FORMULATION OF CARBAMAZEPINE AND SODIUM VALPROATE FIXED DOSE COMBINATION FOR MANAGEMENT OF EPILEPSY

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DECLARATION

I, **Mmakgomo Seabi**, hereby declare that the work on which this study is based is original, except where acknowledgements indicate otherwise.

This thesis is submitted for the degree **Master of Pharmacy (Pharmaceutics)** at the University of Limpopo. Neither the whole work nor any part of it has been submitted before for any degree or examination at this or any other university.

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This dissertation is dedicated to God Almighty and to my family.

"You don't choose your family. They are God's gift to you, as you are to them" Desmond Tutu

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ABBREVIATIONS AND ACRONYMS

ADHD	Attention Deficit Hyperactivity Disorder
AEDs	Antiepileptic Drugs
API	Active Pharmaceutical Ingredient
BA	Bioavailability
BCS	Biopharmaceutical classification system
BP	British Pharmacopoeia
cGLP	current Good Laboratory Practice
cGMP	current Good Manufacturing Practice
CYP 450	Cytochrome P450
DSC	Differential Screening Calorimetry
FDA	Food and Drug Administration
FDC	Fixed Dose Combination
FTIR	Fourier transform infrared spectroscopy
GTCS	General tonic-clonic seizures
HCV	Hepatitis C Virus
ICH	International Council for Harmonisation
LOD	Limits of detection
LOQ	Limits of quantification
PgP	P-glycoprotein
QbD	Quality by design
SSRI	Selective serotonin reuptake inhibitors
TAM	Thermal Activity Monitor

TPP	Target product profile
TREC	Turfloop Research and Ethics Committee
UGT2B7	UDP-Glucuronosyltransferase-2B7
USP	United States Pharmacopoeia
Vd	Volume of distribution

ABSTRACT

Epilepsy is the fourth most common neurological disorder after migraine, stroke and Alzheimer's disease and it affects about fifty million people worldwide. Careful consideration should be taken when deciding to initiate treatment in epilepsy as it should consider the balance between the possibility of further seizures and their associated risks, including the possible risk of sudden expected death, inconvenience and the risks of taking regular medication for each individual. In the early 1980's, the first-line treatment for epilepsy was polytherapy. This was due to findings that smaller doses of two drugs rather than larger doses of one drug can achieve synergistic effects or less drug toxicity. However, following more trials on the treatment of epilepsy, this was later changed to monotherapy as first-line treatment. Despite the change, patients remain uncontrolled on a single anti-epileptic drug, thus they are initiated on polytherapy, one such combination being carbamazepine in combination with sodium valproate. The use of these in combination has pharmacological threats such as compliance, the control of side effects and the achievement of synergistic effects. The development of a Fixed Dose Combination (FDC) has often been used to resolve pharmacological threats, and this study aims to develop a fixed dose combination tablet of carbamazepine and sodium valproate to resolve the pharmacological threats in epilepsy.

Samples of carbamazepine and sodium valproate and a physical mixture (1:1 w/w) of both drugs and excipients were prepared for compatibility with thermal analysis and spectroscopy techniques. Data was analysed by comparing the DSC curves, FTIR spectra, XRPD peaks and TAM analysis of carbamazepine and sodium valproate alone and in their physical mixture (1:1 w/w) and with excipients. Both carbamazepine and sodium valproate were evaluated for flowability using angle of repose, tapped and bulk density, compressibility index and particle size distribution. To formulate the proposed FDC tablet of carbamazepine and sodium valproate, direct compression and wet granulation methods were employed. The tablets were then evaluated for official and non-official post formulation parameters (weight variation, crushing strength, friability, diameter and thickness, and disintegration) according to BP and USP standards. A standardised HPLC method was developed and validated for analytical procedures. Dissolution studies were conducted

according to USP methods to verify and quantify the release of the APIs from the FDC tablet.

Carbamazepine and sodium valproate were tested for compatibility with excipients using DSC, FTIR, XRPD and TAM analysis. The overall results confirmed that carbamazepine and sodium valproate are compatible, with each other and the excipients used in the study. Powder flow of carbamazepine and sodium valproate was poor, hence they were subjected to granulation prior to compression to improve flowability. The specifications of the fixed-dose combination were developed in accordance with the FDA's quality by design concept and WHO recommendations. The tablets were subjected to non-official and official pharmacopoeial tests, and passed all the tests. Dissolution studies according to a USP method were conducted to verify and quantify the release of the APIs in the fixed-dose combination. The initial dissolution rate (DRi) of carbamazepine and sodium valproate in the SLS dissolution medium was rapid as required for an immediate release formulation.

The study aimed at developing a fixed dose combination of carbamazepine and sodium valproate to try to reduce the burden of taking more than one tablet for epilepsy. Based on the results obtained from preformulation studies to assay of the final product, the study was successful.

Key words: Fixed dose combination, carbamazepine, sodium valproate, epilepsy, compatibility

CHAPTER 1:
INTRODUCTION

1.1. INTRODUCTION AND BACKGROUND

According to Smith and Chadwick (2001), careful consideration should be taken when deciding to initiate treatment in epilepsy. It should consider the balance between the possibility of further seizures and their associated risks, including the possible risk of sudden expected death, inconvenience and the risks of taking regular medication for each individual.

In the early 1980's, the first-line treatment for epilepsy was polytherapy. This was due to findings that smaller doses of two drugs rather than larger doses of one drug can achieve synergistic effects or less drug toxicity (Lee and Dworetzky, 2010). However, following more trials on the treatment of epilepsy, this was later changed to monotherapy as first-line treatment. Despite the change, patients remain uncontrolled on a single anti-epileptic drug, thus they are initiated on polytherapy (Lee and Dworetzky, 2010: Rossiter, 2016).

There are a number of available combinations in relation to the type of epilepsy they treat, one such being carbamazepine and sodium valproate in the management of focal seizures (Mani, 2013). In a study by Sirmagul, Atli, & Ilgin (2012), a comparitive study on antiepileptic combination therapy between carbamazepine, sodium valproate and phenytoin revealed that combinations of carbamazepine and sodium valproate are better tolerated than carbamazepine/phenytoin and sodium valproate/phenytoin combiantion therapy. Currently carbamazepine and sodium valproate are available as single entities. The use of these in combination has pharmacological threats such as compliance, the control of side effects and the achievement of synergistic effects (Koo, 2010).

The development of a Fixed Dose Combination (FDC) has often been used to resolve pharmacological threats (Desai, 2013). The use of fixed dose combination (FDC) drug products is common in most therapeutic areas. FDC formulations that are available commercially are oral, parenteral and even inhalations (Albsoul-

Younes, *et al*., 2016). The use of FDC drug therapies provides better safety and clinical effectiveness, improved patient compliance and convenience, and reduced treatment cost for patients compared to single drug treatment. Moreover, patients will adhere more to FDC therapy and eventually achieve improved disease treatment and management (Moon & Oh, 2016).

The use of FDCs has been widely demonstrated in the treatment of a number of conditions such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), malaria, tuberculosis and psychotic disorders such as epilepsy. They have advantages when there is a distinguishable patient population for whom treatment with a particular combination of actives in a fixed ratio of doses has been shown to be safe and effective. Furthermore, all of the actives contribute to the overall therapeutic effect as in the case of carbamazepine and sodium valproate (World Health Organisation, 2005).

One of the major factors in drug formulation to be considered is the Biopharmaceutics Classification System (BCS). The BCS focusses on aqueous solubility and intestinal permeability of the Active Pharmaceutical Ingredient (API), which is classified into four classes (Gouws, 2015; Chavda, *et al*., 2010):

Class I: High Solubility- High permeability,

Class II: Low Solubility- High permeability,

Class III: High Solubility- Low permeability and

Class IV: Low Solubility- Low permeability

Carbamazepine and sodium valproate are antiepileptic drugs which are often used together/or as alternates for numerous diseases. Carbamazepine is a class I antiepileptic and sodium valproate is a class II. They are both used in the treatment of trigeminal neuralgia, epilepsy, bipolar disorder, neuropathic pain and have been proved to have therapeutic value in breast, colorectal and prostate cancer (Martin, *et al.,* 2015; Abou-Khalil, 2017; Buoli, Serati, & Altamura, 2014; Prisco, *et al.,* 2011).

This study explored pharmaceutical parameters which resulted in the formulation of a FDC containing carbamazepine and sodium valproate. The formulation of this combination was in line with the development of a FDC where the individual

components have been successfully used simultaneously in practice. However, due to the fact that carbamazepine and sodium valproate affect each other's metabolism, blood levels must be monitored regularly (Rossiter, 2016). This will require the individual doses to be adjusted, thus creating a need for different strengths of the FDC which will not form part of the study

1.2. PROBLEM STATEMENT

For effective management of epilepsy, antiepileptic drugs are administered for a lifetime. To date, monotherapy remains the mainstay for the initial treatment of epilepsy. However, a large number of patients do not respond to the use of a single antiepileptic drug (Perucca, 2005). This then results in the burden of taking multiple medications, increased health-care costs and decreased patient compliance, ultimately increasing the risk of treatment failure (Maher, Hanlom and Hajjar, 2013).

Carbamazepine and sodium valproate is one of the many combinations available in the management of uncontrollable epilepsies (Perucca, 2005). However, currently there is no fixed dose combination (FDC) with this combination on the market, which is necessary to resolve problems associated with the use of multiple drugs in epilepsy.

1.3. RESEARCH QUESTIONS

- Are carbamazepine and sodium valproate compatible with each other?
- Which method will be suitable for manufacturing a fixed dose combination tablet containing carbamazepine and sodium valproate?

1.4. AIM OF THE STUDY

The aim of this study was to formulate a fixed dose combination containing carbamazepine and sodium valproate.

1.5. OBJECTIVES OF THE STUDY

The objectives of the study were as follows:

- To determine the compatibility of carbamazepine and sodium valproate.
- To identify a suitable method of manufacturing a FDC tablet
- To manufacture a fixed dose combination of carbamazepine and sodium valproate.

1.6. IMPORTANCE OR SIGNIFICANCE OF THE STUDY

Successful development of a fixed dose combination (FDC) formulation of carbamazepine and sodium valproate will encourage research and development to pursue interest in this study and research further to establish if it is possible to have it in the market. With the formulation in the market, patients will have a reduced therapeutic burden.. This will help health practitioners manage epilepsy by improving patient's acceptance and adherence to the medication.

1.7. SUMMARY

This chapter provided a background and rational of the study in detail. It profusely defined both the research questions and objectives as outlined in this chapter.. The next chapter, which is the literature review, will provide an extensive review of combination therapy of carbamazepine and sodium valproate in the management of epilepsy. Physicochemical profiles of the two drugs are compared to determine whether they can be formulated as a fixed dose combination.

CHAPTER 2:
LITERATURE REVIEW

2.1. INTRODUCTION

In this chapter, the literature provided recent studies related to the current study. There is an increase in the development of fixed dose combinations in therapeutic areas such as HIV/AIDS, diabetes and cardiovascular diseases where polytherapy is a major factor in treatment failure (Koo, 2010). These have led to an interest in studies of possible FDC in psychotic disorders where polytherapy is becoming a common practice.

2.2. OVERVIEW OF EPILEPSY

Epileptic seizures normally involve excessive firing and synchronization of neurons. This interferes with the normal functioning of the part of the brain that is involved, thus causing symptoms and semiology of epilepsy (Jefferys, 2002).

Initiation and progression of epilepsy involves the glutamatergic molecular mechanisms (figure 2.1). These include upregulation of glutamate receptors, elevation of extracellular glutamate concentration, abnormalities in glutamatergic transporters and autoimmune mechanism. These mechanisms may cause the excessive glutamatergic activity, which is involved in the hyper-excitability of neurons and epilepsy (Yin, Ahmad and Makmor-Bakry, 2013).

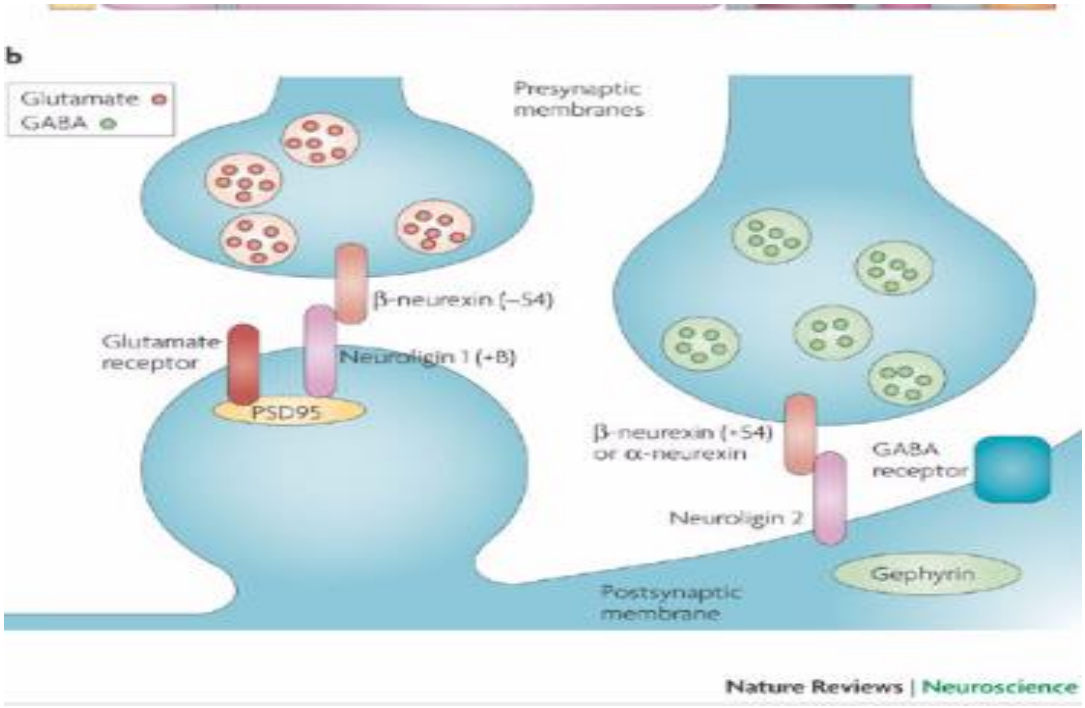

Figure 2.1: Pathogenesis of epilepsy

Source: Yin, Ahmad and Makmor-Bakry (2013).

The National institutes of Health, (2015) classify epilepsy according to the kind of seizures a patient present with. There are two major categories of seizures, namely: Partial (also focal) and generalized seizures.

2.2.1. Partial seizures

Generally, partial seizures (also focal seizures) are localized, and may originate from one hemisphere (Scheffer, Berkovic, Capovilla, Connoly, Guilhoto, Hirsch, Moshe, Nordli, Zhang and Zuberi, 2012). They may further be subdivided into simple and complex partial seizures. In simple partial seizures, the affected part is the motor context causing repeated convulsion of certain muscles. Voluntary control of the affected parts is lost but the patient remains conscious (Mukhopadhyay, Kandar, Das, Ghosh and Gupta, 2012). Complex partial seizures are characterized by impairment of consciousness at onset. The affected part is the temporal lobe and the seizure remains focused there. This may lead to involuntary muscle contractions, effects mood and behavior as well as abnormal sensory experiences (Henry, 2012).

2.2.2. Generalized seizures

Generalized seizures are also subdivided into myoclonic seizures, absence seizures (petitmal), clonic seizures, tonic seizures and tonic-clonic seizures (grandmal) (Mukhopadhyay *et alet al*., 2012). Myoclonic seizures consist of sudden, short, muscle contractions that may occur in one limb, more widespread or bilateral. They are either single jerks or repeated jerks over long period (Dekker, 2002). Absence seizures are more common in adolescence and childhood. They are characterized by a brief (seconds) loss of consciousness, during this type of seizure, patients often stare into space and stand still, hence it is often discovered later (Scotland, 2010). Tonic clonic seizures involve the combination of tonic and clonic seizures. The tonic phase includes the stiffening of the body and irregular breathing. The clonic phase includes jerks and spasms. The patients often make grunting noises, bite their tongues or cheeks, or suffer incontinence. The jerking may last a couple of minutes before the patient recovers. After recovery, they may feel tired, sleepy and confused for some time afterwards (Scotland, 2010).

2.2.3. Management of epilepsy

According Smith and Chadwick (2001) treatment of epilepsy is guided by three principles, which are:

- To minimize risk of acute allergic and dose related toxicity, initiate monotherapy with caution;
- Gradually increase the dose of the drug to maximum allowed dose, given that seizures continue with the initial dose. If this fails, switch to an alternative drug;
- Lastly, having tried both principles (1) and (2) without reducing the seizures, combination therapy should be introduced.

The management of epilepsy is guided by the type of seizure the patient presents or is diagnosed with. The four main antiepileptic drugs (AEDs) can effectively manage the most common type of epilepsy, general tonic-clonic seizures (GTCS), namely: phenobarbitone, phenytoin, carbamazepine and sodium valproate (Perucca, 2005). The most commonly used medications being sodium valproate and carbamazepine

(Appleton and Cross, 2015). Pharmacological factors influence the choice of drug in each seizure type according to the efficacy, toxicity and ease of use (Smith and Chadwick, 2001), as shown in table 2.1 below.

Table 2.1: Summary of seizure types and treatment (Guerreiro, 2008)

Seizure type or epilepsy syndrome	Choice of drugs (according to preference)
Adults with partial-onset seizures	Carbamazepine, phenytoin Sodium valproate Gabapentin, lamotrigine, oxcarbazepine, phenobarbitone, topiramate and vigabatrin
Children with partial-onset seizures	Oxcarbazepine Carbamazepine, phenobarbitone, phenytoin, topiramate and sodium valproate.
Elderly with partial-onset seizures	Gabapentin, lamotrigine Carbamazepine
Adults with generalized tonic-clonic seizures	Carbamazepine, sodium valproate, phenytoin, phenobarbitone, oxcarbazepine, lamotrigine and topiramate
Children with generalized tonic-clonic epilepsy	Sodium valproate, ethosuxamide and lamotrigine

2.2.3.1. Monotherapy in epilepsy

Research shows that monotherapy has always been mainstay of therapy in epileptic patients for over years. About 70% of epileptic patients are likely to become seizure free and go into long-term remission shortly after initiation on a single antiepileptic drug (Nolan, Sudell, Weston, Tudur Smith, & Marson, 2014). Although monotherapy is preferable for majority of patients with epilepsy, it favours certain patient populations (see table 2.1). Monotherapy may be ineffective when the AED choice is suboptimal for a particular patient type thus raises a need for polytherapy to treat the seizures effectively (Erik, William, & Thomas, 2009).

2.2.3.2. Polytherapy in epilepsy

Combination between antiepileptics in contrast to monotherapy has been found to be successful in about 30% of patients (Jukka, Maria, Jani, Tapani, Elham, & Anss, 2008). Literature has shown that polytherapy is more effective in patients who failed on monotherapy for the control of seizures. However, a combination of two or more antiepileptic drugs can be disadvantageous (Sirmagul, Atli, & Ilgin, 2012). Maher *et al*., (2013) mentioned increased health-care costs and decreased patient compliance to be the most prominent disadvantages of polytherapy. In a study conducted by Poolos, Warner, & Humphreys (2012), efficacy of AED combinations were compared, the most prescribed combination was carbamazepine and sodium valproate.

Table 2.2: Comparative efficacy of individual AED combinations (Poolos *et al*., 2012)

AED Combination	No. of patients
CBZ	**66**
CBZ/VPA	**54**
VPA	**50**
VPA/PHT	**41**
LTG/VPA	**40**

Similar results were obtained in one of the studies conducted by Joshi, Tripathi, Gupta, Gulati, & Gupta (2017). In their results, carbamazepine was prescribed more than the other AEDs in combination either with or greater than three AEDs. Frequency of use other AEDs, including sodium valproate were significantly high in polytherapy (Joshi *et al*., 2017). To reduce the burden that comes with the increasing application of polytherapy in epilepsy, as seen in other treatment modalities such HIV/AIDS, diabetes and hypertension (Moon & Oh, 2016), FDC formulation of the most frequently used AEDs seems to be a solution.

2.3. FIXED DOSE COMBINATION

Fixed dose combination (FDC) formulation is a combination of two or more active ingredients with different mechanisms of action into one entity (i.e. tablet). The development of a FDC product has to take into consideration the safety and efficacy of final product. The single components should remain effective even when in the form of a FDC. Although it is not always possible to achieve synergistic effects, it is important that the formulation not to interfere with the effectiveness of single components (Modi and Patel, 2011).

Development of a FDC regimen can arise from a number of concepts, which can be categorized, into 4 (four) (WHO, 2004). A FDC generic product bioequivalent to an existing FDC, a new FDC developed by combining components that are already well studied, and the simultaneous use of all the individual active components have been well characterized safe and effective. The dosage regimen of the components given individually and the dosage regimen of the FDC are the same. A new FDC product developed from individual components that are well characterized for safety and efficacy when used as monotherapy, but the efficacy and safety of their simultaneous use is not well established or two or more well characterized individual products are combined using a novel dosage regimen. Finally, a FDC that is developed by incorporating one or more new molecular entities.

The development of FDC products bases its rationale on potential benefits that are founded on effective therapeutic principles and validated by clinical evidences. Therefore, the issue of how to rationalise combination products, either individually or in combination, with respect to category of therapeutic benefits, class of pharmacokinetic and pharmacodynamics interaction and type of combination effects is first discussed (Moon & Oh, 2016). Table 2.3 illustrates examples of FDC products and the rationale behind their development.

Table 2.3: Examples representing the rationale for the development of FDC products (Kota, Ayalavajjala & Sivasubramanian, 2015)

Rationale	Example of FDC products
Treatment synergy (complimentary mechanism of actions)	Short term Treatment (Acute therapy) Artemether/Lumefantrine (Malaria) Everolimus/Cyclosporine (Immune suppression) Long term Treatment (Chronic therapy) Ramipril/Felodipine (Hypertension) Atenolol/Amlodipine (Hypertension) Azidothymidine/Lamivudine/Abacavir/Nevirapine (HIV infection) Pioglitazone/Metformin (Diabetes) Metformin/Glipizide (Diabetes)
Bioavailability Enhancement	Lopinavir/ritonavir (Lopinavir is a CYP and PgP substrate; Ritonavir inhibits gut CYP and Pgp resulting in higher oral BA of lopinavir) Amoxicillin/Clavulenate (Bacterial infection) Levodopa/Carbidopa/Entacapone(Parkinson's)
Multiple Indications (co-morbid disease states)	Amlodipine/Atorvastatin (Hypertension and Hyperlipidemia) Diclofenac/Chlorzoxazone (Inflammation and muscle sprain)
Adverse Event Management	Ibuprofen/Famotidine (Co administration of proton pump inhibitor to overcome hyperacidity related side effects of ibuprofen) Morphine/Methylnaltrexone (To overcome morphine induced constipation by methylnaltrexone)

2.3.1. Advantages of FDC

The main advantage of FDC is improved patient's acceptance and adherence to the medication. A FDC is also more economic and some products can enhance the effect of the other in the combination as in FDC tablet containing tenofovir,

emtricitabine and efavirenz (in HIV) and an FDC tablet containing levodopa, carbidopa and entacapone (in Parkinson's disease) respectively (Davies, 2013: Seeberger and Hauser, 2009). FDC lead to a reduced chance of drug abuse and multi-dose therapy like in pain medication containing codeine (ibuprofen, paracetamol and codeine (Seedat, 2008). It provides the ability to treat many ailments with the same pill and it also reduces the risk of resistance as in cases of co-trimoxazole (trimethoprim and sulphamethoxazole) and co-amoxiclav (amoxicillin and clavulanic acid) (Snehal, 2008).

2.3.2. Disadvantages of FDC

Like any other drug, there are disadvantages associated with the use of an FDC tablet. The main disadvantage is the inability to change the dosing once the formulation is a particular dose. If side effects occur, it cannot be specifically identified which drug causes them. In other cases, if the formulation is in a tablet form, it may be too big for the patient to swallow (Desai, 2013).

2.3.3. Types of fixed dose combination tablets

Types of FDC tablets include multilayer tablets, compression coated tablet and monolithic FDC tablet. Multi-layered tablets formulators insert an inert layer, which acts as a barrier between two matrices to prevent interaction. This method is useful when manufacturing an FDC tablets that contain incompatible matrices (Gupta *et al*., 2012). Although high compression force degrades certain actives this method still enables formulators to formulate two or more drugs with different release patterns, thereby resolving the issue of instability and incompatibility (Snehal, 2008).

2.3.4. Multi-layered tablets

Incompatible substances can be separated by formulating them in separate layers as a two-layer tablet or separating the two layers by a third layer of an inert substance as a barrier between the two. Two-layer tablets may be designed for sustained release; one layer for immediate release of the drug and the second layer for extended release, thus maintaining a prolonged blood level. Layers may be coloured differently to identify the product.

2.3.5. Compression coated tablet

Compression coated tablet (tab in tab) is prepared through a dry process in which the tablet will contain two parts. The first part is the internal core, which is a small porous tablet; it is then coated with a powder that will form the external tablet. The more powder added on the internal core the bigger the tablet gets. This results in a tablet within a tablet (Powar, Jaimini, Chauhan and Sharma, 2014).

