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# Design and Evaluation of a New Fixed-Dose Immediate Release Capsule of Atenolol, Enalapril Maleate and Hydrochlorothiazide

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors NAP and ASA designed the study, performed the statistical analysis and together with author DAB wrote the protocol. Authors NAP and ASA wrote the first draft of the manuscript. Authors NAP, ASA and DAB managed the analyses of the study. Authors NAP, EBM and ASA managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim and Objectives:** To design, formulate and perform *in vitro* studies of a fixed dose combination (FDC) immediate release solid dosage form of the antihypertensive drugs atenolol, enalapril and hydrochlorothiazide. The objectives of the study were to perform pre-formulation studies, design an immediate-release FDC capsule dosage form, and evaluate the prepared dosage form.
**Methods:** Differential Scanning Calorimetric (DSC) analysis of physical mixtures of drugs and drug-excipient combinations was used to assess compatibility. Binary mixtures of atenolol and

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enalapril form a eutectic mixture indicating that a tablet dosage form, in which the drugs would be highly compressed together, may not be stable. Hard gelatin shell encapsulation was therefore employed. Atenolol and enalapril were separately dry-granulated with hydrochlorothiazide and excipients and mixed together at appropriate therapeutic proportions and encapsulated in size 1 hard gelatin capsules (HGC). Preliminary screening produced five formulations each with similar proportions of the 3-drug FDC but with 5 different levels of disintegrant. Capsule weight variation, disintegration time and dissolution rate were determined as output variables, following USP procedures. For the dissolution studies, a reverse phase high-pressure liquid chromatographic (RP-HPLC) method was developed for simultaneous analysis of the three drugs.

**Results:** Granules possessed good fluidity (compressibility index 8.53 to 11.63 and tapped bulk density 1.09 to 1.132). Capsule disintegration time was generally $\leq$ 2 minutes while 80% of each drug dissolved within 20 minutes. Capsules showed no sign of physical instability or adverse effect during a period of 1 year shelf storage at room temperature (average temperature 25°C).

**Conclusions:** Granules possess adequate fluidity and compressibility for filling into HGC. Results generally indicated that an immediate release FDC capsule of atenolol, enalapril and hydrochlorothiazide was stable for more than 1 year of observation. Capsule disintegration time and dissolution rate were generally within the USP specification.

*Keywords: Atenolol; enalapril; hydrochlorothiazide; fixed-dose combination; eutectic mixture.*

## 1. INTRODUCTION

Hypertension is considered a major contributing factor to death and a heavy health care burden globally [1]. Consistent elevation of blood pressure (BP) leads to progression of abnormalities of the heart, kidneys, brain and blood vessels. This leads to premature development of comorbid diseases such as heart failure, post myocardial infarction, diabetes, chronic kidney disease, recurrent stroke, kidney failure, visual impairment and cardiovascular diseases due to target organ damage [1-3]. The choice of agents used in treatment of elevated BP is greatly influenced by co-morbid conditions. Patients with or without comorbidities often require combinations of specific antihypertensive agents [4], since evidence suggests that combination of drugs tend to be more efficacious than individual components [5]. Currently and in accordance with the JNC8, at least two or more antihypertensive agents are being used to achieve target blood pressure readings [6] ultimately increasing the complexity of drug regimen. This complexity is sometimes associated with poor adherence to therapeutic regimen, which is a major factor affecting patients with hypertension with comorbid conditions.

Initial treatment should begin with one of four primary drug classes: thiazide-type diuretics, calcium channel blockers (CCB), angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARBs). The latter two classes should not be used in the same patient [4] as evidence has shown that such combination does not produce any marked improvement in symptoms or quality of life of patients [7]. The Eighth Joint National Committee (JNC 8) guideline for the management of high blood pressure in adults recommends that therapy be assessed within a month of treatment. If treatment goals are not achieved, a second drug from the four primary classes is added to the drug regimen. If BP goals are still not achieved, a third drug from the four classes is added [4]. Other drug classes may be added as needed if BP goals are not achieved with a three drug regimen [4]. The rationale for combination therapy is to increase the antihypertensive effectiveness by utilizing drugs with differing mechanisms of action that are complementary to each other [3]. One such combination comprises hydrochlorothiazide (thiazide diuretic) and bisoprolol (β blocker) with an established outcome of benefits in the treatment of hypertension. The benefit of this combination lies in the fact that bisoprolol suppresses rennin and angiotensin II production resulting in fluid and sodium retention. Sodium retention is reversed by the hydrochlorothiazide resulting in a greater reduction in blood pressure than if both ingredients were given separately [3]. Similarly, the initial treatment of heart failure or a previous myocardial infarction includes a beta blocker and ACEI. A modest effect on left ventricular remodeling may be achieved by the ACEI while the beta-blocker produces substantial improvement in ejection fraction. Beta-blockers

are anti-ischaemic and effective in reducing the risk of sudden cardiac death, thereby reducing overall mortality rate [8].

Fixed dose combination (FDC) products decrease medication non-compliance issues by combining drugs of different pharmacological classes in one dosage form, [9] reducing the complexity of drug regimen and decreasing the pill burden. Such designs have improved compliance issues and produced favorable clinical outcomes [10] by reducing the risk of medication non-compliance by 24% to 26% [11]. There are many such combinations on the market that have proven to be quite beneficial. An FDC is a physically inseparable delivery of multiple products (usually active pharmaceutical ingredients, API) that is contained in a dosage form in fixed proportions [9,12]. It may also extend to any type of dosage form such as liquids and is not limited to tablets and capsules [13].

Many formulations and manufacturing issues may arise when combining different APIs in a unit solid dosage form such as a tablet or capsule. Thorough investigation must be done to ensure that, upon mixing the formulation ingredients, there are no interactions between drugs and excipients that could compromise product stability and effectiveness [14]. Common compatibility issues observed in combination drug products include drug-drug chemical interactions, drug-excipient interactions and drug-excipients-drug interactions. Manifestations include physical instability resulting in changes in the physical appearance of the product (e.g. precipitation, color changes); chemical instability exhibited as changes in drug content and presence of impurities; and functional instability resulting in changes in the release of the drug [15].

Other challenges such as the development of new analytical procedures may arise due to differing physical and chemical properties of each component such as wavelength maxima and log P values [13,16]. Such procedures are needed to assess potency, uniformity, quality and purity for multiple drugs in the presence of excipients.

