**An-Najah National University**

**Faculty of Graduate Studies**

# Extemporaneous Compounding and Physiological Modeling of Amlodipine/Valsartan Suspension

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**This Thesis was defended successfully on 13 / 5 /2018 and approved by:**

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# Dedication

I dedicate this to Allah Almighty my guardian and my source of inspiration and wisdom.

To the honest heart who prayed in the dark nights so I can follow the light and shine with the sunrise, you'll always be the dearest gift I ever dreamed of.

To any person who has ever thought highly enough of me to offer me any sort of help or advice.

This is only the beginning of my journey…

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To all my friends, thank you for the encouragement during my moments of crisis. You kept pushing me up towards achieving my goals, your friendship made me stronger; you'll always be in my heart.

Wafa Aabed

# الإقرار

أنا الموقع أدناه موقع الرسالة التي تحمل العنوان:

**Extemporaneous Compounding and Physiological Modeling of Amlodipine/Valsartan Suspension**

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

# Declaration

The work provide in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

**Student's name:** **اسم الطالب:**

**Signature:** **التوقيع:**

**Date:** **التاريخ:**

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# List of Abbreviations

| | |
|---|---|
| **ACAT** | Advanced Compartmental Absorption and Transit model |
| **ACN** | Acetonitrile |
| **ADME** | Absorption, Distribution, Metabolism, Excretion |
| **AML** | Amlodipine |
| **ARB** | Angiotensin Receptor Blocker |
| **AUC** | Area Under the Curve |
| **BCS** | Biopharmaceutical Classification System |
| **BE** | Bioequivalence |
| **BP** | Blood Pressure |
| **CACO-2** | Human colon carcinoma cell line |
| **CFU** | Colony Forming Unit |
| **cm** | Centimeter |
| **$cm^2/s$** | Centimeter square per second |
| **$C_{max}$** | Maximum serum concentration |
| **CNS** | Central nervous system |
| **ºC** | Degree celsius |
| **Cp** | Viscosity |
| ***f*1** | Difference factor |
| ***f*2** | Similarity factor |
| **FDA** | Food and Drug Administration |
| **GI** | Gastrointestinal |
| **g/gm** | Gram |
| **H2 blocker** | Histamine 2 blocker |
| **Hr/s** | Hour/s |
| **HCl** | Hydrochloric acid |
| **HPLC** | High performance liquid chromatography |
| **IR** | Immediate Release |
| **IV** | Intravenous |
| ***IVIVC*** | *In vitro-In vivo* Correlation |
| **Kg** | Kilogram |
| **L** | Liter |
| **M1** | Metabolite1 |
| **MeOH** | Methanol |
| **mg** | Milligram |
| **min** | Minute |
| **ml** | Milliliter |

| | |
|---|---|
| **µL** | Micro liter |
| **Mm** | Mellimeter |
| **µm** | Micro meter |
| **N** | Normality |
| **n** | Number of samples |
| **ng** | Nano gram |
| **nm** | Nanometer |
| **PDA** | Photo diode array |
| **PE** | Prediction Error |
| **$P_{eff}$** | Effective permeability |
| **pH** | Potential of Hydrogen |
| **PK** | Pharmacokinetic |
| **$pk_a$** | Acid dissociation constant |
| **PTFE** | Polytetrafluro ethylene |
| **R1** | Percentage of drug dissolved at each time point of the reference drug |
| **RH** | Relative Humidity |
| **RLD** | Reference Listed Drug |
| **rpm** | Round per minute |
| **SD** | Standard deviation |
| **sec** | Second |
| **SUPAC** | Scale Up and Post Approval Changes |
| **T1** | Percentage of drug dissolved at each time point of the test drug |
| **$t_{max}$** | Time to maximal concentration |
| **USP** | United States Pharmacopeia |
| **UV** | Ultraviolet |
| **VAL** | Valsartan |
| **Vd** | Volume of distribution |
| **S. aureus** | *Staphylococcus aureus* |
| **P. aeroginosa** | *Pseudomonas aeroginosa* |
| **C. albicans** | *Candida albican* |

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## Abstract

Background: In case of absent liquid dosage form, crushing a tablet or dispersing a capsule would be the most convenient option for using these drugs in patients with dysphagia difficulties. However, no bioequivalence or stability studies are conducted for these extemporaneous preparations, which leads to confusion regarding its efficacy and safety. *In silico* and *in vitro* tools have proven to be useful in predicting the *in vivo* performance of drugs depending on its physicochemical properties and it's *in vitro* dissolution profiles. No liquid formulation of combination Amlodipine and Valsartan is available in the pharmaceutical market for use in pediatric population with hypertension.

Purpose: The aim of the present study was to prepare an extemporaneous suspension of Amlodipine and Valsartan from available commercial tablets, and to evaluate the stability and dissolution properties of the compounded suspension.

Method: Amlodipine/Valsartan extemporaneous suspension was prepared from available commercial tablets Valzadepine®. The dissolution profiles

for the extemporaneous preparation and the commercial tablet was determined in different pH media. The physical, chemical and microbial stability of the compounded formulation was evaluated over one month period at room temperature. Moreover, *In silico* modeling using GastroPlus ™ software was used to build absorption models for both drugs based on the *in* vitro dissolution data. The simulated plasma profile for both active ingredients were compared with the *in vivo* plasma profile to examine the similarity of the extemporaneous suspension and the commercial tablets.

Results: The Amlodipine/Valsartan extemporaneous suspension was successfully prepared with acceptable organoleptic properties. The suspension was stable for four weeks period preserving its physical and chemical features. The release profiles of valsartan and Amlodepine from the suspension were similar to that from source tablet Valzadepine®. *In silico* modeling predicted similarity of the extemporaneous suspension and the commercial tablets.

Conclusion: Amlodipine/Valsartan extemporaneous suspension could be prepared from available commercial tablets. Moreover, GastroPlus™ can be applied along with the *in vitro* dissolution in order to affirm similarity in extemporaneous compounding situations.

# Chapter One
# Introduction

Among all pharmaceutical formulations; oral preparations are still the most popular and convenient. When considering pediatrics and geriatrics with swallowing difficulty, liquid preparations are the most preferred formulations due to the ease of administration, flexibility of the administered doses. In case of absence of liquid preparation of an active ingredient; health care providers tend to split or crush the oral solid dosage form ignoring its safety and efficacy to get access to the required dose [1]. However, this may be associated with the risks of loss of effectiveness, safety, and stability problems, since, these extemporaneous preparations are not generally assessed for their safety, stability, efficacy and bioavailability. Therefore, there is an urgent need to develop efficient and stable extemporaneous liquid dosage forms starting from the commercially available solid pharmaceutical products such as tablets and capsules.

## 1.1 Tablet scoring

There are different reasons for splitting a tablet into halves or quarters, for example healthcare providers tend to split a tablet to get access to smaller doses that are not available and still needed, for tapering or titrating a dose, or to ease the administration of large tablets especially in children and elderly patients with swallowing difficulties. Cost saving is another common reason for tablet splitting especially for patients with chronic conditions [2, 3].

FDA recommends that the generic product must follow the RLD regarding scoring manner, tablet scoring is considered as a sign for patients and healthcare providers for splitting a tablet in order to fraction a dose assuming content uniformity of the fractions

Nevertheless, there are many limitations in splitting a tablet; unsuitable dosage form such as controlled release, sustained release and film coated tablets, another is loss of fragments due to the poor techniques used, tablets tend to shatter when split, then weight uniformity and accordingly, content uniformity of the subdivided tablets cannot be guaranteed all the time even for scored tablets. Moreover, elderly patients with weak muscles and vision and poor focus find it difficult to cut tablets into halves even for scored ones [4].This attempt may lead to the administration of the incorrect doses, especially when there are many available commercial tablets have failed the weight uniformity test which leads to serious complications especially in case of narrow therapeutic window drugs [5].

On the other hand, many of available medications are not stable in liquid vehicles, and for pharmaceutical companies to produce such preparations it is considered economically ineffective, especially when liquid preparations have a shelf-life of two years from the date of production, this time is mostly lost in distribution system and waiting on the shelves for the time of administration. Another reason for the lack of pediatric preparations is the small size of the targeted population of children that make it financially unattractive for pharmaceutical companies to produce a liquid preparation of each medication [6]. Moreover such formulations require adequate

studies on pediatric patients concerning its safety and efficacy in such population which means additional costs and increased liability concerns [7].

### 1.2 Extemporaneous compounding

Extemporaneous compounding is the art of remediation of drugs and excipients into new doses or dosage forms that are not available in the market in order to match up with specific individual needs [8].

For neonatal and unconscious patients who cannot swallow even halves or quarters of a tablet, health care providers go after the off-label medications by preparing extemporaneous suspensions from available commercial solid dosage form [9].

Extemporaneous preparations are referred to as off-label medications because they are used out of the license limits that is approved by Food and Drug Administration (FDA), while registered medications follow the internationally recommended standards of good manufacturing practices, extemporaneous preparations are compounded manually with the traditional techniques that are lacking any of these standards [8].

#### 1.2.1 Formulation for extemporaneous suspension

Extemporaneously prepared suspensions range from simply crushing a tablet or opening a capsule then the addition of water or any other liquid to its complex formulations with the addition of preservatives and organoleptic enhancers.

A successful formulation of an extemporaneous suspension usually starts with crushing available commercial tablets or opening a capsule to be suspended in a vehicle. However, most of available medications are not soluble in water and hence suspending agents such as methylcellulose or others are needed. Anti-oxidants is another component to be added to improve the stability and ensure that the extemporaneous suspension is safe and effective during the treatment period. Sweeteners, colors and flavoring agents could be added as well to enhance the palatability and organoleptic properties and accordingly the compliance, preservatives, to prohibit microbial growth in the suspension [10].

As a result, the final suspension must be rapidly dispersed upon brief shaking in order to get the accurate doses upon administration. Furthermore, it must be palatable with acceptable taste and odor. It has to be stable over the intended period of treatment, and easy to prepare and store, taking into consideration that filtration has to be avoided to prevent loss of active ingredient [7].

Most of drugs are poorly soluble in water, although intravenous preparations could be an alternative option to the crushed tablets but limitations like high cost and poor oral bioavailability of some drugs restrict such option, moreover, intravenous preparations contain excipients such as propylene glycol or others that are not preferred to be administered in large amounts or for long periods [10].

A large number of medications that are not available in liquid dosage forms are prepared by unprofessional caregivers through crushing the tablets and mixing them with food or beverages at time of administration, this action may include errors in the preparation and incomplete administered doses. Therefore, the pharmacist or any other professional healthcare provider is preferred to prepare an extemporaneous suspension suitable to cover an extended period of time by containing multiple doses to meet patient's needs [7].

There were several attempts to prepare extemporaneous oral liquid dosage forms from commercially available products [11-13]. An extemporaneous suspension containing Amlodepine (AML) was prepared and a comparative bioavailability study was conducted in which bioequivalence was proved between the tablet and the extemporaneous preparation [14].Another attempt was to prepare valsartan (VAL) extemporaneous suspension; which was successfully prepared from available commercial tablets without hindering its chemical stability or dissolution profile [15, 16] but no bioavailability study was conducted. However, there was no effort done for preparing the combined (amlodipine/valsartan) suspension.