2.3.6. Monolithic fixed dose tablet

Monolithic FDC tablet is a tablet composed of active ingredients that have the same release rate and are compatible with each other. The powders are mixed together into a single tablet without any layering. For the tablet to remain intact, the distance between the adjacent molecules of the powder should be greatly reduced in order to avoid brittleness of the tablet. They should also be mixed in the same base (Powar *et al*., 2014). The benefits of monolithic FDC tablet are ease of manufacture and smaller tablets can be obtained thus making the FDC convenient for the patients (Koo, 2010).

2.4. FACTORS TO BE CONSIDERED WHEN DEVELOPING A FDC

The formulation of FDCs is dependent on the pharmacodynamics, pharmacokinetics and chemical and physical compatibility factors to ensure its success. Pharmacodynamic effects will lead to reduced efficacy or enhanced toxicity of the FDC. Pharmacokinetic properties may lead to the FDC formulation having peak efficacy at different time, whereas chemical and physical incompatibility may lead to a decreased shelf-life of the formulation (Gupta & Ramachandran, 2016). To rule out pharmacokinetic/pharmacodynamic interactions between the individual components, it is important to understand the pharmacokinetic and pharmacodynamic properties of the components of the proposed FDC (Kota, Ayalavajjala, & Sivasubramanian, 2015). In some cases, to enhance therapeutic efficacy, a FDC formulation is developed based on the pharmacokinetic or pharmacodynamic interaction. An example of such is either by maximizing the intestinal uptake of the drug or by protecting the drug from presystemic degradation by combining with inhibitors of enzyme or efflux transporter to enhance the bioavailability of the drug. This strategy

is especially prevalent in HIV and hepatitis C virus (HCV) treatment, where for example, ritonavir is used as an enzyme inhibitor to enhance the bioavailability of other anti-retroviral drug

To determine whether a FDC is possible between the drugs, they have to undergo a compatibility check-up, as shown in figure 2.2 below:

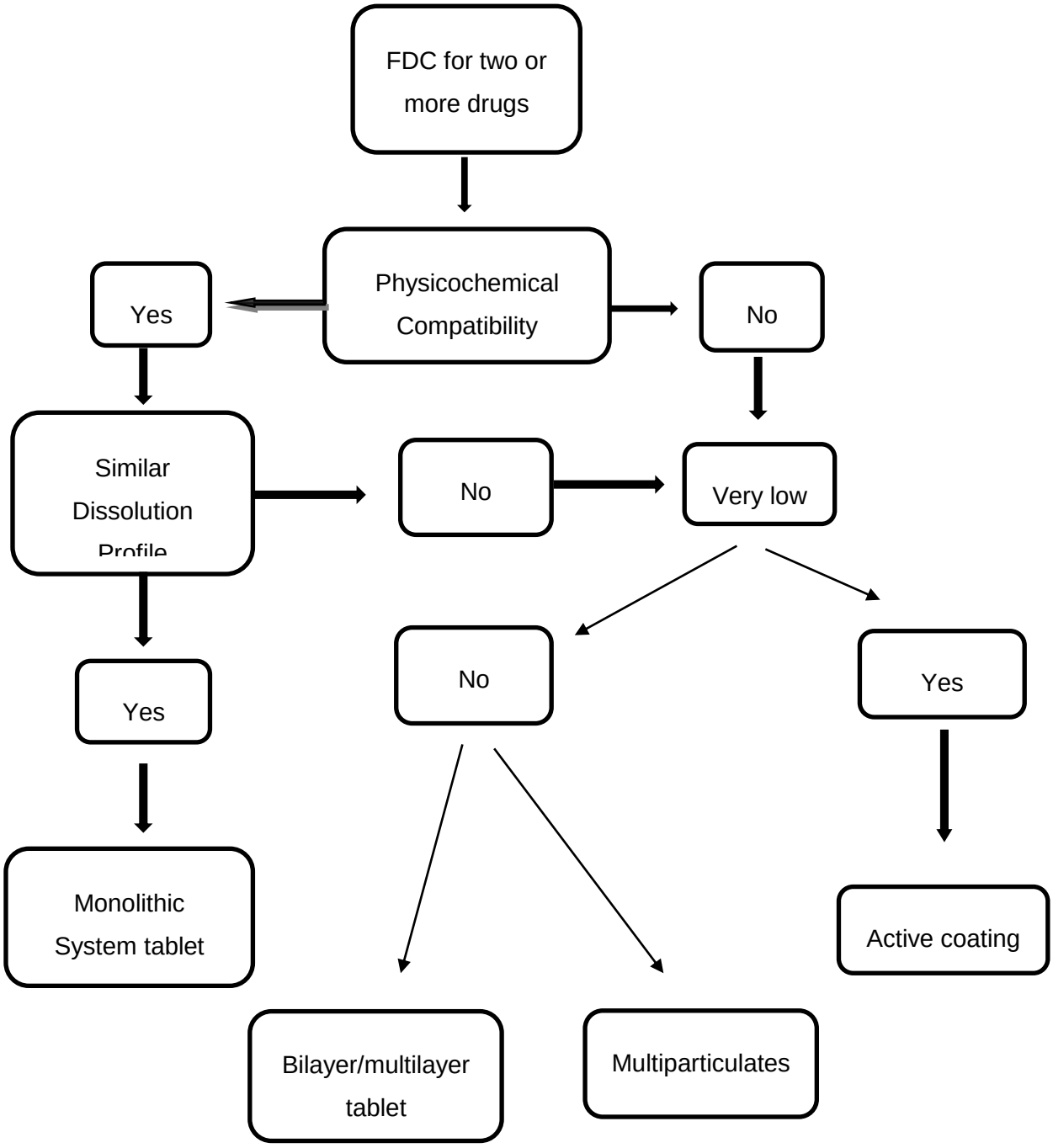

Figure 2.2: Compatibility check for FDC formulation (Moon & Oh, 2016)

2.4.1. Biopharmaceutical classification system

Biopharmaceutical classification system (BCS) correlates the *in vitro* solubility and permeability to the *in vivo* bioavailability (Jouyban, 2010). Although it is simple in developing formulation and manufacturing process, the suitability of the drugs in monolithic systems should be thoroughly investigated in terms of this correlation. In case of FDC drugs where the drugs combined have different absorption mechanisms such as the combination of BCS Class I and BCS Class II drugs, the dissolution rate of poor soluble drug, may be decreased in the FDC formulation (Moon & Oh, 2016). Formulation design can improve absorption of a class II drug to be more like that of a class I drug, provided that a class II drug can be maintained in a solubilized state in the lumen of the gut (Pouton, 2006) see figure 2.3.

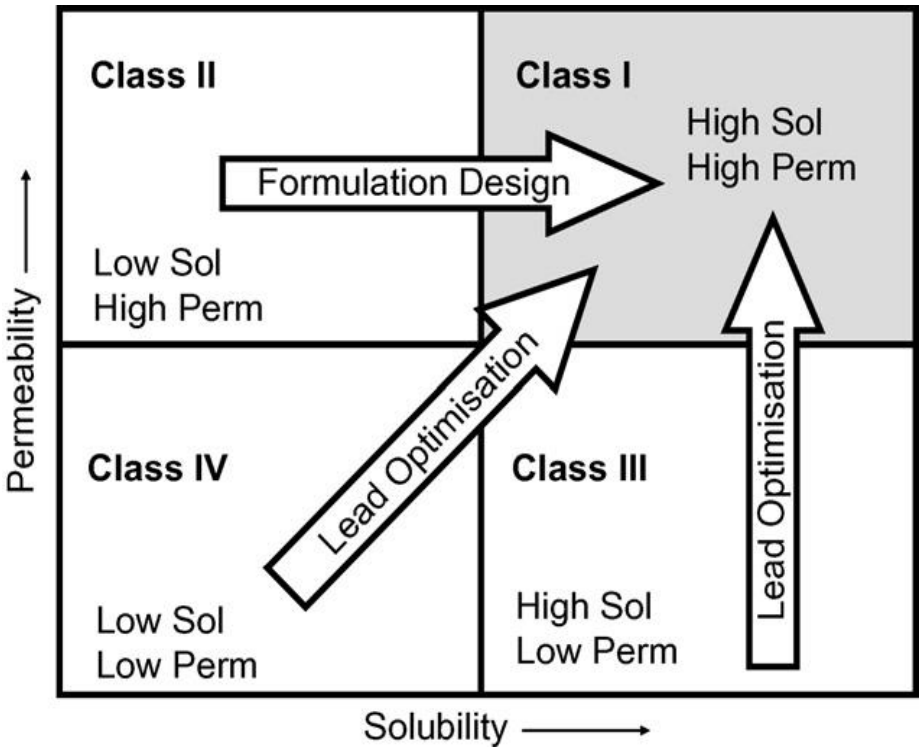

Figure 2.3: Representation of the biopharmaceutical classification system

Source (Pouton, 2006)

Formulation strategies can do little to improve the absorption of classes I and III drugs which are limited by poor membrane permeability (Jouyban, 2010). Carbamazepine and sodium valproate belong to different BCS classes and that

makes them both suitable for fixed dose combination formulation. Sodium valproate belongs to class I, which represents drugs with high solubility and high permeability and carbamazepine belongs to class II of low soluble but high permeable drugs (Chan, et al., 2016).

2.4.2. Formulation design

Formulation design is defined as the processes in which different chemical substances for instance, active chemical substances are combined together to produce a medical compound (Hassan, 2012). Formulation design incorporates drug design, which is the process of formulating a new medical product, completely based on the knowledge of biological target (Hassan, 2012; (Bartlett, et al., 2017). When developing an FDC dosage form, a quality by design approach is of dire importance for a robust formulation and manufacturing process. Application of a quality by design approach is often similar to the single application for a single API formulation, but the presence of multiple APIs makes the formulation a bit more complicated (Yu, et al., 2014). The first and most important step is to define the target product profile, which describes the use, safety and efficacy of the product. Target product profile (TPP) forms an integral part in the basis of design for the development of the product. Considerations for inclusion in the TPP could include the following (Zhang & Mao, 2017):

- Intended use in a clinical setting, route of administration, dosage form, and delivery system(s)
- Dosage strength(s)
- Container closure system
- Therapeutic moiety release or delivery and attribute affecting pharmacokinetic characteristics (e.g., dissolution and aerodynamic performance) appropriate to the drug product dosage form being developed
- An in depth understanding of the formulation, excipients and process (which will reduce the amount of experimentation and analytical testing required and consequently, the manufacturing and testing costs)

- Drug product quality criteria (e.g., sterility, purity, stability, and drug release) appropriate for the intended marketed product

Following TPP is to design the formulation and identify the critical quality attributes of the final product that must be controlled to meet the TPP. It is important to identify and control critical process parameters to achieve the final products critical quality attributes (Krishna, et al., 2016). A control strategy is essential during the process of formulation, from the development stage to commercialization. The control strategy should include raw material and API controls (for particle size distribution, moisture, polymorphs and impurities amongst others, process controls (such as hardness, thickness, friability, tablet weight during compression and others) and design space around individual or multiple unit operations (such as granulation, compression, coating, encapsulation and packaging (Haleem, et al., 2015). (Rantanen & Khinast, 2015) Further reiterated that it is significant that the controls are monitored and the processes are updated to ensure target product profile by quality by design approach. To achieve successful target product profile quality by design approach, it is important to use design of experiment and process analytical technology. A quality by design approach provides the following advantages (Krishna *et al*., 2016; Zhang & Mao, 2017):

- Negligible chance of batch failure because the batches are manufactured in a design space defined during product development.
- Enhanced understanding of the formulation and manufacturing processes
- The development of a robust process that leads to greater regulatory confidence
- Continuous improvement in the manufacturing process during development, validation and post commercialisation in a defined design space doesn't require submission to the FDA
- Increased product quality improved yields, reduced investigations and testing, and lower manufacturing costs
- Guaranteed therapeutic equivalence of each batch of generics manufactured
- A better less expensive, and safer drug product

By using a QbD approach, the researcher can develop safe and effective FDC formulation of carbamazepine and sodium valproate to optimize the pharmacokinetics, bioavailability and therapeutic effect of the drugs as single

entities. This as mentioned in *Chapter 1* will benefit patients, particularly since epilepsy requires long term management therapies. The drug profiles of carbamazepine and sodium valproate are discussed below.

2.5. PHARMACOLOGICAL OVERVIEW OF CARBAMAZEPINE

Carbamazepine is a tricyclic iminostilbene derivative used as a first line agent for the management of GTCS epilepsy. It has a chemical name Benzo[b] [1] benzazepine-11-carboxamide and chemical formula $C_{15}H_{12}N_2O$ and a molecular weight of 236.269 g/mol. The chemical structure is depicted below (British Pharmacopoeia, 2014):

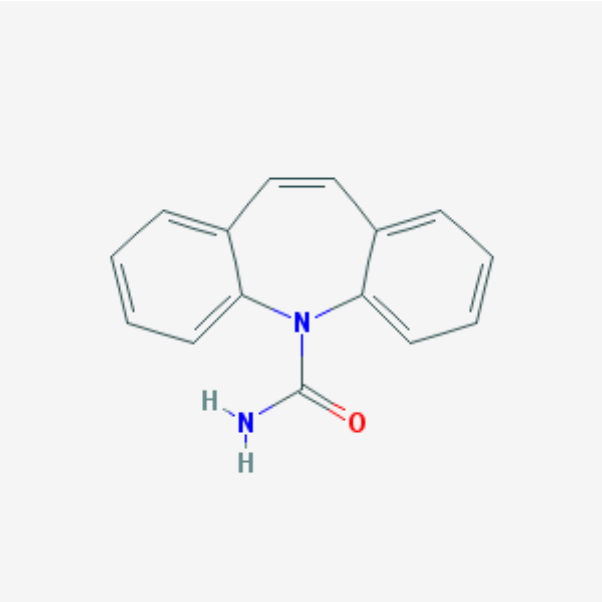

Figure 2.3: Chemical structure of carbamazepine

Source: Adapted from British Pharmacopoeia, (2014)

2.5.1. Physicochemical properties

Carbamazepine is a white to off-white crystals with a melting point of 190.2 degrees Celsius. It has a got a bioavailability of 75-85% with a volume of distribution (Vd) of 0.8 to 1.2 l/kg. It is practically insoluble in water but slightly soluble in ethanol. The solubility of carbamazepine in different mediums is summarised in table 2.4 below.

Table 2.4: Solubility of carbamazepine in different mediums (Pubchem, 2005)

Solubility medium	Solubility
Acetone	Soluble
Propylene glycol	Soluble
Water	Practically insoluble
Chloroform	Soluble
Dimethylformamide	Soluble
Methanol	Soluble
Ethanol	Slightly soluble
Glacial acetic acid	Slightly soluble

2.5. 2. Clinical pharmacology

According to Ambrósio, Soares-da-Silva, Carvalho and Carvalho, (2002) carbamazepine may interact with different types of channels and receptors but the main targets are voltage-dependent sodium channels. It reduces the occurrence of sustained repetitive firing of action potentials in cultured mammalian central neurons. It is now considered to be the drug of choice for the treatment of partial and tonic-clonic seizures (Brunton *et al*., 2011). It can also be used in the management of attention deficit hyperactivity disorder (ADHD), schizophrenia and post-traumatic stress disorder as a mood-stabilizing agent, as well as phantom limb syndrome, complex regional pain syndrome, paroxysmal extreme pain disorder and neuromyotonia disorder (Tolou-Ghamari, Zare, Habibabadi and Najafi, 2013).

The common side effects of carbamazepine are sedation, dry mouth, dizziness, ataxia, and gastrointestinal effects. Carbamazepine interacts with other anticonvulsants, antiretroviral drugs, cimetidine, dextropropoxyphene, erythromycin and other macrolides, SSRIs, verapamil, diltiazem, isoniazid, lithium, monoamine oxidase inhibitors, oral contraceptives, corticosteroids, doxycycline, midazolam, neuroleptics, theophylline, tricyclic antidepressants and warfarin. The use of carbamazepine is contraindicated in patients suffering from Antrioventricular heart block, history of bone marrow depression, and porphyria (Rossiter, 2016).

2.5.3. Clinical pharmacokinetics

2.5.3.1. Absorption

Although the rate of absorption of carbamazepine varies extensively among patients, almost complete absorption occurs in all. Following administration, peak levels are usually achieved after 6–8 hours. Giving the drug after meals slows absorption; however, it helps the patient tolerate larger total daily doses (Katzung, Masters and Trevor, 2010).

2.5.3.2. Distribution

Carbamazepine binds to serum proteins at an extent of 70-80%. The concentration of unchanged substance in the saliva reflects the non-protein-bound portion present in the serum (20-30%) (Product monograph: Tegretol®, 2014). Carbamazepine has a large volume of distribution (Vd), projected to be 1 to 2 L/kg (Islam, Al Aidarous, Jan and Dehlawi, 2013).

2.5.3.3. Metabolism

The main pathway of carbamazepine metabolism in humans involves conversion to its active metabolite carbamazepine-10, 11 epoxide. This metabolite is as active as the parent compound. It is further metabolized primarily into inactive compounds such as carbamazepine 10, 11-transdiol, 9-hydroxymethyl-10-carbamoyl-acridan, various monohydroxylated compounds and the N-glucuronide of carbamazepine produced by UDP-Glucuronosyltransferase-2B7 (UGT2B7). CYP3A4 is primarily responsible for the biotransformation of carbamazepine (Brunton *et al*., 2011).

2.5.3.4. Elimination

Whether administered as a single or in repeated doses, only 2-3% of carbamazepine is excreted in the urine in an unchanged form. Approximately 30% of carbamazepine is renally eliminated via the carbamazepine-10, 11-epoxide pathway with carbamazepine 10, 11 -trans-diol as the main urinary metabolite (Islam *et al*., 2013).

2.6. PHARMACOLOGICAL OVERVIEW OF SODIUM VALPROATE

It is a synthetic derivative of 2-propylpentanoic acid with antiepileptic properties and potential antineoplastic and antiangiogenesis activities. It has a molecular formula of $C_8H_{15}NaO_2$ and a molecular weight of 166.196 g/mol (Pubchem, 2005). Its chemical structure is depicted in figure 2.4 below

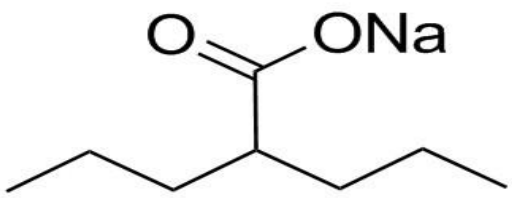

Figure 2.4: Chemical structure of sodium valproate

Source: Adapted from British Pharmacopoeia (2014)

2.6.1. Physicochemical properties

Sodium valproate is a colourless crystal with a melting point of 219.5 degrees Celsius. It has almost similar solubility properties as carbamazepine with slightly better solubility properties. The solubility of sodium valproate in different mediums is summarised in table 2.5 below.

Table 2.5: Solubility of sodium valproate in different mediums (Pubchem, 2005)

Solubility medium	Solubility
Organic solvents	Very soluble
Sodium hydroxide	Freely soluble
Water	Practically insoluble
Chloroform	Freely soluble
Acetone	Freely soluble
Methanol	Freely soluble
n-heptane	Slightly soluble
Benzene	Freely soluble

2.6.2. Clinical pharmacology

At therapeutically relevant concentrations, sodium valproate inhibits sustained repetitive firing induced by depolarisation of mouse cortical or spinal cord neurons. The action appears to be mediated by a prolonged recovery of voltage-gated sodium channels from inactivation. In neurons isolated from the nodose ganglion, valproate also produces small reductions of calcium ions currents at clinically relevant but slightly higher concentrations than those that limit sustained repetitive firing (Brunton *et al*., 2011). Sodium valproate is indicated for all forms of epilepsy. It is the drug of choice in the treatment of tonic-clonic seizures as part of the syndrome of primary generalized epilepsy. It is also used for prophylaxis of migraine and for control of the acute-manic phase of bipolar disorder (Rossiter, 2016).

The common side effects of valproate are nausea, vomiting, diarrhoea and constipation. Valproate interacts with carbamazepine, central nervous system depressants, carbapenems, ethusixamide, lamotrigine, phenobarbital, phenytoin, warfarin, aspirin and dipyridamole as well as zidovudine. The use of sodium valproate is contraindicated in patients suffering from pre-existing liver disease and porphyria (Rossiter, 2016).

2.6.3. Clinical pharmacokinetics

2.6.3.1. Absorption

Following oral use, sodium valproate is well-absorbed and 80% bioavailable. Peak blood levels occur within 2 hours. Absorption may be delayed by food and if the drug is given after meals, toxicity may be decreased. However, this does not affect the extent of absorption (Katzung *et al*., 2010).

2.6.3.2. Distribution

The apparent volume of distribution of sodium valproate is relatively slow (approximately 0.15- 0.21/kg). Extravascular distribution is limited by its high binding affinity for plasma albumin. Sodium valproate has a half-life ranging between 8-20 hours. It is usually shorter in children. It is approximately 90% bound to plasma proteins but only 60% to albumin. Distribution of sodium valproate is rapid and most possibly limited to the circulation and rapidly exchangeable extracellular water. Cerebrospinal fluid and breast milk levels were found to be 5 to 15% and about 1 to 10% of plasma levels, respectively (Loscher, 1999).

2.6.3.3. Metabolism

Sodium valproate's metabolism is complex; the major elimination pathway is by glucuronidation (40-60%). The remainder is largely metabolised via oxidation pathways, β-oxidation accounting for 30-40% and the remaining fraction being w-oxidation (cytochrome P450 dependent). Only 1 to 3% of the ingested dose is found to be excreted unchanged in the urine (Brunton *et al*., 2011).

2.6.3.4. Elimination

Sodium valproate is almost completely metabolised prior to excretion. Plasma half-life is variable but generally appears to be 8 to 12 hours (range 3.84 to 15.77 hours). It may be shorter in patients receiving other anticonvulsants or in children and patients receiving the medicine for long periods. In cases of overdose, plasma half-life up to 30 hours has been reported (Loscher, 1999).

2.7. SUMMARY

This chapter provided the overview and management of epilepsy. The literature on the use of carbamazepine and sodium valproate individually and in combination provided significance for the formulation of the two drugs into a fixed dose combination tablet. This was reiterated by checking the pharmacological aspects and pharmacokinetic data which was found in existing data. The next chapter, which is experimental methods, will provide the methods, materials and apparatus used to formulate the fixed dose combination tablet of carbamazepine and sodium valproate

CHAPTER3: EXPERIMENTAL METHODS, MATERIALS AND APPARATUS

3.1. INTRODUCTION

The experimental materials, procedures and apparatus used in the different experiments to formulate a fixed dose combination tablet of carbamazepine and sodium valproate are described in this chapter.

Carbamazepine and sodium valproate were chosen for the purpose of the study because both of them are drugs of choice for the treatment of epilepsy. They are both on the same regimen which is a preliminary requirement for the development of FDC. The following materials will be used in the study:

Table 3.1: Materials used in the study

Material	Lot number	Manufacturer
Carbamazepine	BN20180618	DB Fine Chemicals, Johannesburg, RSA.
Sodium valproate	BN20180619	DB Fine Chemicals, Johannesburg, RSA.
Magnesium stearate	21203	Kirsch Pharma, Isando, Johannesburg, RSA.
Ac-Di-Sol®	49825612PO	FMC Corporation, Philadelphia, Pennsylvania, USA.
Kollidon®VA64 Fine	554707188PO	BASF Aktiengesellschaft, Ludwigshafen, Germany.
Pharmacel® 101	B0551203047	FMC Corp., Cork, Ireland.
Emcompress®	D9214C	FMC Corp., Cork, Ireland.

3.2. STUDY DESIGN

This was a quantitative research project with a true experimental design. Quantitative research refers to a formal, objective and systematic process in which numerical data are used to obtain information from groups or experiments. This research method is used to describe variables, to examine relationships among

variables and to determine cause-and-effect interactions between variables (Brink, van Rensburg and van der Walt, 2012). When using a true experimental design, the researcher will have to a control group and an experimental group. The controls that were used in this study were pure carbamazepine and sodium valproate, which were positive controls as they are the active pharmaceutical ingredients in this study.

3.3. PREFORMULATION STUDIES

The most important thing when handling a drug is powder flow and compatibility between the drugs and the excipients. There are number of methods existing that can be used to evaluate powder flow and compatibility and some of them were chosen and used for this study. They are extensively explained and the methods used are outlined below.

3.3.1. Evaluation of starting materials and granules

The starting materials and granules were evaluated for pre-compression parameters such as angle of repose, bulk density, tapped density and Carr's index

3.3.1.1. Particle size distribution

Particle size analysis of the powders and granules were conducted by means of laser diffraction, using a Malvern® Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK), fitted with a sample suspension unit. The dispersion unit contained water and cyclohexane during the particle size distribution measurements of carbamazepine and sodium valproate respectively.

The calculation of the span of a powder sample is made to determine its particle size distribution. The span gives an indication of the width of the distribution based on the 10th, 50th and 90th percentile. The span can be calculated by using the following equation (Malvern Instruments Ltd, 2012):

$$Span = \frac{Dv.0.9 - Dv.0.1}{Dv.0.5}$$ Equation 3.1

3.3.1.2. Angle of repose

Angle of repose is a characteristic related to inter particulate cohesion between the particles, which was used as an indirect method to quantify the flow properties of powders (Aulton, 2007). Static angle of repose was determined according to the fixed funnel and freestanding cone method whereby the powder was accurately weighed and carefully poured though the funnel with its tip 2 cm high, until the apex of the conical heap formed reach the tip of the funnel. The angle of repose (θ) was calculated using equation 3.2:

$$Tan\ \theta = \frac{H}{R} \qquad \text{Equation 3.2}$$

where H is the height of the tip of the funnel and R is the radius of the base of the powder cone.

