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Formulation and physicotechnical evaluation of a fixed dose combination of amlodipine, metformin and glibenclamide in the management of hypertension-diabetes mellitus comorbidity

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Abstract

The concept of fixed dose combination (FDC) dates as far back as the early 17th century and still finds relevance till today. Almost every disease condition has at least one approved FDC commercially available for its treatment. The prevalence of hypertension-diabetes mellitus (HTN-DM) comorbidity is high globally. However, there are no available FDC formulation approved to be used in managing patients with this comorbidity. This study was designed to fill this gap by formulating an FDC of Amlodipine (antihypertensive), Metformin (antidiabetic) and Glibenclamide (antidiabetic), which are already being used clinically as monotherapies in management of HTN-DM comorbid patients. The APIs were granulated using wet granulation method with the aid of the excipients, after which the granules properties were examined and subsequently compressed into tablets. The physicotechnical properties and *in-vitro* release profile were evaluated on the tablets. The results of granule properties such as angle of repose (22.8°), Hausner ratio (1.16) and Carr's compressibility index (13) for the 8% binder batch show excellent flow properties and is indicative of forming tablets with good qualities. "8% binder" tablets batch has the following properties; hardness (kgF), friability (0.55%), disintegration time (220 secs), all within the acceptable official requirements. The release profile showed that the drugs were bioavailable from the tablets in good time. It can be concluded that the formulation of an FDC for HTN-DM comorbidity was a success and studies to confirm the compatibility of the APIs and excipients are recommended.

Keywords: Fixed dose combination; Hypertension; Diabetes Mellitus; Comorbidity; Amlodipine; Metformin; Glibenclamide

1. Introduction

A fixed dose combination (FDC) product is defined as a combination product that includes two or more active pharmaceutical ingredients with similar or different pharmacological activity and different mechanisms of action combined in a single dosage form, which is manufactured and distributed in fixed doses [1]. The decision of the active pharmaceutical ingredients (APIs) to be selected for FDCs formulation is usually based on factors such as approval status of the APIs, clinical experience, manufacturing feasibility, pharmacological mechanisms, biopharmaceutical properties, pharmacokinetics, metabolic pathways, drug-drug interactions and the required doses of the individual APIs [2]. To qualify as acceptable candidates for FDC formulation, the APIs to be combined shall have different mechanisms of action, have similar pharmacokinetic properties, have minimal drug-drug interactions and treat closely related

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diseases or same disease using different mechanisms of action [3]. Fixed-dose combinations provide significant advantages over monotherapy, with respect to improved efficacy, reduced adverse event frequency and severity, improved compliance, reduced treatment costs, and a shorter time to attain targeted treatment plan [4]. Globally, there are up to 75% adults with hypertension-diabetes mellitus (HTN-DM) co-morbidity [5]. The incidence is consistently on the increase worldwide [6]. Hypertensive patients with diabetes mellitus co-morbidity have increased mortality rate by 7.2 times [7,8]. Presence of hypertension in diabetic patients accelerates the progression of microvascular (retinopathy, nephropathy and neuropathy) and macrovascular (atherosclerotic) complications [9], thus drastically reducing the quality of life of the patients. Jeffrey and Kirchner [10] confirmed that many diabetic patients also have hypertension and stated that Calcium channel blockers (a class of antihypertensive drugs, e.g. Amlodipine) have been shown to be effective for treating both hypertension and ischemic heart disease, as well as for preventing renal complications in patients with diabetes. Amlodipine is a dihydropyridine Calcium channel broker (CCB) that inhibits the slow channel transmembrane influx of calcium ions into vascular smooth muscle and, to a lesser extent, into cardiac muscle. It is a peripheral arterial vasodilator that acts directly on vascular smooth muscle, resulting in reduced peripheral vascular resistance and lowered blood pressure [11]. Amlodipine displays near linear pharmacokinetics at therapeutic doses and while the absolute bioavailability of orally administered amlodipine is estimated to be around 64–90 %, absorption is gradual, with the drug having a t_{max} of approximately 6–12 hours. With once-daily dosing, steady state plasma concentrations are reached after 7–8 days [11, 12]. Metformin belongs to the biguanide class of oral hypoglycemic agent and is the first-line drug of choice in the management of type II diabetes. It is considered an antihyperglycemic drug because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia [13]. Metformin has an absolute oral bioavailability of 40 to 60%, and gastrointestinal absorption is apparently complete within 6 hours of ingestion. It is rapidly distributed following absorption and does not bind to plasma proteins. Metformin undergoes renal excretion and has a mean plasma elimination half-life after oral administration of between 4.0 and 8.7 hours. Glibenclamide, also called glyburide is a second-generation sulfonylurea used to treat patients with diabetes mellitus type II. It is typically given to patients who cannot be managed with the standard first line therapy, metformin. Glibenclamide stimulates insulin secretion through the closure of ATP-sensitive potassium channels on beta cells, raising intracellular potassium and calcium ion concentrations. It is indicated alone or as part of combination product with metformin, as an adjunct to diet and exercise, to improve glycemic control in adults with type 2 diabetes mellitus [14]. The absorption of glibenclamide in elderly patients reaches a C_{max} of 211-315 ng/mL with a T_{max} of 0.9-1.0 hour, while in younger patients it reaches a C_{max} of 144-302ng/mL with a T_{max} of 1.3-3.0 hours. Patients taking glibenclamide have and AUC of 348ng*h/mL. Elderly patients have a volume of distribution of 19.3-52.6L, while younger patients have a volume of distribution of 21.5-49.3L. Glibenclamide is 99.9% bound to protein in plasma with >98% accounted for by binding to serum albumin and is extensively metabolized in the liver via the cytochrome P450 system [15]. This study is designed to formulate a fixed dose combination of Amlodipine, Metformin and Glibenclamide for use in the management of hypertension-diabetes mellitus comorbidity and to evaluate the physicotechnical properties of the new formulation.

