed that there is no effect on the moisture content if the concentration of the disintegrant is changed.

Results of in process test of tablets i.e., weight variation, hardness, thickness, friability were also within limit. However, the data of disintegration test shows that H1 failed to disintegrate within the required time i.e. 15 min and H3 had the lowest disintegration time. This shows that increasing the amount of disintegrating agent decreases the disintegration time (Repka *et al*. 2007).

In dissolution test of amlodipine besylate H9 exhibited best dissolution rate. Graph represents that almost 50 % dissolution occurred within first 15 min of test. The release rate continued to increase up to 30 min. Data shows that amlodipine layer showed immediate release behavior as drug release occurred within 30 min time. The

Differential scanning calorimetry

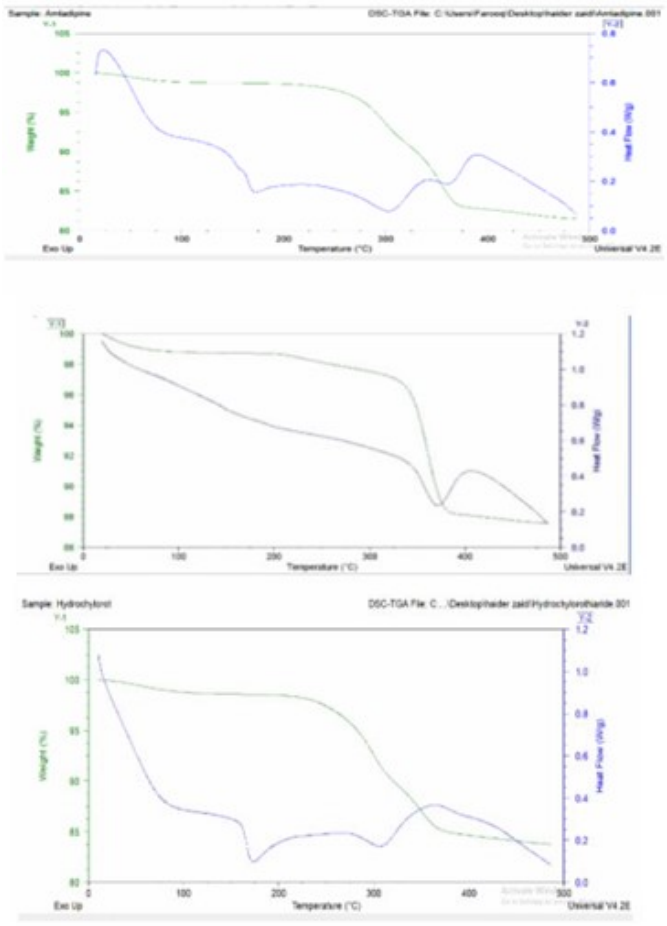

Fig. 11: DSC thermogram of amlodipine besylate, rosuvastatin calcium and hydrochlorothiazide

release was fitted to both zero order and First Order. It was found that all the formulations followed Zero Order Kinetics which indicates that drug release rate is independent of concentration. When the release was fitted to Krosmeyer Pappas model determine the value of diffusion exponent (n), it was found that all formulations except H8 followed non Fickian release. Only H8 followed Super Case II release.

In dissolution test of rosuvastatin calcium H2 exhibited best dissolution rate. Graph represents that almost 50 % dissolution occurred within first 15 min of test. The release rate continued to increase up to 30 min. Data shows that amlodipine layer showed immediate release behavior as drug release occurred within 30 min time. The release was fitted to both zero order and First Order. It was found that all the formulations followed Zero Order Kinetics. When the release was fitted to Krosmeyer Pappas model to determine the value of diffusion exponent (n), it was found that all formulations except H7 followed non-Fickian release. Only H7 followed Super Case II release.

In the dissolution test of Hydrochlorothiazide H2 exhibited best dissolution rate. Graph represents that almost 50 % dissolution occurred within first 15 min of test. The release rate continued to increase up to 30 min. Data shows that hydrochlorothiazide layer showed immediate release behavior as drug release occurred within 30 min time. The release was fitted to both zero order and First Order. It was found that all the formulations followed Zero order Kinetics. When the release was fitted to Krosmeyer Pappas model determine the value of diffusion exponent (n), it was found that all formulations except H8 followed non-Fickian release. Only H8 followed Super Case II release.