Several analytical methods have been used to determine compatibility issues between formulation ingredients. One such method is thermal analysis. This method of analysis is cost effective and requires less time per run when compared to other methods such as isothermal stress testing, which generally give indications only of chemical, and not physical stability issues [17,18]. Differential scanning calorimetry (DSC) is a thermal analytical technique used to rapidly evaluate possible incompatibilities, such as eutectic formation in physical blends of solid ingredients. Incompatibility challenges may be addressed once the cause is clearly identified. Physically separating the incompatible ingredients using techniques such as microencapsulation, pelletizing of particles, coating, granulation, encapsulation and multilayered tablets designs [15] is often employed to circumvent such challenges.

Many classes of drugs are used in various combinations to effect better clinical outcomes. It has been proven that combination therapies provide superior BP control than monotherapy. The use of FDC therapies tend to show fewer side effects with added therapeutic effects. In many cases, drugs of FDC products are usually administered at lower doses resulting in fewer side effects experienced, thereby maximizing potential benefits. Mahmud and Feely have reported that the administration of a FDC product of low doses of the drugs amlodipine, atenolol, bendrofluazide and captopril was more efficacious than a standard single dose of each agent individually [19]. Additionally, FDC products of enalapril and hydrochlorothiazide have been approved by the Food and Drug Administration at starting dose of 5 mg and 12.5 mg respectively to maximum dose levels of 20 mg to 50 mg respectively. The FDA approved combination product of atenolol and the thiazide-like diuretic Chlorthalidone was approved for hypertension at a starting dose 50 mg and 25 mg respectively. Three drug based FDC products recently approved by the FDA for hypertension contain drugs from the primary drug classes which include hydrochlorothiazide at a starting dose of 12.5 mg in all three products [20].

As such, the aim of this study was to formulate and perform in vitro studies of a fixed dose combination of an immediate release dosage form of the antihypertensive drugs atenolol, enalapril maleate and hydrochlorothiazide. Although preliminary studies were not done to establish optimal potency levels of each drug, the amount of each drug was chosen based on dose levels for hypertension as recommended by established clinical dosing guidelines. Atenolol (ATL) is a beta adrenergic blocking agent that is used in the treatment of hypertension, ischemic

heart disease, arrhythmias and myocardial infarction. The usual oral dose is 50-100 mg daily as a single dose. The fixed dose used in this formulation was 50 mg per dosage unit [21]. ATL is chemically described by the British Pharmacopoeia (BP) as a benzene acetamide, 4-[21 – 4icromer – 31 – [(1- methyl ethyl) amino] propoxy] [22].

Enalapril maleate (ENL) is an angiotensin converting enzyme (ACE) inhibitor that blocks angiotensin converting enzyme (ACE) producing vasodilation and reduces peripheral resistance [23]. The fixed dose for this formulation was 10 mg per dosage unit. An initial oral dose for hypertension of 5 mg daily with a maintenance dose of 10-20 mg daily [21] is often suggested. According to the BP the chemical description of ENL is (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid (*Z*)-butenedioate.

Hydrochlorothiazide (HCTZ) is a thiazide diuretic that is used in the treatment of edema and hypertension. It promotes water and electrolyte excretion via the kidney. The initial dose of 12.5 mg is often prescribed titrating to maintenance dose of up to 25-50 mg daily either alone or in combination treatment with other antihypertensives [21]. Hydrochlorothiazide is chemically described as 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide [22]. The fixed dose that was used in this formulation was 12.5 mg per unit dosage unit. . The structural formula for each of the three drugs is as shown in Fig. 1.

The objectives of this study were to perform pre-formulation studies on the chosen APIs and usual formulation excipients using Differential Scanning Calorimerty techniques, to design an immediate release fixed-dose combination dosage form (tablet) of atenolol, enalapril maleate and hydrochlorothiazide, to evaluate the fluidity and compactibility of the formulation, to evaluate the physical properties of the formulated dosage form and to evaluate the *in vitro* drug release properties of the formulation.

## 2. METHODOLOGY

### 2.1 Materials and Reagents

The ingredients atenolol, enalapril maleate and hydrochlorothiazide used in this study were purchased from Medical Export Co. Ltd. (Northants, UK). The British Pharmacopoeia Chemical Reference Substance (BPCRS) atenolol, enalapril maleate and hydrochlorothiazide were purchased from British Pharmacopoeia Commission Laboratory. The formulation excipients used were: spray dried lactose (Pharmco-AAPER), sodium stearyl fumarate (JRS Pharma) and sodium starch glycolate (JRS Pharma, Viva Star).

### 2.2 Compatibility Studies

#### 2.2.1 Differential scanning calorimetry

A Mettler Toledo Differential Scanning Calorimeter (DSC, model TC 15 TA controller, USA) was used. A sample of approximately 4-5 mg was weighed directly in a pierced aluminum 40 µL pan. The lid was crimped and the pan was placed in the sample holder; a sealed empty aluminum pan was placed in the reference holder. Samples were heated at a rate of 10°C/min, from 50°C to 300°C, under a dynamic nitrogen atmosphere. Thermograms were produced were examined.

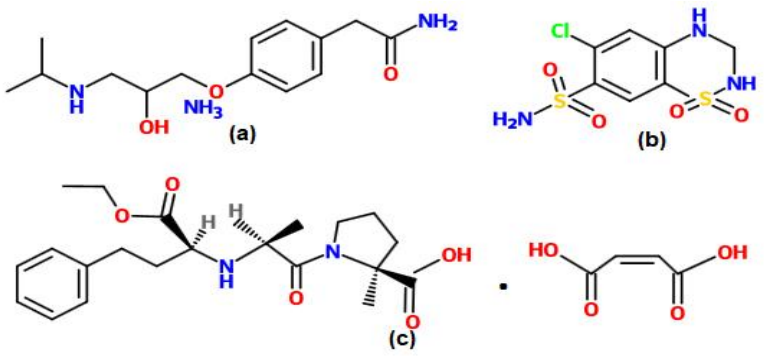


**Fig. 1. Structure of (a) Atenolol (ATL), (b) Hydrochlorothiazide (HCTZ) and (c) Enalapril Maleate (ENL)**

*2.2.1.1 Sample preparation*

A 1:1 binary mixture ratio of drug to drug and a ternary mixture ratio of all three drugs were prepared. In addition, a 1:1 binary mixture ratio of each API to each excipient of the formulation was prepared. Approximately 300 mg of each mixture blend were compressed using Carver hydraulic press (force of 3 tons, dwell time 1 minute). Samples were left for several hours. Compacts obtained were size reduced and weighed aliquots placed in aluminum pans and scanned and thermograms compared with that of pure ingredients.