2. Material and methods

Amlodipine besylate was received as a gift in support of the research from Juhel Pharmaceuticals Enugu. Metformin and Glibenclamide were also received as gifts in support of the research from May and Baker Pharmaceuticals Lagos. Microcrystalline cellulose, calcium hydrogen phosphate, sodium starch glycolate and magnesium stearate were purchased from a pharmaceutical excipients' vendor.

The formula for the formulation is as shown in Table 1 below

Table 1 Formula for FDC formulation

Batch	Amlodipine	Metformin	Glibenclamide	M. cellulose	Starch	C.B.P	Mg. Stearate 1%w/w
A	10 mg	500 mg	5 mg	4% (2.68 g)	3.35 g	5.45 g	0.67 g
B	10 mg	500 mg	5 mg	6% (4.01 g)	3.35 g	4.11 g	0.67 g
C	10 mg	500 mg	5 mg	8% (6.07 g)	3.35 g	2.77 g	0.67 g

M. cellulose = Micro-crystalline cellulose; C.B.P = Carbon Biphosphate; Mg. Stearate = Magnesium Stearate

2.1. Preparation of granules

Wet granulation method as described by Osonwa *et al.,* [16] was used to prepare the granules of the FDC. Quantities of the APIs and excipients as listed in Table 1 were carefully weighed out.

Batch A: 2.68 g of the binder (microcrystalline cellulose) was completely spread and slightly moistened with water in the mortar, using pestle. The respective quantities of the APIs and excipients were added in aliquots one after the other and properly triturated in the mortar with intermittent addition of drops of water in order to obtain a uniform and cohesive mix. The wet mass was wet-screened using a mesh-12 sieve to form the granules which were subsequently dried in an oven (Genlab, England) at 60 ^{o}C for 15 mins. The dry granules were dry-screened using a mesh-20 sieve to obtain a uniformly sized and shaped granules, which were properly stored in an air-tight, water-proof container. The same procedure was used to prepare the batches B and C granules.

2.2. Evaluation of granules' properties

The granule properties such as flow rate, angle of repose, bulk and tapped densities, Hausner quotient, Carr's compressibility index, percentage fine was determined using the methods as described by Manek *et al.,* [17] and Osonwa *et al.,* [16].

Granules of weight 20 mg were weighed into a 10 ml measuring cylinder and tapped on the desk three times. The volume occupied by the granules was recorded. The bulk density was calculated from the result using the formula as shown by Equation 1 [16].

$$Bulk\ Density = \frac{weight\ of\ granules\ (grams)}{volume\ before\ tapping\ (milliliters)} \quad \ldots..Equation\ 1$$

The cylinder containing the granules was tapped 100 times on a smooth surface. The final volume occupied was recorded and used to calculate the tapped density according to the Equation 2 formula [16].

$$Tapped\ Density\ = \frac{weight\ of\ granules\ (grams)}{volume\ after\ tapping\ (milliliters)} \quad \ldots\ldots.\ Equation\ 2$$

Hausner ratio was calculated as the ratio of tapped density to bulk density of the samples as shown by Equation 3 while Carr's compressibility index was calculated using the formula shown in Equation 4.

$$Hausner\ quotient = \frac{Tapped\ Density}{Bulk\ Density} \quad \ldots\ldots\ldots\ Equation\ 3$$

$$Carr's\ Compressibility\ Index\ = \frac{Tapped\ Density\ -\ Bulk\ Density}{Tapped\ Density}\ X\ \frac{100}{1} \ldots\ldots\ldots\ldots.Equation\ 4$$

Also, a simple method whereby weighed quantity of granules (50 mg) from each batch was allowed to flow through an orifice (funnel) of diameter 1cm at a fixed height (15 cm) was used to determine the flow rates of the granules. The time taken for the weighed granules to flow out completely from the orifice was recorded [17]. This was performed in triplicate. Flow rate was obtained by the equation 5 below

$$Flow\ rate\ = \frac{weight\ of\ granules\ (grams)}{time\ of\ flow\ (seconds)} \quad \ldots\ldots\ldots\ldots.Equation\ 5$$

Furthermore, the angle of repose (θ) was determined by calculating $\tan^{-1}$ of the height and radius of the cone formed by the granules as they flowed out of the orifice.

$$Angle\ of\ repose\ (\theta) = tan^{-1}\frac{Height\ of\ cone\ (cm)}{radius\ of\ cone\ (cm)} \quad \ldots\ldots\ldots\ldots..Equation\ 6$$

The percentage fine was obtained by weighing 50 mg granules of the various batches and then passing the granules through a sieve no 44 (0.355 mm). The coarse granules and fine granules were then separated and reweighed for each batch. From the results, the percentage of fines was calculated using the formula in equation 7 below [16];

$$Percentage\ fine\ = \frac{Weight\ of\ fine}{Total\ weight\ of\ granules\ (fine\ +\ coarse)}\ X\ {}^{100}/_{1} \ldots\ldots\ldots\ldots\ Equation\ 7$$

2.3. Compression of the granules into tablets

The granules were blended with the 1%w/w of the lubricant (magnesium stearate). The blend was compressed using a 10-station rotary tablet press (Proton multiple punch rotary press). The die-volume was set so as to obtain the calculated final weight of the formulation. The compression was done individually for all the batches.