Generally, for most of extemporaneous preparations no bioequivalence studies are conducted, which leads to further confusion whether these crushed tablets preserve its efficacy or this action may lead to serious complications.

### 1.2.2 Risks associated with extemporaneous compounding

The risks associated with extemporaneous compounding cannot be underestimated, some are due to weighing and calculation errors, other risks are related to mistakes in selecting the appropriate formula and the right excipients. In extreme cases, these errors may lead to the death of the patient [8, 17].According to a prospective study conducted in a children's hospital, pediatric patients were identified as the most vulnerable population in suffering adverse reactions in such situations [18].

Moreover, those extemporaneous suspensions do not follow any stability or bioavailability testing. The pharmacokinetics of these preparations may vary because of the different behavior of the different dosage forms and the type of excipients used. Accordingly adverse reactions or toxicity might occur [19]. In a study involving preparing an alcohol-free extemporaneous suspension of spironolactone for pediatric use, different excipients were used to come up with four different formulations. Those formulations were investigated regarding their dissolution profiles and their physical, chemical and microbiological stability; only one of the four extemporaneous suspensions preserved the optimum conditions required for a safe and effective dosage form [20].

The bioavailability of an $H_2$ blocker; Nizatidine was investigated in two different extemporaneous solutions and compared with that of a commercially available oral syrup and Nizatidine capsule. The two extemporaneous solutions were prepared one in infant formula and the

other in an apple juice. The study showed that the bioavailability of Nizatidine in apple juice was markedly retarded, whereas; for Nizatidine in infant formula and the commercially prepared solution they were bioequivalent to the capsule [21]

### 1.2.3 Stability of extemporaneous formulations

Stability of an extemporaneous suspension is another challenge. A medication is considered to be stable over a specific period of time when this product retains its particular specifications of identity, quality and purity over a specific period of time (shelf-life) [22].

Regarding the stability of a medication, there are different aspects to consider: physical, chemical, microbiological and therapeutic stability.

-*Chemical Stability*

A pharmaceutical product is considered chemically stable when its active ingredient preserves its chemical integrity and labeled potency over a specified period of time (shelf life). Usually, the shelf life of a drug product can be considered as the time taken for the drug concentration to be reduced to 90% of the original concentration. The shelf life of a formulation needs to be determined at the realistic storage temperature, normally at room temperature or in a refrigerator.

- *Physical Stability*

Physical stability is confirmed by retaining the original properties such as, appearance, palatability, uniformity and suspendability over the shelf-life. Physical instability in suspensions is expressed as caking of sediment or particle growth

- *Microbiological Stability*

Microbiological stability refers to the absence of bacterial growth in the formulation during the specified period. Microbial instability may lead to spoilage in the product's appearance and change in its organoleptic properties. Furthermore, the presence of microorganisms in the formulation may render it ineffective or even toxic.

- *Therapeutic stability*

Therapeutic stability means that the product should remain effective during the shelf-life period [22].

Stability testing is the study of the effect of different environmental conditions (temperature, humidity, light) on the quality of a drug product over time, as well as to set a re-testing period for the active ingredient or a shelf life for a drug product including the recommended storage conditions for each starting material and drug product [23].Stability testing is a legal requirement before the registration of a new drug product, in order to ensure that the medication remains within acceptable limits of safety efficacy and good quality until a patient consumes the last dose [24].

There are different types of stability testing; long term stability testing, intermediate stability testing and accelerated condition stability testing. They are described in **table** 1.

**Table 1: Types of stability studies and their storage criteria [23].**

| Stability Study | Storage Criteria | Time Period* |
|---|---|---|
| Long term | (25±2) ºC / (60±5) % RH<br>Or:(30±2) ºC / (65±5) % RH ** | 12 months |
| Intermediate | (30±2) ºC / (65±5) % RH | 6 months |
| Accelerated | (40±2) ºC / (75±5) % RH | 6 months |

- * Minimum time required to be covered by data at submission.
- ** If (30±2) ºC / (65±5) % RH is the long term condition, then there is no intermediate condition

When a drug product fails to meet its specifications it is considered as a significant change, if such a condition occurred in the accelerated stability study and the long term testing was conducted at (25±2) ºC / (60±5) % RH, then intermediate stability testing should be carried out [23].

Stability of extemporaneous preparations is an add on challenge, it's important to consider the stability of the entire formulation than the active ingredient alone, where some extemporaneously prepared suspensions have increased stability due to the introduction of antioxidants into its formulations, however there are some cases where stability of these suspensions is inversely affected as a result of interaction between the active ingredient and the excipients used more than the degradation of the drug substance by oxidation or hydrolysis means [19].

## 1.3 Lack of Bioequivalence/Bioavailability data

Bioequivalence means that there is no significant difference in rate and extent of absorption between the test and the reference listed product. Bioequivalence is usually assessed in terms of peak plasma concentration ($C_{max}$), time to reach $C_{max}$ ($t_{max}$) and area under the concentration time curve (AUC). In terms of regulatory guidance, two formulations can be considered bioequivalent, if the 90% confidence interval for either $C_{max}$ or AUC falls within the limits of 80–125%. For licensed medicine, it relies on the manufacturer to prove that anew generic drug product is bioequivalent to the listed drug product, however, few bioequivalence data are available in the literature for the extemporaneous preparations compared to the licensed product. In some cases, the extemporaneous formulations were not bioequivalent to the reference medications. Bioequivalence studies are cost expensive and time consuming. The development of the *in vitro* and *in silico* methods can help in the prediction of the *in vivo* absorption profiles of drugs and adoption of *in vitro in vivo correlation* (*IVIVC*). As a result ,several regulations were put to waive the need for bioequivalence studies for a large number of drugs when specific criteria are met. (Biowaiver) which is based on the biopharmaceutical classification system BCS [25], classifies the drugs into four groups considering their solubility and permeability (Figure 1); with the high solubility and permeability are combined in BCS1, and the lowest are combined in BCS 4, BCS 2 lacks in solubility, and BCS 3 lacks in permeability [26]. *In silico* and *in vitro* methods can help in predicting the bioequivalence of extemporaneously

prepared formulations without the need of expensive and time consuming bioequivalence studies.

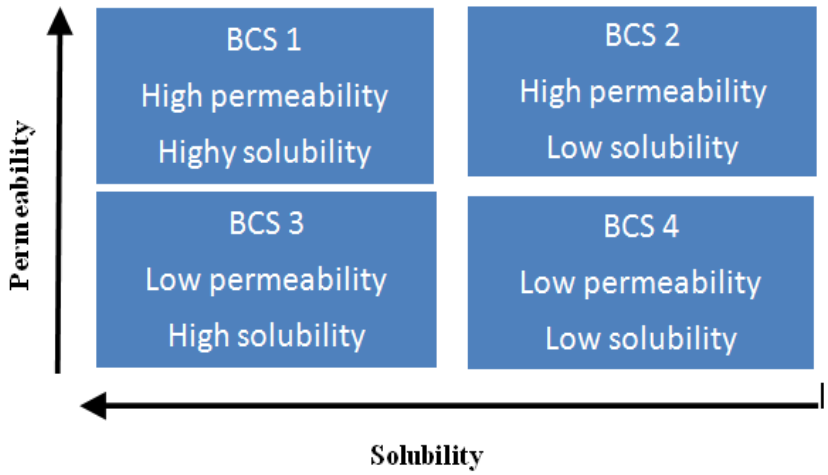


**Figure 1:** The BCS as defined by Amidon [26].

## 1.4 *In vitro* Dissolution Testing

For a pharmaceutical drug product, the rate and extent of absorption is primarily controlled by its dissolution behavior from its specific dosage form. Accordingly, for a drug to be effective, it must be released from the dosage form and dissolved in the gastrointestinal fluids as a first and essential step before being absorbed into blood circulation [27].

Differences in dissolution behavior among drugs have a great impact on their bioavailability, which leads to different therapeutic responses that ranges from toxicity to sub therapeutic levels [28].

*In vitro* dissolution testing is a distinctive tool that illustrates the release behavior of a drug by reducing human exposure without abandoning product quality. For pharmaceutical drug products, dissolution testing is

routinely performed for quality control and quality assurance purposes. It is used in the drug development stages and for commercial pharmaceutical manufacturing as well [29, 30].

*In vitro* dissolution testing is a regulatory requirement in the development and assessment of new pharmaceutical formulations; it ensures batch to batch consistency, helps in evaluating stability of the drug product during its shelf life period, and to confirm product quality in scale up post approval changes (SUPAC) for means of bioequivalence studies [31].

In addition, *in vitro* dissolution is appreciated as time and money saving method since it is considered an FDA-approved surrogate for *in vivo* studies (Biowaiver), [30].The dissolution characteristics of a pharmaceutical dosage form can affect its bioavailability. Formulations with different release rates can produce different pharmacokinetic (PK) profiles of the same drug substance, potentially resulting in bioavailability differences. Evaluation of the dissolution behavior of an extemporaneous preparation is an important quality-control parameter.

### 1.5 *In silico*

With the evolution of combinatorial chemistry, a large series of related chemical compounds are prepared with the same reaction and a variety of reagents. However these compounds have to run through high throughput screening and only few of them are chosen to complete with for further reactions and testing. To keep up with such dramatic increase in chemical compounds capacity, there is an insistent need of developing new methods

to facilitate the screening of absorption, distribution, metabolism, excretion (ADME), and so the need of new tools and equipment [32].

Recently, *in silico* modeling play an important role in the prediction of *in vivo* behavior based on *in vitro* data [33], by the estimation of specific parameters. The computational simulation technology has proven its usefulness in their ability of predicting the rate and extent of drug absorption using the properties predicted from the chemical structure alone. This method give pharmaceutical companies invaluable opportunity to estimate and assess the capacity of absorption before compounds being actually synthesized [34].

Nowadays, several commercial software for *in silico* simulations of oral drug absorption are available. GastroPlus™ software is an example, which employs the Advanced Compartmental Absorption and Transit (ACAT) model and the BCS principles to establish *IVIVC*, assess biowaiver studies and facilitates the evolution of new formulations and dosage forms through which saving time and budget of pharmaceutical companies [33]. GastroPlus™ simulate the pharmacokinetics of the drug and its absorption in gastro intestinal tract. ACAT model consists of nine compartments (stomach, duodenum, jejunum 1, jejunum 2, ileum 1, ileum 2, ileum 3, caecum, and ascending colon) to mimic the human GI tract. Beside human physiology, models for rat, cat, rabbit or dog are available as well, taking into consideration the physicochemical properties of the drug such as solubility, pKa, lipophilicity, and permeability, beside formulation characteristics in addition to pharmacokinetic properties. GastroPlus™ was

proved for its powerful efficiency in predicting plasma concentration profile for many drugs [35].

In a study on calcium channel blocker agent (Nifedipine); *In silico* modeling was coupled with *in vi*tro dissolution for the prediction of *in vivo* behavior of the drug [36].

For another study, GastroPlus™ was applied to predict oral bioavailability of newly developed high permeability low solubility CNS drug followed by *in vivo* study on beagle dogs in order to build a preclinical formulation through which a simple oral dosage form gave the acceptable pharmacokinetic parameters without the need of complex formulations and hence considerable budget saving was achieved [37].