Table 3.2: Flow properties and corresponding angle of repose (British Pharmacopoeia, 2014)

Flow properties	**Angle of repose**
Excellent	25-30
Good	31-35
Fair	36-40
Passable	41-45
Poor	46-55
Very poor	56-65
Poorest	>66

3.3.1.3. Bulk and tapped density

A known quantity of each sample (25 g) was weighed on a Precisa® analytical balance (model 240A, OERLIKON AG, Zurich) and poured through a funnel into a 100 ml graduated cylinder. The cylinder was then lightly tapped twice to collect all the powder sticking on the wall of the cylinder. The volume was then read directly from the cylinder and used to calculate the bulk density. For tapped density, the

cylinder was tapped from a height of 15 cm, 50 times on a wooden bench top to attain a constant volume reading from the cylinder. The bulk density was calculated using equation 3.3

$$Pbulk = \frac{mass}{volume} \qquad \text{Equation 3.3}$$

Equation 3.4 was used to calculate the tapped density

$$Ptapped = \frac{mass}{volume} \qquad \text{Equation 3.4}$$

3.3.1.4. Hausner ratio and Carr's index

Carr's index and the Hausner ratio previewed the degree of densification, which could occur during tableting. The Carr's index was calculated using 3.5:

$$Compressibility\ \% = \frac{Ptapped - pbulk}{Ptapped} \times 100 \qquad \text{Equation 3.5}$$

The Hausner ratio was calculated using equation 3.6

$$Hausner\ ratio = \frac{ptapped}{pbulk} \qquad \text{Equation 3.6}$$

Table 3.3: BP scale of flowability for Carr's compressibility index and the Hausner ratio (British Pharmacopoeia, 2014)

Flow character	Carr's compressibility index (%)	Hausner ratio
Excellent	≤10	1.00 – 1.11
Good	11 – 15	1.12 – 1.18
Fair	16 – 20	1.19 – 1.25
Passable	21 – 25	1.26 – 1.34
Poor	26 – 31	1.35 – 1.45
Very poor	32 – 37	1.46 – 1.59
Very, very poor	≥38	≥1.60

3.3.2. Simultaneous Thermal Analysis

The starting materials were evaluated for physico-chemical compatibility using Differential Scanning Calorimetry (DSC) in combination with Thermogravimetric Analysis (TGA), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Powder Diffraction (XRPD) and Thermal Activity Monitor (TAM) method.

3.3.2.1. Differential Scanning Calorimetry (DSC)

A DTG simultaneously measures the mass loss (TGA) and heat flow (DSC) of a sample during the heating process. With this combined technique, information about the mass loss, melting point, glass transition, solid-state transformation(s), loss of solvents and degradation of a sample can be obtained (Brown, 2001).

A Mettler DTG 3^+ (Mettler Toledo, Greifensee, Switzerland) was used to record the DSC and TGA thermograms. DTG was used to investigate the physicochemical compatibilities and solid interaction of carbamazepine and sodium valproate and the excipients. Powder samples weighing approximately 5-8 mg were placed in open aluminium crimp cells (100 µl) and heated to an end temperature dependant on the

melting point of the APIs and the excipients, at a heating rate of 10 ^{o}C/min, with a nitrogen gas flow of 35ml/min.

Experimental conditions

The set DTG temperature for carbamazepine was between 30^{o}C to 350^{o}C to accommodate all the excipients. All the weighed quantities of the materials are outlined in table 3.2 below

Table 3.3: Samples for compatibility study

Sample	Amount weighed (mg)
Magnesium stearate	5.62
Ac-Di-Sol®	5.36
Combilac®	5.51
Pharmacel® 101	5.26
Carbamazepine/Magnesium stearate (1:1)	8.45
Carbamazepine/Ac-Di-Sol® (1:1)	8.04
Carbamazepine/Combilac® (1:1)	8.53
Carbamazepine/Pharmacel® (1:1)	8.68
Sodium valproate/Magnesium stearate (1:1)	8.66
Sodium v*valproate/Ac-Di-Sol® (1:1)	8.79
Sodium valproate/Combilac® (1:1)	8.65
Sodium valproate/Pharmacel® (1:1)	8.69

3.3.2.2. Fourier Transform Infrared Spectroscopy (FTIR)

This method was used to confirm the identity of carbamazepine and sodium valproate as well as detecting the interaction between the two drugs. Samples were placed on the Nicolet disc, and was done using pressed pellet method where samples will be thoroughly mixed with. The mixture was compressed to form a disc which was placed in the spectrophotometer for recording.

Experimental conditions

The IR-spectra was recorded on a Nicolet nexus 4709- Fourier transform infrared spectroscopy (FTIR) spectrometer (Nicolet instrumentation corporation, Madison USA) over a range of 0-400cm-1.

3.3.2.3. X-ray Powder Diffraction (XRPD) method

X-ray powder diffraction was used as an effective method to distinguish between different solid phases in different or the same powdered samples. The X-ray powder diffractogram produces information about the diffraction characteristics of the sample (Watts, Maruyoshi, Hughes, Brown, & Harris, 2016). These diffraction characteristics include the intensities of the maximum diffraction peaks and the angles at which they occur (Bunaciu, Udristioiu, & Aboul-Enein, 2015). The XRPD traces of the powders were compared with regard to peak position and relative intensity, peak shifting and the presence or lack of peaks in certain regions of º2θ values.

Experimental conditions

Approximately 1g of carbamazepine and sodium valproate were placed into the aluminium sample holder using a glass side to assure a flat upper surface. XRPD patterns of the materials were determined on a Philips PW 1380 X-Ray diffractometer with high intensity Cu-Kα radiation (λ = 1.54 Å, 40 kV and 40 mA) and a graphite monochrometer at a scanning rate of 0.025 s-1 ranging from 4 to 64 degrees 2-theta, where theta is the diffraction angle. The raw count data was captured using Philips Automatic Powder diffraction software which was then converted into ACSII format and imported into a Microsoft® Excel spreadsheet.

3.3.2.4. Thermal Activity Monitor (TAM)

Compatibility between the different APIs was established with a 2277 Thermal Activity Monitor (TAM III; TA Instruments, United States of America), equipped with an oil bath with a stability of ± 100 µK over 24 h. The calorimeter's temperature was maintained at 40°C. During the compatibility studies the heat flow was measured for the single components, as well as for the mixtures. The TAM is a sensitive and accurate instrument for detecting incompatibilities and instabilities between APIs.

Masses of samples:

Carbamazepine 1: 100.43 mg; carbamazepine 2: 100.53 mg

Sodium valproate 1: 100.05 mg; sodium valproate 2: 100.19 mg

Combination 1: 100.68 mg; Combination 2: 94.59 mg

3.4. FORMULATION STUDIES

The FDC tablets were manufactured using direct compression method and wet granulation method.

3.4.1. Direct compression

Formulations of carbamazepine/sodium valproate monolithic FDC tablet was prepared by direct compression. All the raw materials for carbamazepine and sodium valproate were weighed inside a 250 cm^3 glass container to obtain a mass of 100 g. The glass containers were fitted with a screw cap prior to mixing. All mixing procedures employed a Turbula®-mixer (model T2C W.A. Bachofen, Basel, Switzerland) at 130 rpm for 5 minutes as the standard method to ensure a proper distribution of powder particles.

Compression settings employed upper punch settings of between 8.25 and 8.5 and the filling volume of the die was altered by the adjustment of the lower punch setting to 5.25 and 5.5. Round shaped die set was utilised to manufacture tablets that presented a round surface. A Korsch® XP 1 Research tablet press (Berlin, Germany) was employed during all tableting procedures. After manufacturing was completed, tablets were stored in sealed glass containers away from light at room temperature for 24 hours preceding further analysis.

3.4.2. Preparation of wet granulated tablets

Carbamazepine and sodium valproate, Ac-Di-Sol®, Kollidon® VA64 fine and Emcompress® were weighed together inside a 250 cm^3 glass container. The glass container was fitted with a screw cap prior to mixing. All mixing procedures employed a Turbula®-mixer (model T2C W.A. Bachofen, Basel, Switzerland) at 130 rpm for 5

minutes as the standard method to ensure a proper distribution of powder particles. The mixture was wetted with ethanol and granulated. The granules were forced through a 841 µm mesh stainless steel sieve, dried, and kept in a desiccator for 24 hours at room temperature. After 24 hours, the primary granules were forced through 250 µm mesh stainless steel sieve. Magnesium stearate was added to the granules and mixed for 2 minutes using a Turbula®-mixer (model T2C W.A. Bachofen, Basel, Switzerland) prior compression.

Compression settings employed upper punch settings of between 8 and 8.5 and the filling volume of the die was altered by the adjustment of the lower punch setting to 5 and 5.25. Round shaped die set was utilised to manufacture tablets that presented a round surface. A Korsch® XP 1 Research tablet press (Berlin, Germany) was employed during all tableting procedures. After manufacturing was completed, tablets were stored in sealed glass containers away from light at room temperature for 24 hours preceding further analysis.

3.4.3. Mixture preparation of the fixed dose combination

3.4.3.1. Selection of excipients

Excipients were carefully selected in advance based on theoretical compatibility, theoretical stability and to eliminate disturbances during the analytical process of HPLC. Both APIs and excipients were studied prior formulation for compatibility by employing differential scanning calorimetry, infrared absorption spectroscopy and x-ray powder diffraction to identify excipient incompatibilities.

3.4.3.2. Fillers

Fillers are used in tablet formulations to help in free flowing of granules from hopper to die cavity and to minimize friction between particles (Karthik, 2016). Emcompress® was selected as the preferred filler to Pharmacel® and Combilac® for direct compression method. Emcompress® was the only filler between the three that could produce tablets that could withstand crushing upon handling, and increased flowability whilst Pharmacel® and Combilac® produced tablets that broke upon handling. Pharmacel® was the preferred filler for wet granulation as the

3.4.3.3. Lubricants

Lubricants are used in tablet formulation to help decrease friction at the interface between a tablet's surface and the die wall during ejection so that the wear on punches and dies are reduced. They also prevent sticking of tablets to punch faces (Li & Wu, 2014). Magnesium stearate was selected as the preferred lubricant mainly because it is commonly used in the pharmaceutical manufacturing of tablets.

3.4.3.4. Disintegrants

Disintegrating agents are included in tablet formulations to help the tablet break down in to small particles and promote moisture penetration of the matrix of the dosage form in dissolution fluids (Karthik, 2016). Ac-Di-Sol® was the preferred disintegrant as it is highly active in relatively low concentrations compared to other disintegrants, thus the amount used in all the formulations was not going to affect dissolution of the tablets.

3.4.3.5. Binders

Binders are employed to impart cohesiveness to the granules, thus to ensure the tablet remain intact after compression (Patil, et al., 2014).

3.5. POST FORMULATION STUDIES

Tablet dimensions were evaluated to establish the applicability of the manufacturing processes for the oral fixed-dose combination in terms of tablet weight variation, friability and physical proportions. British Pharmacopoeia (2014) methods were used to evaluate the dosage form. The crushing strength, friability, diameter and thickness of the tablets are non-official pharmacopoeial tests and were conducted for data gathering purposes.

3.5.1. Tablet crushing strength, diameter and thickness

Twenty tablets were randomly selected and measured for crushing strength, diameter and thickness by using Pharma Test® (model PTB-311) tablet test unit (Pharma Test, Switzerland).

3.5.2. Average weight of the dosage unit

The weight variation test of tablets is an official British Pharmacopoeia (2014) test. Twenty tablets from each batch were dusted and weighed using a Precisa® analytical balance (model 240A, OERLIKON AG, Zurich) which was used to determine weight variation. Weight values were reported in milligrams and were measured against the BP limits for tablet weight variation.

3.5.3. Friability test

Twenty tablets were weighed individually from each batch. The tablets were placed in a Roche friabilator and exposed to 25 rotations in 4 minutes. The tablets were dedusted and reweighed. Equation 3.5 was used to calculate the percentage friability.

$$\%F = \frac{wb-wa}{wb}\ x\ 100 \qquad \text{Equation 3.6}$$

Where:

W_a - weight of tablets before rotation

W_b - weight of tablets after rotation

3.5.4. Disintegration studies

The disintegration equipment and method used are described in the British Pharmacopoeia (2014). The disintegration times of six tablets from each formulation were determined using an Erweka® GmbH tablet disintegration test unit (Type ZT503, Heusenstamm, Germany). A set limit of 15 minutes was employed. The

disintegration medium was distilled water and was maintained at a temperature of 37± 2°C by a thermostat.

3.5.5. Assay of tablets

The HPLC system was equipped with a variable wavelength UV detector and an integrator. In this instance a Hewlett Packard Agilent 1100 equipped with a variable wavelength UV detector was used. The parameters and conditions are summarized in table 3.4.

Table 3.4: The instrument parameters

Column	Restek Ultra 18 column, 250 x 4.6mm, 5µm (Restek, Bellefonte,PA)
Mobile phase	Acetronitrile/water with 0.2% orthophodphoric acid 70:30
Stop time	8 minutes
Flow rate	1.0 ml/min
Injection volume	20 µl
Detection	UV at 214 nm
Retention time (carbamazepine)	3.13 minutes
Retention time (sodium valproate)	4.74 minutes

The peak areas of the responses obtained from the chromatograms for the withdrawn dissolution samples were measured to calculate the percentage content, with reference to the raw powder standard preparations of carbamazepine and sodium valproate.

3.5.5.1. HPLC method development and validation

To validate the analytical method, the method has to be developed in terms of Specificity, Linearity, Accuracy, Precision (repeatability, intermediate precision, and reproducibility), Range, limits of detection (LOD) and limits of quantification (LOQ), robustness and system suitability testing. The validation has to be performed according to the ICH guidelines (ICH, 2015).

3.5.5.1.1. Specificity

Specificity is the ability to evidently assess the analyte in the formulation, which may be expected to be present in that formulation or sample. Normally these might include impurities, degradants, matrix, etc. To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, an assay is used for determining an accurate result in order to provide specific and precise information on the content and potency of the analyte in a sample (ICH, 1996).

3.5.5.1.2. Linearity

The linearity of an analytical procedure is defined as the ability to obtain results that are directly proportional to the concentration of drug used in a sample. E.g. the difference between a 10mg concentration must be proportional to that of 5mg looking at the absorbance obtained during test (ICH, 1996). Linearity in this study was confirmed by dilutions that were prepared from the standard solution which had the concentration of 0.01µg, 0.10 µg, 0.51 µg, 1.52 µg, 5.06 µg,15.18 µg, 25.30 µg, 50.60 µg and 101.20 µg for carbamazepine and 0.01 µg, 0.10 µg, 0.51 µg, 1.54 µg, 5.12 µg,15.36 µg, 25.60 µg, 51.20 µg and 102.40 µg for sodium valproate in 100ml volumetric flask. These solutions were then transferred into an HPLC-vial through the 0.45µ filter and analyzed. The average peak areas from three injections on the chromatograms were used to plot the standard curves using Microsoft Excel TM

3.5.5.1.3. Range

The range of an analysis for the assay of an active substance is usually between 80 to 120 percent of the test concentration (ICH, 1996). The range of concentrations used to determine standard linearity of carbamazepine and sodium valproate was between 0.0001mg/100ml up to 04 mg/100ml and covered the entire anticipated range of concentrations expected to elicit a pharmaceutical response. This is established by confirming that the analytical procedure provides an acceptable degree of linearity.

3.5.5.1.4. Robustness

Robustness is a measure to determine the ability of the analytical procedure to remain unchanged by slight or deliberate variations in method parameters used. It provides an indication of its reliability during a test procedures or analytical run (ICH, 2015). When working with HPLC, typical variations that can occur include the following: Flow rate of the mobile phase, columns (different suppliers, brand and dimensions), Temperature of the column, pH of a mobile phase and composition of mobile phase (Yanamandra, Chaudhary, Bandaru, Sastry, Patro, Murthy & Ramaiah, 2011).

3.5.5.1.5. Accuracy

The accuracy of an analytical method states the similarity between the value which is accepted either as a conventional true value or an accepted reference value and the found value. To determine the accuracy of a method, the reference material must be subjected to the analytical procedure. (FDA, 2001; ICH, 2015). Three injections of varying concentrations were analyzed in triplicate in this study to determine if they all result in similar area under the chromatogram or closest to the first injection.

3.5.5.1.6. Precision

Validation of assay tests methodology includes an investigation of precision. The precision of an analytical procedure expresses as the closeness of agreement between a series of measurements acquired from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered in three levels, these include repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be explored using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually stated as the variance, standard deviation or coefficient of variation of a series of measurements (ICH, 2015)

Twenty tablets were weighed individually, then placed in the mortar and powdered with a pestle. An amount equivalent to 200mg of both carbamazepine and sodium valproate was extracted with 100ml of 0.1 M hydrochloric acid, sonication for 15

minutes. The solution was filtered through a filler of 0.45 µm pore size, properly diluted with 0.1 M hydrochloric acid and measured the drug content using HPLC.

3.5.6. Dissolution studies

In-vitro drug release were studied using paddle dissolution apparatus, with 900 ml water containing 1.0% sodium lauryl sulphate used as medium maintained at 37±0.5°C at the rotation speed of 75 rpm. 1.5 ml of samples were withdrawn after 10, 15, 20, 30, 45 and 60 minutes, and was replaced by an equal volume of fresh dissolution medium of same pH**.** The collected samples were filtered and transported to HPLC wells. The samples were analysed by employing HPLC with UV-visible spectrophotometer at a measured wavelength of 285nm which was used to calculate the drug release. To comply with dissolution test 3: in 15 minutes between 60.0 and 75.0% of the drug must have dissolved and not less than 75.0% must have dissolved in 60 minutes.

3.6. DATA ENTRY AND ANALYSIS

All numerical data was captured and analysed using Design Expert® software. Statistical analysis of data was performed using a one-way repeated analysis of variance (ANOVA) with Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA) to determine the significant ($p < 0.05$) between direct compression method and wet granulation method. The average and standard deviation were calculated to indicate reproducibility.

3.7. ETHICAL CONSIDERATIONS

No humans or animals were involved in this study. Ethical clearance was obtained from the University of Limpopo's research and ethics office. Experimental part of the project commenced after obtaining the ethical clearance certificate. All laboratory experiments conducted were in accordance with current Good Laboratory Practice (cGLP) and current Good Manufacturing Practice (cGMP). Current Good Laboratory Practice prescribes a laboratory to work according to a system of procedures and protocols to ensure the uniformity, consistency, reliability, reproducibility, quality and integrity of pharmaceuticals. These included standards for laboratory safety and

suitable means of disposing wastes. Good manufacturing practice provide guidance for manufacturing, testing and quality assurance in order to ensure that drug product meet the minimum standards a prescribed by BP and/or USP.

3.8. SUMMARY

This chapter provided the experimental methods, materials and apparatus used to formulate the fixed dose combination tablet of carbamazepine and sodium valproate. The methods used are official methods from the British Pharmacopoeia and ICH guidelines. The methods were categorised into three phases, starting with preformulation studies that were used to ensure that the raw materials used in the study were suitable and appropriate for the formulation of a FDC tablet. The second phase was formulation studies which encapsulated different methods of formulating the conventional FDC tablet. The last phase, which was post formulation studies, consisted of numerous tests (official and non-official) that were used to determine if the tablets complied. The next chapter will provide the results and discussion of the first phase, which is preformulation studies.

CHAPTER 4: PRE-FORMULATION STUDIES: RESULTS AND DISCUSSION

4.1. INTRODUCTION

The results obtained in chapter 3 are presented in this chapter and discussed in detail. The results described below comprise several imperative factors to ultimately deliver a product with suitable flow and compressibility properties. Particle flowability and compressibility are two critical process parameters tested when a material is designed for direct compression. The interpretations of the results obtained compatibility studies and the evaluation of power are discussed in this chapter.

4.2. COMPATIBILITY STUDIES

The starting materials were evaluated for physico-chemical compatibility using Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Powder Diffraction (XRPD) method.

4.2.1. Differential Scanning Calorimetry (DSC)

The appearance, shift or disappearance of endothermic or exothermic peaks on the DSC thermograms were monitored as a crucial aspect of drug-drug compatibility or interaction. In this study, the thermograms of the carbamazepine and sodium valproate mixture was compared to the different drugs alone and the excipients.

Figure 4.1 shows the DSC thermogram of carbamazepine (blue) with a single endothermic peak at 190.74 º C, corresponding to its melting point of 191.5º C (Yadav & Lariya, 2017). DSC thermogram of sodium valproate (red) showed a single endothermic peak of 110.02º C.

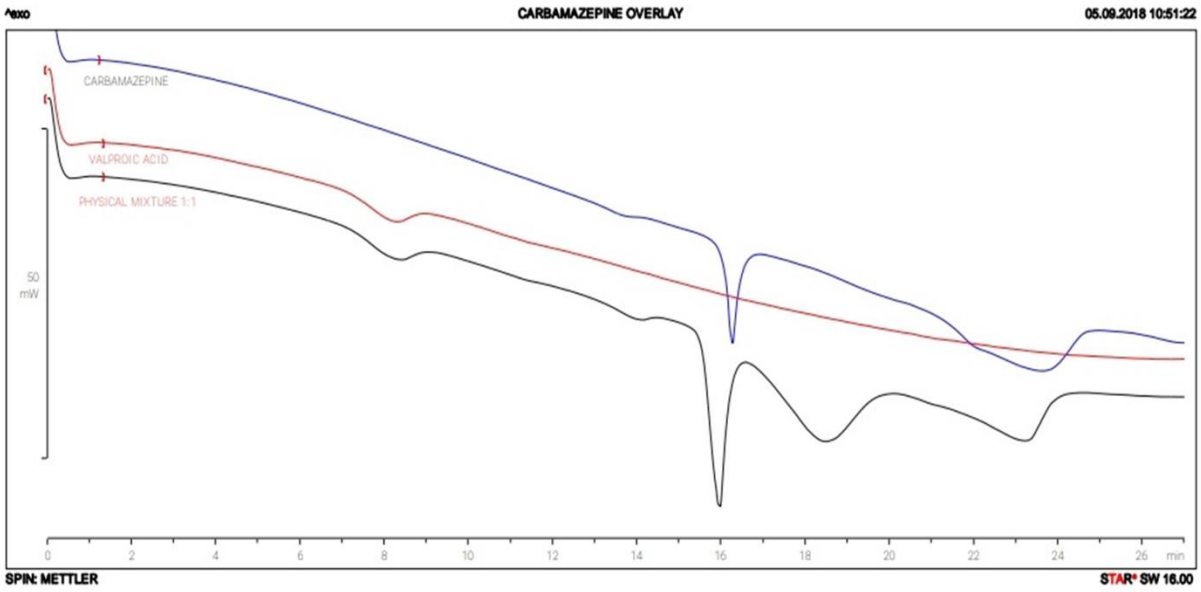

Figure 4.1: DSC thermograms of carbamazepine (blue), sodium valproate (red) and their physical 1:1 mixture (purple)

The DSC thermograms of carbamazepine-sodium valproate mixture (purple) is depicted in figure 4.1. The thermogram indicate a reduction shift of approximately 3º C in the endothermic peaks of both carbamazepine and sodium valproate. The shifts are from 190.74º C to 187.63º C and 110.02º C to 112.94º C for carbamazepine and sodium valproate respectively. The results suggest that sodium valproate and carbamazepine are compatible.

Figure 4.2 to figure 4.7 shows the compatibility check for carbamazepine and/or sodium valproate with the excipients that were used in this study. The DSC thermogram in figure 4.2 shows the DSC thermogram of carbamazepine (red) with a single endothermic peak at 190.74 º C, corresponding to its melting point of 191.5º C (Yadav & Lariya, 2017). DSC thermogram of Ac-di-sol® (black) shows a single exothermic peak at of 300º C, corresponding to its melting point of >250 (Varma & Begum, 2012)

The DSC thermograms of carbamazepine-ac-di-sol® mixture (blue) is depicted in figure 4.2. The thermogram indicate a shift increment of less than 1º C in the endothermic peak of carbamazepine and approximately 4º C for Ac-Di-Sol®. The shifts are from 190.74º C to 190.83º C and 300.00º C to 304.64º C for

carbamazepine and Ac-Di-Sol® respectively. The results suggest that carbamazepine and Ac-Di-Sol® are compatible.