2.4. Evaluation of the tablets' properties

Compendial and non-compendial tests were conducted on the tablets to assess the quality and performance of the batches with different binders in comparison with one another. These tests include hardness, uniformity of weight, friability, uniformity of active ingredient, disintegration test and dissolution time test.

2.5. Tablet hardness test

Ten (10) tablets were randomly selected from each of the batches and their crushing strength evaluated on individual tablet basis using Monsanto Hardness Tester (PI-91/12125, Coslab, India). The pressure taken to crush the tablet was recorded in kgF. The mean and standard deviation of the tablet hardness were determined [18].

2.6. Uniformity of weight test

Twenty (20) tablets were randomly selected from each batch and weighed individually after which the average weight and % deviation was calculated. According to pharmacopoeia, the following limits are given for the weight variation of tablets.

Table 2 Pharmacopeial limit for weight variation of tablets

Average weight	% Deviation
80 mg	± 10 %
More than 80mg but less than 250 mg	± 7.5 %
250 mg or more	± 5 %

2.7. Friability test

Twenty (20) tablets were randomly selected, weighed and placed in the Roche friabilator and exposed to rolling and repeated shocks as they fall 6 inches in each turn within the apparatus. After 4 minutes of 100 revolutions, the tablets were weighed and the final weight obtained. Percentage (%) friability was calculated (Equation 8) as a function of the initial and final weights of the tablets.

$$\%friability = \frac{Initial\ weight - final\ weight}{Initial\ weight} X\ {}^{100}/_{1} \quad \ldots\ldots\ldots\ldots\ldots Equation\ 8$$

2.8. Disintegration time test

Disintegration time of the tablets was determined by the use of tablet disintegration test apparatus (ZT 320, Erweka, Germany). One tablet was introduced into each tube and the disc was placed into each tube. The whole assembly was suspended in the beaker containing distilled water at 37 ± 2 ^{o}C. The apparatus was operated until no residue remained on the screen or adhered to the surface of the disc and the disintegration time was recorded.

Table 3 Pharmacopeial limit for disintegration time of tablets

Tablet type	Disintegration time
Uncoated tablet	Not more than 15 mins
Film coated tablet	Not more than 30 mins
Enteric coated tablet	Not more than 45 mins

2.9. Dissolution Time Test

This test measures the amount of time required for a given percentage of the drug substance in a tablet to go into solution under a specified set of conditions. It is intended to provide a step toward the evaluation of the physiological availability of the drug substances.

2.10. For amlodipine

Drug release studies of the batches was carried out using tablet dissolution test apparatus at 75 rpm. 500 ml of 0.01N HCl was used as the dissolution medium with temperature maintained at 37 ± 2 °C in all experiments.

10 mL of sample was withdrawn at 5 min interval for times 5, 10, 15, 20, 25 and 30 minutes respectively and replaced with fresh medium to maintain sink conditions. Samples withdrawn were analyzed at 237nm for percentage drug release using Shimadzu UV-Visible spectrophotometer. The concentrations were then calculated using the constant K obtained from Beer's calibration: A = K * C. Where: A = Absorbance, C = Concentration K = constant.

2.11. For metformin

Drug release studies of the batch were carried out using tablet dissolution test apparatus at 100 rpm. 1000 ml of phosphate buffer at pH 6.8 was used as the dissolution medium with temperature maintained at 37 ± 2 °C in all experiments.

10 mL of sample was withdrawn at intervals for times 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes respectively and replaced with fresh medium to maintain sink conditions. Samples withdrawn were analyzed at 234 nm for percentage drug release using Shimadzu UV-Visible spectrophotometer. The concentrations were then calculated using the constant K obtained from Beer's calibration: A = K * C. Where: A = Absorbance, C = Concentration, K = constant.

2.12. For glibenclamide

Drug release studies of the batch were carried out using tablet dissolution test apparatus at 75 rpm. 900 ml of phosphate buffer at pH 6.8 was used as the dissolution medium with temperature maintained at 37 ± 2 °C in all experiments. 10 mL of sample was withdrawn at intervals for times 5, 10, 15, 20, 30, 45, and 60 minutes respectively and replaced with fresh medium to maintain sink conditions. Samples withdrawn were analyzed at 239 nm for percentage drug release using Shimadzu UV-Visible spectrophotometer. The concentrations were then calculated using the constant K obtained from Beer's calibration: A = K * C. Where: A = Absorbance, C = Concentration, K = constant.