Ajay Saxena and others have established an *In vitro- In silico- In vivo* (*IVISIV)* correlation using GastroPlus™ to predict the absorption of weak basic drugs that undergo pH dependent solubility, thus growing liability assessment in early drug development stages [38].

### 1.6 Antihypertensive Medications

Globally; cardiovascular diseases are one of the leading causes of mortality[39]. Hypertension, also known as high or elevated blood pressure is one of the risk key factors of cardiovascular diseases [40].

Nowadays, there is an increasing interest in developing new formulations of marketed agents to keep up with the market need. Anti-hypertension medications are among the most common drugs that pharmaceutical market

still of continuous need especially with the lack of liquid preparations of these agents.

### 1.6.1 Amlodipine

AML as besylate is a long acting, 3rd generation dihydropyridine derivative of calcium channel blockers group, amlodipine chemically: $C_{26}H_{31}ClN_2O_8S$ (Figure2).

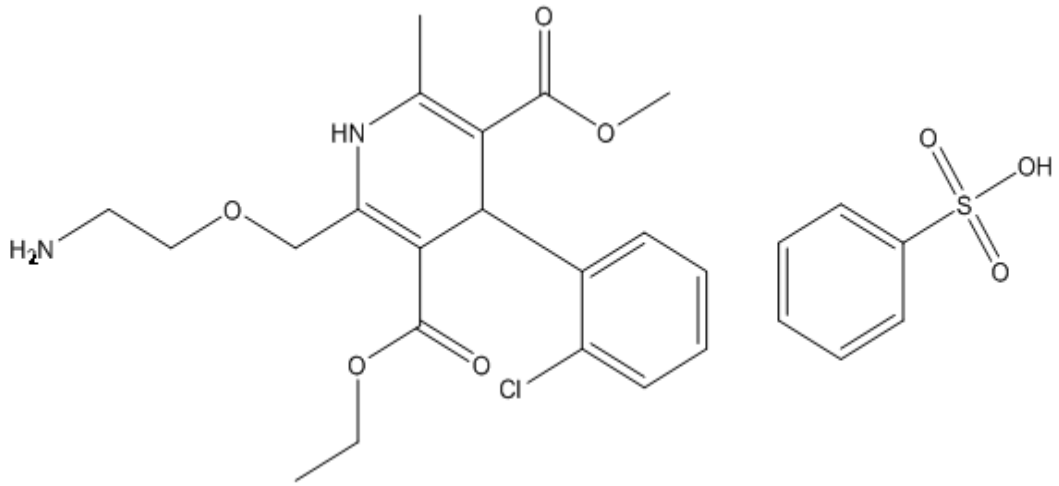


**Figure 2:** AML Besylate chemical structure.

AML blocks the influx of calcium through the “slow” channels in both coronary and peripheral blood arteries causing them to dilate and subsequently reducing blood pressure [41].

AML either 5 or 10 mg tablets have proved its efficacy as an anti-hypertensive agent either as a monotherapy or combined with other classes of medications [42]. It’s used for the treatment of hypertension and of angina as well [41].

AML has a partition coefficient of 2.66 at pH 7.4.It is a basic drug with a pKa value of 8.7 ,which keeps AML in its ionized form at physiological pH [43]. According to BCS, AML is considered as class 1 [44], with high solubility of 0.774 mg/ml and high permeability with $0.0743 \times 10^{-4}$ cm/sec (caco-2) [45].

AML is 98% bound to plasma proteins and has a volume of distribution (Vd) of 21 L/Kg and a bioavailability of 60-80%and a clearance is 7 ml/min/kg [46, 47]. AML was 62% recovered from urine and 23% from feces after IV administration [48].

Although AML is extensively metabolized in the liver, this process is considered relatively slow with retarded elimination rate that results in prolonged elimination half- life (40-60) hrs, such properties make AML substantially unique drug if compared to other calcium channel blockers of dihydropyridines and non dihydropyridines [49, 50].

### 1.6.2 Valsartan

VAL chemically:$C_{24}H_{29}N_5O_3$ (Figure 3) is angiotensin II receptor blocker (ARB).

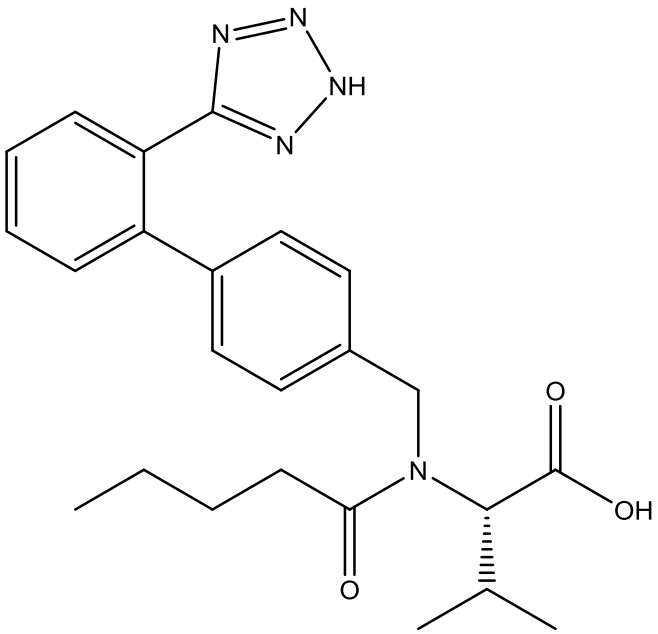


**Figure 3:** VAL chemical structure.

VAL is available in different strength 40, 80, 160 and 320 mg tablets, it acts by preventing angiotensin from binding to angiotensin receptors, and because angiotensin is known by its ability to constrict blood vessels; then blocking these receptors leading blood pressure to be reduced [51].

VAL has a distribution co efficient of -0.34 at pH 7 [52]. VAL is a weak acid that has pH dependent solubility. It has 2 pKa values (3.9 and 4.73) [53, 54], while solubility of VAL is limited below pH 3.VALsolubility increases with increasing pH whereas permeability decreases at the same range. Accordingly, some papers assign it as BCS class 2 and others consider VAL as a BCS class 3 drug with high solubility of 16.8 mg/ml at pH 8 and low permeability of $0.262\times10^{-4}$ cm/sec (in rat) [26, 55]

VAL has a bioavailability of 39%, elimination half-life 9.5 hrs and a Vd of 16.9 L [56].The main dose of VAL is excreted unchanged through faecal

route and to a lower extent in urine, about 9% of VAL is recovered as inactive metabolite M1 [57].

AML and VAL are considered as a safe and effective combination; as it is well tolerated in most patients with minimum adverse reactions and reduced peripheral oedema incidence [58, 59]. Furthermore, the combination therapy of AML and VAL was significantly more effective in lowering BP than using AML or VAL alone [60, 61].

Provided that both AML and VAL are safe and effective in treatment of HTN in children from 1 years and older [62-64]; this affords that liquid formulation (amlodipine/valsartan) will provide additional value for this group of patients as well.

AML and VAL as a combination is available in the pharmaceutical market as a film coated tablets. However, no liquid formulation of this combination of active ingredients is available. Therefore, crushing of the tablet is the only choice for using these drugs in patients with swallowing difficulties.

There were several attempts to make VAL extemporaneous suspensions [65, 66], moreover, and AML extemporaneous suspension from available commercial tablets [14], but there was no efforts done for preparing the combined (AML/VAL) suspension. No bioequivalence studies are conducted in such situations, which leads to further confusion whether these crushed tablets preserve its efficacy or this action may lead to serious complications.

## 1.7 Aims of this study

The main aim of this study was to develop an extemporaneous suspension of AML and VAL as a combination using crushed commercial tablets (Valzadepine® 5/80) for use in patients with swallowing difficulties.

**The Specific goals were to:**

1. To formulate an oral liquid dosage form of both (AML and VAL) from commercially available tablets (Valzadepine® 5/80).

2. To evaluate the chemical, physical and microbial stability of this extemporaneous suspension.

3. To determine the *in vitro* release behavior of this combination from the different formulations (the extemporaneous suspension and the film coated tablets).

4. To ensure the bioequivalence of the extemporaneous suspension obtained from crushed tablet with the tablet swallowed as whole, using simulation technology to predict the *in vivo* behavior of this formulation and compare it with the observed profile of the whole tablet based on the *in vitro* dissolution data.

**The objectives of this study**

In this thesis, an extemporaneous preparation of an oral suspension (AML and VAL)was developed for use in pediatric population with hypertension. In the literature review, the extemporaneous suspension and the stability

aspect of these preparations are discussed. In the experimental part of this work, an extemporaneous suspension containing a combination of AML and VAL was prepared from crushed oral dosage form. The stability and the *in vitro* dissolution properties were investigated. Furthermore, Simulation technology was used to predict the *in vivo* behavior of this extemporaneous suspension.

# Chapter two
# Methodology

## 2.1 Materials, Equipment and Dosage form.

Valzadepine® film coated tablet, containing 5 mg AML and 80 mg VAL, was used in this study (Pharmacare PLC, Palestine,Batch 036B16; Expiry date 02\2018). AML and VAL United States pharmacopeia (USP) reference standards, and all the excipients and materials (aspartame, mannitol, tri-sodium-citrate, guar gum, potassium dihydrogen phosphate, sodium hydroxide, glacial acetic acid); were kindly donated by Pharmacare PLC, Ramallah, Palestine.

All chemicals and reagents that used were of analytical grade and no further purification was needed.

HPLC grade solvents; acetonitrile (ACN) (Sigma-Aldrich), methanol (MeOH) (LAB-SCAN, Ireland), triethyl Amine (Merck) and phosphoric acid (Frutarom). Highpurified water was prepared by using a Millipore Milli-Q plus water purification system.

- *Equipment and tools:*

Equipment used are: balance (Ohous balance), viscometer (Brookfield), pH meter (Mettler Toledo MP225), dissolution apparatus (ERWEKA DT70), HPLC (HITACH), sonicater (BRANSON 8510), GastroPlus™software (version 9.0, Simulation Plus Inc, Lancaster, CA, USA).

- *Media for Dissolution study:*

- Phosphate buffers (USP) for pH= 6.8 and pH= 4,5 was prepared by dissolving 47.6 g of potassium dihydrogen phosphate and 6.272 g of sodium hydroxide in 7 L of water, pH was adjusted to 6.8 using 0.2 N sodium hydroxide and to pH 4.5 using phosphoric acid.

- 0.1 N HCl (USP) pH 1.2

**2.2 Preparation of the extemporaneous suspension**

In this study, an extemporaneous suspension containing (AML 5 mg/VAL 80 mg) , was prepared from commercial tablet Valzadepine® (5/80). The Detailed method of preparation are clarified in the following steps:

**1.** 100 tablets of Valzadepine® (AML 5/VAL 80) mg were crushed to a fine powder.

**2.** Then all the excipients in (Table 2) were weighed and mixed with the powder to achieve a final concentration of 0.2 mg/gm.