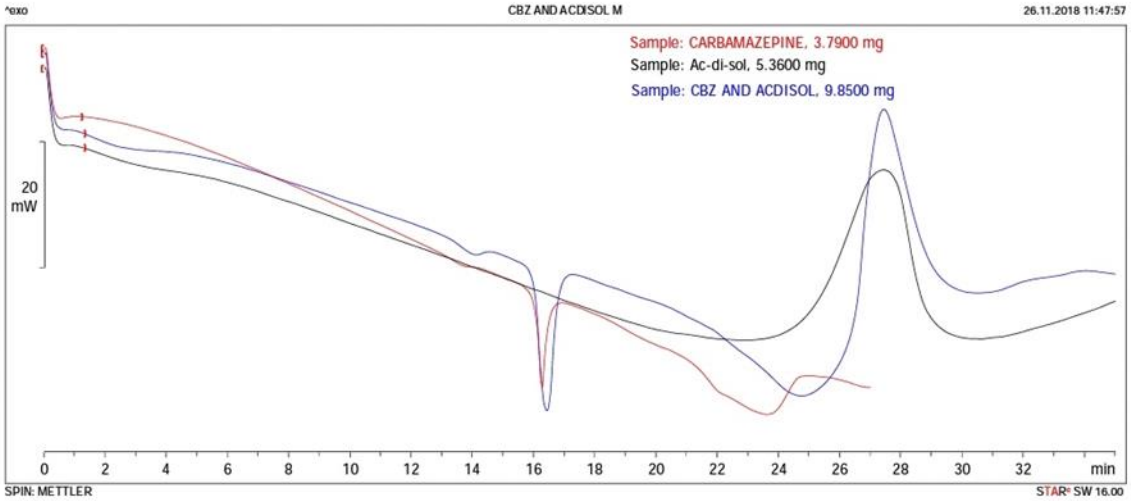

Figure 4.2: DSC thermogram of carbamazepine (red) Ac-Di-Sol® (black) and carbamazepine-ac-di-sol® mixture (blue)

The DSC thermograms of sodium valproate (red) and Ac-di-sol® (black) are shown in figure 4.3. Sodium valproate shows a single endothermic peak of 110.02º C and Ac-di-sol® shows a single exothermic peak of 300º C.

The single thermograms were compared to the DSC thermogram of sodium valproate-ac-di-sol® mixture (blue) in figure 4.3. The thermogram indicate a reduction shift of approximately 5º C in the endothermic peak of sodium valproate and no peak shift for Ac-Di-Sol®. The shifts are from 110.02º C to 105.35º C for sodium valproate and Ac-di-sol® retained its exothermic peak at 300º C. The results suggest that carbamazepine and Ac-Di-Sol® are compatible.

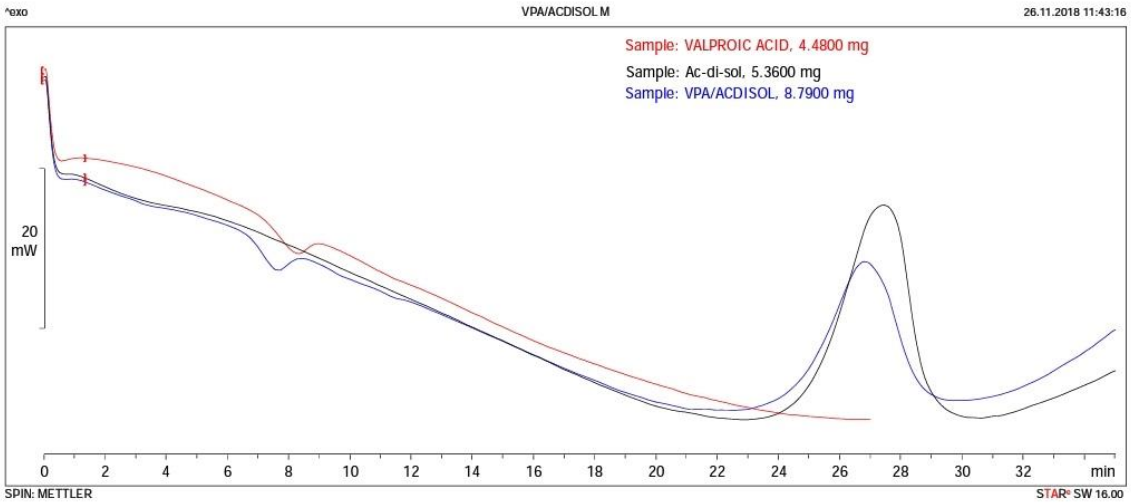

Figure 4.3: DSC thermogram of sodium valproate (red) Ac-Di-Sol® (black) and sodium valproate-ac-di-sol® mixture (blue)

In the DSC thermogram for magnesium stearate (blue) shown in figure 4.4, two peaks were observed. The first occurring at a temperature of 110.40º C and the second occurring at 125.08º C. The DSC thermogram of carbamazepine (black) shows a single endothermic peak of 190.74º C.

The DSC thermogram for carbamazepine and magnesium stearate were compared to the DSC thermogram of carbamazepine-magnesium stearate mixture (red) in figure 4.4. Four peaks were observed in carbamazepine-magnesium stearate mixture, the first one occurring at 87.86º C, characteristic of magnesium stearate melting point (Li & Wu, 2014). There was a reduction peak shift of approximately 2º C in the second and a shift increment of less than 1º C for carbamazepine. The shifts are from 110.40º C to 109.03º C and 125.08º C to 123.32º C for magnesium stearate and from 190.74º C to 190. 81º C for carbamazepine. Given the results, carbamazepine is compatible with magnesium stearate.

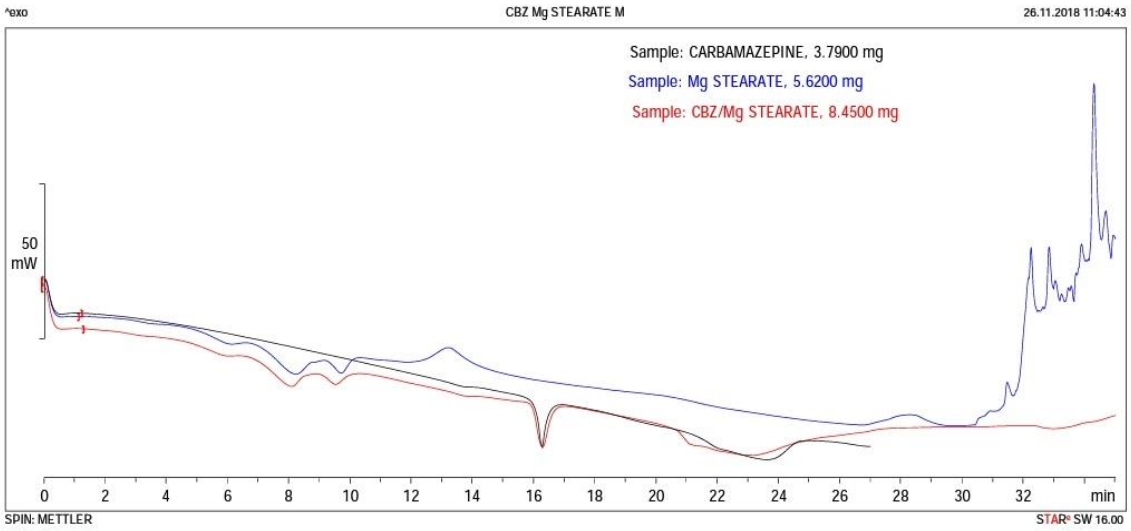

Figure 4.4: DSC thermogram of carbamazepine (black) magnesium stearate (blue) and carbamazepine-magnesium stearate mixture (red)

In the DSC thermogram for magnesium stearate (black) shown in figure 4.5, two endothermic peaks were observed. The first occurring at a temperature of 87.80º C and the second occurring at 125.08º C. The DSC thermogram of sodium valproate (red) shows a single endothermic peak of 110.02º C.

The DSC thermogram for sodium valproate and magnesium stearate were compared to the DSC thermogram of sodium valproate-magnesium stearate mixture (blue) in figure 4.5. Three peaks were observed in carbamazepine-magnesium stearate mixture. There was a shift increment between 1º C to 5º C for magnesium stearate and a shift increment of approximately 1º C for sodium valproate. The shifts are from 87.86º C to 88.94º C and 125.08º C to 129.66º C for magnesium stearate and from 110.02º C to 110.17º C for sodium valproate. Given the results, sodium valproate is compatible with magnesium stearate.

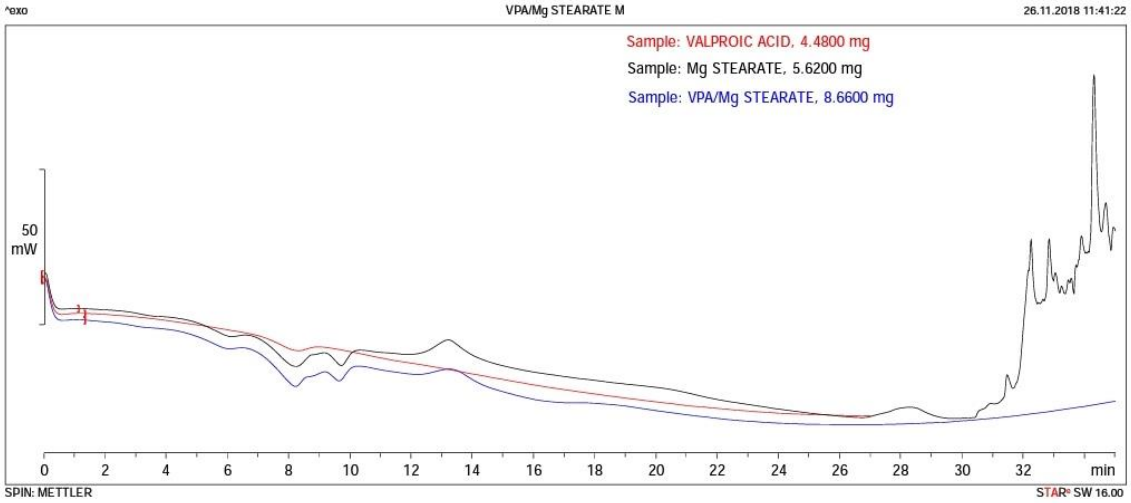

Figure 4.5: DSC thermogram of sodium valproate (red) magnesium stearate (black) and sodium valproate-magnesium stearate mixture (blue)

The DSC thermogram for pharmacel® (blue) in figure 4.6 shows a single endothermic peak of 338.44 º C, which deviated from its melting point of 260-270º C (Kharismi & Suryadi, 2018). The DSC thermogram of carbamazepine (black) shows a single endothermic peak of 190.74º C.

The DSC thermogram for carbamazepine and pharmacel® were compared to the DSC thermogram of carbamazepine-pharmacel® mixture (red) in figure 4.6. Three peaks were observed in carbamazepine-pharmacel® mixture. There was a shift increment of approximately 1º C for carbamazepine and approximately 4 º C for pharmacel®. The shifts are from 190.74º C to 191.5º C and 338.44 º C to 342.76º C for carbamazepine and pharmacel® respectively. Given the results, carbamazepine is compatible with pharmacel®.

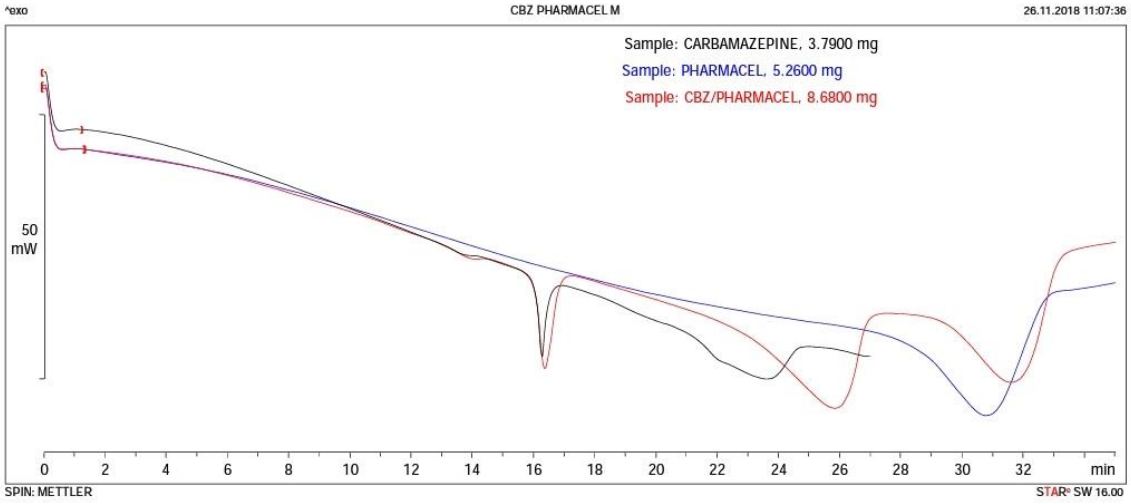

Figure 4.6: DSC thermogram of carbamazepine (black) pharmacel® (blue) and carbamazepine- pharmacel® mixture (red)

The DSC thermogram for pharmacel® (black) in figure 4.7 shows a single endothermic peak of 338.44 º C, which deviated from its melting point of 260-270º C. The DSC thermogram of sodium valproate (red) shows a single endothermic peak of 110.02º C.

The DSC thermogram for sodium valproate and pharmacel® were compared to the DSC thermogram of sodium valproate-pharmacel® mixture (blue) in figure 4.7. The sodium valproate-pharmacel® mixture showed a single endothermic peak of 105.65 º C which was characteristic to sodium valproate. There was a shift increment of approximately 4 º C for sodium valproate, from 110.02º C to 105.65º C. Although sodium valproate was not affected in the sodium valproate-pharmacel® mixture, the absence of pharmacel® peak in the mixture can be attributed to decomposition. Phaemacel® decomposes at its melting point 260-270º C (Pubchem, 2005; Kharismi & Suryadi, 2018). The results suggested that carbamazepine is compatible with pharmacel®.

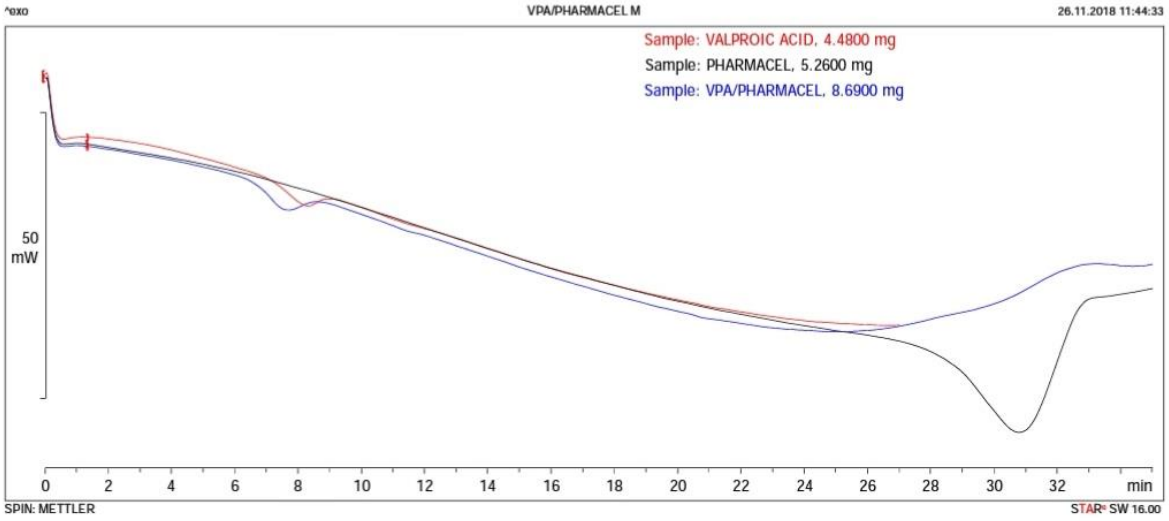

Figure 4.7: DSC thermogram of sodium valproate (red) pharmacel® (black) and sodium valproate- pharmacel® mixture (blue)

4.2.2. Infrared Spectroscopy (IR)

Figure 4.12 shows the FTIR spectrum of carbamazepine (blue), which shows absorption bands at 3463.72 cm^{-1} and 3157.25 cm^{-1} attributed to the amine group (N-H), 1676.23 cm^{-1} and 1594.25 cm^{-1} attributed to the alkene group (C=C). The carbonyl group (C=O) was represented between 1488.15 cm^{-1}, 1461.74 cm^{-1}, 1382.30 cm^{-1}, 1306.66 cm^{-1} and 1244.64 cm^{-1}. At 1115.05 cm^{-1}, the ether group (C-O) together with an alcohol group (C-OH) were observed whilst the aromatic ring was represented between 870 cm^{-1}, 800 cm^{-1}, 724 cm^{-1}, 646 cm^{-1} and 623 cm^{-1}. All these functions groups are represented in the chemical formula of carbamazepine, $C_{15}H_{12}N_2O$, and the spectrum conformed to the spectrum in literature (British Pharmacopoeia, 2014).

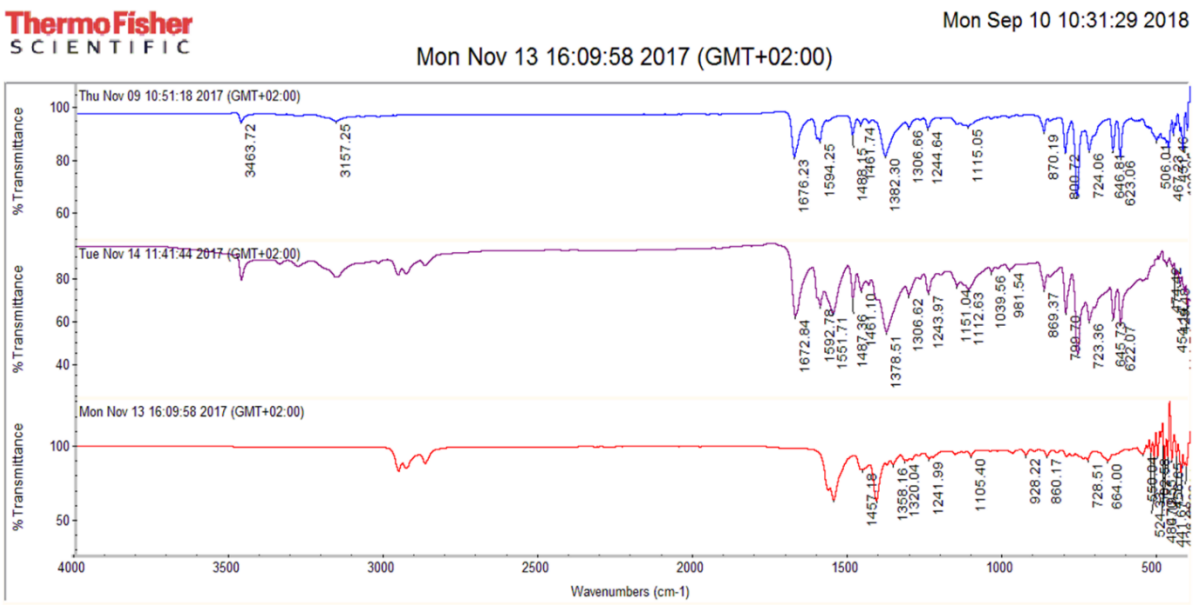

Figure 4.8: FTIR spectrum of carbamazepine (blue), sodium valproate (red) and their physical 1:1 mixture (purple)

The FTIR spectrum of sodium valproate (red) showed absorption bands at 1594.60 cm^{-1} attributed to the alkene group (C=C), 1457.18 cm^{-1}, 1411.20 cm^{-1}, 1358.16 cm^{-1}, 1320.04 cm^{-1}, 1241.99 cm^{-1}, 928.22 cm^{-1} and 860.17 cm^{-1} representing the carbonyl group (C=O). The ether group (C-O) and the alcohol group (C-OH) were observed at 1105.40 cm^{-1} whilst the aromatic ring was represented between 7258.51 cm^{-1} and 664.00 cm^{-1}. All these functions groups are represented in the chemical formula of sodium valproate $C_8H_{15}NaO_2$ and the spectrum conformed to the spectrum in literature (British Pharmacopoeia, 2014).

The FTIR spectrum of carbamazepine (blue) was compared with that of carbamazepine-sodium valproate mixture (purple). Characteristic bands of carbamazepine were observed at 1672.84 cm^{-1} and 1592.78 cm^{-1}(-C=C), 1487.36 cm^{-1}, 1461.10 cm^{-1}, 1378.51 cm^{-1}, 1306.62 cm^{-1}, 1243.97 cm^{-1} and 869.37 cm^{-1} that represented the carbonyl stretch (C=O), 1151.04 cm^{-1} attributed to the ether (C-O) and the alcohol (C-OH) groups. The aromatic ring stretch was observed at 799.70 cm^{-1}, 723.36 cm^{-1}, 645.73 cm^{-1} and 622.07 cm^{-1}.

Similarly, the FT-IR spectrum of sodium valproate alone (red) was also compared to that of carbamazepine-sodium valproate mixture (purple). Sodium valproate showed characteristic absorption bands at 1551.71 cm^{-1} (-C=C). The carbonyl group (C=O)

was represented at 112.63 cm^{-1}, 1039.56 cm^{-1}, 987.54 cm^{-1}. The aromatic ring stretch was observed between 723.36 cm^{-1} and 645.73 cm^{-1}. Since both spectra were observed in the 1:1 physical mixture, this suggests that carbamazepine and sodium valproate are compatible.

4.2.3. X-ray Powder Diffraction analysis (XRPD)

The XRPD diffractogram of carbamazepine in figure 4.18 demonstrated sharp peaks at 2θ=15.1º, 15.7º, 16.4º, 17.7º, 19.8º, 21.7º, 22.6º, 23.70º, 27.8º, 28.9º, 31.0º, 31.6º and 37.3º. These results were consistent with those described in the literature (Pinto, Ambrozini, Ferreira, & Cavalheiro, 2014)

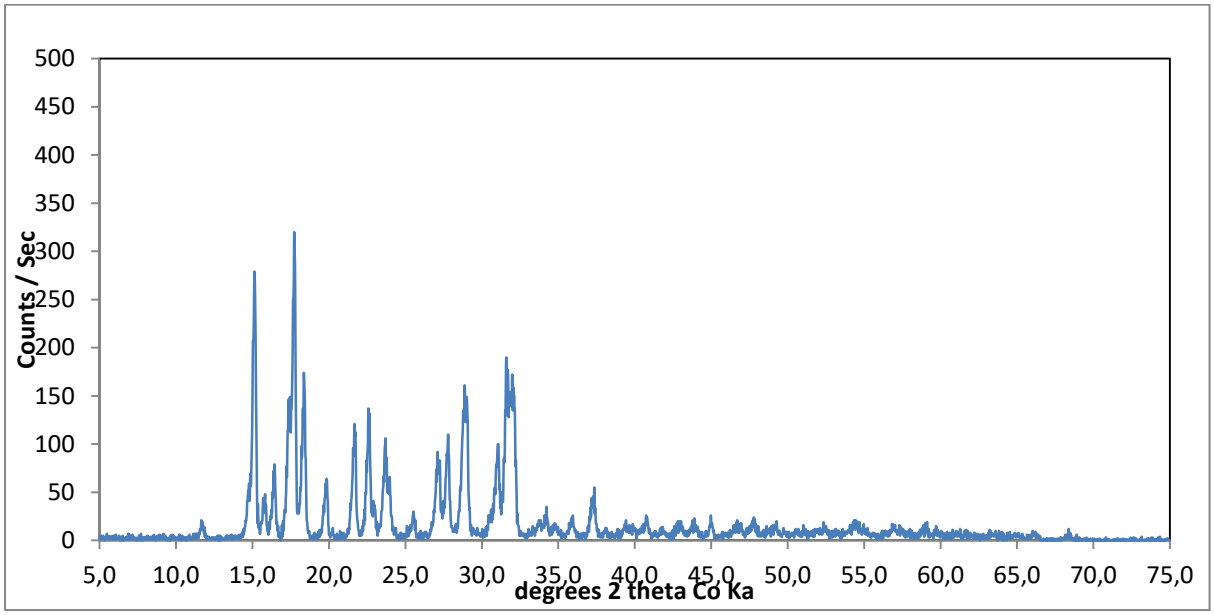

Figure 4.9: XRPD diffractogram of carbamazepine

Sodium valproate in figure 4.19 showed sharp peaks at 2θ=7.2º, 8.6º and 16.9º. Shallow peaks were observed at 2θ=23.7º, 25.7º, 26.3º and 70.0º. These results also conformed to those described in the literature (Dicaire, Perras, & Bryce, 2014)

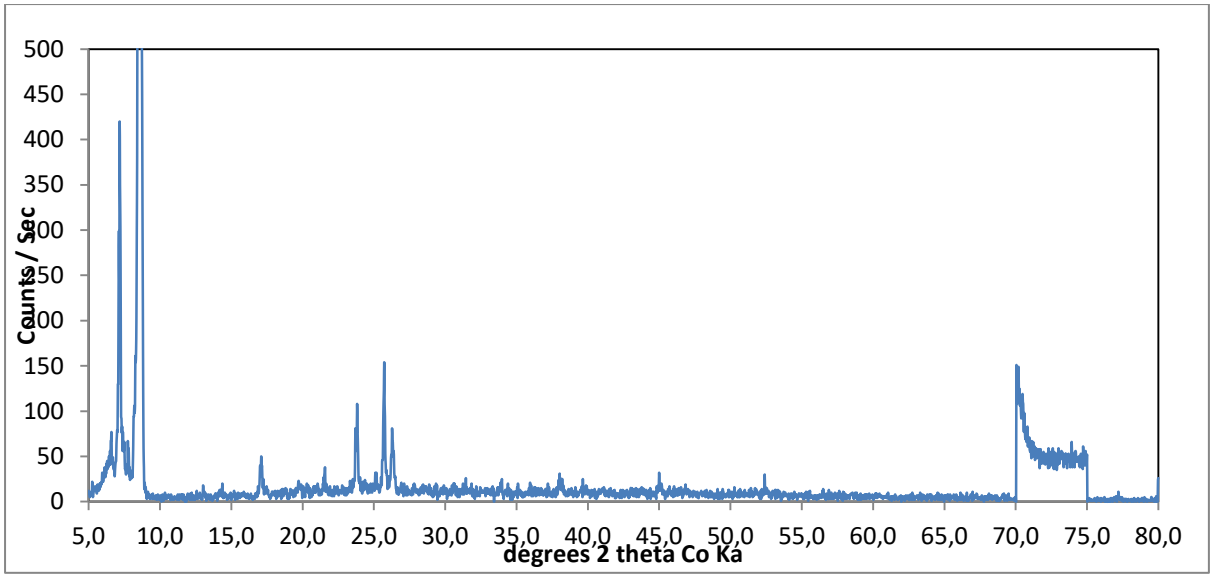

Figure 4.10: XRPD diffractogram of sodium valproate

The XRPD diffractogram of carbamazepine was compared to the 1:1 physical mixture of carbamazepine-sodium valproate in figure 4.20 and similar patterns were observed at 2θ=15.0º, 15.6º, 161º, 17.6º, 21.5º, 22.4º, 23.5º, 25.5º, 27.6º, 28.2º, 30.9º 31.4º and 36.9º. Although there were peak shifts, they were not that significant, hence rendering carbamazepine and sodium valproate compatible.