2.13. Assay of Active Ingredient

2.13.1. Amlodipine:

The buffer, mobile phase, standard solution and sample solutions were prepared following the procedures recommended by United State Pharmacopeial National Formulary [19] for assay of Amlodipine besylate.

Buffer: 7.0 mL of triethylamine was added into a 1000-mL flask containing 900 mL of water. The solution with adjusted to a pH of 3.0 using phosphoric acid ▲▲ (USP 1-May-2021), diluted with water to volume, and properly mixed.

Mobile phase: This was prepared by mixing methanol, acetonitrile, and buffer at the ratio of 35:15:50 respectively.

Standard solution: Solution of 0.0275 mg/mL of USP Amlodipine Besylate RS was formed using Mobile phase as the solvent.

Sample solution: Nominally 0.02 mg/mL of amlodipine in Mobile phase prepared as follows. Five (5) tablets of the FDC were placed in a suitable volumetric flask, and added sufficient quantity of Mobile phase to disintegrate the tablets. The dispersion was shaken for 30 min, and diluted with Mobile phase to volume. The sample was passed through a syringe tip filter of 0.45-µm pore size. The first few milliliters of the filtrate were discarded.

Chromatographic system: For the assay, the chromatographic system parameters were set as follows:

Mode: LC

Detector: UV 237 nm. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2021)

Column: 3.9-mm × 15-cm; ▲4- or▲ (USP 1-May-2021) 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: Not less than 3 times the retention ▲time▲ (USP 1-May-2021) of amlodipine

After the analysis, the percentage of the labeled amount of amlodipine in the FDC tablets was calculated using the equation 9 below

$$Result = \left(\frac{rU}{rS}\right) \times \left(\frac{CS}{CU}\right) \times \left(\frac{Mr1}{Mr2}\right) \times 100 \ \ldots\ldots\ldots\ldots . Equation\ 9$$

rU = peak response of amlodipine from the Sample solution
rS = peak response of amlodipine from the Standard solution
CS = concentration of USP Amlodipine Besylate RS in the Standard solution (mg/mL)
CU = nominal concentration of amlodipine in the Sample solution (mg/mL)
Mr1 = molecular weight of amlodipine, 408.88
Mr2 = molecular weight of amlodipine besylate, 567.05
Acceptance criteria: 90%–110%▲▲ (USP 1-May-2021)

2.13.2. Glibenclamide:

The buffer, mobile phase, diluent, standard stock solution, standard solution, system suitability solution 1 and system suitability solution 2 and sample solutions were prepared following the procedures recommended by United State Pharmacopeial National Formulary [20] for assay of Glibenclamide.

Buffer: 28.8 g/L of monobasic ammonium phosphate

Mobile phase: This was formed by mixing acetonitrile and buffer at a ratio of 40:60. The pH was adjusted to 5.3 using 1 N sodium hydroxide.

Diluent: Acetonitrile and water were mixed together at the ratio of 50:50

Standard stock solution: 0.25 mg/mL of USP Glibenclamide RS was prepared as follows. An amount of USP Glibenclamide RS to make 0.25 mg/ml was weighed and transferred into a suitable volumetric flask and dissolved first in the acetonitrile, using 50 % of the final volume, and then diluted with water to volume.

Standard solution: 0.025 mg/mL of USP Glibenclamide RS in Diluent, from the Standard stock solution

System suitability solution 1: Solution containing 0.025 mg/mL of USP Glyburide Related Compound A RS was prepared in Diluent. 50 µL of this solution was transferred to a 50-mL volumetric flask, and diluted with Standard solution to volume.

System suitability solution 2: 5.0 mg/mL of USP Metformin Hydrochloride RS in System suitability solution 1

Sample solution: Five (5) tablets were dissolved in Diluent by stirring with a magnetic stirring bar for at least 1 h and diluted to obtain a solution containing 0.025 mg/mL of glibenclamide, based on the label claim. A portion of this solution was centrifuged at 3000 rpm for 10 min and the clear supernatant used for the assay.

Note: A portion of this solution was retained for the Assay for Metformin Hydrochloride.

Chromatographic system: For the assay, the chromatographic system parameters were set as follows:

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 100 µL

Run time: 1.25 times the retention time of glibenclamide

After the analysis, the percentage of the labeled amount of glibenclamide in the FDC tablets was calculated using the equation 10 below

$$Result = \left(\frac{rU}{rS}\right) \times \left(\frac{CS}{CU}\right) \times 100 \quad \ldots\ldots\ldots\ldots\ldots \; Equation\ 10$$

rU = peak response of glibenclamide from the Sample solution
rS = peak response of glibenclamide from the Standard solution
CS = concentration of USP glibenclamide RS in the Standard solution (mg/mL)
CU = nominal concentration of glibenclamide in the Sample solution (mg/mL)
Acceptance criteria: 90.0 %–110.0 % of the labeled amount of glibenclamide

2.14. Metformin hydrochloride

Buffer: 1.0 g each of sodium heptanesulfonate and sodium chloride was transferred to a 2000-mL volumetric flask. 1800 mL of water was added into the volumetric flask and adjusted with 0.06 M phosphoric acid to a pH of 3.85 and diluted with water to volume.

Mobile phase: Acetonitrile and Buffer were mixed at a ratio of 10:90

Diluent: Acetonitrile and water were mixed at a ratio of 1:40

Standard solution: 0.25 mg/mL of USP Metformin HCl RS in Diluent and sonicated to achieve

complete dissolution.