## 2.3 Preparation of Immediate Release Capsules

### 2.3.1 Preparation of granules

Amounts of each ingredient per capsule are shown in Table 1. Each formulation was prepared in two equal portions, A and B. Portion A (60 g) comprised of atenolol and formulation excipients while portion B (60 g) contained enalapril maleate, hydrochlorothiazide and formulation excipients. Ingredients for both portions (except lubricant) were weighed and dry blended separately for ten minutes. Lubricant was then added and mixed for an additional three minutes.

Powder blend for each portion was compressed at 4 tons using a single punch Carver press and a round flat-faced 13 mm die (Perkin Elmer) with dwell time 1 minute. Slugs obtained were stored in tightly closed glass container for 48 hours. They were size reduced in a porcelain mortar and size separated using size 653 µm sieve. Granules of both portions A and B were then combined by dry blending for 5 minutes with lubricant.

### 2.3.2 Evaluation of granules

*2.3.2.1 Micromeritic properties of granules*

The following micromeritic properties were evaluated using standard methods of the United States Pharmacopoeia [24]. These include bulk and tapped density, Carr's index and Hausner's ratio.

$$\textit{Bulk Density} = \frac{\boldsymbol{weight\ of\ the\ blend\ (g)}}{\boldsymbol{bulk\ volume\ of\ the\ blend\ (mL)}} \quad (1)$$

$$\textit{Tapped Density} = \frac{\boldsymbol{weight\ of\ the\ blend\ (g)}}{\boldsymbol{Tapped\ volume\ of\ the\ blend\ (ml)}} \quad (2)$$

$$\textit{Carr's Index (CI)} = \frac{\boldsymbol{Tapped\ Density - Bulk\ Density}}{\boldsymbol{Tapped\ Density}}\ \boldsymbol{x}\ 100\% \quad (3)$$

$$\textit{Hausner's Ratio} = \frac{\boldsymbol{Tapped\ Density}}{\boldsymbol{Bulk\ Density}} \quad (4)$$

*2.3.2.2 Flow through orifice testing*

A truncated glass funnel 5.8 cm in diameter, was mounted at a height of approximately 12.7 cm above a top loading balance using a clamp and stand and a glass beaker used to collect the granules. Orifices with four (4) different diameters were placed at the opening of the funnel which was blocked by means of an adjustable shutter which remained closed during the filling process. A sample of approximately 10 g was allowed to flow through the funnel. Time taken for the powder to flow through each orifice was recorded. The flow rate was calculated using the following equation:

$$\textit{Flow rate} = \frac{\boldsymbol{mass\ (g)}}{\boldsymbol{Time\ (sec)}} \quad (5)$$

*2.3.2.3 Moisture content of powder blend*

The moisture content of the formulated granules was obtained by determining the loss in weight (% w/w) resulting from water and any volatile material that may be removed by heat. A Fisher Scientific (Isotemp®, Model 281A) vacuum oven was used in this test. Weighed samples of granules, approximately 2 g, were placed in a glass vial that was previously dried and weighed. Vials were dried at the same temperature used in this test. The vials were placed in the vacuum oven and the samples were dried at 60°C. The weight of the samples was recorded until a constant value was obtained. This method was adapted from a method in the literature and percentage loss on drying was obtained using the equation following [25]. The test was done in triplicate.

$$\%\ Loss\ on\ drying = \frac{\boldsymbol{W2 - W3\ (or\ Wn)}}{\boldsymbol{W2 - W1}}\boldsymbol{x}\ \boldsymbol{100\%} \quad (6)$$

Where:

W1 = Weight of empty bottle (g)
W2 = Weight of bottle with sample (g) before drying
W3 = Weight of bottle with sample (g) after drying (as time specified)
Wn= Weight of bottle with sample (g) after additional 1 hour drying (or constant weight)

**Table 1. Compositions of capsule formulations**

| Formulation | ATL (mg) | ENP (mg) | HCTZ (mg) | SSG (mg) | SSF (mg) | SDL (mg) | Total |
|---|---|---|---|---|---|---|---|
| F1 | 50 | 10 | 12.5 | 4 | 2 | 321.5 | 400 mg |
| F2 | 50 | 10 | 12.5 | 8 | 2 | 317.5 | 400 mg |
| F3 | 50 | 10 | 12.5 | 12 | 2 | 313.5 | 400 mg |
| F4 | 50 | 10 | 12.5 | 16 | 2 | 309.5 | 400 mg |
| F5 | 50 | 10 | 12.5 | 20 | 2 | 305.5 | 400 mg |

*Note. ATL = atenolol, ENL = enalapril maleate, HCTZ = hydrochlorothiazide, SSG= sodium starch glycolate, SSF= sodium stearyl fumarate, SDL = spray dried lactose*

*2.3.2.4 Encapsulation of granules*

The granules obtained were encapsulated by manually filling size #1 capsules using a manual Cap. M. Quick size 1/0 capsule filling machine with tamper and filler unit (Empty Capsule Co. Ltd., USA, #055871) with capacity to fill 50 capsules per filling. A sample of 100 capsules was prepared per formulation and stored in air tight glass containers.

**2.3.3 Evaluation of formulated capsules**

*2.3.3.1 Disintegration test*

One capsule was placed in each of the six tubes of the basket-rack assembly with supported guided disc. The disintegration medium (distilled water) was placed in a 1000 mL beaker that was placed in thermostatic water capable of maintaining the bath at 37 (± 2)°C. Time taken for the capsules to disintegrate completely, leaving only a soft non-palpable mass, was recorded [24].

*2.3.3.2 Weight variation test*

Twenty capsules were randomly selected and weighed individually using an analytical balance taking care to preserve the identity of each capsule. The individual weights were recorded. The shells were then carefully emptied and individually weighed and recorded. The net weight of the contents of each capsule was calculated by subtracting the corresponding weight of the capsule shells. The mean weight of the individual capsule content and the standard deviations were calculated using the equation following [26]:

$$\textit{Weight variation} = \frac{(W_{avg} - W_{individ}) x\ 100}{W_{avg}} \quad (7)$$

Where:

$W_{avg}$ = Average weight of capsule content

$W_{individ}$ = Individual weight of capsule content

The deviation of individual capsule content from the mean was then determined and compared with the acceptance value as outlined in the USP (2006) method for uniformity of dosage units. The requirements for dosage uniformity are met if the weight deviation is less than 15% [24].