FTIR results indicate that active ingredients are not compatible with each other in the form of a mixture; rather they can only be combined in the form of layers. Therefore, triple layered tablet is the most suitable option for this combination of active ingredients.

SEM results of amlodipine besylate indicate that particles found are spherical or round in shape. Particle size was determined through calibrated scale mentioned below the

images. Particle size ranges from 1000µm to 1250µm. The variation in particle sizes is very small. Reason behind small variation of particle size is that the grains formulated after wet granulation were passed through same mesh.

SEM results of rosuvastatin calcium layer show that Particles are oblong shaped. Particle size ranges from 20µm to up to 400 µm size. As the particle size is small it indicates that very fine grains were formed after dry granulation. Large particles are of rosuvastatin calcium as it is a granular material. Small particles are of excipients.

SEM results of hydrochlorothiazide show that particles are spherical in shape. Originally hydrochlorothiazide exists in lamellar crystals however this characteristic feature of hydrochlorothiazide disappeared in formulation which indicates change of hydrochlorothiazide crystal structure during the granulation process (Natrajan *et al*. 2010). Large sized spherical particles are uniformly distributed size of particles. Particle size ranges from 2000µm to 3000µm. large sized particles indicate that binding of drug and excipients was good and large grains were formed after wet granulation.

Thermal behavior of amlodipine layer shows that, characteristic thermal events like water loss, melting and decomposition have occurred during DSC test. Melting point of amlodipine is 205ºC (Nikolić *et al*., 2010). DSC thermogram of amlodipine layer should exhibit endothermic peak within this temperature range. However, the thermogram shows a broadened endothermic peak at 60 to 100ºC which represents water loss from drug polymer matrix. An endothermic peak is found at 160ºC which clearly shows the melting of mannitol because at this temperature mannitol start to melt. Other endothermic peaks are found at 300ºC and 360ºC temperatures which shows shift in position of endothermic peak of Amlodipine. Finally, an exothermic peak is found at about 400ºC which exhibits decomposition of drug at this temperature.

DSC studies of rosuvastatin calcium layer were conducted to observe any interaction in drug and excipients. Melting point of rosuvastatin calcium is 147-156°C. DSC thermogram of rosuvastatin layer should exhibit endothermic peak within this temperature range. However, a sharp endothermic peak was observed at about 375°C which shows shift in the position of endothermic peak of rosuvastatin calcium. The results suggest that rosuvastatin got entrapped in excipients and existed in amorphous state.

Thermal behavior of Hydrochlorothiazide layer shows that characteristic thermal events like water loss, melting and decomposition have occurred during DSC test. Melting point of hydrochlorothiazide is 274ºC. If DSC test of pure hydrochlorothiazide was conducted it would certainly give a sharp endothermic peak at this temperature. But in this study DSC test of Hydrochlorothiazide layer was conducted which contained API as well as excipients. Therefore, the thermogram shows a broadened endothermic peak at 60ºC to 100ºC which represents water loss from drug polymer matrix. An endothermic peak is found at 160ºC which clearly shows the melting of mannitol because at this temperature mannitol start to melt. Other endothermic peak is found at 320ºC which shows shift in position of endothermic peak of hydrochlorothiazide. Finally, an exothermic peak is found at about 375ºC which exhibits decomposition of drug at this temperature.

CONCLUSION

Formulation development of triple layer tablets were fabricated by or polymers along with APIs i.e., amlodipine besylate, rosuvastatin calcium and hydrocholorothiazide. *In vitro* studies of granules were performed to determine angle of repose, loss on drying, bulk density, tapped density and hausner ratio. Results of all these parameters are quite in range, which showed that change in disintegrant concentration does not affect the flow ability of granules to much extent. After compression tablets were further subjected to physicochemical investigation like weight variation, hardness, thickness, friability, disintegration, dissolution studies and FTIR. Drug release data of all formulations were studied which showed that all the formulations exhibited First order kinetics. FTIR analysis revealed that those active ingredients are not compatible with each other in the form of a mixture; rather they can only be combined in the form of layer. Formulation H8 had the best results in terms of physicochemical properties, assay and dissolution studies. Hence it can be concluded that this research proved to an ideal product in terms of patient compliance and cost effectiveness can be developed.

