# DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF RUFINAMIDE BULK MATERIAL AND DOSAGE FORMS

**A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Pharmacy in Pharmaceutical Analysis of the University of Nairobi**

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**November 2016**

# DECLARATION

This research thesis is my original work and has not been presented elsewhere for award of any degree.

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| Title of the work | Development and validation of a stability indicating liquid chromatographic method for the analysis of rufinamide bulk material and dosage forms. |

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# DEDICATION

This research thesis is dedicated to my dear wife, Caroline Gathoni and my daughters, Bianca Muthoni and Nadia Wambura, for believing in my abilities and enduring my constant absence from home during the research period.

To my mother, Beatrice Gathoni and my siblings, Peris Wambui and Patrick Ngumo, for their unending encouragement, prayers and strong believe in my work.

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# LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| ACN | Acetonitrile |
| API | Active Pharmaceutical Ingredient |
| BDS | Base deactivated silanol |
| BP | British Pharmacopeia |
| CNS | Central Nervous System |
| CV | Coefficient of variation |
| CYP | Cytochrome P450 |
| °C | Degree Centigrade |
| DMSO | Dimethylsulfoxide |
| DP | Degradation product |
| EEG | Electroencephalogram |
| g | Gram |
| GABA | Gamma-amino butyric acid |
| GIZ | Gesellschaft für International Zusammenarbeit GmbH |
| g/mol | Grams per mole |
| HPLC | High Performance Liquid Chromatography |
| HPMC | Hydroxypropyl methylcellulose |
| IBE | International Bureau for Epilepsy |
| ICH | International Conference on Harmonization |
| ILAE | International League Against Epilepsy |
| IUPAC | International Union of Pure and Applied Chemistry |
| LC | Liquid Chromatography |

| | |
|---|---|
| LGS | Lennox-Gastaut Syndrome |
| LOD | Limit of Detection |
| LOQ | Limit of Quantitation |
| Ltd | Limited |
| MCC | Microcrystalline cellulose |
| mg | Milligram |
| mg/L | Milligram per Litre |
| mL/min | Millilitre per minute |
| mm | millimeter |
| nm | nanometer |
| ODS | Octadecylsilane |
| RH | Relative Humidity |
| RP | Reversed Phase |
| RRCA | Rufinamide related compound A |
| RRCB | Rufinamide related compound B |
| RSD | Relative standard deviation |
| RS | Related substances |
| RUF | Rufinamide |
| THF | Tetrahydrofuran |
| µL | microliter |
| µg/mol | Micrograms per mole |
| USA | United States of America |
| US-FDA | United States Food and Drug Administration |

| | |
|---|---|
| USP | United States Pharmacopeia |
| UV | Ultraviolet |
| UV/VIS | Ultraviolet/visible |
| WHO | World Health Organization |

# ABSTRACT

Rufinamide is a triazole anti-epileptic used together with other medications/therapies in the management of seizures linked to Lennox Gastaut Syndrome (LGS) and various epileptic disorders in adults, as well as in children above four years of age. Rufinamide is classified as an orphan drug by the World Health Organization. Currently, rufinamide dosage forms like tablets and suspension are available in the United States and European Union markets. Kenya and other East African countries have to import rufinamide products from these markets. Therefore, there is need to ensure local production of good quality generic rufinamide dosage forms for use by the East African population suffering from LGS. Liquid chromatography is the analytical procedure of choice for the analysis of rufinamide. The current published methods have various drawbacks including the fact that a majority of them are not stability indicating.

In the current study, an isocratic, specific, precise, robust, sensitive and accurate reversed phase high performance liquid chromatographic method was developed for the analysis of rufinamide both in bulk material and dosage formulation. The effect of chromatographic factors were investigated in the process of method development through use of inorganic buffer, ion-pairing agent, organic modifier, pH modification and temperature variation. A Phenomenex Hyperclone BDS C-18 chromatography column 250 mm and 4.6 mm dimensions, particle size of 5 µm maintained at a temperature of 35 °C was used. Methanol-0.1 M octane sulphonic acid-0.1 M dipotassium hydrogen phosphate pH 6.5-water (30:10:5:55, % v/v/v/v) mobile phase composition delivered at a flow rate of 1.0 mL/min was used. The pH of the mixture of buffer and ion pairing agent was brought to 6.5 using an equimolar solution of 0.1 M *ortho*-phosphoric

acid. The analytes were detected by ultra-violet absorption spectroscopy at 210 nm wavelength. Rufinamide retention time was found to be about 9.4 min.