*2.3.3.3 In vitro dissolution test*

A six station VanKel 700, Alliance Analytical, USA, dissolution machine (USP Apparatus 1) with stainless steel basket with stirring element at 100 rpm was used. Dissolution medium comprised 900 mL of 0.1 M hydrochloric acid maintained at 37 (± 0.5)°C. A dosage unit was placed in the dry basket, which was carefully lowered into the medium. Samples (10 mL) were withdrawn at time points 1, 5, 10, 15, 20, 30, 45 and 60 minutes, immediately replaced with fresh dissolution medium, filtered (0.45 µm nylon filter) and assayed using the developed RP-HPLC method. Dissolution data was analyzed by one way analysis of variance (ANOVA).

## 2.4 RP-HPLC Method Development

Reverse phase high performance liquid chromatography (RP-HPLC) was developed and optimized for simultaneous identification and quantification of all three APIs using a Schimadzu LC-20 AP Prominence Preparatory Liquid Chromatography system (SchimadzuCorporation, Japan), consisting of binary pump with UV-VIS detector, photodiode array detector and refractive index detector and an autosampler and prep column organizer capable of full-loop or partial loop injection. The separation was achieved using a column BDS C18 of 4.6 x 150 mm dimension and 5 µm particle packing (Thermo Scientific). Analyses were performed by LabSolutions LC Multi-PDA software.

#### 2.4.1 Optimization of developed RP-HPLC method

Chromatographic conditions include a mobile phase of 25 mM phosphate buffer solution of pH 3.0 ± 0.05 (pH adjusted using 85% orthophosphoric acid), acetonitrile and methanol. Various mobile phase compositions were tested at 227 to 230 nm. Analysis was done in isocratic mode at flow rate of 0.7 mL/min with injection volume of 20 µL for 15 minutes.

*2.4.1.1 Preparation of standard stock solutions of drug samples*

A 10-mL standard stock solutions of atenolol, enalapril maleate and hydrochlorothiazide 1 mg/mL respectively were prepared by dissolving 10 mg of each drug in sufficient methanol. From this standard stock solution working standard solutions were prepared using the mobile phase.

*2.4.1.2 Determination of linearity and range*

To evaluate linearity, calibration curves were obtained for all three APIs at ten concentrations (1-100 µg/mL) using the developed RP-HPLC method. Peak area concentration was subjected to a least square regression analysis.

*2.4.1.3 Method precision (repeatability)*

Instrumental precision was evaluated by repeatedly injecting (n = 5) solution of ternary mixture containing 250 µg/mL of atenolol, enalapril maleate and hydrochlorothiazide.

## 3. RESULTS AND DISCUSSION

### 3.1 Compatibility Studies Using DSC Analysis

The DCS thermal curve of atenolol showed a sharp endothermic peak of its melting at 155°C (Fig. 2). The DSC curves of the binary mixtures of atenolol with each excipient: spray dried lactose (SDL), sodium stearyl fumarate (SSF) and sodium starch glycolate (SSG) showed a general depression of melting points of the pure compounds. The DSC endothermic peak corresponding to melting point of enalapril maleate was 150.1°C (Fig. 2). Thermal analysis of the binary mixture of enalapril maleate and excipients also demonstrated a general lowering and broadening of thermoprofiles compared to the pure compounds. Broadening and lowering of endothermic peaks was also observed in binary physical mixture of hydrochlorothiazide and excipients. The endothermic peak corresponding to melting point of hydrochlorothiazide was 270.6°C (Fig. 2). These broadening and lowering of thermograms are thought to be due to the mutual contamination of each ingredient by the other. Therefore, It is not expected that compatibility issues will arise with these ingredients.

The endothermic peak of atenolol shifted towards a lower temperature when the binary physical mixture of atenolol and hydrochlorothiazide was analyzed (Table 2). Analysis of the binary physical mixture of enalapril maleate and hydrochlorothiazide resulted in a downward shift of the endotherm. Thermoprofiles observed from analysis of the binary physical mixture of atenolol and enalapril maleate indicated the disappearance of endothermic peaks for both drugs and the emergence of an unknown broad peak (Fig. 3). Analysis of Atenolol, Enalapril Maleate and Hydrochlorothiazide ternary mixture shows the disappearance of individual drug's endothermic peaks and the emergence of a new peak (Fig. 3). Summary of thermoanalytical data is illustrated in Table 2.

DSC analysis revealed a physical incompatibility between atenolol and enalapril, which manifested as a eutectic mixture. The lowest melting point of the mixture was 97.4°C at binary composition of 40% (w/w) atenolol and 60% (w/w) enalapril. Although eutectic mixtures of several drug substances (occurring at specific ratios) have been reported in the literature, eutectic behaviour between an ACEI (enalapril) and β-blocker (atenolol) appear to be new. In order to co-formulate these drugs with HCTZ, granulation of individual drug and encapsulation in HGC were employed.

Thermal analysis of drug to excipient binary mixtures showed a general decrease in melting temperatures due to lowering of the purity of each component in the mixture resulting in the depression of the melting points of individual components. However, there were no observed solid-solid interaction between the drugs and formulation excipients.

A solid-solid incompatibility was seen between the active ingredients atenolol and enalapril maleate. Physical blends of the drugs produced a wet mass upon mixing and liquefied a few