**3.** 16.02 gm were weighed and diluted with water in two steps up to 50 ml.

Through which each 5 ml of suspension contains one crushed tablet with 5 mg AML and 80 mg VAL, and hence for the 50 ml bottle 10 crushed tablets are needed, given that each tablet weighs 0.2 g, then 2 g of crushed tablets are needed for each 50 ml bottle and 40 gm for 20 bottles.

**Table 2: The composition of the AML/VAL 5/80 suspension formula.**

| Material | Function | mg/g | g/50ml bottle | Gram |
|---|---|---|---|---|
| Valzadepine® crushed tab 5/80 | Active ingredients | 0.20 | 2.00 | 40.00 |
| Aspartame | Sweetening agent* | 0.01 | 0.10 | 2.00 |
| Mannitol | Flavoring agent * | 1.36 | 13.60 | 272.00 |
| Tri-sodium citrate | pH modifier/Buffering agent * | 0.016 | 0.16 | 3.20 |
| Sodium hydroxide | pH modifier /Buffering agent * | 0.001 | 0.01 | 0.20 |
| Guar gum | Suspending agent * | 0.015 | 0.15 | 3.00 |
| Total weight | | 1.602 | 16.02 | 320.40 |

- * are from [67]

- The average weight of Valzadepine® 5/80 mg tablets is 0.2 g.

The resulting powder was divided into 20 amber glass bottles (16 gm powder in each 50 ml bottle), which were ready for reconstitution to form the 5/80 mg AML/VAL extemporaneous suspension (to be completed up to 50 ml water and to be shaken well before use).

### 2.3. pH measurements:

The pH of the different media as well as the reconstituted suspension was determined using Mettler Toledo MP225 pH meter, each measurement was done in triplicate.

### 2.4 Viscosity measurements

The rheological behaviour of the extemporaneous suspension was measured using Brookfield viscometer over a shear rate 90-100 s-1). The

viscosity measurement was performed at 25°C in duplicate and the rheogram was obtained for the selected formula.

## 2.5 Stability study

### 2.5.1Chemical stability

The stability study was conducted by storing 10 containers containing 50 ml of the extemporaneous suspension at room temperature. Another 10 bottles, containing the initial powder were kept for further analysis. The suspensions were analyzed using HPLC in duplicates in a weekly manner over a period of one month. The stability of the extemporaneous suspension was determined by calculating the percentage of the drugs remaining at the end of every week.

### 2.5.2 Physical stability

The formulated suspension was tested for its physical properties such as: pH, viscosity, appearance, and its organoleptic properties. They were tested at the time of preparation and at the end of every week over one month at room temperature.

### 2.5.3 Microbiological stability

#### *2.5.3.1 Preparation of culture media*

28g of nutrient agar dehydrated powder was dissolved in 1L of distilled water. The prepared suspension was heated until boiling while being mixed roughly. The solution was placed in the autoclave at 125°C for 15 minutes

in order to get it sterilized. After sterilization, the solution was poured in already sterilized petri dishes. The petri dishes were placed in the refrigerator for 24 hrs.

#### *2.5.3.2 Microbiological analysis*

After 24 hrs, 0.1 ml of each reconstituted suspension was placed on one of the petri dishes and they were placed in the incubator at 37 °C for 48 hrs. The analysis includes: total bacterial count and examine the presence of mold and yeast, *Staphylococcusaureus*, *Pseudomonas aeroginosa* and *Candida albican.*

### 2.6 Drug release study

#### 2.6.1 Dissolution

Dissolution rotating paddle apparatus II (Erweka dt70, Germany) was used to study the release of AML/VAL from the tablets as well as the extemporaneous suspension. 1000 ml medium was used for each vessel of the paddle apparatus that was rotating 75 rpm for 30 minutes, the temperature was set at (37 °C ± 0.5 °C).

10 ml samples were withdrawn at predetermined time points; 5, 10, 15, 20, 30 minutes and replaced with fresh media, the samples were taken from the midway between the surface and the top of the rotating paddles not less than 1 cm from the vessel wall. Each sample was filtered through a 0.45-mcm microporous PTFE syringe filter, then they were introduced to HPLC analysis to figure AML/VAL concentration in the samples [68]

### 2.6.2 Statistical Analysis

Similarity and difference factors ($f_2$ and $f_1$respectively*)* were used to assess the dissolution data as reported in equations 1 and 2 below.

The $f_2$ factor is a measure of the closeness of two profiles while $f_1$ is a measure of the difference between two profiles:

$$f_1 = \left\{ \left[ \sum_{t=1}^{n} |R_t - T_t| \right] \Big/ \left[ \sum_{t=1}^{n} R_t \right] \right\} \times 100 \quad \text{..... (1)}$$

$$f_2 = 50 \cdot \log \left\{ \left[ 1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad \text{..... (2)}$$

where $R_t$ and $T_t$ are the percentages of drug dissolved at each time point for the reference and test products, respectively. When $f_1$ value is greater than 15; this indicates no similarity, and when $f_2$ value is greater than 50; then there is a significant similarity between the two products.

### 2.7 The HPLC analysis

#### 2.7.1 Instruments, Solutions and Chromatographic Conditions

The HPLC system consisted of Lachrom (Merck-Hitachi) equipped with model L-7100 pump, L-7200 autosampler, L-7300 column oven, DADL-7450 photo diode array (PDA) detector, and D-7000 software HSM version 3.1 (Merck Hitachi, Kent, England).

Weights were measured using Ohous balance, pH was identified using Toledo pH meter.

The HPLC experimental conditions were optimized on a stainless steel column (250 cm ×4.6 mm) packed with octadecylsilyl silica gel for chromatography ( 5 µm).

Mobile phase was prepared by mixing 2 solutions; solution A: solution B (1:1) in which solution A is: Methanol, Acetonitrile, and Buffer (175:75:250), and solution B is: Water, Acetonitrile, Glacial acetic acid (150:350:0.5), and the buffer was prepared by adding 7.0 ml of triethylamine into 1000 ml flask containing 900 ml of water, the pH of this buffer was adjusted to 3.0 $\pm$ 1 with phosphoric acid, then diluted with water to the final volume of 1000 ml.

The mobile phase was filtered through a 0.45-mcm microporous filter and degassed by sonication prior to use, the flow rate was 1.0 ml/minute with injection volume of 20 µL, and the UV-detector was set to 220 nm.

The diluent was: Acetonitrile: Water (1:1)

**2.7.2 Standard stock solution**

The standard solution of AML was prepared by dissolving 27.74 mg of AML besylate reference standard in diluent till reach 200 ml, the standard solution of VAL was prepared by dissolving 80 mg of VAL reference standard in 40 ml diluents then sonicated till dissolved and the volume completed to 50 ml with the diluent. Then the standard solution of the combination was prepared by taking 5 ml of each standard solution to 50 ml volumetric flask together and completed to 50 ml with the mobile phase.

### 2.7.3 Sample stock solution

Sample stock solution was prepared by taking 5.5 gram of the suspension to 50 ml volumetric flask with 10 ml of water, 30 ml of diluent was added, stirred and sonicated then completed to the volume with the diluent, 5 ml of this sample stock solution was taken and diluted to 50 ml with the mobile phase, each sample was filtered through 0.45-µm syringe tip filter.

The peak quantification was obtained by comparing sample & standard peak area ratios as a function of concentration.

## 2.8 Gastrointestinal simulation

GastroPlus™ software (version 9.0, Simulations Plus Inc., Lancaster, CA, USA), which based on the Advanced Compartmental Absorption and Transit (ACAT) was used in this study. The approach used was to develop and verify absorption models for both AML and VAL from Valzadepine® tablet). The *in silico* models were initially constructed for immediate release (IR) tablet, and were afterwards implemented, to predict the *in vivo* profiles for both drugs from the extemporaneous suspension.

Therefore, Two databases were established: one for AML and the other for VAL. Each database consists of two records; one for the tablet and the other for the suspension.

GastroPlus™ as a single simulation mode was used to run the gastrointestinal simulation depending on the physicochemical, physiological, and the pharmacokinetics properties of AML and VAL, as

well as the *in vitro* dissolution data from both the tablet and the suspension. GastroPlus™ includes three modules: compound, physiology, and pharmacokinetics. For the compound and pharmacokinetics modules; the input data were collected from the literature. In the physiology module, the simulations were conducted using The Human Physiology Fasted mode. All the physiological parameters were fixed at default values. In the pharmacokinetic module: two compartment kinetics were followed for AML and for VAL as well, both exhibited zero order absorption and first order elimination[69].

The simulations were conducted using the Johnson model as a dissolution model. (IR tablet) mode, in GastroPlus™ was selected for simulations. The model for IR tablet was verified by comparing the simulated profiles to the observed *in vivo* pharmacokinetic profiles of (Valzadepine® tablet), which was obtained from Pharmacare Ltd (Table 3).The developed model for the "IR tablet" dosage form was then employed for predicting the *in vivo* performance of the suspension. The simulation of the suspension was performed using the "IR suspension" as the selected dosage form and by introducing the dissolution data for the formulated suspension.

**Table 3: Plasma concentration-time profile of AML and VAL of Valzadepine® 5/80 tablets obtained from Pharmacare pharmaceutical company.**

| Time (hr) | AML (ng/ml) | VAL (ng/ml) |
|---|---|---|
| **0.0** | 0.00 | 0.00 |
| **0.5** | - | 153.75 |
| **1.0** | 0.6 | 458.45 |
| **1.5** | - | 611.95 |
| **2.0** | - | 689.80 |
| **2.5** | 1.8 | 747.30 |
| **3.0** | - | 689.35 |
| **3.5** | - | - |
| **4.0** | 2.7 | 552.70 |
| **5/0** | - | 425.60 |
| **6/0** | 3.2 | 338.65 |
| **7/0** | - | 280.8 |
| **8.0** | 2.8 | 224.8 |
| **12.0** | 2.5 | 162.6 |
| **15.0** | 2.2 | - |
| **18.0** | 1.8 | - |
| **24.0** | 1.4 | 59.65 |
| **48.0** | - | 15.7 |
| **72.0** | - | 13.4 |
| **96.0** | 0.6 | - |
| **120.0** | 0.3 | - |
| **144.0** | 0.1 | - |

The experimental *in vitro* dissolution profiles for both active ingredients from Valzadepine® tablet and suspension in the different pH media were

incorporated in the corresponding model. The summary of all input parameters for simulation is given in Table 4.