System suitability stock solution: 25 µg/mL each of USP Metformin Related Compound B RS and USP Metformin Related Compound C RS in Diluent

System suitability solution: 0.5 mL of the System suitability stock solution was transferred to a 50-mL volumetric flask, and diluted with Standard solution to volume.

Sample solution: The retained portion of the Sample solution from the Assay for Glibenclamide was diluted with water to obtain 0.25 mg/mL of metformin hydrochloride based on the label claim.

Chromatographic system: For the assay, the chromatographic system parameters were set as follows:

Mode: LC

Detector: UV 218 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 5 µL

After the analysis, the percentage of the labeled amount of glibenclamide in the FDC tablets was calculated using the equation 10 above.

rU = peak response of metformin from the Sample solution
rS = peak response of metformin from the Standard solution
CS = concentration of USP Metformin Hydrochloride RS in the Standard solution (mg/mL)
CU = nominal concentration of metformin hydrochloride in the Sample solution (mg/mL)
Acceptance criteria: 90.0%–110.0% of the labeled amount of metformin hydrochloride

2.15. Stability Studies

The FDC tablets were stored in an airtight container under atmospheric humidity and room temperature for a period of 12 months during which the quality control parameters were evaluated and recorded.

3. Results

3.1. Evaluation of granules' properties

The results of the granules' properties such as flow rate, angle of repose, bulk and tapped densities, Hausner quotient, Carr's compressibility index, percentage fine as evaluated are as shown in Table 4.

Table 4 Granules' properties

Batches	Flow rate (g/sec)	Angle of repose (°)	Percentage fine (%)	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratio	Carr's index
4 % Binder	0.94	34.6	26.7	0.64	0.75	1.17	15
6 % Binder	1.16	28.8	21.2	0.50	0.61	1.22	18
8 % Binder	1.55	22.8	13.6	0.45	0.52	1.16	13

Mass of the granules used for the flow rate is 61g

3.2. Evaluation of FDC tablet properties

The results of the physicotechnical properties of the tablets such as hardness test, % friability test and disintegration test as evaluated were recorded and presented graphically as shown by Figure 1 while uniformity of weight test is presented with Figure 2.

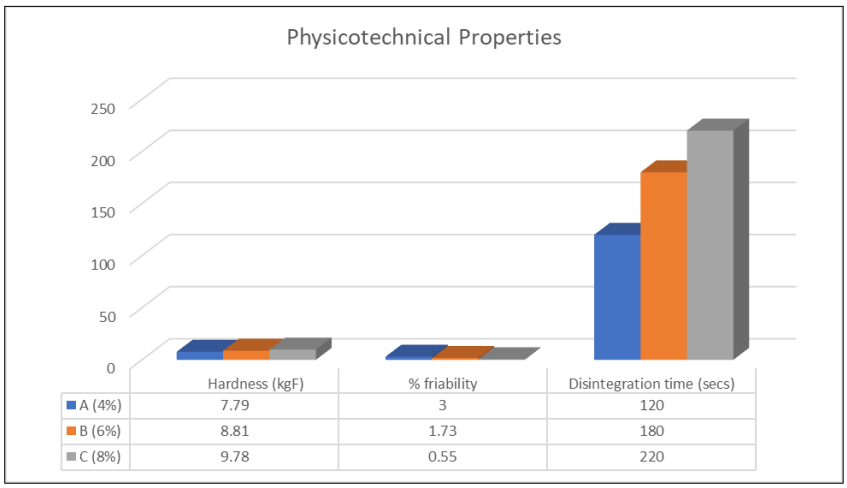

Figure 1 Graphical presentation of the physicotechnical properties of the FDC tablet batches

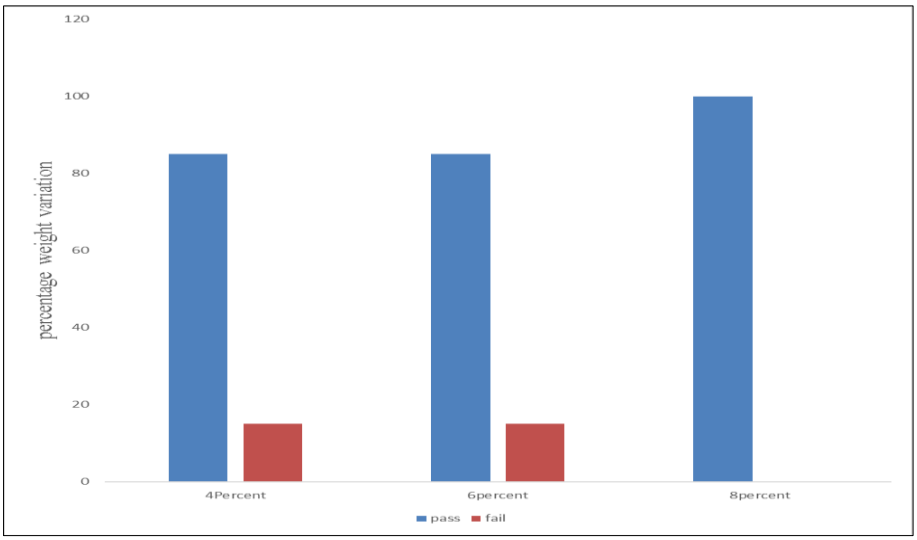

Figure 2 Graphical presentation of the weight variation properties of the FDC tablet batches

3.3. Dissolution profile of the FDC tablet

Dissolution profiles of the various APIs from the FDC tablets were evaluated by plotting the graphs of percentage (%) cumulative drug release against time and are shown by Figures 3 to 5. Figure 6 shows the comparison between the release profiles of the three (3) APIs.