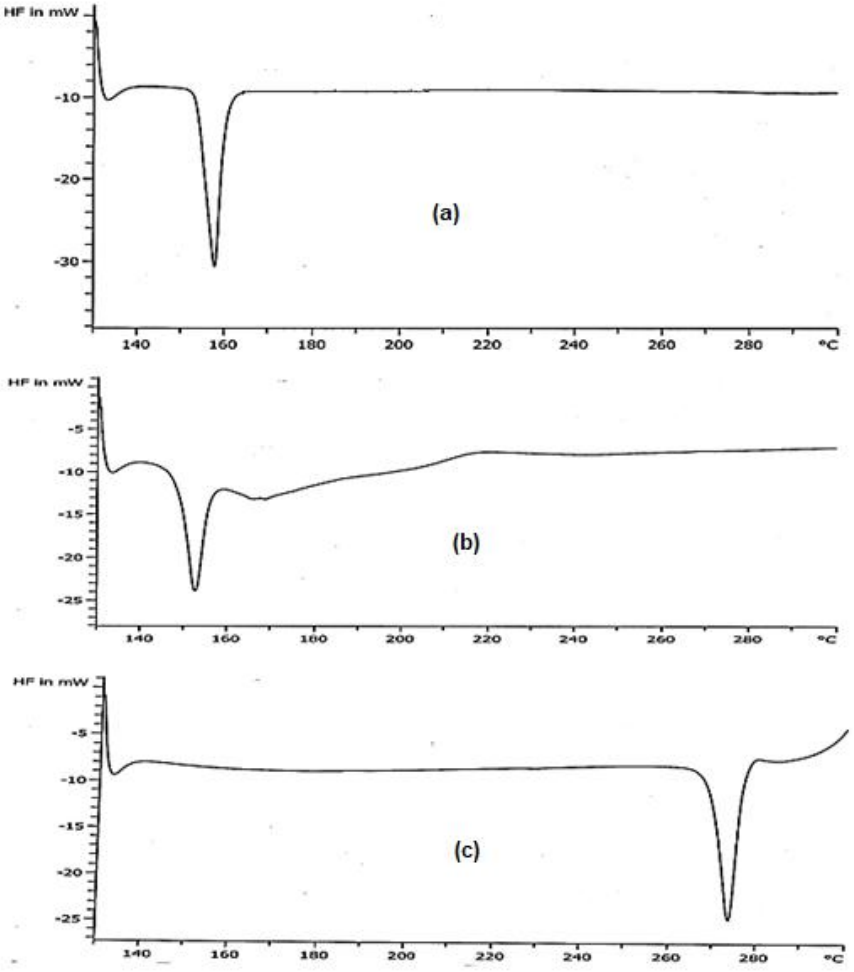


**Fig. 2. DSC thermoprofiles of (a) Atenolol (ATL), (b) Enalapril Maleate (ENL) and (c) Hydrochlorothiazide (HCTZ)**

**Table 2. Thermoanalytical data of Atenolol (ATL), Enalapril Maleate (ENL) and Hydrochlorothiazide (HCTZ) using differential scanning calorimetry**

| Substance | DSC temperatures | | |
|---|---|---|---|
| | $T_{onset}$ ($^0$C) | $T_{peak\ DSC}$ ($^0$C) | Nature of the process |
| ATL | 150.2 | 151.2 | Melting |
| ENL | 148.7 | 149.8 | Melting |
| HCTZ | 269.5 | 270.5 | Melting |
| ATL + HCTZ | 138.7;306.5 | 140;308 | Melting; decomposition |
| ATL + ENL[a] | 189.5 | 190.5 | - |
| ENL + HCTZ | 144.6;304.3 | 146;305 | Melting; decomposition |
| ATL + ENL + HCTZ[a] | 193.095 | 194 | - |

*Note. [a]Absence of the drugs' melting event or undefined peak*

hours after compression indicative of eutectic formation. Once components are not in intimate contact, eutectic melting will not be observed. Granulation and encapsulation of drug mixtures in hard gelatin capsule shell were selected to reduce this stability issue. No observable incompatibility was seen between physical binary blends of either drug with hydrochlorothiazide.

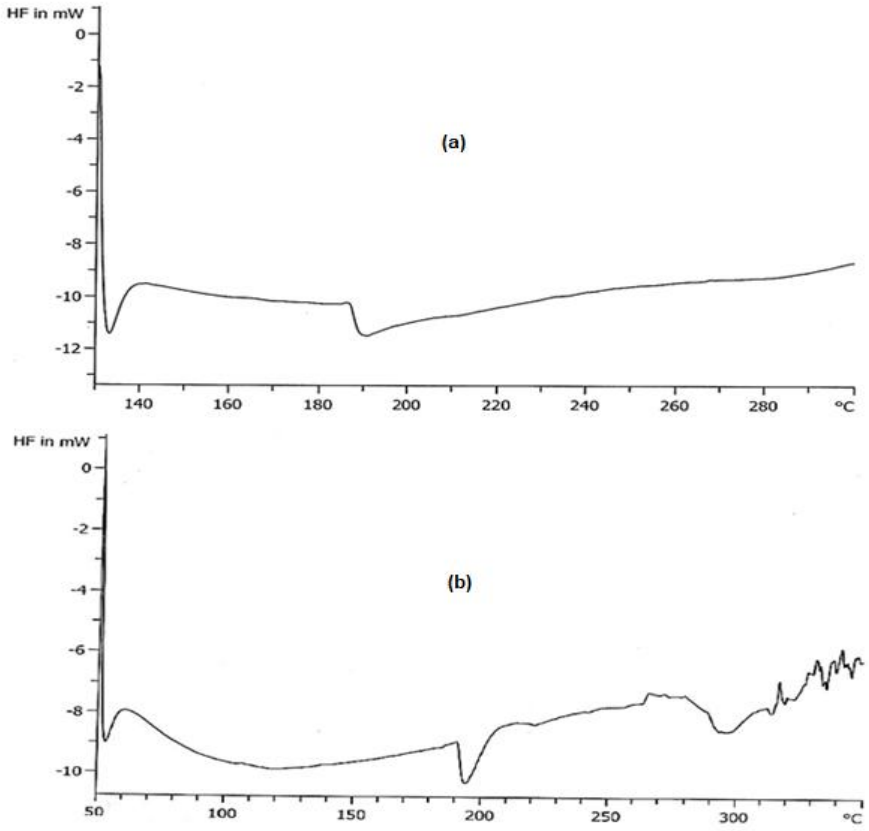


**Fig. 3. DSC thermoprofiles of (a)1:1 (w/w) binary mixtures of Atenolol (ATL) and Enalapril Maleate(ENL) and (b) 1:1:1 ternary mixture of ATL, ENL and Hydrochlorothiazide (HCTZ)**

## 3.2 Evaluation of Granules

### 3.2.1 Micromeritic properties of granules

The flow properties of the granules were evaluated using the parameters bulk density, tapped density, compressibility index and Hausner's ratio (Table 3). Formulation 1, 3, 4 and 5 all have compressibility index below 10 and Hausner's ratio all below 1.12 indicating excellent flow properties while formulation 2 showed good flow properties with Compressibility Index