The developed method was validated using the International Conference on Harmonization guidelines. The parameters investigated include linearity, range, sensitivity, precision, accuracy, specificity and robustness. Method validation showed that it exhibited good linearity over the 50% to 150% range of the analytical concentration with a linear regression coefficient ($r^2$ value) of 0.9997. The detection limit and quantitation limit were found to be 7.81 µg and 15.53 µg, respectively. The method exhibited good precision with the same day analysis coefficient of variation of 0.96% while different day analysis coefficient of variation of 0.64%.

The developed method was applied in analyzing five samples, one of them being a commercial sample, Inovelon®, obtained from the United Kingdom market and the other four locally formulated tablets in the Department of Pharmaceutics and Pharmacy Practice of the University of Nairobi. Results obtained indicated that the samples complied with the United States Pharmacopeia 2015 specifications for the assay of rufinamide tablets. From the assay data, the developed method may be utilized and adopted for routine quality analysis of rufinamide dosage formulations. It can also be used in stability studies by the pharmaceutical industry and drug regulatory authorities in assuring a high quality of rufinamide in the market. The method can also find application in monitoring the quality of rufinamide products during post market surveillance.

# CHAPTER ONE

# INTRODUCTION AND LITERATURE REVIEW

## 1.1 Epilepsy

Epilepsy is a collection of neurological disorders marked by paroxysmal cerebral dysrhythmia. It presents as short episodes of disturbance of consciousness, convulsions, sensory or psychiatric manifestations (Tripathi, 2003; Katzung, 2007). Epilepsy is defined by two or more unprovoked seizures appearing longer than 24 hours apart, one provoked seizure and another likely seizure almost the same to the general recurrence risk following two unprovoked seizures (Fisher *et al*., 2005; Fisher *et al*., 2014; Panayiotopoulos, 2011).

Epilepsies and epileptic syndromes are classified based on the underlying causes, which mainly determine the clinical course and prognosis (Engel, 2006). International League Against Epilepsy (ILAE) Commission classifies epilepsy into four; epilepsy associated with genetic origin, symptomatic epilepsy associated with gross or pathological abnormalities, provoked epilepsy due to specific systemic or environmental factors and cryptogenic epilepsy in which the cause has not been identified (Berg *et al*., 2010; Shorvon, 2011). Sub-categories reflect recent technological and scientific advances.

## 1.2 Epilepsy syndromes

Epilepsy is categorized into syndromes based on the specific features present. The features are the age when seizures start, the type of seizure and electroencephalogram (EEG) presentation. Identification of an epileptic syndrome is important in determining the actual cause and the anti-epileptic medication to be utilized (ILAE, 2014; National Clinical Guideline Centre, 2012).

Epilepsy is categorized into specific syndromes mainly in children because of the early onset of seizures (Neligan *et al*., 2012). Severe epileptic syndromes with diffuse brain dysfunction are known as epileptic encephalopathies. The severe syndromes are characterized by frequent seizures resistant to anticonvulsant therapy and cognition difficulties. Examples of the severe epileptic syndromes are Lennox-Gastaut syndrome (LGS) and West syndrome. Genetics may have a link in the onset of these severe epileptic syndromes (Neligan *et al*., 2012; Nordli, 2012).

## 1.3 Lennox-Gastaut Syndrome

Lennox-Gastaut syndrome was the first established epileptic syndrome to promote the concept of syndromic classification. This classification is dependent on a combination of special clinical manifestations and EEG findings (Oguni, 2010). It is one of the severe forms of childhood-onset epileptic syndromes. Lennox Gastaut Syndrome is comprised of a triad of multiple types of seizures, cognitive dysfunction or a learning disability (Arzimanoglou *et al*., 2009; Buck, 2009). The LGS can also occur due to a brain insult like perinatal anoxia, cerebral dysgenesis or cryptogenic in a child who was normal previously. It presents 1-4% of childhood epilepsy. However, LGS comprises a bigger proportion of all refractory epilepsy (American Epilepsy Society, 2009).

The prevalence of LGS is one in ten of all childhood epilepsy. The incidence rate of LGS in all new cases of epilepsies is about 0.6% (Feoli, 2011). It is more common in males than in females. However, in day-to-day clinical set up new cases of LGS are relatively uncommon (Carmant and Whiting, 2012; Rantala and Putkonen, 1999). This wide incidence range makes it difficult to interpret a classic criteria in diagnosing LGS (Markand, 2003).