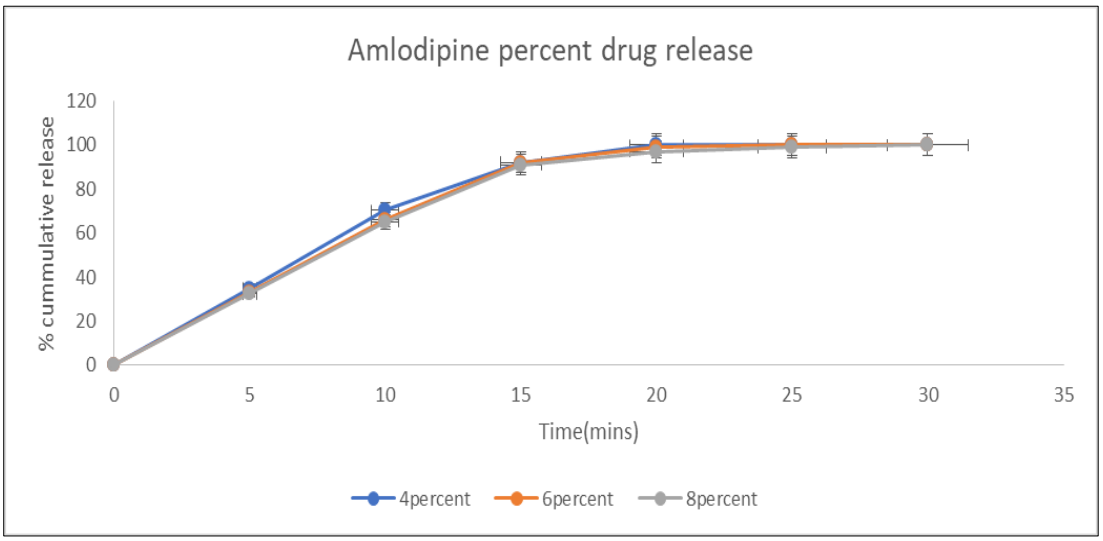

Figure 3 Release profile of Amlodipine from the FDC tablet

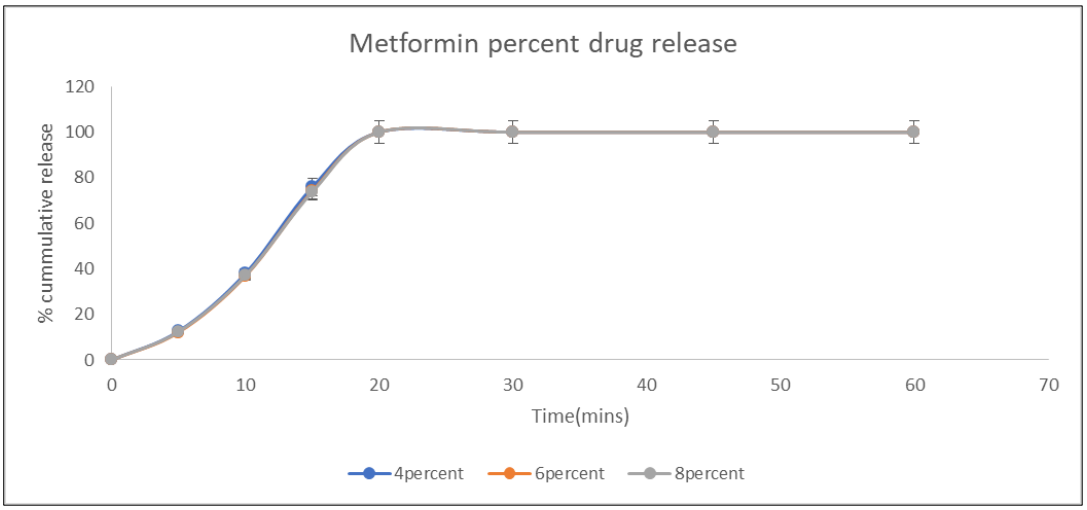

Figure 4 Release profile of Metformin from the FDC tablet

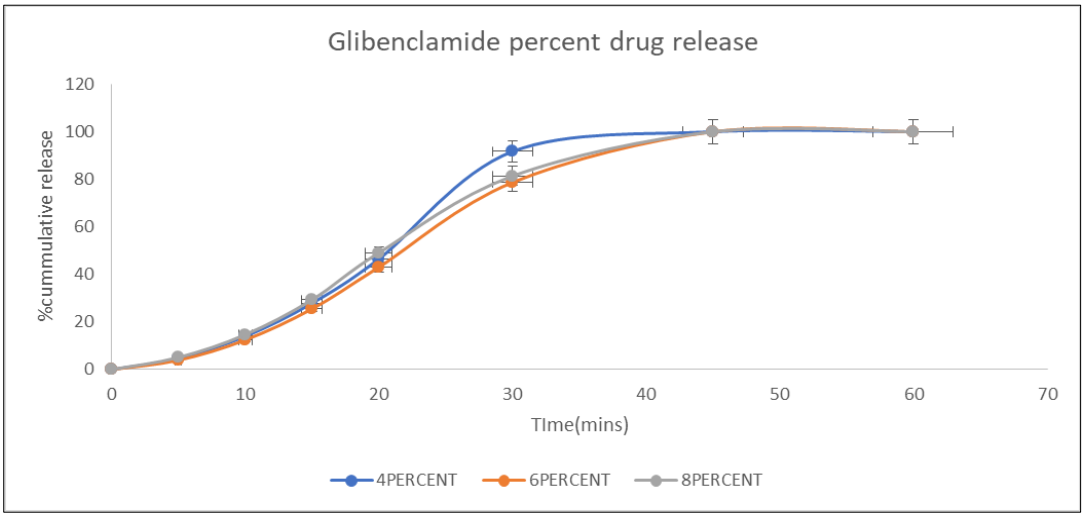

Figure 5 Release profile of Glibenclamide from the FDC tablet

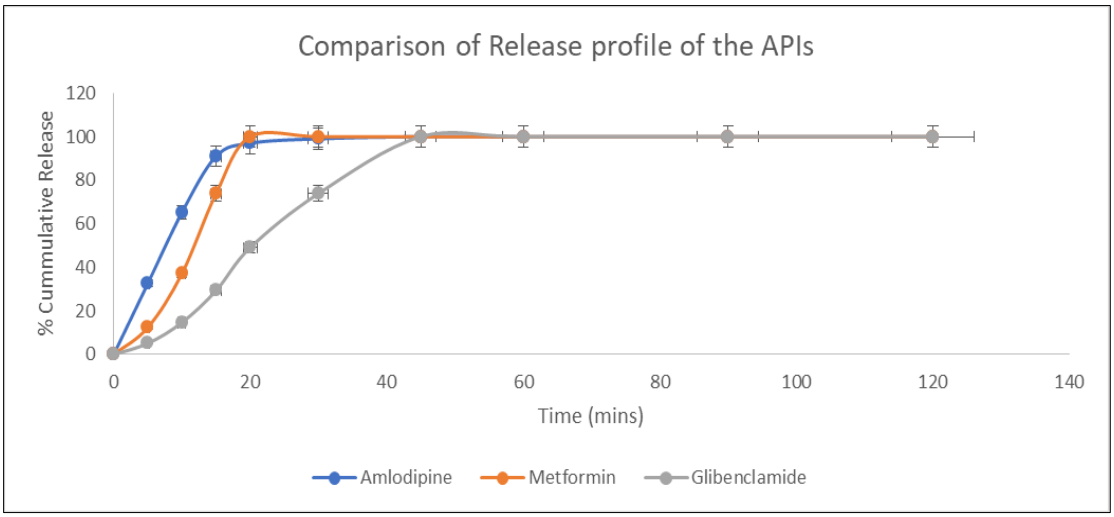

Figure 6 Comparison of the release profiles of Amlodipine, Metformin and Glibenclamide from the FDC tablet

3.4. Assay of Active Ingredient

The key parameters from the Assay of active ingredient using the HPLC are tabulated in Table 5. The response, the standard deviation, the relative standard deviation and % amount of labeled active ingredient in the formulation were determined.

Table 5 HPLC Assay of Active Ingredient

		Average Response	Standard Deviation	Relative Standard Deviation	% Amount of labelled active ingredient
Amlodipine	Standard solution	6213558	76086.37	1.22	102.596 %
	Sample solution	6258965	62551.64	0.99	
Metformin	Standard solution	2740.842	13.406	0.489	101.931 %
	Sample solution	2793.779	98.152	3.513	
Glibenclamide	Standard solution	462.744	1.652	0.356	109.673 %
	Sample solution	507.508	11.561	2.278	

3.5. Stability Studies