**Table 3. Densities, Carr's compressibility index and Hausner ratio of formulated granules**

| Formulation | Bulk density[a] | Tapped density[b] | CI[†] | HR[‡] |
|---|---|---|---|---|
| 1 | 0.698 ± 0.0001 | 0.763 ± 0.0111 | 8.527 ± 1.343 | 1.093 ± 0.016 |
| 2 | 0.698 ± 0.0001 | 0.790 ± 0.0002 | 11.628[§] | 1.132[§] |
| 3 | 0.683 ± 0.0155 | 0.750 ± 0.0001 | 9.060 ± 2.067 | 1.100 ± 0.025 |
| 4 | 0.688 ± 0.0092 | 0.757 ± 0.0111 | 9.146 ± 2.194 | 1.101 ± 0.027 |
| 5 | 0.709 ± 0.0093 | 0.783 ± 0.0113 | 9.380 ± 0.262 | 1.104 ± 0.002 |

*[†]A small CI indicates good flow properties. Values ≤ 10 indicate excellent, 11-15 good, 16-20 fair and > 23 poor flow properties. An HR value of < 1.25 indicates free flowing and > 1.25 poor flow properties. Adapted from "Compression Physics of Pharmaceutical Powders: A Review," by S. Mohan, 2012, International Journal of Pharmaceutical Sciences and Research, Vol. 3(6), p 1582.*

*All values represent mean values ± standard deviation (SD) n= 3.*

*[†]Compressibility Index (CI)- calculated based on average densities*

*[‡]Hausner Ratio (HR)-calculated based on average densities*

*[§] Standard deviation (SD) values are too small to record in table*

and Hausner's ratio values of 11.6 and 1.13 respectively when compared to literature Values [27]. Compressibility index (CI) and Hausner ratio (HR) have become the simple, fast, and popular methods of predicting powder flow characteristics. The CI has been proposed as an indirect measure of parameters such as bulk density, size and shape of powder particles, surface area, moisture content, and cohesiveness of materials [28]. Observed CI and HR values indicate good to excellent flow properties as compared with literature results [26]. Good flow properties are usually attributed to more uniform particle shape and size. Acceptable flow properties exhibited by the granules may reflect uniformity of capsule filling, during encapsulation, which is required for the uniformity of content of active ingredients [29].

#### 3.2.2 Flow through orifice

Data on flow through orifice are tabulated in Table 4 and is based on the average of five replicate samples. Orifice diameter is directly proportional to the rate of flow of a powder once the height of the powder bed is consistently greater than the diameter of the orifice [30]. This was a general observation with the samples. Flow behavior of powders may be affected by factors such as the physical properties of the material as well as the equipment used for handling [31]. Forces of cohesion and adhesion acting on the surface of particles will cause flowability challenges [30]. Flow may also be affected by variation in particle size distribution. Generally, fine particles will have greater surface area and greater cohesive forces when compared to larger coarser particles. Coarser particles tend to flow better due to greater influence of gravitational forces. Particles with varying shapes and sizes exhibit different flow properties due to irregular surfaces and textures causing arches and bridges within the powder bed, causing interlocking of the particles impeding flow [30]. This could explain the decrease in the flow rate for formulations 2, 3 and 4. Other factors such as moisture content, temperature and humidity may also influence the observed flow properties of the formulations. Compressibility index (CI) is an indirect measure of bulk density, size and shape of powder particles, surface area, moisture content, and cohesiveness of materials [28]. Observed CI and Hausner ratio (HR) values indicate good to excellent flow properties as compared with literature results [26] reflecting uniformity of capsule filling, during encapsulation. Good flow properties are usually attributed to more uniform particle shape and size.

### 3.3 RP-HPLC Method Development

Optimization of chromatographic conditions resulted in a mobile phase of composition acetonitrile, methanol and 25 mM phosphate buffer pH 3.0 (± 0.05, adjusted using 85% orthophosphoric acid), in the ratio 5: 10: 85 by volume respectively. Elution of the analytes was done using isocratic technique with an injection volume of 20 µL and 15 minutes run time. Satisfactory separation was achieved with good peak symmetry for all three drugs (Fig. 4).

Replicate samples of the ternary mixture of all three drugs were injected and analyzed using the optimized method. Linear correlation was obtained between the peak areas and concentrations of all three drugs in the range of 1-100 µg/mL (Fig. 5). Summary of the regression analysis, limit of detection and limit of quantification may be found in Table 5. The precision data of the method expressed as the percentage relative standard deviation (% RSD) are listed in Table 6.

The optimized chromatographic method, produced excellent resolution of the active ingredients producing satisfactory separation with good peak symmetry. Methods in the literature were modified and optimized to achieve the chromatographic conditions used for the simultaneous separation and quantification of the drugs in standard preparations as well as in the formulated capsules [32-38]. Regression analysis of the calibration data showed good correlation between the peak areas and concentration for all three drugs. The RSD values of less than 5% for method precision indicated that the method is repeatable. An RSD value of ≤6 for multiple analytes is considered satisfactory [39]. Although the RP-HPLC method produced excellent resolution of the analytes and offers satisfactory precision, the method was not a stability indicating assay and as such specificity was not evaluated. Therefore, interference between the three drugs and degradation products was not determined. Although the developed method was optimized, it was not validated, as this was beyond the scope of this study.

**Table 4. Flow rates of granules through orifices 5.1 mm, 6.4 mm, 7.8 mm and 9.4 mm**

| Flow rate (g/second) of granules/Orifice diameter (mm) | | | | |
|---|---|---|---|---|
| **Formulation** | **5.1 mm** | **6.4 mm** | **7.8 mm** | **9.4 mm** |
| 1 | 0.796 ± 0.15 | 1.605 ± 0.33 | 2.536 ± 0.38 | 2.881 ± 0.61 |
| 2 | 0.651 ± 0.10 | 1.846 ± 0.40 | 2.815 ± 0.40 | 2.533 ± 0.87 |
| 3 | 0.626 ± 0.17 | 1.533 ± 0.40 | 2.43 ± 0.19 | 2.18 ± 0.21 |
| 4 | 0.562 ± 0.12 | 1.707 ± 0.26 | 2.664 ± 0.49 | 2.358 ± 0.74 |
| 5 | 0.688 ± 0.17 | 1.570 ± 0.11 | 2.466 ± 0.49 | 2.895 ± 0.75 |