## 1.4 Management of Lennox-Gastaut syndrome

Since only a few patients diagnosed with LGS attain seizure free-state (American Epilepsy Society, 2009), the main objective of drug therapy is reduction in the frequency and severity of seizures thus enhancing the standards of life. A rise in the frequency of seizures affect both the patient and the caregiver with respect to the quality of life (Gallop *et al*., 2009). A decrease in the frequency of seizures can result in higher alertness, improvement in behavioural and cognitive ability (Arzimanoglou *et al*., 2009), less body injuries, minimal interruption of school attendance and little effect on social relationships (Gallop *et al*., 2010).

Many patients with LGS require a multi-drug regimen with antiepileptic drugs such as hydantoins, barbiturates (Carl and Smith, 1992), phenyltriazines, benzodiazepines, gamma-aminobutyric acid (GABA) analogs (Rho *et al*., 1997; Foster and Kemp, 2006), dibenzazepines and carbamates (Kwan and Brodie, 2004; Shorvon, 2009; Mazza *et al*., 2007) as well as corticosteroids or immunoglobulins. In addition to pharmacotherapy, other means of managing LGS include ketogenic diet, vagus nerve stimulation and surgical resection (Arzimanoglou *et al*., 2009; Feoli, 2011). The mode of therapy to be utilized is based on the presentation of the syndrome, gender and side effects in case of pharmacotherapy (Feoli, 2011). Despite an

aggressive approach to treatment, many patients continue to have frequent seizures. This unmet medical need has continued to spur research into newer and more effective antiepileptic drugs. Several new agents are under investigation for the treatment of LGS (Rogawshi, 2006). One of the clinically useful drugs in the management of LGS is rufinamide.

## 1.5 Rufinamide

### 1.5.1 Description

Rufinamide was developed in 2004 by Novartis Pharmaceuticals (Hakimian *et al*., 2007; Arroyo, 2007) and approved as a prescription drug by the United States Food and Drug Administration (US-FDA) in 2008 for the management of seizures associated with LGS. Due to the rarity of LGS and dearth of effective medicines for its treatment, rufinamide was classified as an orphan drug in the USA and European Union by World Health Organization (WHO) (Arroyo, 2007). Inovelon® is manufactured by Eisai Pharmaceuticals (Hakimian *et al*., 2007) under license from Novartis Pharmaceuticals. Rufinamide is commercially formulated as coated tablets and oral suspensions. Rufinamide is used in conjunction with other therapies to manage seizures associated with LGS and various other seizure disorders in adults, as well as in children above four years (Muneer *et al*., 2012; Annapurna *et al*., 2012). Rufinamide has also been studied clinically in the management of refractory partial seizures (Hakimian *et al*., 2007; Arroyo, 2007).

### 1.5.2 Mechanism of anti-convulsant action

The precise mode of anti-convulsant action of rufinamide is not well understood. However, it is believed to act through stabilizing excitability of neurons (Rogawski, 2006). This is achieved mainly by prolonging the inactive state of the voltage-gated sodium ion channels; effectively

keeping the ion channels closed, thereby making the neurons less likely to depolarize (Annapurna *et al*., 2012; Patel *et al*., 2011). Clinical studies have shown that rufinamide raises the seizure's threshold and thus prevents subsequent spread of seizure (Patel *et al*., 2011).

### 1.5.3 Chemistry

Rufinamide is an orally active triazole derivative structurally distinct from other antiepileptic drugs (Patel *et al*., 2011). It is composed of substituted phenyl and triazole rings linked by a methylene group as shown in Figure 1.1. The International Union of Pure and Applied Chemistry (IUPAC) name of rufinamide is 1-[(2,6-difluorophenyl)methyl]-1*H*-1,2,3-triazole-4-carboxamide with molecular formula $C_{10}H_8F_2N_4O$ and a molecular weight of 238.19 g/mol.

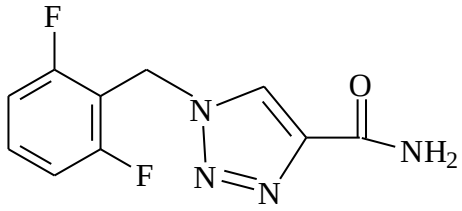



**Figure 1.1: Chemical structure of rufinamide.**

Rufinamide is a white to off-white odourless crystalline powder with low solubility in gastric fluid, intestinal fluid and water. It is slightly soluble in tetrahydrofuran (THF), methanol, ethanol and acetonitrile (ACN). However, it is soluble in dimethylsulfoxide (DMSO). Rufinamide has no ionizable functional group. It freely forms agglomerates and consequently has a low bulk density and poor flow properties. Rufinamide exhibits polymorphism and four polymorphic forms A, B, C and R-5 have been distinguished. Polymorph A is thermodynamically stable and is the commercially used form.