Similarly, the comparison of the XRPD diffractogram of sodium valproate to that of 1:1 physical mixture in figure 4.20 depicted similar patterns. The peaks corresponding to the ones found in figure 4.19 were observed at 2θ=7.0º, 8.4º, 17.2º, 23.5º and 26.9º. Due to the similarity in patterns between sodium valproate alone and sodium valproate in combination with carbamazepine, sodium valproate was found to be compatible with carbamazepine.

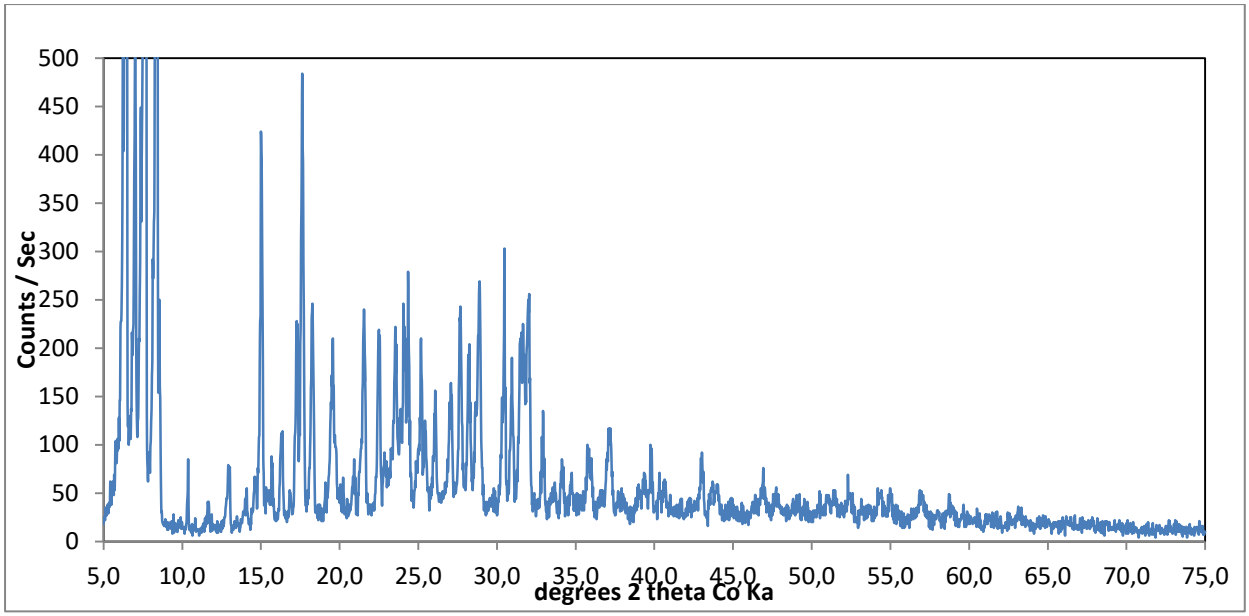

Figure 4.11: XRPD diffractogram of carbamazepine/sodium valproate

4.2.4. Thermal Activity Monitor (TAM)

Both samples of carbamazepine and sodium valproate in combination with one another showed to be compatible. Figure 4.16 depicts the heat flow curve obtained with the first combination. The fact that the measured heat flow and the theoretically calculated heat flow correlate very well allows the conclusion that the two compounds are compatible with one another. The average interaction heat flow was measured to be 430.7 nW/g.

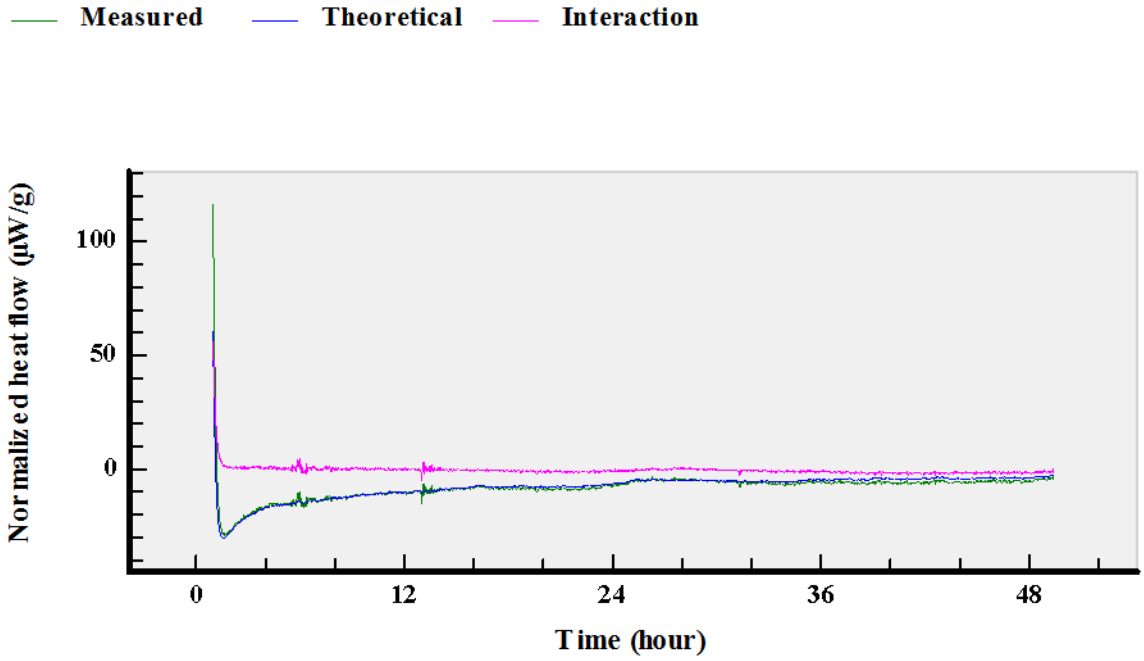

Figure 4.12: Heat flow graph of carbamazepine in combination with sodium valproate in a 50:50 weight ratio

The same applied to the second combination sample of carbamazepine and sodium valproate (50:50 weight ratio). The heat flow curves are depicted in Figure 4.13. The average interaction heat flow was measured as 386.23nW/g, thereby correlating well with that of the first sample set.

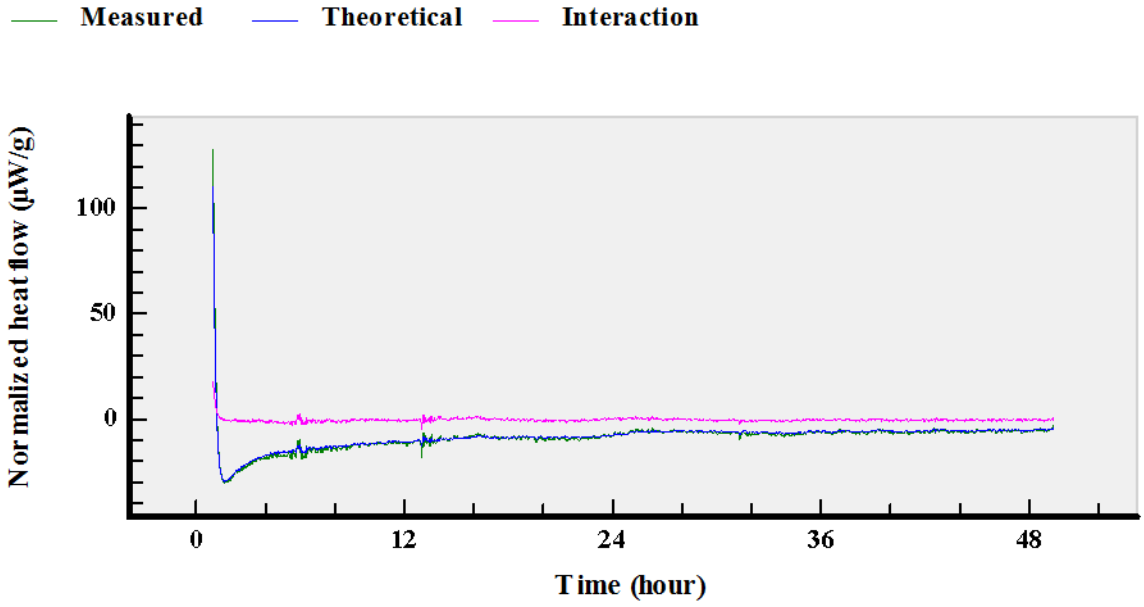

Figure 4.13: Heat flow graph of carbamazepine in combination with sodium valproate in a 50:50 weight ratio

4.3. POWDER CHARACTERIZATION

Powder was characterised in terms of particle size distribution**,** flowability and density. This information is essential in determining the need for milling or size enlargement of the starting material, appropriate excipients to be used in the formulation and appropriate method of formulation.

4.3.1. Particle size distribution

The particle size distribution was determined using the Malvern® Mastersizer 2000 and the results are summarised in table 4.2. Carbamazepine had a mean median particle size (D_{50}) of 70.840 µm approximately double of that of sodium valproate (D_{50} of 31.702). To ensure that the FDC tablet to be formulated will have uniform particle size with the difference observed between carbamazepine and sodium valproate powders, granulation would be required prior compression. Carbamazepine powder exhibited a narrow particle size distribution (figure 4.14) and sodium valproate showed a wide particle size distribution. According to the BP classification of powder by fineness (table 4.1), both carbamazepine and sodium valproate were considered very fine powders.

Table 4.1: Classification of powder according to its measure of fineness (British Pharmacopoeia, 2014)

Classification of powders by fineness	
Descriptive terms	***X_{50}*** **(µm)**
Coarse	**>** 355
Moderately fine	180 – 349
Fine	125 – 179
Very fine	< 125

Table 4.2: Particle size distribution parameters of carbamazepine

Sample	D(0.1) (µm)	D(0.5) (µm)	D(0.9) (µm)
Carbamazepine	16.929±2.155	70.840±1.550	199.320±2.362
Sodium valproate	7.286±2.252	31.702±4.800	350.384±23.334

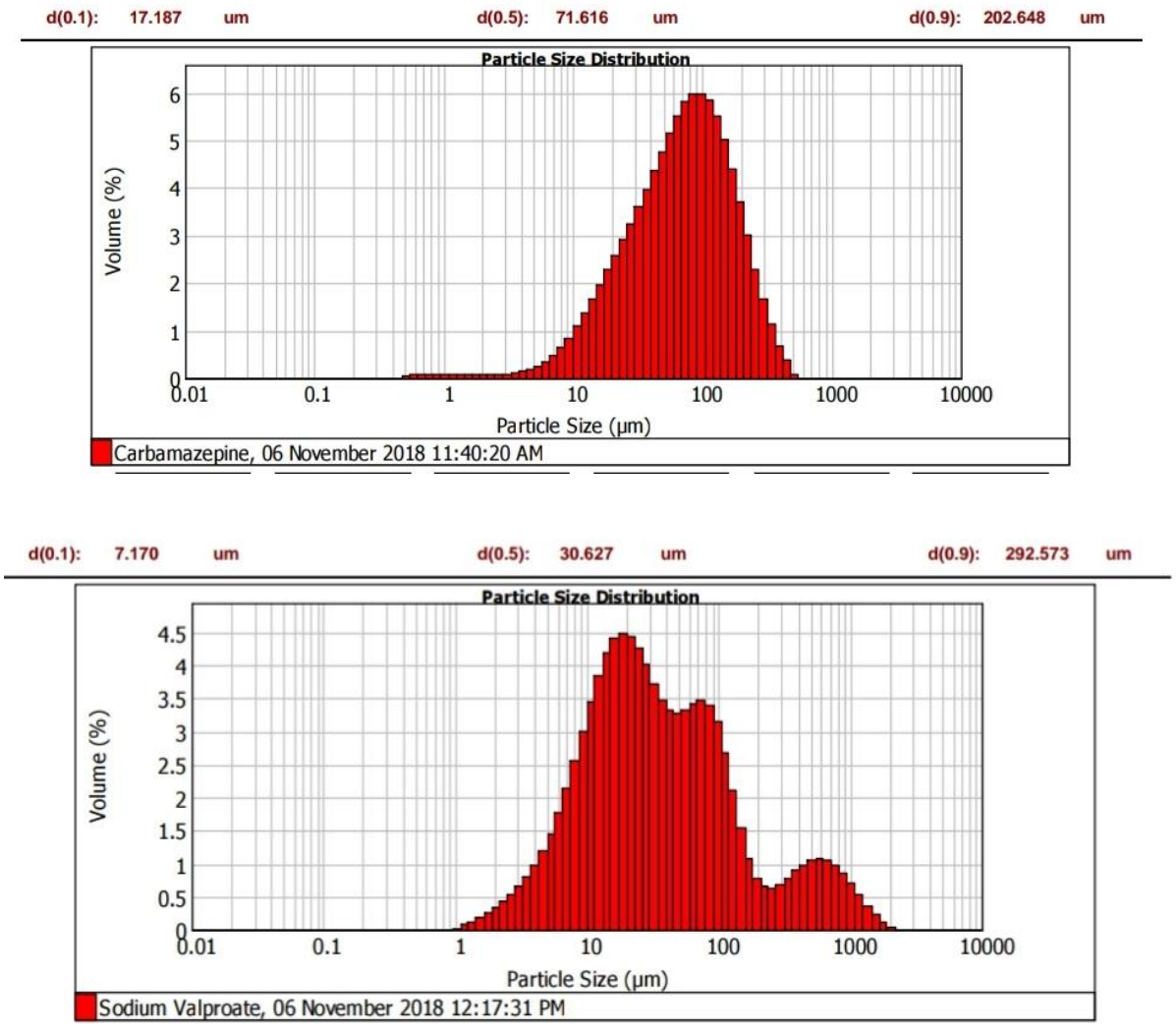

Figure 4.14: Histograms generated from the particle size analysis of carbamazepine sample (top) and sodium valproate powder (bottom)

Carbamazepine powder revealed that it had small particles that promotes powder homogeneity and dissolution rate. However, small particles may have a negative

impact on flowability of the material (Etzler & Uddin, 2013). For powders with small particles, size enlargement techniques have to be applied to improve the flow properties of the material. This information was also helpful in the selection of appropriate excipients, which were used to aid flowability of the material. Sodium valproate exhibited a wide particle size distribution, which may be subjected to segregation during handling due to different particle size thus hindering homogeneity of the product. The information was essential in the selection of the appropriate method that will ensure that particle size is uniform and enlarged, and the required method would be granulation prior compression.

4.3.2. Angle of repose

The angle of repose was determined according to the fixed funnel and freestanding cone method. Angle of repose was calculated to be 38.55° for carbamazepine. According to BP, carbamazepine denoted fair flowability. Flowability of the powder ensure that the tablet weight remains uniform across. The angle of repose for sodium valproate could not be determined due to its poor flow properties. Sodium valproate is cohesive and hygroscopic and the angle of repose test is suitable for 'free flowing' powders (Manyama, 2011). The appropriate formulation method for powders with poor flow properties is granulation. This study employed wet granulation method to increase particle size and improve flowabilty.

4.3.3. Bulk and tapped density

The bulk density was calculated to be 0.48 g/ml for carbamazepine and 0.36 g/ml for sodium valproate. Tapped density was calculated to be 0.62 g/ml carbamazepine and 3 and 0.47 g/ml for sodium valproate. These values were used to calculate hausner ratio and carr's index.

4.3.4. Hausner ratio and Carr's index

The hausner ratio for carbamazepine was calculated to be 1.31 and for sodium valproate it was calculated to be 1.29. Both hausner ratio showed that both drugs flow properties fall within the criteria of passable. Compressibility index was found at 23.40% and 22.58% for carbamazepine and sodium valproate respectively. As the

hausner ratio, the carr's index also showed that the flow for both drugs are passable. Given the results, there was surely a need for a flow aid to the FDC formulation of carbamazepine and sodium valproate. This also showed that direct compression alone won't be adequate for the formulation but granulation will be a required method for the formulation.

Table 4.4: Flow parameters of carbamazepine and sodium valproate powder

Material	Densities (g/ml)		Compressibility index
	Bulk	Tapped	
Carbamazepine powder	0.48 ± 0.06	0.62 ± 0.09	23.40 ± 1.42
Sodium valproate powder	0.36 ± 0.03	0.47 ± 0.05	22.58 ± 1.38

Table 4.5: Summary of powder evaluation parameters

Property	Carbamazepine	Sodium valproate
Mean particle size (µm)	94.692 ± 5.070	114.675 ± 5.374
Tapped density (g/ml)	0.62 ± 0.09	0.47 ± 0.05
Bulk density (g/ml)	0.48 ± 0.06	0.36 ± 0.03
Hausner ratio	1.31 ± 0.05	1.29 ± 0.04
Carr index	23.40 ± 1.42	22.58 ± 1.38
Angle of repose	38.55	

4.4. SUMMARY

To formulate a fixed dose combination, APIs and excipients need to be tested for compatibility in order to determine a suitable formulation. The four methods corroborated the results and revealed that there was no drug-drug nor drug-excipient incompatibility. Many pharmaceutical processes depend on the flowability of the

powder formulation and as such, powders with better flow are always preferred. Both carbamazepine and sodium valproate exhibited poor flow properties. To improve the flow properties, a flow aid would be required when manufacturing the tablet and the suitable method to manufacture the tablet would be granulation methods.

CHAPTER 5: FORMULATION STUDIES RESULTS AND DISCUSSION

5.1. INTRODUCTION

In this chapter the results of the evaluation of the physical properties of the tablets of the oral fixed-dose combination of carbamazepine and sodium valproate will be presented. The tablets were evaluated in terms of physical dimensions, weight variation, friability, and disintegration to establish the applicability of the manufacturing process. The quality of the compressed tablets was evaluated according to standards set by the British Pharmacopoeia.

5.2. EVALUATION OF THE TABLETS

Tablets were evaluated in terms of tablet weight variation, friability and crushing strength as described in Chapter 3. Three batches were successfully formulated and their composition are presented from table 5.1 to table 5.3. The diameter of the tablets was approximately 14 mm and the thickness was approximately 4 mm.

Table 5.1: Tablet composition of formulation 1

Ingredient	Purpose	Amount per tablet (%w/w)	Quantity per tablet (mg)
Carbamazepine	Active ingredient	25	200
Sodium valproate	Active ingredient	25	200
Ac-Di-Sol®	Disintegrant	3	24
Magnesium stearate	Lubricant	0.5	4
Kollidon® VA 64	Binder	3	24
Emcompress®	Filler	43.5	348
Total		100	800

Table 5.2: Tablet composition of formula 2

Ingredient	Purpose	Amount per tablet (%w/w)	Quantity per tablet (mg)
Carbamazepine	Active ingredient	25	200
Sodium valproate	Active ingredient	25	200
Ac-Di-Sol®	Disintegrant	2	16
Magnesium stearate	Lubricant	0.5	4
Kollidon® VA 64	Binder	3	24
Pharmacel®	Filler	44.5	356
Total		100	800

Table 5.3: Tablet composition of formula 3

Ingredient	Purpose	Amount per tablet (%w/w)	Quantity per tablet (mg)
Carbamazepine	Active ingredient	25	200
Sodium valproate	Active ingredient	25	200
Ac-Di-Sol®	Disintegrant	2	16
Magnesium stearate	Lubricant	2	4
Kollidon® VA 64	Binder	5	40
Emcompress®	Filler	41	328
Total		100	800

5.2.1. Physical strength: Friability and Crushing strength

Friability and crushing strength are both attributed to the physical strength of the tablet, hence their results are discussed together. Friability was determined using the Roche friabilator and crushing strength using the Pharma Test® and the results are summarised in table 5.5 and table 5.6. The total weight of the tablets before rotation

was 154.5 g, 153.9 g and 151.1 g for formulation 1, 2 and 3 respectively. After rotation the average mass of the tablets was 154.3 g, 153.6 g and 151.0 g and the percentage friability was 0.129% for formulation 1, 0.195% for formulation 2 and 0.066% for formulation 3. The results showed that all the formulations were in accordance to BP specifications. The BP specifications states that the tablets should not lose more than 1% of their total weight (British Pharmacopoeia, 2014).

The results for crushing strength complimented the friability results. The tablets showed a crushing strength of 121.4 N, 113.6 N and 118.6 N for formulation 1, 2 and 3 respectively. According to Ayorinde & Itiola (2012), the recommended crushing strength for tablets is 40 N to 150 N, which deem the results for all the formulations successful. Accordingly, it can be concluded that the tablets were strong enough to withstand abrasion.

Table 5.5: Crushing strength of the tablets

Formulation	Crushing force (N)
Formula 1	121.4 ± 11.34
Formula 2	113.6 ± 15.84
Formula 3	118.6 ± 10.74

Table 5.6: Percentage friability of the three formulations

Formulation	Total weight before rotation (g)	Total weight after rotation (g)	% Friability
Formulation1	154.5	154.3	0.129
Formulation 2	153.9	153.6	0.195
Formulation 3	151.1	151.0	0.066

5.2.2. Weight variation of the tablets

Weight variation helps to establish if the final tablets contains relatively correct amount of the API. Weight variation results are summarised in table 5.7. The average tablet weight were 782.5 mg, 756.2 mg and 758 mg for formulation 1, 2 and 3 respectively. The tablets mass ranged from 740 mg to 810 mg, 730mg to 780 mg and 730 mg to 800 mg for formulation 1, 2 and 3 respectively. The acceptable weight range was 743.375 mg - 821.625 mg for formulation 1, 718.675 mg – 803.325 mg for formulation 2 and 720.175 mg – 795.90 mg for formulation 3, calculated according to the BP acceptable weight variation guideline in table 5.8. Only one tablet deviated from the average weight by more than 5% and for formulation 1 (5.431%) and 3 (5.541%), and none deviated from the acceptable range in formulation 2. According to BP specification not more than two of the individual weights are allowed to deviate from the average weight. From the results, the tablets from all the formulations passed the weight variation test.

Table 5.7: Mass variation results of the tablets

Formulation	**Weight Variation (mg)**	**BP acceptable range**
Formulation 1	782.5 ± 2.635	743.375 – 821.625
Formulation 2	756.5 ± 1.652	718.675 – 803.325
Formulation 3	758 ± 2.640	720.175 – 795.9

Table 5.8: British Pharmacopoeia (2014) limits for weight variation

Average weight of tablet	**Percentage deviation**
80 mg or less	10
More than 8 0mg and less than 250 mg	7.5
250 or more	5

5.2.3. Disintegration

To establish whether the tablets, made from the three batches of carbamazepine and sodium valproate powders, would undergo disintegration after administration, disintegration tests were performed, as described in Chapter 3. The results are summarised in Table 5.9 and graphically presented in Figure 5.1. Formulation 1 contained 3.00% Ac-Di-Sol® (50:50 intragranula:extragranula ratio), formulation 2 (70:30) and formulation 3(100:0) contained 2.00% Ac-Di-Sol® concentration. For formulation 1, the tablets disintegrated at approximately 10 minutes, which was approximately double the time it took for the 100:0 ratio (6.17 minutes) to disintegrate. This was expected as the ratio difference between the two formulations was double. For formulation 2, the tablets disintegrated at approximately 3 minutes, approximately one third of formulation 1 and half of formulation 3. All of the tablets from the three batches disintegrated within the prescribed 15 minutes, as per the BP specification. However, the tablets needed to contain at least a 70:30 weight distribution for a total of Ac-Di-Sol® concentration of 2.00% w/w to render rapid tablet disintegration. Based on the results from the crushing strength, it was expected that tablets of formulation 2 would render a rapid disintegration times compared to formulations 1 and 3. The disintegration time of tablets increases with an increase in physical strength (Juban, Briancon, Puel, Hoc & Lehon, 2017).

Table 5.9: The ratio of the internal granular disintegrant percentage against the external percentage Ac-Di-Sol® per tablet mixture against disintegration time. (Ac-Di-Sol® at 2.00% and 3.00% w/w of total powder mixture).