**Table 4: Simulation input data**

| **Parameter** | **Value** | |
|---|---|---|
| | **Amlodipine (as besylate)** | **Valsartan** |
| Molecular weight (g/mole) | 567.051 | 435.53 |
| Partition/Distribution coefficient | 2.66 (pH=7.4) [a] | -0.34 (pH=7) [b] |
| $PKa_1$ | 8.7[c] | 3.9 [d] |
| $PKa_2$ | - | 4.73[d] |
| Solubility (mg/ml) | 0.774 (pH 7.4) [e] | 16.8 (pH=8) [f] |
| $P_{eff}$ (Human jejunal permeability) (cm/sec) | $0.0743 *10^{-4}$ [g] (caco-2) | $0.262*10^{-4}$ [h] (rat) |
| Dose (mg) | 5 | 80 |
| Dose volume (ml) | 250 | 250 |
| Mean precipitation time (sec) | 900 [i] | 900 [i] |
| Diffusion coefficient ($cm^2/s$) | $4.2*10^{-8}$ [j] | $1.1*10^{-8}$ [k] |
| Drug particle density (g/ml) | 1.2[i] | 1.2 [i] |
| Blood plasma concentration ratio | 1[i] | 1[i] |
| Body weight (kg) | 70 | 70 |
| Unbound percent in plasma (%) | 2 [l] | 5 [m] |
| Clearance (l/hr) | 28 [n] | f [n] |
| Volume of distribution, Vc(L/Kg) | 17 [n] | 0.23 [n] |
| Elimination half-life (h) | 27.03 [o] | 5.58 [o] |
| Simulation time (hr) | 144 | 72 |
| **[a]From [45, 70]** | | |
| **[b]From [52]** | | |
| **[c]From [43, 71]** | | |
| **[d,f]From[53]** | | |
| **[e]From [72]** | | |
| **[g] From[45]** | | |
| **[h]From [73]** | | |
| **[i] From Gastro Plus default values** | | |
| **[j,k]From[74]** | | |
| **[l] From [46]** | | |
| **[m] From [75]** | | |
| **[n] Gastro Plus calculated (using PBPKPlus™ Module)** | | |
| **[o] Gastro Plus calculated (built-in calculation from PK parameters)** | | |

The percent of prediction error of the simulation (% PE) can be calculated by equation 3 below, this represents the percent of error between the predicted values and that of the *in vivo* observed data

$$\%PE = \frac{PK_{predicted} - PK_{\text{observed}}}{PK_{\text{observed}}} \times 100\% \quad \text{.....(3)}$$

# Chapter Three
# Results

## 3.1 The formulation

The AML/VAL suspension was successfully prepared and well suspended upon brief shaking with acceptable appearance, smell and palatable taste. Its pH value was 5.5

## 3.2 Viscosity Determination

The viscosity of the extemporaneous suspension was examined at different shear rates. The behavior is shown to be dilatant, i,e, the viscosity increases with the increase in the shear rate. The data is shown in Table 5 and Figure 4.

**Table 5: The rheological behavior of the extemporaneous suspension over different shear rates.**

| Shear rate(rpm) | 0 | 5 | 10 | 12 | 20 | 30 | 50 | 60 | 100 |
|---|---|---|---|---|---|---|---|---|---|
| Viscosity (Cp) | 0 | 0 | 25.6 | 160 | 377 | 410 | 422 | 425 | 470 |

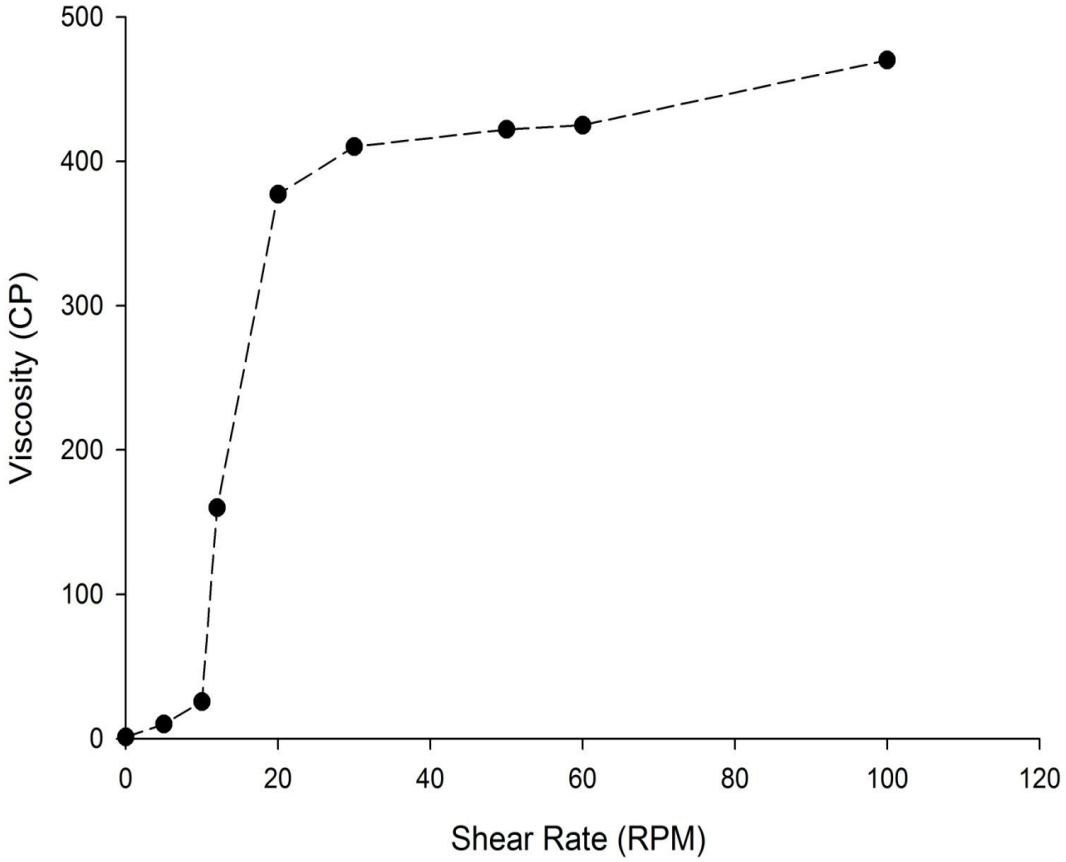


**Figure 4:** The rheological behavior of the extemporaneous preparation over different shear rates

## 3.3. Drug release study

The *in vitro* release of AML from both the IR tablet and the suspension was investigated in media with different pH (1.2, 4.5 and 6.8). The dissolution profiles for AML from both formulations are shown in Figure 5. As can be seen, AML exhibited very rapid dissolution in phosphate buffers (4.5 and 6.8) with more than 85% was dissolved within 15 minutes, and has a rapid dissolution in 0.1 N HCL with more than 85% was dissolved within 30 minutes and an $f_2$value of 51.74.

Whereas for VAL; media pH has shown to have a marked effect on its release from both dosage forms (Figure 6). At pH=6.8, the percentage of VAL released was more than 85% within 15 minutes, however, in pH 4.5 and 1.2 media, the dissolution was much slower. At pH=4.5 less than 70% of the drug released within 30 minutes. Whereas, at pH= 1.2, the apparent amount of VAL released was not more than 26% within 30 minutes from both dosage forms. This decrease in the dissolution rates with the reduction in the media pH reflects the pH-dependent solubility of VAL. $f_1$ and $f_2$ values were calculated for each drug from each dosage form. Where the IR tablet was the reference and the extemporaneous suspension was the test.