### 1.5.4 Synthesis

According to US patent number 4,789,680 by Ciba-Geigy Corporation (www.google.com/US2013184469), synthesis of rufinamide comprises reacting 2, 6-difluorobenzyl chloride with sodium azide ($NaN_3$) in the presence of DMSO. This leads to the formation of 2,6-difluorobenzyl azide, which is treated with propiolic acid in presence of toluene to give a carboxylic acid intermediate. Reaction of this intermediate with thionyl chloride ($SOCl_2$) affords an acyl chloride derivative, which is reacted with methanolic ammonia to yield rufinamide as per the scheme illustrated in Figure 1.2.

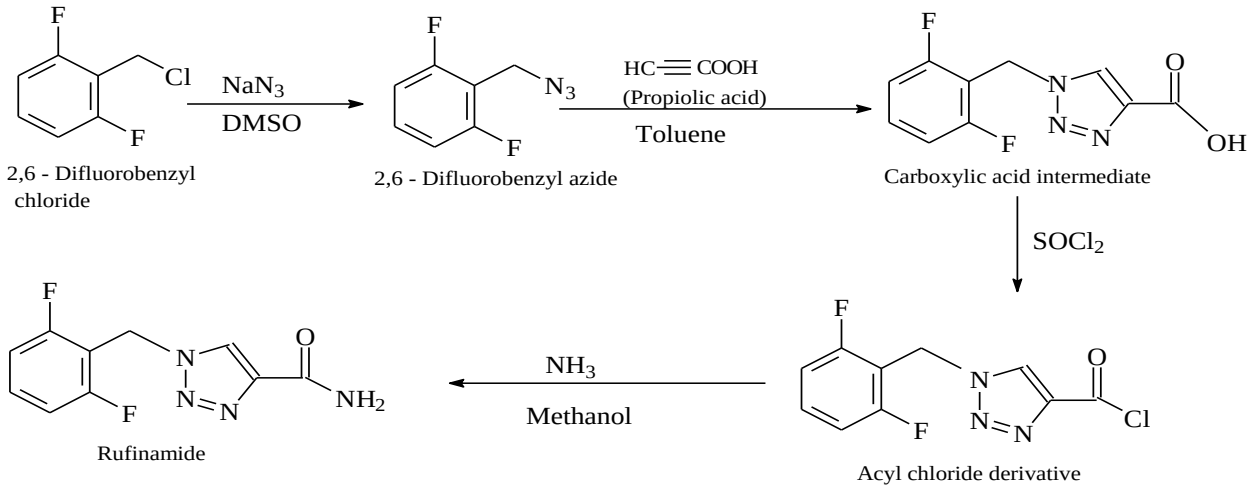
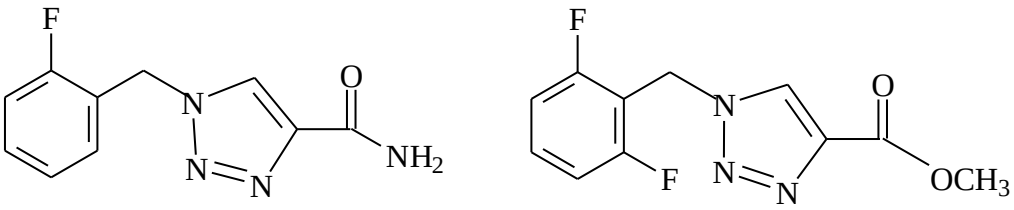



**Figure 1.2: Synthetic scheme of rufinamide.**

From the synthetic scheme, several related substances (RS) may be expected to occur in synthesized samples of rufinamide bulk material. These include 2,6-difluorobenzyl chloride, 2,6-difluorobenzyl azide, the acyl chloride derivative and a dimer of rufinamide. These RS need to be controlled using a suitable sufficiently selective analytical method that should be able to detect the RS in the presence of rufinamide.

The United States Pharmacopeial (USP) Convention further provides for control of two related substances namely rufinamide related compound A (IUPAC name, 1-(2-fluorobenzyl)-1*H*-1,2,3-triazole-4-carboxamide) and rufinamide related compound B (IUPAC name, methyl-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate). The chemical structures of these RS are illustrated in Figure 1.3.