Intragranular disintegrant percentage: Extragranular disintegrant (%)	Disintegration time (minutes)
Formulation 1 50:50	10.22 ± 3.33
Formulation 2 70:30	3.71 ± 1.38
Formulation 3 100:0	6.17 ± 1.36

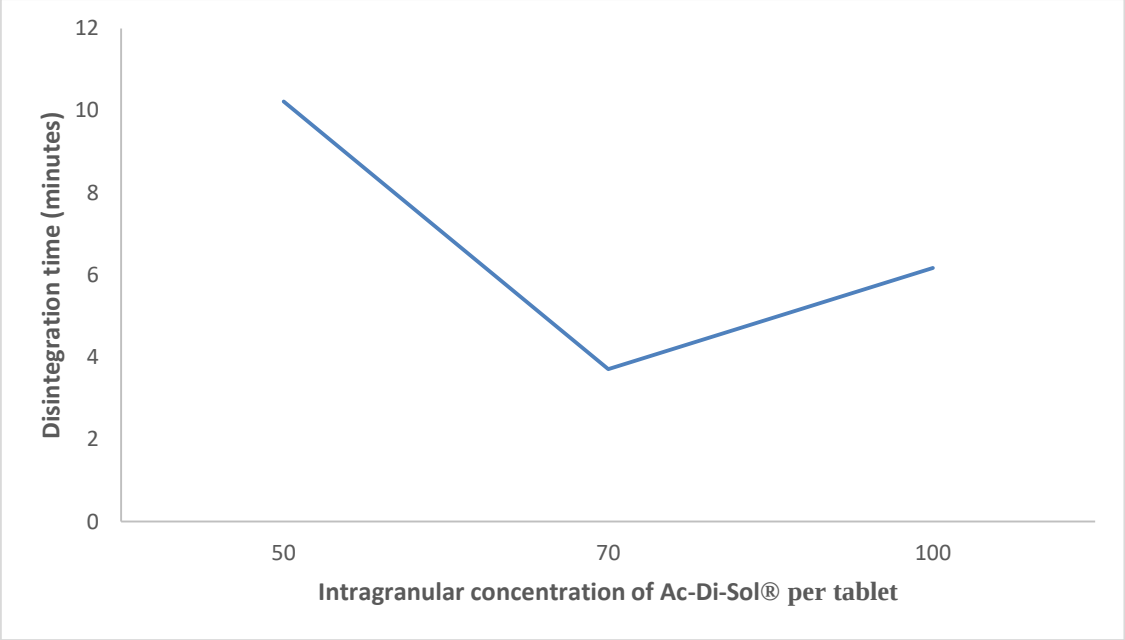

Figure 5.1: The intragranular concentration percentage of Ac-Di-Sol® per tablet against disintegration time

Table 5.10: Summary of the physical properties of the fixed dose combination tablets

	Property	**Value**
Formula 1	Thickness (mm)	3.63 ± 5.93
	Crushing strength (N)	121.4 ± 11.34
	Friability %	0.064%, (complied with BP standards)
	Weight variation	Complied with BP standards
	Disintegration time (s)	613.2 (complied with BP standards)
Formula 2	Thickness (mm)	3.71 ± 0.69
	Crushing strength (N)	113.6 ± 15.84
	Friability %	0.132% (complied with BP standards)
	Weight variation	Complied with BP standards
	Disintegration time (s)	222.6 (complied with BP standards)
Formula 3	Thickness (mm)	3.71 ± 1.34
	Crushing strength (N)	118.6 ± 10.74
	Friability %	0.066% (complied with BP standards)
	Weight variation	Complied with BP standards
	Disintegration time (s)	370.2 (complied with BP standards)

5.3. SUMMARY

The physical tablet properties of the fixed-dose combination of carbamazepine and sodium valproate have shown to be in accordance with the pharmacopoeial standards. The %RSD for the individual mass of twenty tablets for the test for tablet weight variation of the formula was 0.803%, which confirmed excellent powder flow into the tablet die. In addition, the tablets resisted abrasion with a loss of mass of less the 1% during friability testing.

The physical properties of the fixed-dose combination of carbamazepine and sodium valproate tablets showed to be viable for manufacturing purposes. All the formulations passed the disintegration test. However, the establishment of feasible physical properties have no significance unless the integrated drug(s) can carry out is therapeutic function. In the majority of cases, this can only occur when the drug substance has dissolved in the fluids of the gastrointestinal tract. Following in Chapter 6 is the evaluation of the dissolution profiles of the fixed-dose combination.

CHAPTER 6:
HPLC METHOD DEVELOPMENT, VALIDATION AND DISSOLUTION

6.1. INTRODUCTION

To determine if the fixed dose combination possess the antiepileptic function, the drug substance has to dissolve in the gastrointestinal tract fluids and achieve systemic absorption. It is imperative to determine the pharmaceutical availability of the two APIs in the fixed-dose combination. Assay procedures are intended to measure the analyte present in a given sample. In this chapter, the dissolution profiles of carbamazepine and sodium valproate will be presented and discussed as well as the HPLC method that was employed to quantify the concentrations of the two APIs in the dissolution samples.

6.2. HIGH PERFOMANCE LIQUID CHROMATOGRAPHY

A validated HPLC method for the analysis of carbamazepine and sodium valproate was used to quantify the concentration of carbamazepine and sodium valproate in the dissolution samples.

6.2.1. Validation of the HPLC method for carbamazepine and sodium valproate

This method was developed and validated at the Analytical Technology Laboratory, North-West University, Potchefstroom, South Africa.

6.2.1.1. Linearity

The linearity for carbamazepine was determined by performing linear regression analysis on the plot. Standard solutions were prepared in methanol to obtain concentrations ranging from 0.01 µg/ml to 253.00 µg/ml for carbamazepine. The regression value was at 0.9977 and the Y-intercept was 5.6871 for carbamazepine as displayed in Figure 6.1.

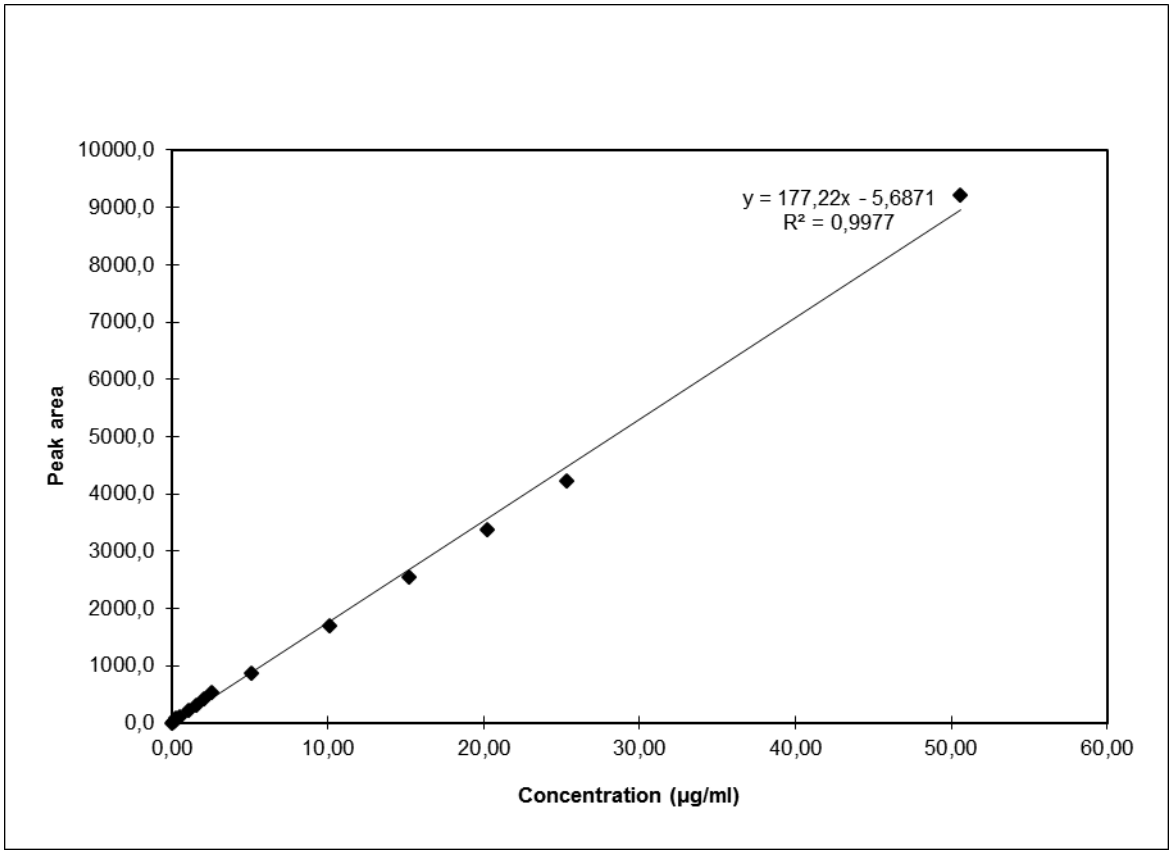

Figure 6.1: The linear regression graph of carbamazepine to determine linearity and range

The linearity for sodium valproate was determined by performing linear regression analysis on the plot. Standard solutions were prepared in methanol to obtain concentrations ranging from 0.01 µg/ml to 256.00 µg/ml for sodium valproate. The regression value (R^2) was greater than 0.9946 and the Y-intercept was 1.7053 for sodium valproate as displayed in Figure 6.2.

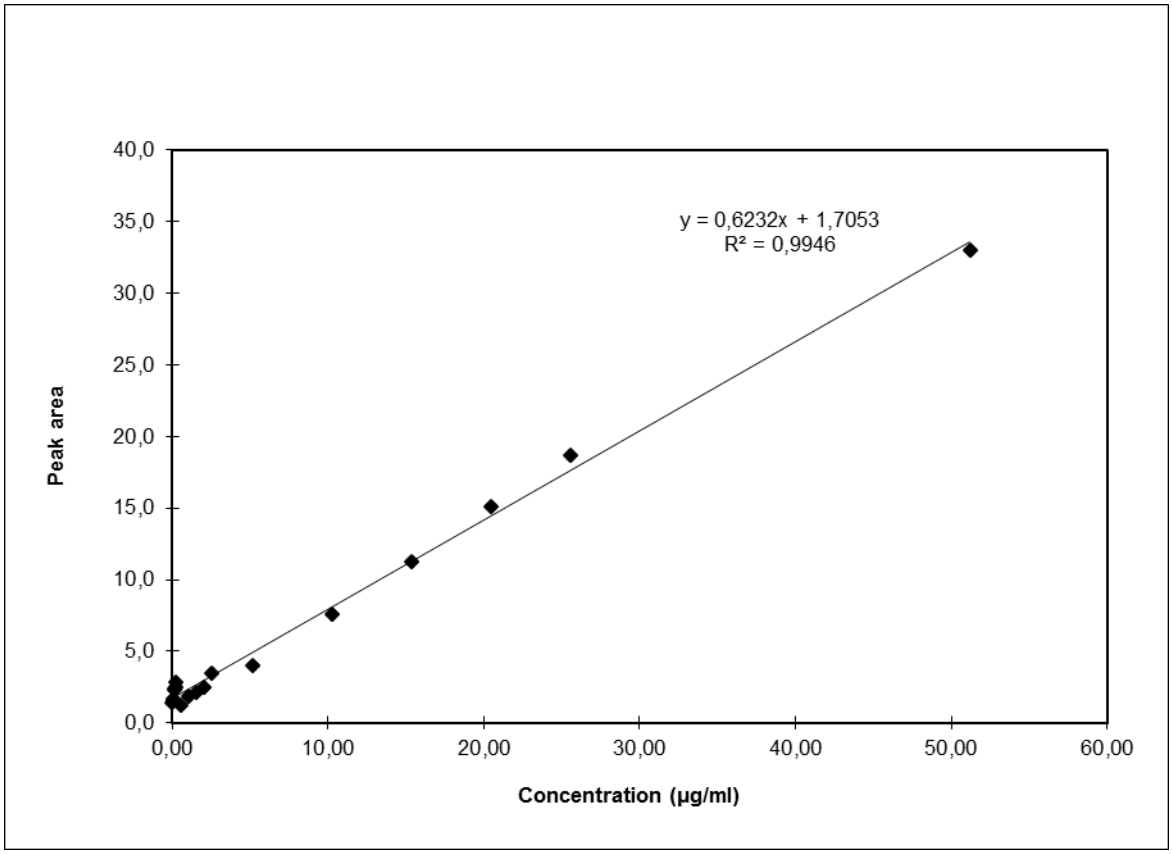

Figure 6.2: The linear regression graph of sodium valproate to determine linearity and range

The regression coefficient of >0.998 is generally considered as evidence of acceptable fit of the data to the regression line (Shabir, 2004). The demonstration coefficient (r^2) obtained for the regression line demonstrates an excellent relationship between peak area and concentration of carbamazepine (figure 6.1). The r2 obtained for the regression line in figure 6.2 demonstrates a good relationship between peak area and concentration of sodium valproate in the FDC formulation. The linearity of the method demonstrated a proportional relationship of response versus analyte concentration over the working range of the FDC formulation.

6.2.1.2. Selectivity

For specificity a solution from the placebo powder similar to the sample solution was prepared in methanol. The placebo did not generate any peaks that interfered with the determination of the active ingredients.

6.2.1.3. Accuracy

Accuracy was determined by, amounts of the placebo equivalent to the amount of sample powder that would contain 80%, 100% and 120% of 100 mg of carbamazepine and sodium valproate were weighed. Quantities of the active ingredients at concentrations of approximately 80%, 100% and 120% respectively of the expected sample concentration, known as spiking, were made up to volume and filtered. The samples were analysed in triplicate by means of HPLC. The results for accuracy are displayed in Table 6.1 and Table 6.2 for carbamazepine and sodium valproate respectively.

Table 6.1: Results for carbamazepine to determine accuracy

Conc. spiked	Area			Mean	Recovery	
µg/ml					**µg/ml**	%
150.60	4751.5	4774.6	4762.9	4763	**166.2**	110.3
148.80	4414.2	4406.7	4409.8	4410	**153.7**	103.3
149.40	4630.6	4631.6	4630.8	4631	**152.3**	101.9
200.8	6189.3	6195.6	6191.1	6192	**203.9**	101.6
198.4	5994.8	5977.3	5715.9	5986	**197.1**	99.3
199.2	6050.3	6061.5	6056.2	6056	**199.4**	100.1
251	7288.8	7295.6	7291.6	7292	**240.3**	95.7
248	7150.4	7098.5	7123.1	7124	**234.8**	94.7
249	7263.5	7279.9	7272.6	7272	**239.6**	96.2

Statistical analysis	
Mean	97.9
SD	2.5
%RSD	2.6

Table 6.2: Results for sodium valproate to determine accuracy

Conc. spiked	Area			Mean	Recovery	
µg/ml					**µg/ml**	%
153.00	104.3	104.7	106	105	**180.5**	118.0
151.20	101.6	101.7	102.7	102	**175.6**	116.1
150.00	104.2	104.2	103.3	104	**180.3**	120.0
204.0	141.4	141.8	142.8	142	**244.9**	120.0
201.6	130.4	128.3	128.3	129	**223.6**	110.9
200.0	133.6	134	134.4	134	**231.3**	115.7
255	156.6	157.1	157.3	157	**271.3**	106.4
252	149.6	149.8	150.6	150	**258.9**	102.7
250	152.8	150.4	152.8	152	**262.2**	104.9

Statistical analysis	
Mean	110.1
SD	6.1
%RSD	5.6

The ICH recommends collecting data from a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g., three concentrations, three replicates each). In the present study, a number of different solutions were prepared with known added amounts of carbamazepine and sodium valproate and injected in triplicate. Percent recoveries of response factor (area/concentration) were calculated. The results of accuracy studies are shown in table 6.1 and table 6.2, and it is evident that the method is accurate within the desired recovery range.

6.2.1.4. Precision

Precision was determined by performing HPLC analysis of a low, medium and high concentration sample for both carbamazepine and sodium valproate. To determine precision, amounts of the placebo equivalent to the amount of sample powder that would contain 80%, 100% and 120% of 100 mg of carbamazepine and sodium valproate were weighed. Quantities of the active ingredients at concentrations of

approximately 80%, 100% and 120% respectively of the expected sample concentration, known as spiking, were made up to volume and filtered. The samples were analysed in triplicate by means of HPLC. The results for precision are displayed in Table 6.3 and Table 6.4 for carbamazepine and sodium valproate respectively. further subdivided into within-run, intra-batch precision or repeatability, which assesses precision during a single analytical run, and between-run, interbatch precision or repeatability, which measures precision with time, and may involve different analysts, equipment, reagents, and laboratories.

Table 6.3: Results for carbamazepine to determine precision

Standard						
µg/ml	Area	Area	Area	Mean		
200.9	21074.8	20918.7	20996.8	20996.750		
Spiked conc						
µg/ml	Area	Area	Area	Mean	Conc. µg/ml	%
605.30	13849.5	14260.8	14055.3	14055.2	168.3	84.1
602.60	13845.4	13676.9	13761.3	13761.2	165.5	82.8
602.90	14545.2	14441.2	14493.2	14493.2	174.2	87.1
754.60	17404.5	17473.9	17439.2	17439.2	167.8	83.9
756.20	17153.9	17421.3	17287.6	17287.6	165.7	82.8
756.70	17471.0	17666.9	17570.2	17569.0	168.3	84.1
908.20	20793.6	20976.6	20885.1	20885.1	166.7	83.3
908.40	21728.0	22085.5	21906.9	21906.8	174.8	87.4
908.00	21805.6	22174.1	21990.0	21989.9	175.5	87.8

Statistical analysis	
Mean	84.82
SD	1.90
RSD%	2.25

Table 6.4: Results for sodium valproate to determine precision

Standard						
µg/ml	Area	Area	Area	Mean		
200	385.35	381.96	383.66	383.655		
Spiked conc						
µg/ml	Area	Area	Area	Mean	Conc. µg/ml	%
605.30	271.0	289.3	280.3	280.2	182.8	91.4
602.60	283.2	288.1	285.5	285.6	187.2	93.6
602.90	282.9	285.6	284.1	284.2	186.2	93.1
754.60	337.5	343.2	340.5	340.4	178.1	89.1
756.20	340.2	350.3	335.1	345.2	180.3	90.1
756.70	347.3	354.7	351.0	351.0	183.2	91.6
908.20	415.7	423.0	419.2	419.3	182.3	91.2
908.40	433.5	437.3	435.7	435.4	189.3	94.6
908.00	429.5	469.6	449.1	449.6	195.5	97.8

Statistical analysis	
Mean	92.48
SD	2.47
RSD%	2.67

Table 6.5: Results for carbamazepine to determine intra-day precision

Carbamazepine concentrations (µg/ml)	Mean % recovered	Standard deviation	%RSD
155.1	98.00	0.69	0.71
157.9			
156.0			
221.8	103.27	0.46	0.44
218.7			
218.3			
272.4	104.06	1.17	1.12
278.6			
278.7			

The acceptance criterion for %RSD was set at 2.0% or less. The intra-day precision for carbamazepine was acceptable with an RSD of 1.12% or less.

Table 6.6: Results for sodium valproate to determine intraday precision

Sodium valproate concentrations (µg/ml)	Mean % recovered	Standard deviation	%RSD
136.8	86.4	0.69	0.80
137.0			
136.0			
110.3	87.34	0.81	0.93
107.8			
107.3			
131.6	86.06	1.05	1.22
135..1			
134.8			

The acceptance criteria for %RSD was set at 2.0% or less. The intra-day precision for sodium valproate was acceptable with an RSD of 1.22% or less.

6.2.1.5. Ruggedness (sample stability)

Three aliquots of each of the low and high concentrations were kept at room temperature for 24 hours to determine stability of the tablet. The mean percentage recovery of carbamazepine as illustrated in Table 6.7 below was 99.2% ± 0.51. The percentage recovered showed that the formulation is stable in room temperature, and carbamazepine was also stable in the formulation. The summarized parameters of the sample stability for carbamazepine are illustrated in Table 6.7 below.

Table 6.7: 24-hour sample stability results for carbamazepine

Mean area	Mean % recovery	Standard deviation	%RSD
7697.5	99.2	0.51	0.51

Three aliquots of each of the low and high concentrations were kept at room temperature for 24 hours to determine stability of the tablet. The mean percentage recovery of sodium valproate as illustrated in Table 6.8 below was 102.7% ± 2.19. The percentage recovered showed that the formulation is stable in room temperature, and sodium valproate was also stable in the formulation. The summarized parameters of the sample stability for sodium valproate are illustrated in Table 6.7 below.

Table 6.8: 24-hour sample stability results for sodium valproate

Mean area	Mean % recovery	Standard deviation	%RSD
161.6	102.7	2.25	2.19

6.2.1.6. Repeatability

The method was performed to substantiate precision. The results obtained for carbamazepine maintained the retention time at 3.506 ± 0.229 minutes.

Table 6.9: Repeatability results for carbamazepine

Mean area	Mean retention times(minutes)	Standard deviation	%RSD
7223.0	3.506	0.008	0.229

The results obtained for sodium valproate maintained the retention time at 7.222 ± 0.231 minutes.

Table 6.10: Repeatability results for sodium valproate

Mean area	Mean retention times(minutes)	Standard deviation	%RSD
149	7.222	0.017	0.231

The HPLC method was validated and was therefore suitable to analyse carbamazepine and sodium valproate in tablets for stability testing, quality control and batch release purposes. No interference was encountered from samples; thus the method can be regarded as being stability indicating.

6.3. THE DISSOLUTION PROFILES OF CARBAMAZEPINE AND SODIUM VALPROATE

A dissolution study of six of the fixed-dose combination tablets was performed for the establishment of in vitro dissolution behaviour. The dissolution conditions are described in Chapter 3.

6.3.1. Results and discussion

6.3.1.1. Carbamazepine

The first range in the dissolution process of tablets in distilled water containing 1.0% SLS is the critical stage as it is necessary for carbamazepine to dissolve from 45 to

75% in the first 15 minutes (USP, 2012). In this fixed dose combination, 20.02% was released within the first 10 minutes. The number gradually increased to 41.28% at 20 minutes and by 30 minutes about 59.98% was already released. This was not entirely undesirable, as an immediate release of the carbamazepine dose was desirable.

According to the USP (2012), the second stage in the dissolution process is that the total amount of carbamazepine dissolved is not less than 75% after 60 minutes. It was found that the dissolution values of the prepared fixed dose combination tablets were more than 95% after 60 minutes. The remainder of carbamazepine dissolved rapidly as expected from 45 minutes onwards as shown in Figure 6.4. This showed that the formulation was conforming to the set standards of the second stage of dissolution process listed in the United States Pharmacopoeia.

Between 60 and 75 minutes, the rotations per minute (rpm) was changed from 75 rpm to 250 rpm to ensure complete dissolution. After 75 minutes, about 100.0% was dissolved, which meant that carbamazepine reached complete dissolution from the prepared tablets.