Properties of the FDC tablets such as content of active ingredient, disintegration test and hardness test were evaluated for the tablets at intervals of zero month, 1, 2, 4, 6 and 12 months while on storage under atmospheric humidity and room temperature. The results are as shown in Table 6 below.

Table 6 Stability studies parameters for the FDC tablet

Quality Control Parameter		Time Interval (Months)					
		Zero	1	2	4	6	12
Hardness (KgF)		9.78	9.78	9.77	9.68	9.31	8.93
Disintegration Test (Secs)		220	219	204	195	183	157
Content of Active Ingredient	Amlodipine	100%	100%	100%	99%	98%	95%
	Metformin	100%	100%	100%	100%	97%	94%
	Glibenclamide	100%	100%	99%	99%	97%	95%

4. Discussion

Angle of repose is a measure of the internal cohesive and frictional forces within the granules. It gives an insight into the flowability of the powder or granule [18]. Its value will be high if the powder is cohesive and low if the powder is non- cohesive. Values of angle of repose less than 30 ^{o} are said to be excellent 25–30, values between 31 ^{o} and 35 ^{o} are classified as good, 36 ^{o} to 40 ^{o} as fair, 41 ^{o} to 45 ^{o} as poor while > 45 ^{o} are considered very poor. The results of the angle of repose as shown in table 4 reveal that the granules have excellent flow properties except the 4 % binder batch which is considered to possess good flow properties.