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REFERENCES

Ahsan SF and Khan MF (2017). Physicochemical properties and pharmacology of amlodipine besylate: A brief review. *BJHS*, **20**(2): 27-31.

Al-Hashemi HMB and Al-Amoudi OSB (2018). A review on the angle of repose of granular materials. *Powder Technology*, **330**(8): 397-417.

Bajaj T and Giwa AO (2020). Rosuvastatin. StatPearls [Internet].

Battu SK, Repka MA, Majumdar S and Madhusudan RY (2007). Formulation and evaluation of rapidly disintegrating fenoverine tablets: effect of superdisintegrants. *Drug Dev. Ind. Pharm.*, **33**(11): 1225-1232.

Bhandwalkar OS, Bhandwalkar MU, Ghadge DM, Dhekale PS and Jamdae UK (2015). Design and development of fast dissolving tablets of hydrochlorothiazide and atenolol co-crystals. *IJPSR.*, **6**(10): 4368.

Bowker MJ and Stahl PH (2008). Preparation of water-soluble compounds through salt formation. The practice of medicinal chemistry, Elsevier, pp.747-766.

Dahima R, Pachori A and Netam S (2010). Formulation and evaluation of mouth dissolving tablet containing amlodipine besylate solid dispersion. *Int. J. ChemTech. Res.,* **2**(1): 706-715.

Harish G, Bhargavi C, Riyajune A, Yasmeen M, Mohammed S, Krishna CM, Jilani S and Nirajana VA (2013). Effect of different disintegrants on ciprofloxacin conventional tablets. *IJRPB,* **1**(3): 281.

Islam MK, Islam R, Akter R, Rahman MH and Shapla NM (2018). An innovative approach of special layered tablet technology. *World J. Pharm. Pharm. Sci.*, **7**(11): 237-246.

Jayaprakash S, Halith SM, Pillai KK, Balasubramaniyam P, Firthhouse PUM and Boopathi M (2011). Formulation and evaluation of bilayer tablets of amlodipine besilate and metprolol succinate. *Der. Pharmacia. Lettre.,* **3**(4): 143-154.

Kapor A, Nikolic V, Nikolic L, Stankovic M, Cakic M, Stanojevic L and Illic D (2010). Inclusion complexes of amlodipine besylate and cyclodextrins. *Open Chemistry,* **8**(4): 834-841.

Nirmal J, Saisivam S, Peddanna C, Muralidharan S, Godwinkumar S and Nagarajan M (2008). Bilayer tablets of atorvastatin calcium and nicotinic acid: Formulation and evaluation. *Chem. Pharm. Bull,* **56**(10): 1455-1458.

Panneerselvam M, Natrajan R, Selvaraj S and Rajendran NN (2010). A novel drug-drug solid dispersion of hydrochlorothiazide losartan potassium. *Int. J. Pharma Bio Sci*, **1**(4): 68-80.

Quan A, Chavanu K and Merkel J (2006). A review of the efficacy of fixed-dose combinations olmesartan medoxomil/hydrochlorothiazide and amlodipine besylate/benazepril in factorial design studies. *Am. J. Cardiovas Drugs,* **6**(2): 103-113.

Salústio PJ, Inácio C, Nunes T, Silva JPSE and Costa PC (2020). Flow characterization of a pharmaceutical excipient using the shear cell method. *Pharm. Dev. Technol.,* **25**(2): 237-244.

Monographs, NPG. United States Pharmacopeia and Nafional Formulary USP 41-NF 36. The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2018

Van den Ban S and Goodwin DJ (2017). The impact of granule density on tabletting and pharmaceutical product performance. *Pharm. Res.,* **34**(5): 1002-1011.