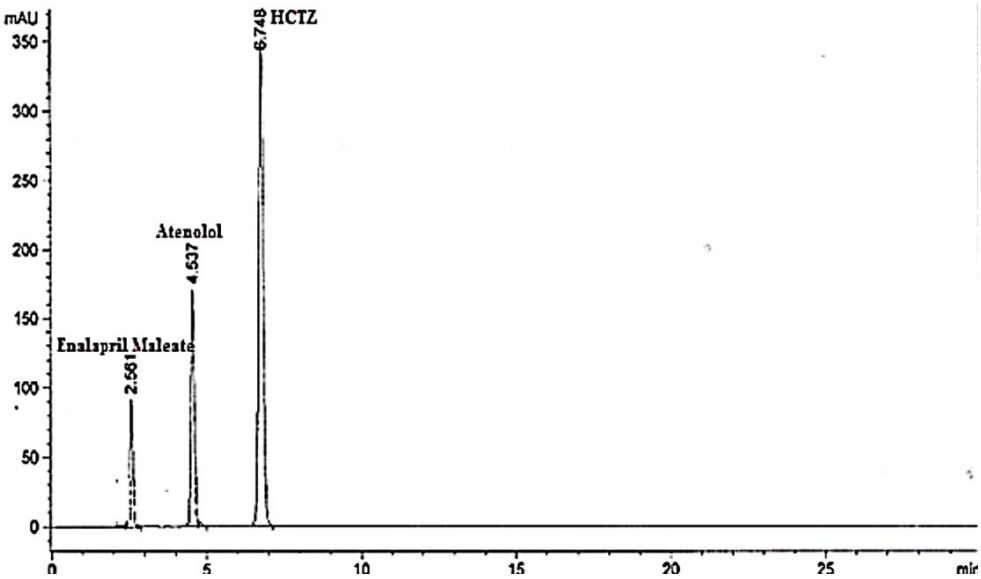


**Fig. 4. Typical chromatogram of ternary mixture of atenolol, enalapril and hydrochlorothiazide showing peak resolution and retention times**

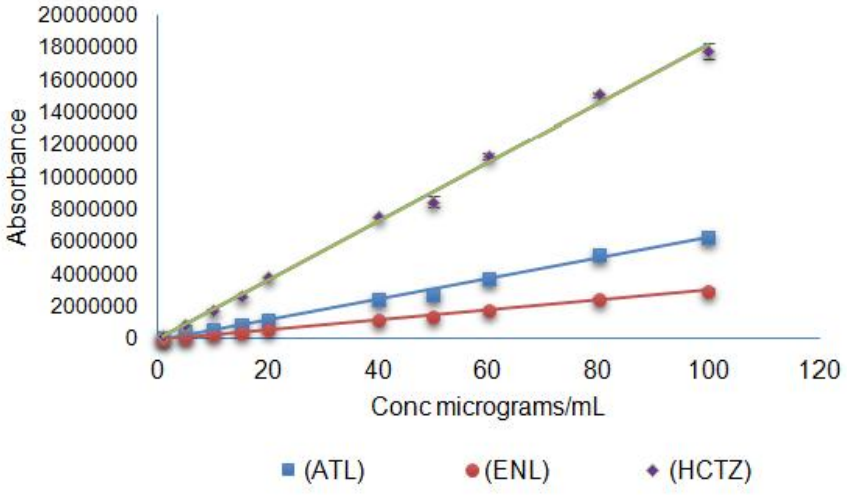


**Fig. 5. Calibration curve of Atenolol (ATL), Enalapril (ENL) and Hydrochlorothiazide (HCTZ) using the optimized RP-HPLC**

**Table 5. Summary of regression analysis of calibration curves of all three drugs using the optimized RP-HPLC method**

| Parameter | ATL | ENL | HCTZ |
|---|---|---|---|
| Linearity range (µg/mL) | 1-100 | 1-100 | 1-100 |
| Detection wavelength (nm) | 227 | 227 | 227 |
| Equation | y= 64700x -111214 | y=30092x - 35739 | y= 184547x – 23941 |
| Correlation coefficient | 0.9964 | 0.9976 | 0.9966 |

*Note. Abbreviations: ATL-Atenolol; ENL- Enalapril Maleate; HCTZ-Hydrochlorothiazide; RP-HPLC- Reverse phase high performance liquid chromatography*

**Table 6. Summary of precision data of the developed RP-HPLC method**

| Drugs | Mean Peak Area +SD† (n=5) | %RSD‡ | Retention Time (min) |
|---|---|---|---|
| ATL | 20746209 ± 372493.8 | 1.8 | 4.57 ± 0.032 |
| ENL | 11725083 ± 386120.8 | 3.3 | 2.67 ± 0.002 |
| HCTZ | 30480111 ± 451180.5 | 1.5 | 7.05 ± 0.005 |

*† SD represents standard deviation and ‡RSD represents relative standard deviation. ATL-Atenolol, ENL-Enalapril and HCTZ- Hydrochlorothiazide*

## 3.4 Evaluation of Capsules

### 3.4.1 Disintegration tests

This test was carried out on six capsules per formulation using the test apparatus outlined previously. The disintegration times are tabulated in Table 7. Disintegration time gives an indication as to how quickly the capsule will break up to present the active ingredients for dissolution and possible absorption. It is expected to decrease from formulation 1 to 5 since concentration of the superdisintegrant sodium starch glycolate was 1%, 2%, 3%, 4%, and 5% in formulations F1, F2, F3, F4 and F5 respectively. The study showed that disintegration time was highest for formulation 1 when compared to formulations 4 and 5. Disintegrants are usually added to capsule formulations to facilitate the breakup and dispersion of agglomerates into smaller particles to aid in dissolution. Super disintegrants such as sodium starch glycolate, allow for faster disintegration at lower concentrations when compared to conventional disintegrants.

### 3.4.2 Weight variation test

The average weight in milligrams and standard deviation for each capsule formulation are shown in Table 7. Each formulation had uniform weight with variation less than 15%, meeting the requirements set by the United States Pharmacopoeia [24]. Each formulation had uniform weight with variation less than 15%, meeting the compendia requirements [24] and indicating uniformity of content. Large variations would result in varied potency of each active pharmaceutical ingredient (API) from the labelled claim from batch-to-batch. This results in either over dosing or under dosing, producing varied therapeutic responses or even toxic effects. This is compounded by the fact that each dosage unit contains three APIs.