Compressibility index of granules is determined so as to assess the ability of the granules to compact and decrease in volume when pressure is applied. This is a measure of the suitability of the granules to form strong tablets that can withstand pressure [21]. Compressibility index is also indicative of the flow properties of granules while Hausner quotient relates to the cohesiveness of the granules [21]. When the percentage compressibility is below 15% the granules have excellent flow properties while cohesive granules with percentage compressibility above 25% indicates poor flow properties. Granules with Hausner ratio below 1.25 have good flow properties. The results show that the granules from the 8 % binder batch will form stronger tablets with less weight variation within the tablets formed with the granules batch. Hardness test, which is a measure of the mechanical strength of tablets aims to assess the ability of tablets to withstand pressure or stress during handling, packaging and transportation. It measures the resistance of tablets to permanent deformation and values of 4 kgF and above have been identified sufficient hardness for a

satisfactory mechanically strong tablet. The hardness test values of the tablet batches are all above the minimum requirement and thus said to be mechanically strong enough tablets.

Percentage (%) friability is another mechanical property of tablets with compendial [22] specification of not more than 1 %. While hardness test assesses the bulk deformation of the tablets, % friability test evaluates the surface deformation which can arise from mechanical shocks during handling and transportation of the tablets. The results of the % friability test as shown in table 4 reveals that while 6 % binder tablet batch is slightly friable with 1.73 % friability, it is only the 8 % binder batch that is not friable with value of % friability at 0.55 %. 4 % binder batch is friable having its value of % friability at 3 %. Disintegration of tablets is a vital step in the release process of the APIs from oral solid dosage pharmaceutical formulations. The rate of disintegration is influenced by the rate of influx of water into the tablets which is also dependent on the porosity of the tablets [16]. All the batches showed a very rapid disintegration time having all disintegrated within 4 mins of commencing the analysis. The *in-vitro* dissolution profile of the FDC tablets was plotted as % cumulative drug release against time (mins). The graphs show that the individual APIs were released from the tablets and went into dissolution in an almost identical manner in all the batches, with the exception of Glibenclamide, whose 4 % binder formulation reached 100 % release of the API at 30 mins while the 6 % and 8 % binder formulations achieved 100 % release after about 45 mins.

A comparison of the release profile of the three drugs as shown in Fig. 6 reveals that Amlodipine has a steeper and quicker release from the formulation and reached about 98 % release before 20 mins and then plateaued until 30 mins when the release finally reached 100 %. Metformin reached 100 % release first before the other two APIs though it had a more gradual release than Amlodipine. Glibenclamide on the other hand, had the slowest but steady release from the formulation and reached 100 % release the latest after 45 mins. All three drugs were 100 % bioavailable before one (1) hour and this is beneficial for formulations where immediate therapeutic outcome is desired. According to the USP NF, the acceptance criteria for the assay of active ingredient is that the calculated percentage of the labeled amount of active ingredient in the formulation should be within 90 % and 110 %. The results of HPLC assay of active ingredients from the FDC formulation reveal that all three drugs (Amlodipine, Metformin and Glibenclamide) are within the acceptable criteria range with percentage amount of labeled active ingredients at 102.596 %, 101.931 % and 109.673 % respectively. This implies that the amounts of the active ingredients present in the FDC formulation are actually what is claimed in the label. Intermediate stability studies were conducted on the tablets by storing them under atmospheric humidity (65 % ± 5 RH) and room temperature (29 ^{o}C ±2) for a period of 12 months, during which the tablets properties such as hardness, disintegration and content of active ingredients were evaluated at intervals. The results as shown in table 6 show that the tablet remained stable for the period of the study under the storage conditions. The mechanical strength of the tablets which is a function of the tablet hardness remained above the minimum requirement of 4 kgF. The acceptable criteria for disintegration time test for conventional uncoated tablets states that the tables must completely disintegrate within 15 mins. The results showed that at 12 months, the disintegration time for the tablets remained well within the acceptable required time for disintegration. The result of the content of active ingredients after 12 months showed that the active ingredients were above 90 % present in the formulation. This implies that the FDC tablets remained stable after 12 months.

5. Conclusion

Granules of the FDC formulated using wet granulation method exhibited excellent flow properties and when compressed into tablet dosage forms, they displayed tablet properties that are within the acceptable criteria for conventional uncoated tablets. The *in-vitro* release study and assay of active ingredient showed that the active ingredients in the formulation are up to 100% bioavailable within the first 45 mins (Amlodipine and Metformin were available within the first 30 mins). It therefore can be concluded that the aim of the study was achieved.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declared discloses no conflict of interest.

Contributions of the Authors

The study was conceptualized by Chukwunonso C. Onwuzuligbo and Charles O. Esimone; designed by Chukwunonso C. Onwuzuligbo and Martins O. Emeje. The study was carried out by Chukwunonso C. Onwuzuligbo, Mmesoma S. Akubude, Chinyere C. Obika and Doris M. Obi. The manuscript was developed by Amarachukwu U. Onwuzuligbo.

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