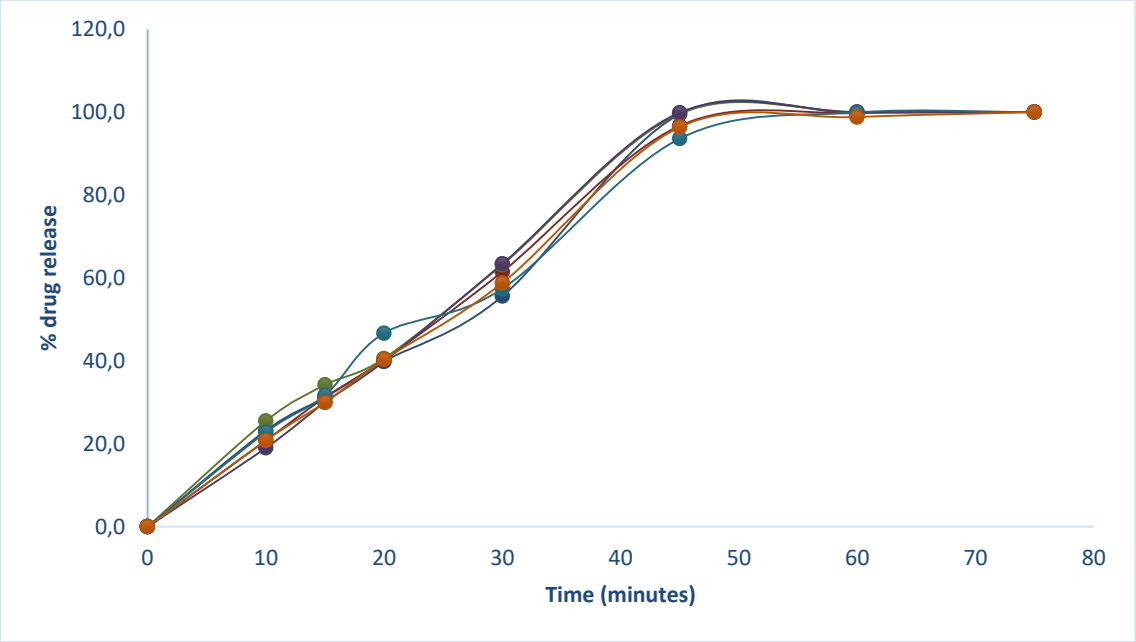

Figure 6.3: The dissolution profile of carbamazepine from six FDC tablets

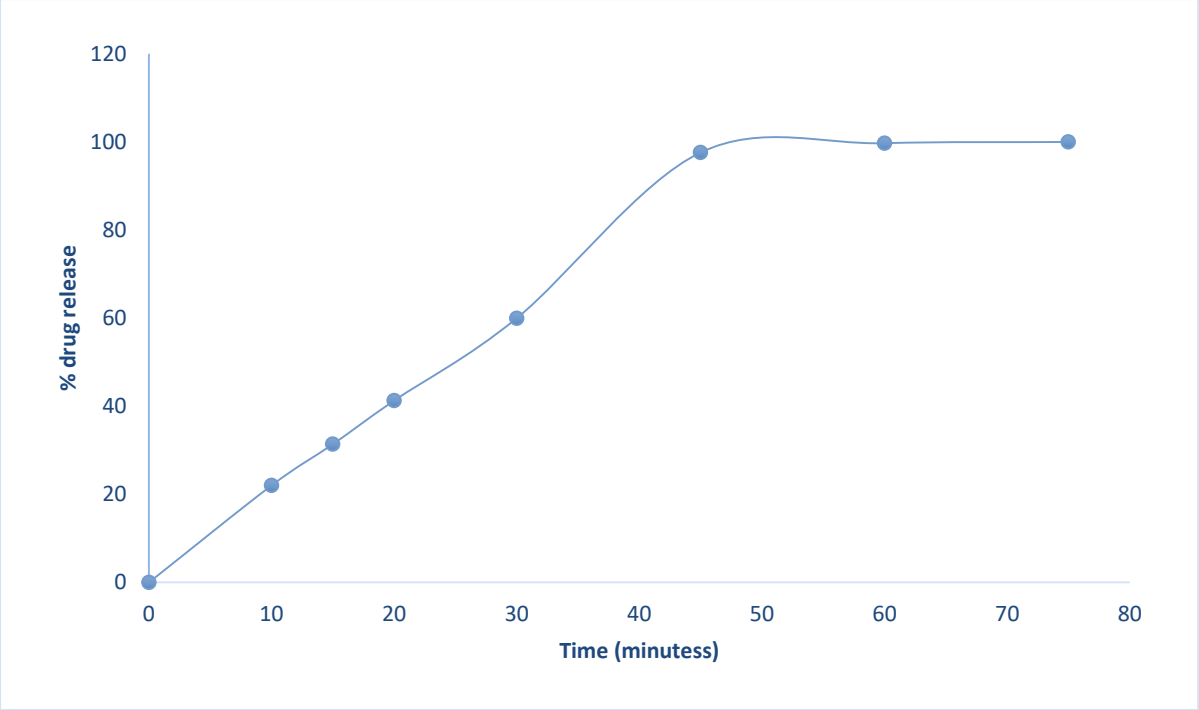

Figure 6.4: The mean percentage of amount of carbamazepine dissolved against time

6.3.1.2. Sodium valproate

In this fixed dose combination, 29.33% was released within the first 10 minutes. The number gradually increased to 39.75% at 20 minutes and by 30 minutes about 47.42% was already released. This was not entirely undesirable, as an immediate release of the sodium valproate dose was desirable.

According to the USP (2012), the second stage in the dissolution process is that the total amount of sodium valproate dissolved is not less than 75% after 60 minutes. It was found that the dissolution values of the prepared fixed dose combination tablets were less than 75% after 60 minutes, with sodium valproate having released only 59.25% at 60 minutes. The rate at which sodium valproate was released from the fixed dose combination was not entirely satisfactory. The desired complete percentage release was expected within 60 minutes at the set dissolution parameters.

Between 60 and 75 minutes, the rotations per minute (rpm) was changed from 75 rpm to 250 rpm to ensure complete dissolution. After 75 minutes, about 100.0% was

dissolved, which meant that sodium valproate reached complete dissolution from the prepared tablets.

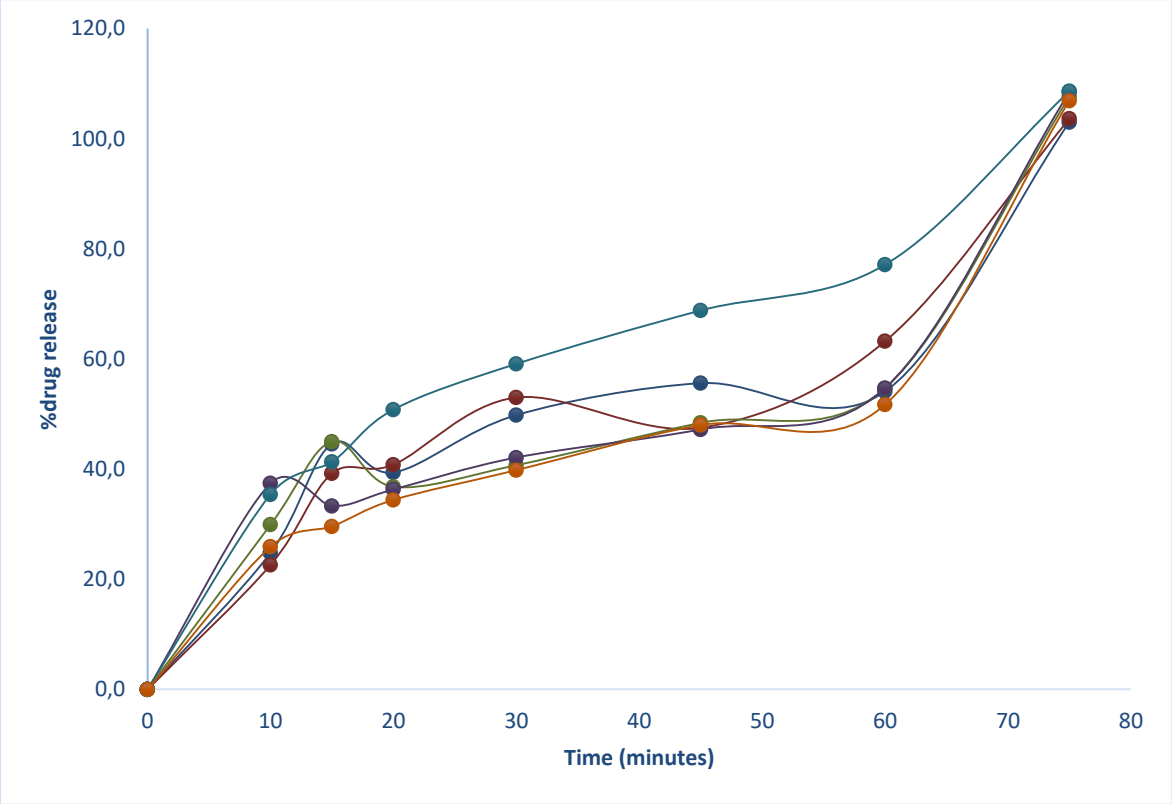

Figure 6.5: The dissolution profile of sodium valproate from six FDC tablets

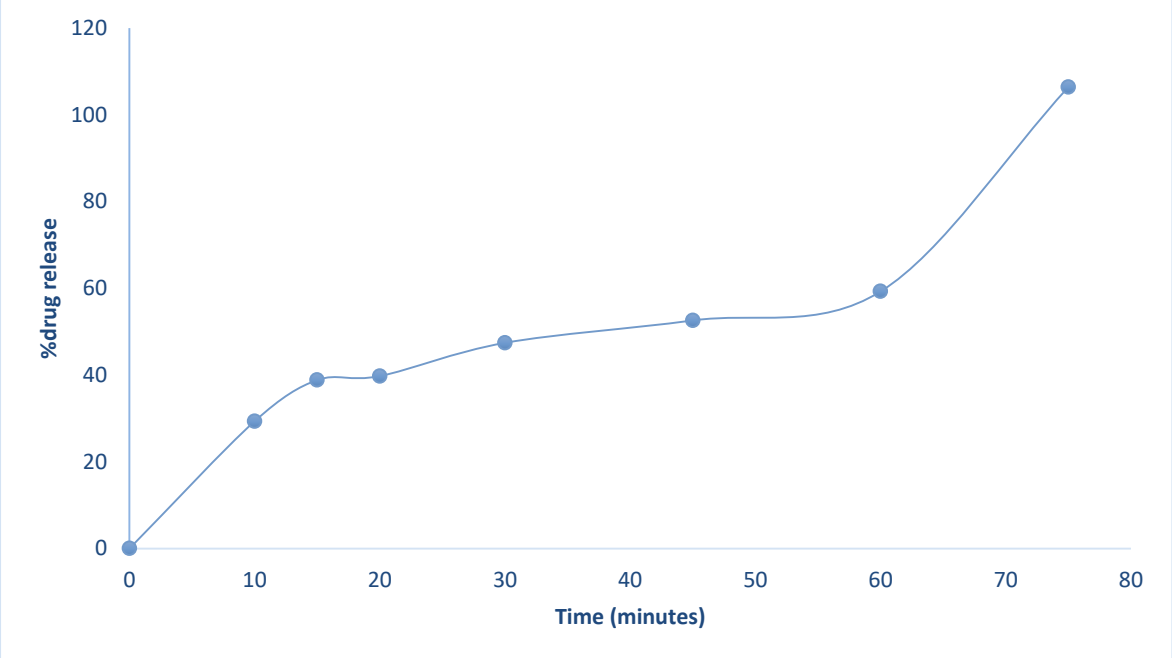

Figure 6.6: The mean percentage of amount of sodium valproate dissolved against time

6.4. SUMMARY

The dissolution profiles of carbamazepine and sodium valproate from the tablets displayed a rapid initial release of both the APIs, followed bya very slow release for sodium valproate, and a secondary release stage for sodium valproate again. The dissolution profile of carbamazepine in 1% SLS revealed complete dissolution within 45 minutes.

An analytical procedure was developed and validated to measure the concentrations of carbamazepine and sodium valproate from the fixed-dose combination released during dissolution. The analytical results presented evidence that the pharmaceutical quality by design concept maximise satisfactory ingredient compatibility, stability and expected dissolution performance.

The method performed well and should be suitable to analyse carbamazepine and sodium valproate in products for stability testing, quality control and batch release purposes. To develop a fixed-dose combination with improved potential absorption rates and oral bioavailability necessitated the study of the dissolution model of the APIs. Following in Chapter 7 is conclusions derived from the overall study, and recommendations for future studies.

CHAPTER 7:
CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

7.1. INTRODUCTION

The focus in this chapter is to draw conclusions from the study with regard to the specific objectives outlined in *Chapter 1*. A brief overview of the content of the dissertation and the summary of the findings will be provided. The limitations of the study will be listed with a conclusion and recommendations for future studies.

7.2.1. Overview of the content of the dissertation

This dissertation consists of seven chapters that was divided into:

- Chapter 1 provided the background and overview of the study, the problem statement, the aim of the study, research questions and objectives of the study.
- Chapter 2 focused on literature review, checking what other studies say about the study and the recent treatment modalities of epilepsy that encouraged the need for the development of a FDC tablet consisting of carbamazepine and sodium valproate.
- Chapter 3 gave an overview of the apparatus and methods that were used in the study
- Chapter 4 provided the results and discussions of the preformulation studies.
- Chapter 5 represented the results and discussions of formulation and post-formulation studies
- Chapter 6 represented the results and discussions of HPLC method development and validation and dissolution studies of the FDC tablet of carbamazepine and sodium valproate that was formulated.
- Chapter 7, which is this one revolves around drawing conclusions from the results obtained and list the limitations of the study with recommendations for future studies.

7.2.2. Conclusions

Epilepsy is one of the common neurological disorders that have social impact on people living with epilepsy. In the 17th and 18th centuries, epilepsy was classified as a mental disorder in Europe and a debate of whether it was a mental or a neurological disorder broke out during those years. The treatment modalities around epilepsy have always been focused on monotherapy. As time went by, monotherapy could not successfully treat epilepsy as was, hence polytherapy was introduced. According to NICE guidelines, polytherapy ensured that the medications that were used were of different classes of antiepileptics and carbamazepine and sodium valproate were the most common drugs prescribed.

The study aimed at reducing the burden of taking more than one tablet for epilepsy and developed a fixed dose combination tablet of carbamazepine and sodium valproate, at the lowest dose of 200mg/200mg per tablet. Following are the conclusions derived on the preformulation studies, formulation and post-formulation studies as well as HPLC method development and dissolution studies

7.2.2.1. Preformulation studies

The preformulation studies that were done considered powder flow, drug-drug compatibility, drug-excipients compatibility, powder compressibility and particle size distribution. From the results that were obtained, the study drew the following conclusions:

- Carbamazepine had fair flowability and sodium valproate had excellent flowability. As the two drugs were mixed together to make the FDC tablet, the flow of the mixture, which included a flow aid was excellent.
- With the four methods that measured drug-drug compatibility, all of them revealed that the drugs were compatible with one another and could be used together with little or no chemical interactions.
- The drug-excipient compatibility was measured using only one method, that being DSC. The results revealed that the two drugs were compatible with the excipients that were used. One method however, was not enough to critic the compatibility between the drugs and the excipients used hence, commercially

available tablets of carbamazepine (sold under the trade name Tegretol®) and sodium valproate (sold under the trade name Epilim®) were checked for the excipients that were used in their development. It was found that the two tablets as the single entities, used the same excipients, and most of the excipients were used in the formulation of the FDC tablet of carbamazepine and sodium valproate in this study.

- The particle size distribution of both powders were good and showed good distribution, which denoted good flowability.

The overall conclusion on the preformulation studies, is that the results provided an insight into what of a formulation would be made for the fixed dose combination between carbamazepine and sodium valproate, which the study opted for a tablet. From the compatibility studies, it was easy to choose the correct and suitable excipients of the FDC tablet, hence the preformulation studies were a success.

7.2.2.2. Formulation studies

The tablets that were formulated used to methods, direct compression method and wet granulation method. The following conclusions were drawn from the results obtained:

- The tablets that were manufactured on first attempt using direct compression method were not smooth, the tablet weight was too little and the batch failed. The flow aid that was added to the mixture was too little, hence the batch failed.
- On the second attempt, using direct compression still, the batch was successful but the tablets were not smooth still, which led to the development of the third batch of the FDC tablets that were manufactured using the wet granulation method.
- The first attempt with the wet granulation method produced very soft tablets that would easily break upon handling, and the observation was that the binder was too little and the lubricant was too much.
- After numerous attempt, tablets that were complying with standards set by the BP and the USP were formulated, using a different filler and adequate proportions of the lubricant and binder.

The overall conclusion on the formulation of the FDC tablets of carbamazepine and sodium valproate is that the formulation was successful.

7.2.2.3. Post-formulation studies

Post formulation studies that were done included non-official and official pharmacopoeial tests. The following are the conclusions that were drawn from the results:

- All the tests that were done complied with the set standards.
- Weight variation test confirmed that the weight of the FDC tablets did not deviate much from the specified tablet weight, which was calculated to be 800 mg, with the average weight of the first batch being at 782.5 mg, the second at 756.5 mg and the third one at 758 mg.
- Although the weight was a bit lower that the specified weight, not more than two of the individual weights of each of the tablet deviated from the average weight by more than the 5% deviation and none deviated by more than twice that percentage. This showed that the batches were successful
- The friability test on all three batches was less than 1% and that meant the tablets passed the test and were good for handling during transportation and were less prone to abrasion.
- The disintegration test was also successful on all three batches, which indicated that the superdisintegrant that was used was adequate and the tablets would render rapid disintegration.
- The tablet dimensions indicated that the tablet will be convenient for patients' acceptability.

The overall conclusion on the post-formulation test revealed that the development of the FDC tablet of carbamazepine and sodium valproate was successful and it met the set BP and USP standards, and further studies can be done to put the product on the market.

7.2.2.4. HPLC method development and validation and dissolution

The method was developed and validated to enable the researcher to check for drug content and perform *in vitro* dissolution studies. The drug content of carbamazepine and sodium valproate in the FDC tablet was checked using the method and the results were satisfactory. A short 24-hour stability was also done using the method and it showed that formulation was stable under set conditions and the method was suitable for the study.

The dissolution study checked for the release profiles of carbamazepine and sodium valproate from the formulation. Carbamazepine is released faster compared to sodium valproate, reaching optimum release within 45 minutes whereas sodium valproate only reached optimum release after an hour at increase rotations per minute (rpm). The USP and the BP don't specify the medium that must be used for running dissolution for sodium valproate, but sodium lauryl sulphate (SLS) is accepted as dissolution medium. Given that the SLS was the most suitable medium for carbamazepine according to USP (2014), the conditions favored the release of carbamazepine more than sodium valproate, hence the marked difference. Despite the different release profiles, both carbamazepine and sodium valproate followed an immediate release profile behavior, which was require of the formulation. The method was therefore suitable for the FDC tablet was successful.

7.2.3. Limitations

There are a few limitations regarding the database employed in the study. The drug-excipients compatibility studies were analyzed using one method. It lacks validation and supporting data that the excipients used in the study are compatible with carbamazepine and sodium valproate. The reason for using one method for drug-excipients compatibility analysis was lack of material to carry out the experiments.

7.2.4. Recommendations

Based on the current FDC development, new technologies for formulation and successful assessments of individual drug components for drug-drug interactions

and drug-excipients interactions, the study recommends the following for future studies:

- The utilisation of more than one method to analyse drug-excipients interactions to validate the results and findings. Use FTIR, XRPD and TAM analysis to complement the DSC data obtained for drug-excipients interactions.
- Include scanning electron microscope (SEM) analysis to determine particle size and shape of carbamazepine and sodium valproate in order to complement particle size analysis and the flow property parameters.
- An investigation in order to determine the in vivo efficacy of carbamazepine and sodium valproate FDC tablet.
- A further study that can design different doses of carbamazepine-sodium valproate FDC tablet for better management of epilepsy.
- An investigation to design other oral fixed-dose combinations of antiepileptics.
- An investigation of stability studies to establish the storage conditions, shelf lives and retest intervals.

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APPENDICES

Appendix 1: Particle size distribution data for carbamazepine sample 1

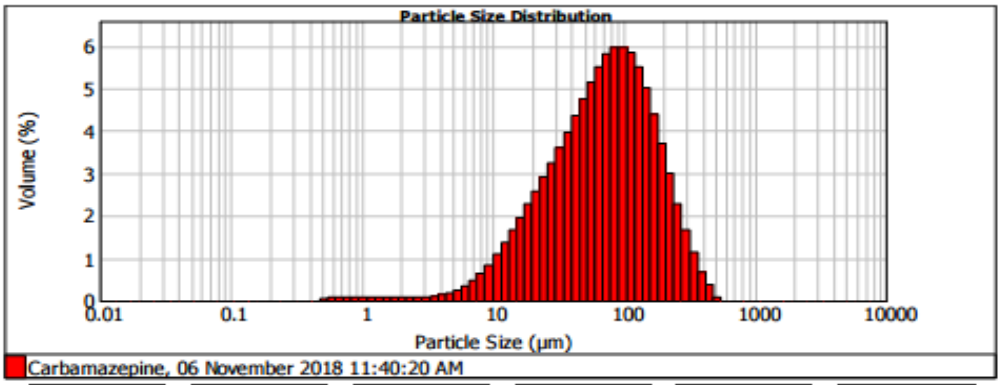
MALVERN INSTRUMENTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: Carbamazepine	**SOP Name:** Makgomo	**Measured:** 06 November 2018 11:40:20 AM	
Sample Source & type: Makgomo	**Measured by:** Neil Barnard	**Analysed:** 06 November 2018 11:40:22 AM	
Sample bulk lot ref: Sample 1	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 10.72 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.310 %	**Result Emulation:** Off
Concentration: 0.0434 %Vol	**Span :** 2.590	**Uniformity:** 0.812	**Result units:** Volume
Specific Surface Area: 0.203 m²/g	**Surface Weighted Mean D[3,2]:** 29.608 um	**Vol. Weighted Mean D[4,3]:** 94.087 um	

d(0.1): 17.187 um **d(0.5):** 71.616 um **d(0.9):** 202.648 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.08	11.482	1.36	120.226	5.50	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.08	13.183	1.66	138.038	5.01	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.08	15.136	1.96	158.489	4.40	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.08	17.378	2.26	181.970	3.71	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.08	19.953	2.59	208.930	3.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.08	22.909	2.92	239.883	2.30	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.08	26.303	3.25	275.423	1.67	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.08	30.200	3.60	316.228	1.12	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.09	34.674	3.80	363.078	0.69	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.11	39.811	3.97	416.869	0.39	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.13	45.709	4.36	478.630	0.07	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.18	52.481	4.76	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.06	5.754	0.24	60.256	5.16	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.07	6.607	0.34	69.183	5.52	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.08	7.586	0.47	79.433	5.80	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.08	8.710	0.64	91.201	5.97	954.993	0.00	10000.000	
0.091	0.00	0.955	0.08	10.000	0.85	104.713	5.98	1096.478	0.00		
0.105	0.00	1.096	0.08	11.482	1.09	120.226	5.82	1258.925	0.00		

Operator notes:

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2018/11/09 10:54:04 AM

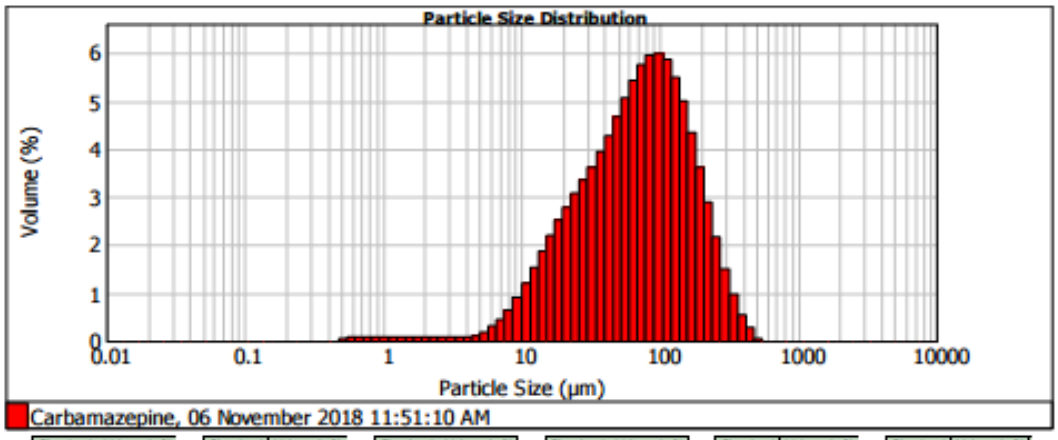
Appendix 2: Particle size distribution data for carbamazepine sample 2

MASTERSIZER 2000

Result Analysis Report

Sample Name: Carbamazepine	**SOP Name:** Makgomo	**Measured:** 06 November 2018 11:51:10 AM	
Sample Source & type: Makgomo	**Measured by:** Neil Barnard	**Analysed:** 06 November 2018 11:51:12 AM	
Sample bulk lot ref: Sample 2	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 10.20 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.275 %	**Result Emulation:** Off
Concentration: 0.0410 %Vol	**Span :** 2.559	**Uniformity:** 0.806	**Result units:** Volume
Specific Surface Area: 0.204 m²/g	**Surface Weighted Mean D[3,2]:** 29.416 um	**Vol. Weighted Mean D[4,3]:** 91.297 um	

d(0.1): 16.671 um d(0.5): 70.063 um d(0.9): 195.991 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.08	11.482	1.53	120.226	5.52	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.08	13.183	1.87	138.038	5.00	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.09	15.136	2.20	158.489	4.35	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.08	17.378	2.51	181.970	3.62	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.08	19.953	2.81	208.930	2.88	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.08	22.909	3.08	239.883	2.15	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.08	26.303	3.35	275.423	1.51	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.07	30.200	3.63	316.228	0.98	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.08	34.674	3.94	363.078	0.56	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.09	39.811	4.26	416.869	0.29	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.13	45.709	4.57	478.630	0.05	5011.872	0.00
0.046	0.00	0.479	0.06	5.012	0.19	52.481	5.07	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.07	5.754	0.30	60.256	5.45	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.08	6.607	0.45	69.183	5.77	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.08	7.586	0.65	79.433	5.97	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.08	8.710	0.90	91.201	6.01	954.993	0.00	10000.000	
0.091	0.00	0.955	0.08	10.000	1.20	104.713	5.86	1096.478	0.00		
0.105	0.00	1.096	0.08	11.482		120.226		1258.925	0.00		

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2018/11/09 10:55:03 AM

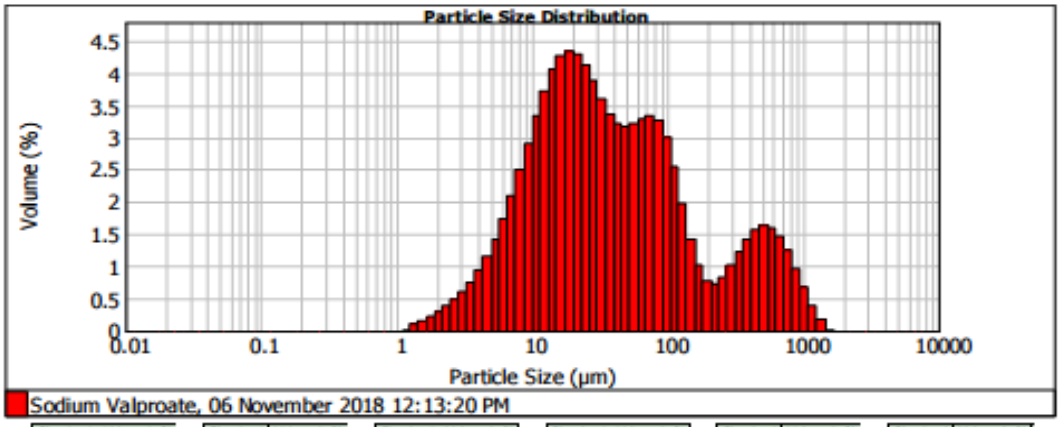
Appendix 3: Particle size distribution data for sodium valproate sample 1