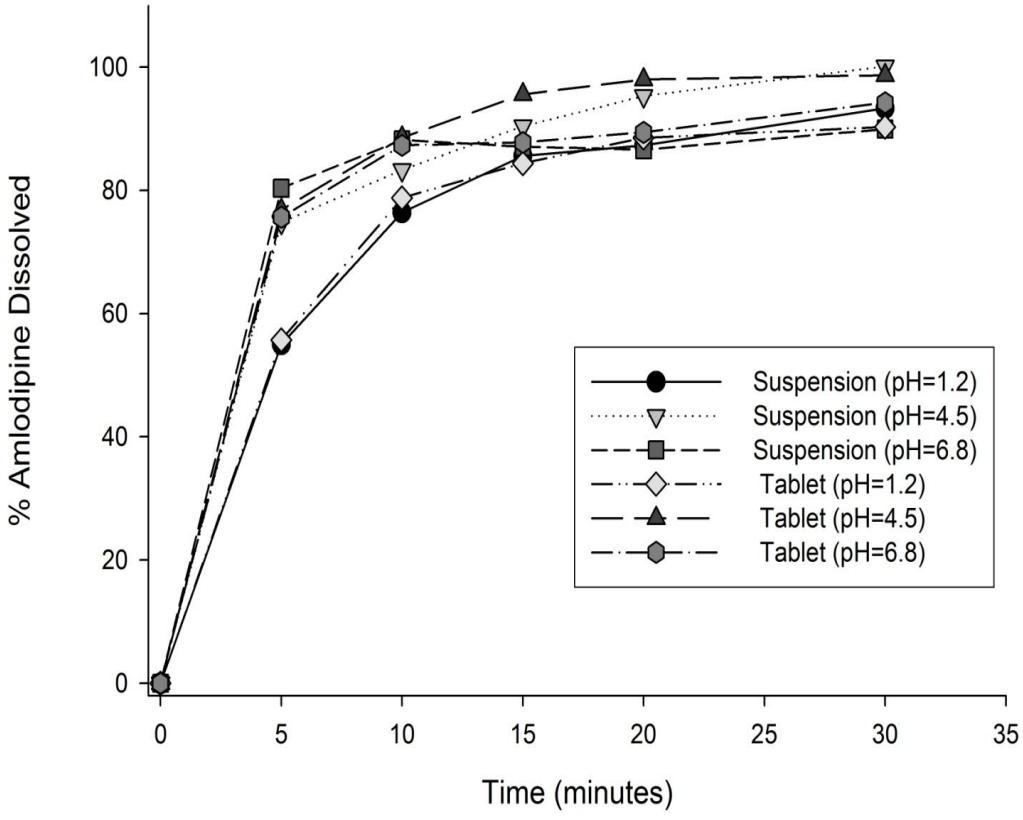


**Figure 5:** Release profiles of AML from the tablet and the suspension at different pH values.

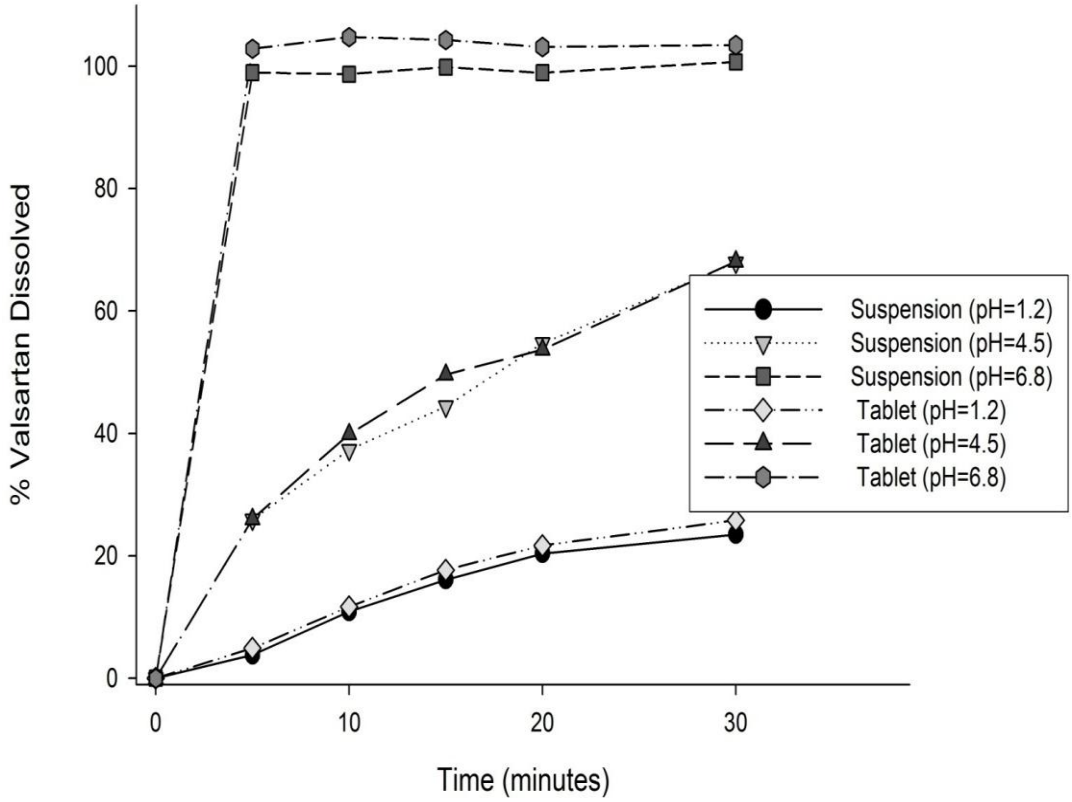


**Figure 6:** Release profiles of VAL from the tablet and the suspension at different pH values.

## Table 6: Dissolution of AML and VAL from Valzadepine® tablets

| **% Dissolved of Amlodipine ± (SD)** | | | | |
|---|---|---|---|---|
| | **Tablet** | | **Suspension** | |
| **Medium** | **15 min** | **30 min** | **15 min** | **30 min** |
| **pH 1.2** | **84.4** ± (2.14) | **90.3** ± (1.81) ($f_2$ =51.74) | **85.6** ± (1.32) | **93.3** ± (1.59) |
| **pH 4.5** | **95.6** ± (1.67) | **98.7** ± (1.73) | **90.4** ± (1.68) | **100.1** ± (1.97) |
| **pH 6.8** | **87.8** ± (1.97) | **94.3** ± (0.78) | **87.1** ± (0.87) | **89.9** ± (1.05) |
| **% Dissolved of Valsartan ± (SD)** | | | | |
| **pH 1.2** | **17.7** ± (2.07) | **25.8** ± (1.91) ($f_2$ =51.80) | **16.1** ± (1.96) | **23.5** ± (1.45) |
| **pH 4.5** | **49.6** ± (2.18) | **68.1** ± (1.89) ($f_2$ =51.63) | **44.4** ± (1.84) | **67.9** ± (2.76) |
| **pH 6.8** | **104.4** ±(1.83) | **103.4** ± (1.22) | **99.8** ± (0.56) | **100.7** ±(1.74) |

The results of similarity were more than 50 for each dissolution showed latency in 85% within 15 minutes indicating the similarity in the release from both formulations. They are shown in Table 6 and Figures 5 and 6.

As pH 6.8 is the recommended media by FDA and USP [68]. For AML, it was very rapidly dissolving with average of 87.3% and 88.1% was dissolved within 10 minutes from the tablet and the suspension respectively. The same in case of VAL; it was very rapidly dissolving with 104.8% and 98.7% dissolved within 10 minutes for the tablet and the suspension respectively, the data are shown in Table 7.

**Table 7: The percentage of AML and VAL released from the tablet and suspension formulations at pH 6.8 as recommended by FDA and USP**.

| **% Dissolved of Amlodipine** | | | | |
|---|---|---|---|---|
| **Time (min)** | **Tablet** | **SD** | **Suspension** | **SD** |
| **5.0** | 75.6 | 1.4 | 80.3 | 2.0 |
| **10.0** | 87.3 | 1.2 | 88.1 | 1.9 |
| **15.0** | 87.8 | 0.9 | 87.1 | 1.9 |
| **20.0** | 89.4 | 1.8 | 86.6 | 1.5 |
| **30.0** | 94.3 | 1.1 | 89.9 | 0.8 |
| **% Dissolved of Valsartan** | | | | |
| **Time (min)** | **Tablet** | **SD** | **Suspension** | **SD** |
| **5.0** | 102.9 | 1.0 | 98.9 | 1.9 |
| **10.0** | 104.8 | 0.3 | 98.7 | 1.8 |
| **15.0** | 104.3 | 0.6 | 99.8 | 1.8 |
| **20.0** | 103.1 | 1.9 | 98.9 | 1.8 |
| **30.0** | 103.4 | 1.7 | 100.7 | 1.2 |

## 3.4. Stability study

### 3.4.1 Physical stability:

There were no changes observed in the appearance, odour, colour and pH.

### 3.4.2 Chemical stability

The suspension was chemically stable throughout the four weeks period. The mean percentages of the remaining active ingredients were over 90% within the four weeks period (Table 8).The mean concentrations of AML and VAL on the thirty day were 97.3% and 101.1% respectively at room temperature.

**Table 8: The mean percentage of the active ingredient in AML/VAL suspension throughout 4 weeks period at room temperature.**

| Week | initial | | Week 1 | | Week 2 | | Week 3 | | Week 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AML | VAL | AML | VAL | AML | VAL | AML | VAL | AML | VAL |
| **% remained** | 102.1 | 106.2 | 101.8 | 105.3 | 99.1 | 102.2 | 98.3 | 101.9 | 97.3 | 101.1 |

### 3.4.3 Microbial Stability

The formulated AML/VAL suspension passed the microbial testing study through the four weeks period. No microbial contamination was observed in the suspension during the study period. The results are described in Table 9.

**Table 9: Microbial study results.**

| Microrganism | Total microbial count |
|---|---|
| Mold and yeast | < 10 cfu/ml.g |
| S. aureus | Negative |
| P. aeroginosa | Negative |
| C. albicans | Negative |

## 3.5 HPLC analysis

AML eluted first at about 4 minutes, and VAL was next at about 11 minutes, standard peaks are shown in Figure 6.

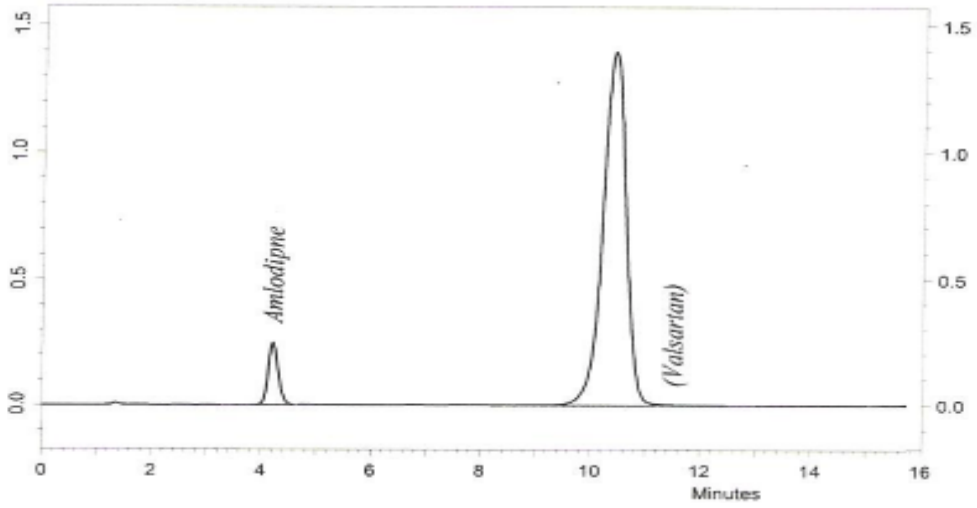


**Figure 7**: standard peaks of AML and VAL as eluted in HPLC analysis.

## 3.6 Drug absorption simulation

### 3.6.1 Gastrointestinal simulation

*In silico* simulation was used to build models describing the *in vivo* absorption of both AML and VAL from IR tablet based upon the physicochemical, physiological and the *in vitro* dissolution data. The simulated plasma profiles for AML and VAL together with the *in vivo* observed curves following the intake of Valzadepine® IR tablet are presented in Figures 8 and 10. The simulated profiles for both drugs from the solid dosage form were in good agreement with the *in vivo* observed curves. The simulated and the *in vivo* pharmacokinetic parameters ($C_{max}$ and $AUC_{0-\infty}$) for both drugs are presented in Tables 10 and 11. The percent

prediction errors obtained were less than 10% for all pharmacokinetic parameters, indicating good predictability. The developed models for the IR tablet dosage form were implemented to predict the *in vivo* performance of the extemporaneous suspension using the *in vitro* dissolution data of the suspension. Figures 9 and 11 for AML and VAL respectively compare the predicted absorption profiles for the suspension and the *in vivo* plasma profile observed for IR tablet. Then *in silico* pharmacokinetic parameters for suspension were compared with that observed *in vivo* for IR tablet. The extemporaneous preparation is predicted to be similar with the IR tablet dosage form, since the 90% confidence intervals for $C_{max}$ and $AUC_{0-\infty}$ for both active ingredients fall within the limits of 80–125% for IR release tablet.

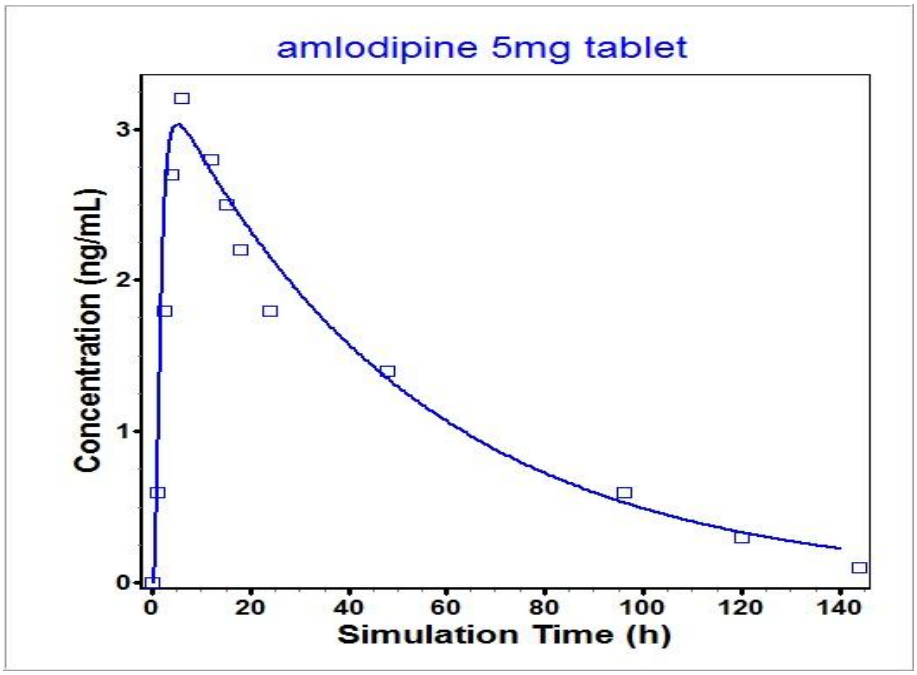


**Figure 8:** The simulated plasma profile of AML from Valzadepine® tablet ( __: *in silico*, : *in vivo* data)

The pharmacokinetics parameters that are predicted by the *in silico* method for AML suspension indicates good predictability with the percent of prediction error maintained less than 10%, the data and predicted profiles of AML suspension are shown in Table 10and Figure 9.