**Table 7. Weight variation and disintegration time of formulated ATL, ENL and HCTZ capsules**

| Formulation | Disintegration time (min) (n= 6) | Weight variation (mg) (n=20) | RSD of weight variation (%) (n=20) |
|---|---|---|---|
| 1 | 2.182 ± 0.156 | 0.456 ± 0.013 | 2.75 |
| 2 | 1.622 ± 0.249 | 0.454 ± 0.006 | 1.31 |
| 3 | 1.892 ± 0.328 | 0.451 ± 0.124 | 2.75 |
| 4 | 1.683 ± 0.281 | 0.455 ± 0.011 | 2.32 |
| 5 | 1.668 ± 0.320 | 0.470 ± 0.024 | 5.09 |

*‡Relative standard deviation (RSD) of weight of capsules*

### 3.4.3 *In vitro* dissolution

*In vitro* drug release of the prepared capsule formulations was studied using the USP Dissolution Apparatus 1 [40]. Aliqout samples were analysed using the optimized RP-HPLC method as described under method development. All formulations had maximum percent release of all three drugs after 60 minutes (see Fig. 6). At the end of 20 minutes at least 80% of atenolol was released and greater than 80% of both enalapril maleate and hydrochlorothiazide was released after 10 minutes from all formulations. Statistical analysis revealed no significant difference in the release of any of the three drugs from the formulations at the end of 60 minutes.

The time required for 25%, 50% and 80% ($T_{25\%}$, $T_{50\%}$, $T_{80\%}$) of the three drugs to be released from all the formulations was evaluated and variation between groups determined. No significant difference in the release of the three drugs between formulations was observed for all three APIs. At least 80% of each drug was released within 20 minutes, 50% below 10 minutes and 25% of each drug was released below 2 minutes for all five formulations. This indicates that all formulations met compendia requirements of not less than 75% of labelled claim for each drug to be released within 30 minutes [40]. No significant difference in the release rate of all three drugs was observed at the end of 60 minutes between all five formulations. Hence, rate of the drug released was not related to concentration of the disintegrant present in the fixed dose combination capsule. It appeared that the disintegrating effect imparted was not significant enough to cause change in the release rate of all three drugs from the dosage form. Contents of the capsules are already in granular form and, as such, will deaggregate into fine particles presenting the drugs for the process of dissolution. Since the surface area of the granules are essentially the same for all formulations, increased concentration of disintegrant had little or no impact on disintegration to cause a significant or discriminant change that could be observed during dissolution.

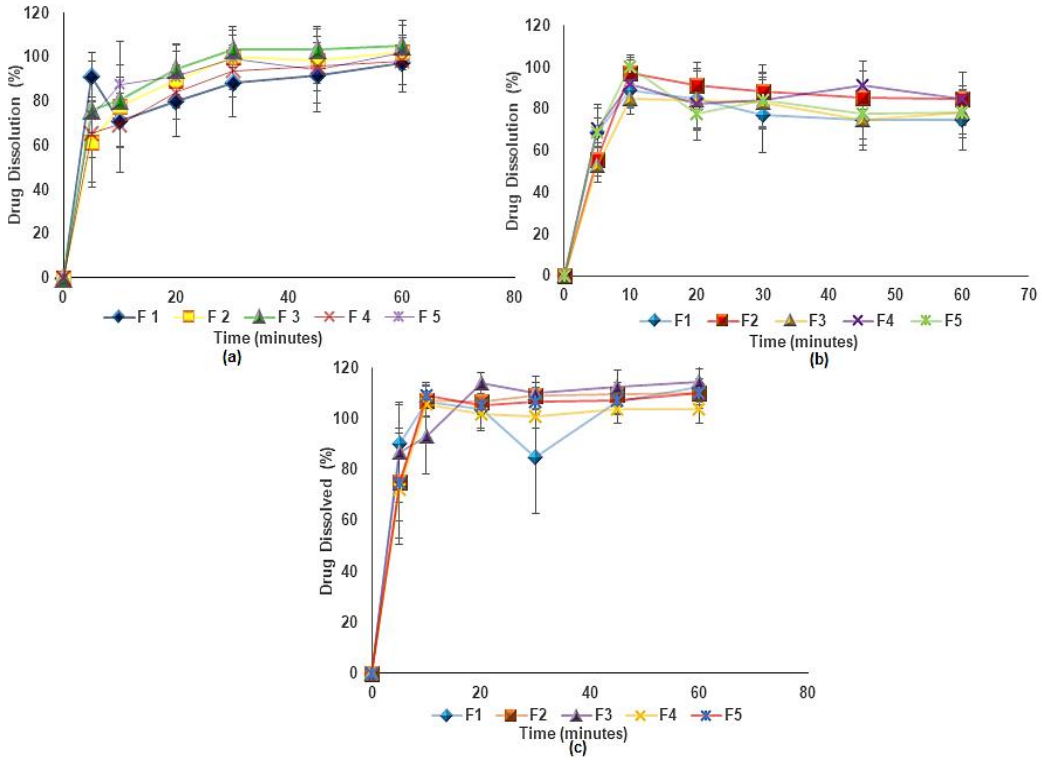


**Fig. 6. Dissolution profile of (a) ATL, (b) ENL and (c) HCTZ in formulated capsule. Comparison of *in vitro* release profile of all three drugs contained in formulated fixed dose combination capsule for formulations F1, F2, F3, F4 and F5. Error bars represent standard error (n=5)**

## 4. CONCLUSIONS

Compatibility studies revealed a physical interaction between atenolol and enalapril maleate. Intimate mixture of both compounds produced a eutectic mixture, which may cause formulation challenges during tableting. To eliminate this stability issue, both ingredients were separated by forming granulates of both drugs with other formulation ingredients using dry granulation technique and granules encapsulated. Formulations generally had good to excellent flow properties and the prepared capsules showed good uniform weight. All formulations had rapid disintegration rates, indicating the potential for making each drug available for absorption within two minutes. In addition, prepared capsules elicited fair *in vitro* release patterns for all three drugs with 80% of each drug dissolved within 20 minutes and thereby meeting the compendia (USP) requirement (≥ 75% dissolved within 30 minutes). The reverse phase HPLC method developed and optimized to simultaneously identify and quantify atenolol, enalapril maleate and hydrochlorothiazide in the formulated capsule, showed promising results and supported the dissolution protocol. The analytical procedures may be further optimized and validated to be used in routine analyses.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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