MASTERSIZER 2000

Result Analysis Report

Sample Name: Sodium Valproate	**SOP Name:** Makgomo	**Measured:** 06 November 2018 12:13:20 PM	
Sample Source & type: Makgomo	**Measured by:** Neil Barnard	**Analysed:** 06 November 2018 12:13:22 PM	
Sample bulk lot ref: Sample 2	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 15.36 %
Dispersant Name: Cyclohexane	**Dispersant RI:** 1.468	**Weighted Residual:** 0.572 %	**Result Emulation:** Off
Concentration: 0.0385 %Vol	**Span :** 12.228	**Uniformity:** 3.17	**Result units:** Volume
Specific Surface Area: 0.344 m²/g	**Surface Weighted Mean D[3,2]:** 17.443 um	**Vol. Weighted Mean D[4,3]:** 119.033 um	

d(0.1): 7.402 um d(0.5): 32.777 um d(0.9): 408.195 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.02	11.482	3.73	120.226	1.96	1258.925	0.19
0.011	0.00	0.120	0.00	1.259	0.11	13.183	4.05	138.038	1.43	1445.440	0.02
0.013	0.00	0.138	0.00	1.445	0.16	15.136	4.26	158.489	1.01	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.22	17.378	4.35	181.970	0.78	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.30	19.953	4.30	208.930	0.74	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.39	22.909	4.12	239.883	0.83	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.49	26.303	3.88	275.423	1.01	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.62	30.200	3.60	316.228	1.23	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.78	34.674	3.36	363.078	1.43	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.94	39.811	3.21	416.869	1.58	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	1.15	45.709	3.16	478.630	1.64	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	1.41	52.481	3.20	549.541	1.60	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	1.73	60.256	3.29	630.957	1.46	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	2.09	69.183	3.34	724.436	1.24	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	2.49	79.433	3.25	831.764	0.97	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	2.91	91.201	2.99	954.993	0.67	10000.000	
0.091	0.00	0.955	0.00	10.000	3.34	104.713	2.54	1096.478	0.40		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925			

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Appendix 4: Particle size distribution data for sodium valproate sample 2

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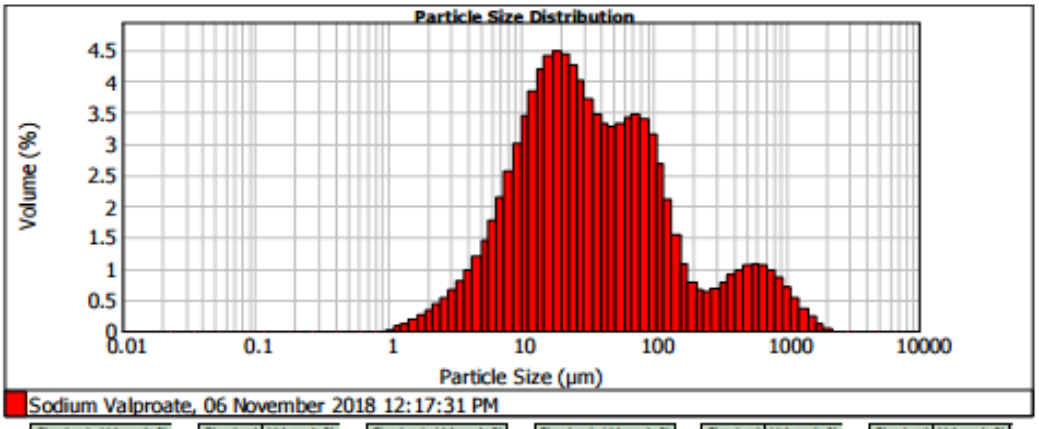
Result Analysis Report

Sample Name: Sodium Valproate	**SOP Name:** Makgomo	**Measured:** 06 November 2018 12:17:31 PM	
Sample Source & type: Makgomo	**Measured by:** Neil Barnard	**Analysed:** 06 November 2018 12:17:33 PM	
Sample bulk lot ref: Sample 3	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 17.00 %
Dispersant Name: Cyclohexane	**Dispersant RI:** 1.468	**Weighted Residual:** 0.556 %	**Result Emulation:** Off
Concentration: 0.0407 %Vol	**Span :** 9.319	**Uniformity:** 3.13	**Result units:** Volume
Specific Surface Area: 0.362 m²/g	**Surface Weighted Mean D[3,2]:** 16.566 um	**Vol. Weighted Mean D[4,3]:** 110.317 um	

d(0.1): 7.170 um d(0.5): 30.627 um d(0.9): 292.573 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.08	11.482	3.86	120.226	2.12	1258.925	0.36
0.011	0.00	0.120	0.00	1.259	0.12	13.183	4.19	138.038	1.54	1445.440	0.23
0.013	0.00	0.138	0.00	1.445	0.18	15.136	4.41	158.489	1.09	1659.587	0.10
0.015	0.00	0.158	0.00	1.660	0.25	17.378	4.49	181.970	0.79	1905.461	0.03
0.017	0.00	0.182	0.00	1.905	0.33	19.953	4.44	208.930	0.65	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.43	22.909	4.26	239.883	0.63	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.53	26.303	4.01	275.423	0.69	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.65	30.200	3.73	316.228	0.79	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.80	34.674	3.48	363.078	0.89	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.97	39.811	3.33	416.869	0.99	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	1.19	45.709	3.28	478.630	1.05	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	1.46	52.481	3.33	549.541	1.07	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	1.78	60.256	3.42	630.957	1.05	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	2.15	69.183	3.48	724.436	0.97	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	2.57	79.433	3.40	831.764	0.85	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	3.01	91.201	3.14	954.993	0.70	10000.000	
0.091	0.00	0.955	0.00	10.000	3.45	104.713	2.69	1096.478	0.53		
0.105	0.00	1.096	0.02	11.482		120.226		1258.925			

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Appendix 5: Crushing strength results of formula 1 tablets

Tablet	**Mass (mg)**	**Crushing force (N)**
1	780	107
2	750	134
3	750	131
4	760	114
5	760	133
6	730	123
7	720	121
8	790	124
9	750	92
10	780	135
Total	**7570**	**1214**
Average	**757**	**121.4**
SD	**22.14**	**13.77**
%RSD	**2.92**	**11.34**

Appendix 6: Crushing strength results of formula 2 tablets

Tablet	**Mass (mg)**	**Crushing force (N)**
1	760	107
2	780	134
3	750	131
4	780	114
5	760	133
6	770	123
7	780	121
8	770	124
9	760	92
10	770	135
Total	**7690**	**1136**
Average	**769**	**113.6**
SD	**11.00**	**18.00**
%RSD	**1.43**	**15.84**

Appendix 7: Crushing strength results of formula 3 tablets

Tablet	**Mass (mg)**	**Crushing force (N)**
1	780	121
2	760	123
3	780	135
4	780	115
5	750	101
6	790	118
7	780	121
8	770	95
9	760	135
10	770	122
Total	**7720**	**1186**
Average	**772**	**118.6**
SD	**12.30**	**12.74**
%RSD	**1.60**	**10.74**

Appendix 8: Mass variation results of formula 1

Tablet	Mass (mg)	%variation	Results
1	800	2,498	Complied
2	800	2,498	Complied
3	770	-1,597	Complied
4	780	-0,319	Complied
5	780	-0,319	Complied
6	800	2,236	Complied
7	770	-1,597	Complied
8	780	-0,319	Complied
9	780	-0,319	Complied
10	750	-4,153	Complied
11	780	-0,319	Complied
12	760	-2,875	Complied
13	780	-0,319	Complied
14	790	0,958	Complied
15	800	2,236	Complied
16	820	4,792	Complied
17	760	-2,875	Complied
18	740	-5,431	Complied
19	800	2,236	Complied
20	810	3,514	Complied
Total	15650		
Average	782,5		
STDEV	20,61907367		
%RSD	2,635025389		

Appendix 9: Mass variation results of formula 2

Tablet	Mass (mg)	%variation	Results
1	750	-0,859	Complied
2	770	1,852	Complied
3	760	0,463	Complied
4	750	-0,859	Complied
5	730	-3,503	Complied
6	770	1,852	Complied
7	780	3,106	Complied
8	750	-0,859	Complied
9	750	-0,859	Complied
10	750	-0,859	Complied
11	760	0,463	Complied
12	750	-0,859	Complied
13	750	-0,859	Complied
14	780	3,106	Complied
15	760	0,463	Complied
16	760	0,463	Complied
17	740	-2,181	Complied
18	750	-0,859	Complied
19	760	0,463	Complied
20	760	0,463	Complied
Total	15130		
Average	756,5		
STDEV	12,49561327		
%RSD	1,651766459		

Appendix 10: Mass variation results of formula 3

Tablet	Mass (mg)	%variation	Results
1	780	2,902	Complied
2	750	-1,055	Complied
3	750	-1,055	Complied
4	760	0,264	Complied
5	760	0,264	Complied
6	730	-3,694	Complied
7	730	-3,694	Complied
8	790	4,222	Complied
9	750	-1,055	Complied
10	780	2,902	Complied
11	740	-2,375	Complied
12	730	-3,694	Complied
13	760	0,264	Complied
14	800	5,541	Complied
15	740	-2,375	Complied
16	760	0,264	Complied
17	750	-1,055	Complied
18	770	-0,859	Complied
19	780	2,902	Complied
20	750	-1,055	Complied
Total	15160		
Average	758		
STDEV	20,01461454		
%RSD	2,640450467		

Appendix 11: The dissolution data for carbamazepine from the FDC tablet

Time	**Carbamazepine % Dissolved**					
(min)	TABLET 1	TABLET 2	TABLET 3	TABLET 4	TABLET 5	TABLET 6
0	0	0	0	0	0	0
10	23.2	20.8	25.5	19.0	22.8	20.8
15	31.2	31.2	34.2	30.0	31.7	30.0
20	39.9	40.3	40.6	39.9	46.7	40.3
30	55.6	61.5	63.2	63.4	57.4	58.8
45	99.5	96.7	99.6	99.9	93.6	96.4
60	99.8	99.8	100.0	100.0	99.9	98.8
75	101.0	101.0	100.0	100.0	99.9	98.8
*AVE DRi**	0.507					
*AVE AUC**	10244.45					

DRi = Initial dissolution rate (%.min^{-1})

AUC = Area under the curve (%.min)

Appendix 12: The dissolution data for sodium valproate from the FDC tablet

Time	**Sodium valproate % Dissolved**					
(min)	TABLET 1	TABLET 2	TABLET 3	TABLET 4	TABLET 5	TABLET 6
0	0	0	0	0	0	0
10	24.8	22.6	29.9	37.4	35.4	25.9
15	44.6	39.2	45.0	33.3	41.3	29.6
20	39.4	40.8	36.8	36.3	50.8	34.4
30	49.8	53.0	40.7	42.1	59.1	39.8
45	55.6	47.5	48.4	47.2	68.8	48.0
60	54.1	63.2	54.7	54.7	77.1	51.7
75	103.0	103.6	107.6	108.5	108.6	106.9
AVE DRi	0.702					
AVE AUC	6851.25					

Appendix 13: Standard deviations of the dissolution data

	Carbamazepine	**Sodium valproate**	**Carbamazepine**	**Sodium valproate**
Time (min)	**STDEV**	**STDEV**	**%RSD**	**%RSD**
0	0	0	0	0
10	2.29	6.23	10.40	21.24
15	1.54	6.19	4.92	15.95
20	2.67	6.66	6.46	16.52
30	3.21	8.63	5.36	18.20
45	2.50	9.41	2.56	17.90
60	0.46	10.34	0.46	17.44
75	0.82	2.05	0.82	2.08

Appendix 14: Standard calibration curve of carbamazepine

Table: Calibration data of carbamazepine at 214 nm

Concentration (µg/ml)	Peak area			Mean area
	1	2	3	
0.01	3.60	3.50	3.40	3.5
0.10	32.70	32.70	32.70	32.7
0.51	107.50	106.80	107.40	107.2
1.52	316.50	319.70	319.70	318.1
5.06	864.80	864.10	864.80	864.5
15.18	2541.40	2550.50	2555.00	2549.0
25.30	4228.60	4224.40	4226.40	4226.5
50.60	9224.00	9206.50	9220.20	9216.9
101.20	17147.10	17168.70	17160.50	17158.8

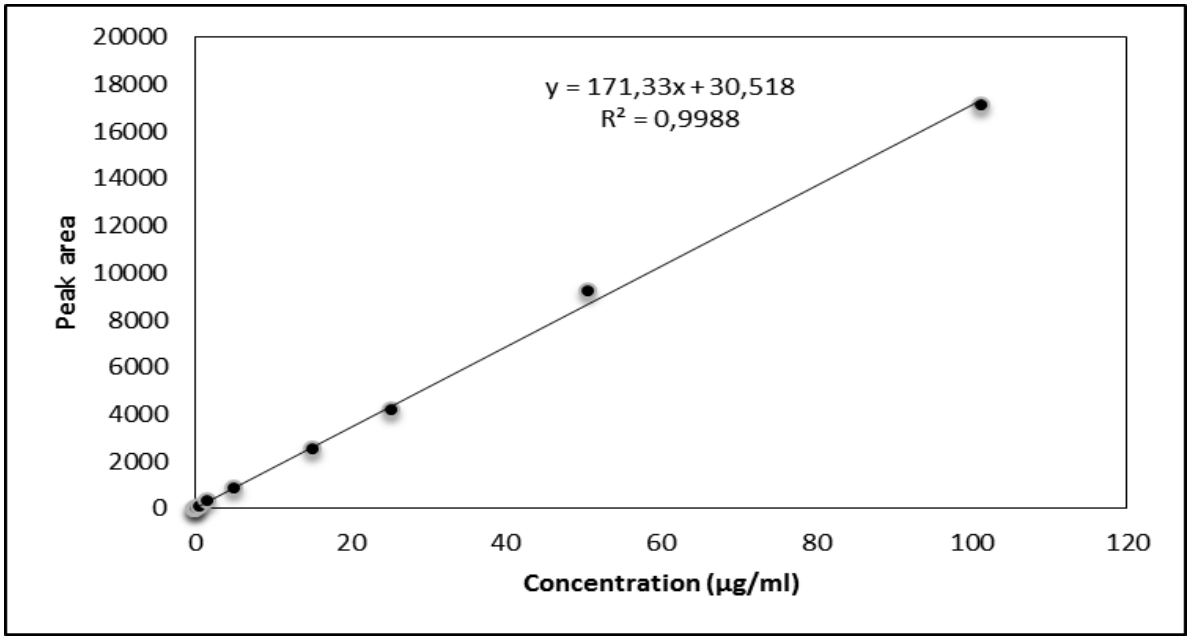


Figure: calibration curve of carbamazepine at 216 nm

Appendix 15: Standard calibration curve of sodium valproate

Table: Calibration data of sodium valproate at 214 nm

Concentration (µg/ml)	Peak area			Mean area
	1	2	3	
0.01	1.60	1.20	1.30	1.4
0.10	2.50	2.30	2.30	2.4
0.51	1.30	1.20	1.30	1.3
1.54	2.10	2.10	2.10	2.1
5.12	4.20	3.90	4.00	4.0
15.36	11.40	11.40	11.20	11.3
25.60	18.80	18.60	18.80	18.7
51.20	33.20	32.90	33.30	33.1
102.40	65.90	66.00	66.00	66.0

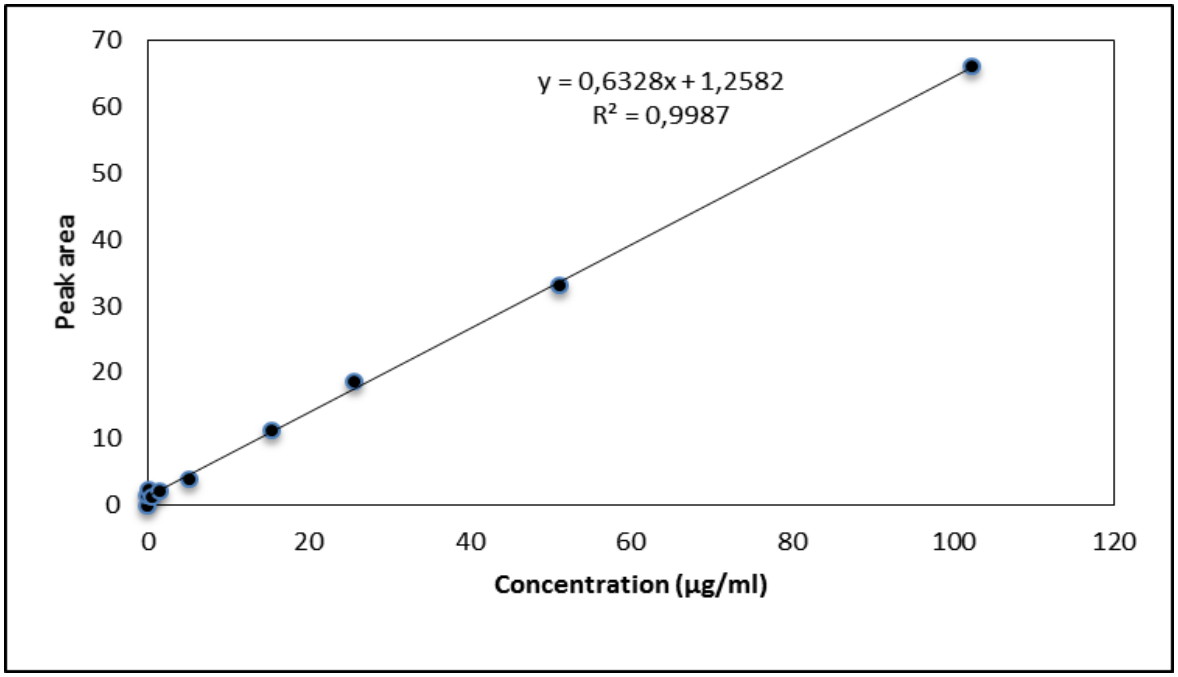


Figure: Calibration curve of sodium valproate at 214 nm

Appendix 16: PRESENTATION AT THE 3rd FACULTY OF HEALTH SCIENCES ANNUAL RESEARCH CONFERENCE 12th SEPTEMBER to 14 SEPTEMBER 2018

Compatibility study of carbamazepine and sodium valproate using thermal analysis and infrared spectroscopy

Authors: Miss Seabi ME, Mr Manyama TL, Mr Tshitake RM and Prof Demana PH
University of Limpopo Turfloop Campus ,P. Bag X 1106,Sovenga 0727
Correspondence: mkseabi@gmail.com

Introduction

Carbamazepine and sodium valproate are antiepileptic drugs which are often used together for epilepsy. This results in the burden of taking multiple medications, increased health-care costs and decreased patient compliance, ultimately resulting in increased treatment failure. To resolve this, the development of a fixed dose combination tablet containing both carbamazepine and sodium valproate is conceptualised, with intention to improve treatment success as seen in HIV/AIDS treatment. However, development of this formulation requires preliminary compatibility studies which would pave a way for successful development of the tablets. In this study, thermal analysis techniques [Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) Spectroscopy and X-ray Powder diffraction (XRPD)] were conducted in order to fulfil this mandatory process.

Aim and Objective

- To determine the compatibility of carbamazepine and sodium valproate.

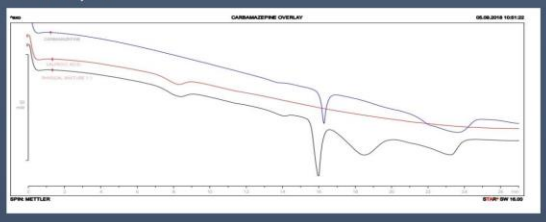
Method

Samples of carbamazepine and sodium valproate and physical mixture (1:1 w/w) of both drugs were prepared for compatibility with thermal analysis and spectroscopy techniques.

Data was analysed by comparing the DSC curves, FTIR spectra and XRPD peaks of carbamazepine and sodium valproate alone with their physical mixture (1:1 w/w).

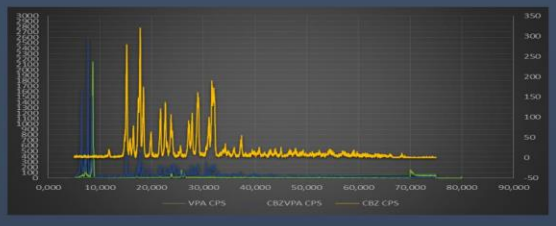
Results and discussions

DSC analysis



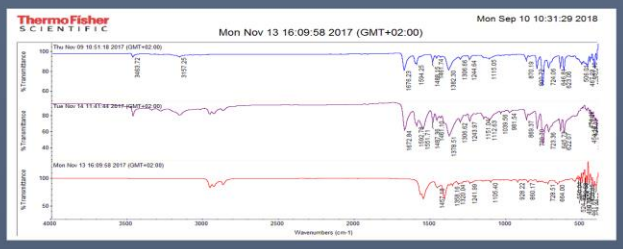
Individual DSC peaks of carbamazepine (blue) and sodium valproate(red) corresponded to their melting points 190.74° C and 110.02° C respectively. The DSC thermograms of the 1:1 physical mixture (grey) corresponded to the individual results with a peak shift to 187.94° C for carbamazepine and 112.63° C for sodium valproate.

XRPD analysis



Individual XRPD diffractograms of carbamazepine (yellow) demonstrated sharp peaks at 2Θ= 15.1°, 15.7°, 17.7°, 21.7°, 28.9°, 31.6° and 37.3°. Sodium valproate (green) demonstrated sharp peaks at 2Θ= 7.2°, 8.6° and 16.9°. The XRPD diffractogram of the physical mixture (1:1 w/w) (blue) corresponded to carbamazepine diffractogram at 2Θ= 15.0°, 15.6°, 17.6°, 21.5°, 28.2°, 31.9° and 36.9° and to sodium valproate at 7.0°, 8.4° and 17.2°

FTIR analysis



FTIR

Individual FTIR spectra of carbamazepine (blue) showed absorption bands at 3463.72 cm^{-1} and 3157.25 cm^{-1} which were attributed to the amine group (N-H), 1676.23 cm^{-1} and 15cm^{-1} attributed to alkene group (C=C), 1488.15 cm^{-1} 1461.74cm^{-1} ,1382.30 cm^{-1}, 1306.66 cm^{-1} and 1244.64 cm^{-1} represented the carbonyl group (C=O), the ether group (C-O) were observed at 1115.05 cm^{-1} and the aromatic ring was represented between 870 cm^{-1}, 800 cm^{-1}, 724 cm^{-1} and 646 cm^{-1}. The FTIR spectra of sodium valproate (red) showed absorption bands at 1594.60 cm^{-1} attributed to the alkene group (C=C), 1457.18 cm^{-1} 1411.20 cm^{-1}, 1358.16 cm^{-1}, 1320.04 cm^{-1}, 1241.99 cm^{-1}, 929.22 cm^{-1} and 860.17 cm^{-1} represented the carbonyl group (C=O), the ether group (C-O) was represented 1105.40 cm^{-1} and the aromatic ring was represented between 728.51 cm^{-1} and 664.00 cm^{-1}. FTIR spectra of the physical mixture (1:1 w/w) (purple) corresponded to carbamazepine 1672.84 cm^{-1}, 1592.78 cm^{-1} (C=C) 1487.36 cm^{-1}, 1461.10 cm^{-1}, 1378.51 cm^{-1}, 1306.62 cm^{-1}, 1243.97 cm^{-1} and 869.37 cm^{-1} represented the carbonyl group (C=O), 1151.04 cm^{-1} represented the ether (C-O). The aromatic ring was observed at between 799.70 cm^{-1}, 723.36 cm^{-1} and 645.73 cm^{-1}. Similarly the FTIR spectra of sodium valproate showed characteristic bands at 1151.71 cm^{-1} (C=C), 112.63 cm^{-1}, 1039.56 cm^{-1} and 987.54 cm^{-1} (C=O) and the aromatic ring at 723.36 cm^{-1} and 645.73 cm^{-1}

Conclusions

Based on the DSC and FTIR data and supported by X-ray diffraction results ,there was no drug/drug incompatibility between the drugs, therefore a fixed dose combination of carbamazepine and sodium valproate into a a conventional tablet can be formulated.

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Appendix 17: Ethical clearance certificate

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TURFLOOP RESEARCH ETHICS COMMITTEE CLEARANCE CERTIFICATE

MEETING:	**04 July 2017**
PROJECT NUMBER:	**TREC/114/2017: PG**
PROJECT:	
Title:	Formulation of carbamazepine and sodium valproate fixed dose combination for management of epilepsy
Researcher:	ME Seabi
Supervisor:	Mr TL Manyama
Co-Supervisor:	Mr RM Tshitake
	Prof PH Demana
School:	Health Care Sciences
Degree:	Masters in Pharmacy

PROF TAB MASHEGO
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: **REC-0310111-031**

Note:
i) **Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.**
ii) **The budget for the research will be considered separately from the protocol.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

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