**Table 10: AML observed and predicted pharmacokinetic parameters with percentage of prediction error.**

| Parameter | Calculated for the tab | | Observed | Calculated for the susp | |
|---|---|---|---|---|---|
| **$C_{max}$ (ng/ml)** | 3.0302 | PE= 5.306% | 3.20 | 3.027 | PE= 5.406% |
| **$AUC_{0-\infty}$ (ng.hr/ml)** | 169,81 | PE= 5.76% | 160.65 | 169.78 | PE= 5.74% |

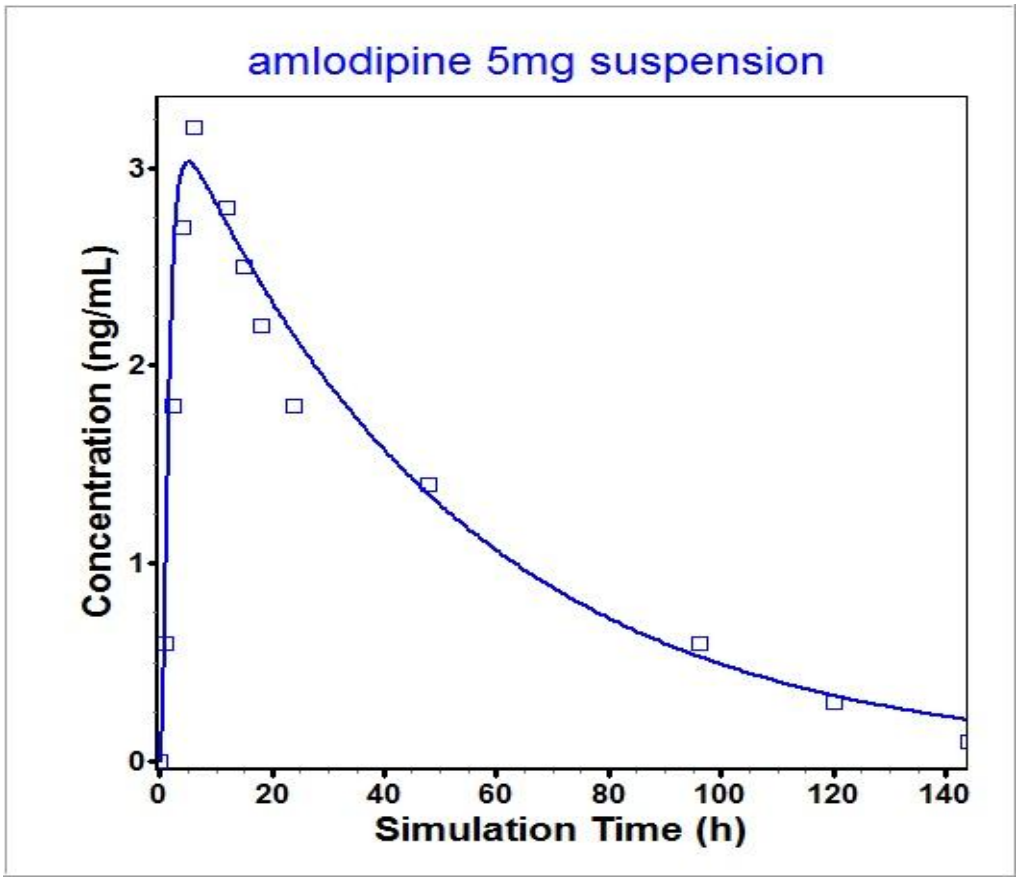


**Figure 9:** The simulated plasma profile of AML suspension ( ___ : *in silico* predicted using *in vitro* data, : *in vivo* data of the tablet).

**Table 11: VAL observed and predicted pharmacokinetic parameters with percentage of prediction error.**

| Parameter | Calculated for the tab | | Observed | Calculated for the susp | |
|---|---|---|---|---|---|
| **$C_{max}$ (ng/ml)** | 704,55 | PE= 5.72% | 747.30 | 707,22 | PE= 5,36% |
| **AUC (ng.hr/ml)** | 8517.70 | PE= 4,826% | 8949.70 | 8517.60 | PE= 4,82% |

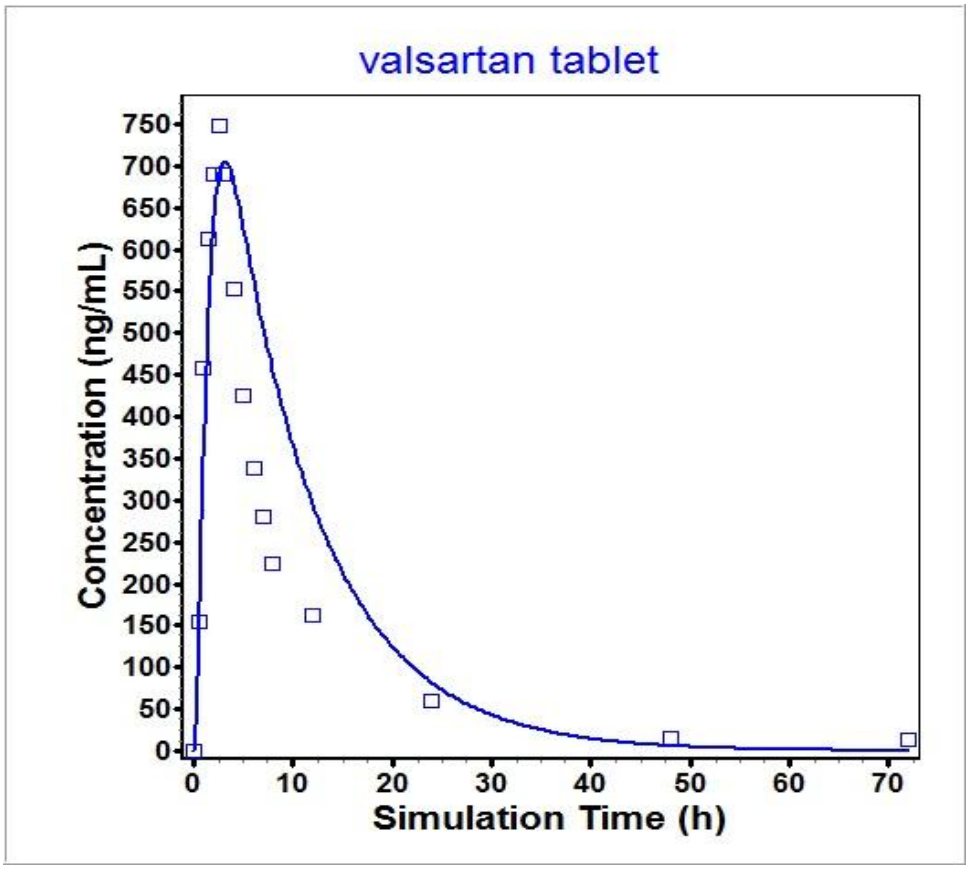


**Figure 10:** The simulated plasma profile of VAL from Valzadepine® tablet ( ___: *in silico*, : *in vivo* data).

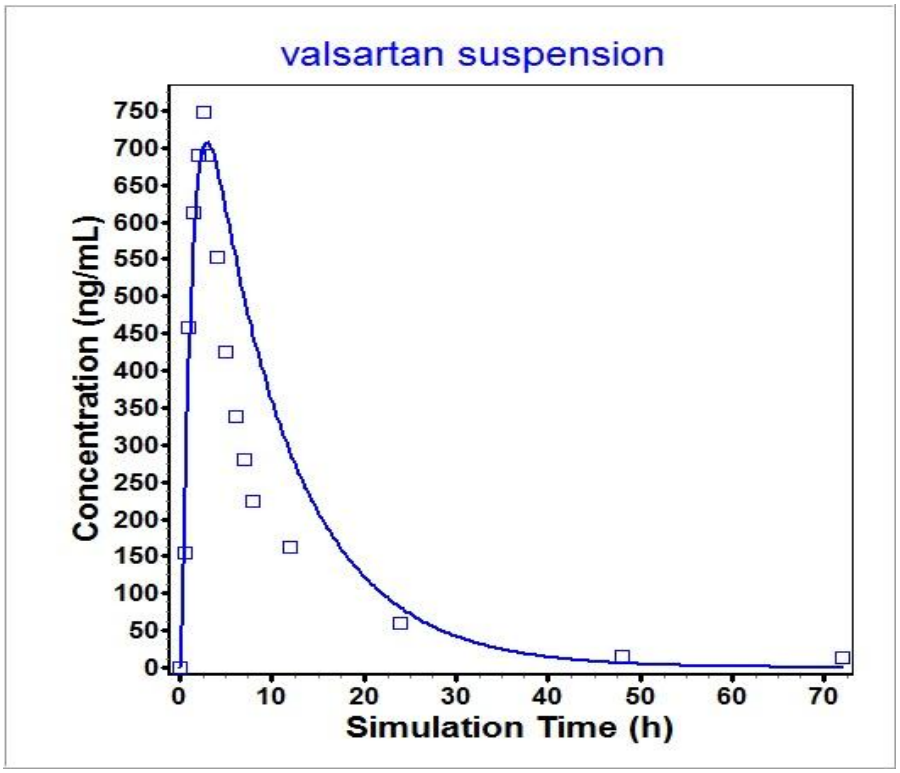


**Figure 11**: The simulated plasma profile of VAL suspension ( ___: *in silico* predicted using *in vitro* data, : *in vivo* data of the tablet).

# Chapter Four
# Discussion

For paediatric or geriatric patients with swallowing difficulties, the liquid preparations are the most convenient ones. A wide variety of medications in the pharmaceutical market are lacking the liquid oral dosage forms. that's why many researchers tend to prepare extemporaneous suspensions to cover up the shortage in the pharmaceutical market especially for paediatric medications [1].

Considering a research conducted by Sharon Conroy et al about 65% of medications that are used in an intensive care unit of children's hospital are off-lable or un-licensed [9].paediatric patients are considered therapeutic orphans especially with the large decrease in medications bearing labels for paediatric administration combined with the insufficient safety data making their prescription and use are limited as off-lable medications [7, 76, 77]. Such medications are not registered or approved by FDA. Moreover, no bioequivalence studies are conducted in such situations, which makes these suspensions questionable in terms of efficacy and safety. The combination of AML and VAL as anti -hypertensive medications is an example of such medications with no liquid oral formulation available.

In the current study, an extemporaneous suspension of a combination of AML and VAL was prepared from available commercial tablets Valzadepine® 5/80 mg as a source of the active ingredients. This

AML/VAL extemporaneous preparation proved its stability in all aspects; physically, microbiologically and therapeutically.

A sugar free 5/80 mg AML/VAL per 5 ml suspension in a 50 ml bottle was adopted upon patient usual dose as well as stability period after reconstitution of the suspension, which is convenient for patients who have a co-existing diabetes as well. The usual daily dose of AML/VAL combination ( 5/80 mg) can be obtained in 5 ml of this extemporaneous suspension, the 50 ml bottle will be sufficient for 10 days period through which the suspension still stable and effective. Provided that the liquid preparations like this suspension provide flexible dosing capacity with the ability of administration of parts of the 5ml dose, the 50 ml volume of the was chosen as a final volume of this suspension in consideration of paediatric hypertensive patients for which the amount will be saved for longer period when parts of the 5 ml dose will be given notifying them to discard the remaining amount at the fourth week after reconstitution.

The stability, efficacy and bioequivalence of these extemporaneous preparations are lacking.In vitro dissolution is considered as a potential surrogate marker of bioequivalence.

*In vitro* dissolution analysis of extemporaneously prepared suspensions coupled with *in silico* modelling can help in predicting the bioequivalence of these preparations. To investigate if the extemporaneous preparation is bioequivalent to the tablet dosage form, GastroPlus was used to build an *in silico* model for both VAL and AML using their respective in

vitro dissolution profiles as input. In this study, the in vitro dissolution of the extemporaneous suspension was conducted against the tablets, where the IR tablets was the reference and the extemporaneous suspension was the test, for the two formulations to be bioequivalent they must have similar dissolution behaviour; either having a very rapid dissolution with $\geq$85% dissolved within 15 minutes, or $\geq$85% dissolved within 30 minutes with similar dissolution profile confirmed with similarity factor $f2 > 50$ And difference factor $f1<15$

Both of AML and VAL release was investigated from both the IR tablet and the suspension in media with different pH (1.2, 4.5 and 6.8). Since AML is a BCS class 1 with high solubility and high permeability, AML exhibited very rapid dissolution in phosphate buffers (pH 4.5 and 6.8) with more than 85% was dissolved within 15 minutes, and has a rapid dissolution in 0.1 N HCL with more than 85% being dissolved within 30 minutes and $f_2$value of 51.74 and $f1$ value of 2.14 confirming the similarity of AML release from the IR tablets and from the extemporaneous suspension.

Whereas for VAL; media pH has shown to have a marked effect on its release from both dosage forms. At pH=6.8, the percentage of VAL released was more than 85% within 15 minutes, however, in pH 4.5 and 1.2media,the dissolution was much slower, with less than 70% of the drug released within 30 minutes in pH 4.5 ($f_2$ and $f_1$ are 51.63and 83.3 respectively).Whereas, at pH=1.2, the apparent amount of VAL released was not more than 26% within 30 minutes from both dosage forms ($f_2$ and

$f_1$ are 51.80 and 8.68 respectively. This decrease in the dissolution rates with the reduction in the media pH reflects the pH- dependent solubility of VAL. These findings are in agreement with previous studies which reported a reduction in VAL solubility at lower pH values. [78-80].

*f1* and *f2* value were calculated for each drug from each dosage form.. The results of similarity were more than 50 for each dissolution in which the release showed latency in 85% within 15 minutes indicating the similarity in the release from both formulations.

According to USP and FDA, dissolution studies of AML/VAL IR tablets have to be conducted in phosphate buffer pH 6.8, the percentage released of both AML and VAL exceeded 85% within 15 minutes in which bioequivalence of the extemporaneous suspension with the IR tablets is guaranteed.

According to BCS, AML which is highly soluble and highly permeable as a BCS class 1 member, then a biowaiver is granted [81].

Whereas in case of VAL, there is a conflict about its BCS classification . Some literature considered VAL as BCS class 2 in which it must have a high permeability and low solubility due to the shortage of VAL solubility at low pH levels [82, 83], others considered VAL as a special case with pH dependent solubility taking into consideration that VAL solubility increase 1000 folds when pH increase from 4 to 6 [84], keeping in mind that VAL site of absorption is the upper gastrointestinal tract where it remains ionized[85] and hence barely absorbed with fraction of dose absorbed and

systemically available after oral administration about 0.23[56]. Then it is more likely to be BCS class 3[55]with high solubility and low permeability [84].

To be more precise, VAL can be identified as intermediate class 2/3 rather than class 2 or class 3 as it is suggested by Chi-Yuan and Wu and Leslie Z. Benet for ciprofloxacin and erythromycin [55] . Similar situation was identified by Arthur Okumu and others for assigning etoricoxib as intermediate class 1/2 [86].

Accordingly, VAL is eligible for biowaiver if the release of VAL exceeds 85% within 15 minutes as it is suggested by BCS [87].

Nevertheless, the extent of VAL release from this extemporaneous suspension is in agreement with AN Zaid et al study conducted on VAL extemporaneous suspension prepared from commercial tablets in which more than 85% of VAL was released within 10 minutes [65].

The *in vitro* dissolution profiles were used in adjacent to *in silico* modelling that was applied to predict the bioavailability of this suspension in order to confirm the bioequivalence of the suspensions with the IR tablets.

Recently, *In silico* modelling developed a new insight in the prediction of bioavailability depending on *in vitro* dissolution testing.[33], in which *in silico* method beside *in vitro* dissolution could be a valuable and reliable tool in predicting the bioavailability of new dosage forms and in this work for our extemporaneous compounded suspension.

GastroPlus™ simulation was used for developing a model for each of AML and VAL in order to predict the absorption of them from the IR tablet and from the extemporaneous suspension. The simulations were carried out using the *in vitro* dissolution profiles of the IR tablet and the suspension. The predicted absorption profiles correspond well with *in vivo* observed data of the IR tablet, for the suspension the simulated profiles were compared with the *in vivo* data of the IR tablets, because there is no available *in vivo* data for the suspension, considering the IR tablets is the reference and the extemporaneous suspension is the test product in a way to test the bioequivalence. Both the IR tablet and the suspension matched well due to the closeness of dissolution profiles with prediction error values for simulated data which indicates good predictability while maintained below 10%. Arthur okumu and others suggested that similar *in vitro* dissolution profiles could justify a biowaiver when they are in compliance with *in silico* predictive profiles [86].

Considering FDA regulations, two products are said to be bioequivalent if the 90% CI of the relative mean $C_{max}$ and $AUC_{0-\infty}$ of the test product to reference product is within 80%-125% range[88]. In this study The 90% CIs of the geometric mean ratios (test: reference) for bioequivalent analysis obtained from pharmacokinetic parameters ($C_{max}$ and $AUC_{0-\infty}$) of AML/VAL 5/80 mg extemporaneous suspension was predicted by GastroPlus™ and compared to that observed for the tablet considering the suspension as the test where the tablet is the reference in order to investigate BE (Table 12).

**Table 12: Confidence interval of pharmacokinetic parameters of AML/VAL suspension.**

| parameter | Test vs Reference Ratio | | 90% confidence interval | |
|---|---|---|---|---|
| | $C_{max}$ | $AUC_{0-\infty}$ | $C_{max}$ | $AUC_{0-\infty}$ |
| **AML** | 0.94593 | 1.05683 | 95% | 106% |
| **VAL** | 0.946366 | 0.951719 | 95% | 95% |

Depending on the simulation data and the *in vitro* dissolution data combined with BE terms that are achieved, then the compounded suspension of AML/VAL appears to be bioequivalent to the commercial tablets Valzadepine® as both are giving similar profiles that gives efficient insight into *in vivo* behaviour of this extemporaneous suspension.

For the compounded anti-hypertensive extemporaneous suspension it must preserve its efficacy and safety over an eligible period of time, for any formulation to be considered stable it must retain > 90% of the initial concentration of the drug, AML/VAL suspension preserves 97.3% and 101.1% respectively of its initial concentration over the 30 days period of time. Moreover, no changes in the appearance, pH, colour or odour was observed, no microbial growth was detected as well throughout the 4 weeks.

## Conclusion

The extemporaneous suspension could be successfully prepared using available commercial tablets as a source of the active ingredients even for the combinations medications. Such suspensions should be carefully evaluated in different aspects; volume, organoleptics, stability and bioavailability which is lacking in such circumstances.

AML/VAL extemporaneous suspension can preserve its stability over four weeks period when stored in room temperature, *in silico* modelling could be applied adjacent to *in vitro* testing to predict PKs and prove similarity of an extemporaneous suspension with the tablets.

Pharmaceutical companies should include a section in their leaflets regarding the compounding and stability of those suspensions when the alternative liquid dosage form in not available in the market which could be a life-saving for a patient in need.

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**كلية الدراسات العليا**

# تركيب معلق فوري من الاملودبين والفالسارتان باستخدام النمذجة الفسيولوجية

**إعداد**

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**قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الصيدلانية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.**

**2018**

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## الملخص

الخلفية: في حالة عدم توفر الدواء على شكل جرعات سائلة، فإن سحق القرص او إذابة الكبسولة سيكون الخيار الأنسب لاستخدام هذه الأدوية في المرضى الذين يعانون من عسر البلع. ومع ذلك، لا يتم إجراء أي دراسات للتكافؤ الحيوي أوثبات صلاحية الأدوية في مثل هذه الحالات، الأمر الذي يؤدي إلى الارتباك فيما يتعلق بفعاليتها وسلامتها. أثبتت البرمجيات الالكترونية على التنبؤ بأداء الأدوية في الجسم الحي اعتمادًا على خصائصه الفيزيائية الكيميائية وعلى سلوكه وذائبيته في المختبر. لا توجد تركيبة سائلة من مزيج الأملودبين والفالسارتان في سوق المستحضرات الصيدلانية لتناسب الأطفال المصابين بارتفاع ضغط الدم.

الهدف: كان الهدف من هذه الدراسة هو إعداد معلق فوري من مزيج الأملودبين والفالسارتان من الأقراص التجارية المتاحة، وتقييم خصائص الثبات و الذائبية للمعلق المركب.

الطريقة: تم إعداد المعلق أملوديبين/ فالسارتان من الأقراص التجارية المتاحة في السوق المحلي Valzadepine®

ودراسة سلوك و ذائبية المعلق في درجات الحموضة المختلفة إضافة إلى تقييم التغييرات الفيزيائية والكيميائية والميكروبية على مدى شهر واحد في درجة حرارة الغرفة لضمان الثبات وتحديد فترة صلاحية المعلق، ثم تم استخدام برنامج التنبؤ الالكتروني GastroPlus™ لبناء نماذج امتصاص خاصة لكل من الأدوية على أساس بيانات الذوبان في المختبر. وتمت مقارنة ملفات سلوك الأدوية في المختبر مع ملفات التنبؤ للتأكد من التكافؤ الحيوي بين المختبر والجسم الحي.

النتائج: تم إعداد تعليق الأملوديبين / فالسارتان بنجاح مع خصائص مقبولة من ناحية اللون والرائحة والطعم. كان المعلق مستقراً لمدة أربعة أسابيع مع الاحتفاظ بخصائصه الفيزيائية والكيميائية، خصائص الذائبية والامتصاص للمعلق كانت مماثلة للأقراص التي هي مصدر المواد الفعالة ومكافئة لما تنبأ به البرنامج الالكتروني من تكافؤ حيوي بين السلوك في المختبر والجسم الحي.

خاتمة: يمكن إعداد معلق فوري من مزيج الاملودبين والفالسارتان من الأقراص التجارية المتاحة. وعلاوة على ذلك، يمكن تطبيق برنامج التنبؤ الالكتروني GastroPlus™ جنبا إلى جنب مع خصائص الذوبان في المختبر من أجل تأكيد التكافؤ الحيوي عند تركيب معلق فوري من مطحون الأقراص المتوفرة.