Rufinamide related compound A  Rufinamide related compound B

**Figure 1.3: Chemical structures of rufinamide related compounds A and B.**

## 1.5.5 Stability

Rufinamide is chemically stable. It does not act as a base or an acid in aqueous solutions. It is not hygroscopic and does not absorb water at up to 95% relative humidity (RH) at 25°C (Annapurna *et al*., 2012). When subjected to common degradative stress conditions in aqueous acidic or alkaline solutions, oxidative, photolytic and thermal degradation, rufinamide is highly resistant (Annapurna *et al*., 2012).

## 1.5.6 Pharmacokinetics and adverse effects

Rufinamide has a comparatively slow rate of absorption. However, it is well absorbed with 85% bioavailability whereby absorption is inversely proportional to increase in dose. The presence of food in the stomach enhances the absorption of rufinamide (Patel *et al*., 2011). After the first

single dose, food raises the bioavailability by about 34% (European Medicines Agency, 2011) but repeat dosing is not affected by food (Patel *et al*., 2011).

Rufinamide is metabolized into a pharmacologically inactive acid derivative through hydrolysis of the carboxamide group catalyzed by carboxyesterases. The drug does not interfere with metabolism of drugs or other substrates metabolized by carboxyl esterases. However, it does play a role in induction of CYP3A4 and increases the metabolism of drugs metabolized by CYP3A4. Rufinamide has no effect on CYP1A1 and CYP1A2 (Patel *et al*., 2011). The resulting metabolites are excreted mainly through the kidneys with minute quantities being observed in faeces.

The most common side effects of rufinamide are somnolence, headache, dizziness and fatigue. It also causes central nervous system effects like convulsion, nystagmus, tremor, status epilepticus, psychomotor hyperactivity and abnormal coordination. Nausea, vomiting, upper abdominal pain, constipation, dyspepsia and diarrhea are the common effects of rufinamide in the gastrointestinal tract (European Medicines Agency, 2013).

In the genito-urinary tract, rufinamide may cause oligomenorrhoea which has an incidence rate of not more than 10%. Back pain has also been reported in the musculoskeletal system. Anemia is the most common hematological adverse effect. In addition, lymphadenopathy, leukopenia and thrombocytopenia may occur but very rarely. Rufinamide may also have some metabolic effects like anorexia, eating disorders, decreased appetite and weight loss. First degree atrioventricular block is the most common effect experienced in the cardiovascular system (Teaneck, 2011).

Multi-organ hypersensitivity reactions including skin rashes and acne have also been reported in a few patients (Cerner Multum, 2009).

## 1.6 Methods of analysis of rufinamide

### 1.6.1 Official method

The USP 2015 monograph for rufinamide describes a high performance liquid chromatographic (HPLC) method on a C-18 column (125 mm × 4.6 mm, 5 μm packing size) with methanol – THF - potassium dihydrogen phosphate buffer (15:5:80 % v/v/v) mobile phase delivered at a rate of 1.0 mL/min with 210 nm ultraviolet (UV) detection. The method is however faced with a number of drawbacks. These include: (1) THF is not a preferred solvent in analytical studies as it penetrates the skin and causes rapid dehydration. It is also suspected to be cancerous; (2) the quantity of the buffer being used is very high and this can cause precipitation in the column in the presence of the organic modifier methanol; and (3) the method is not stability indicating as it does not describe how degradation products arising from rufinamide can be separated from the rufinamide peak.

### 1.6.2 High Performance Liquid Chromatographic methods

Most of the methods reported in the literature for the analysis of rufinamide are based on HPLC. These methods are not included in the USP or British Pharmacopoeia (BP) and suffer some drawbacks that limit their utility. Harisudha *et al.* (2013) developed a reversed phase high performance liquid chromatography (RP-HPLC) method for the analysis of rufinamide using C-18 column (250 mm × 4.6 mm, 5 μm) and a mobile phase containing ACN-potassium dihydrogen phosphate (30:70) adjusted to pH 4.5 detected at 210 nm UV absorption

spectroscopy. This method has the disadvantage of having short retention times for the detection of related substances and it is not stability indicating.

Muneer *et al.* (2012) developed a RP-HPLC method for the analysis of rufinamide using a Symmetry C-18 (150 mm × 4.6 mm, 5 µm packing) column and methanol-water (50:50 v/v) mobile phase, pH 3.0 with 220 nm UV detection. The method has limitations of having a very narrow linearity range and it is not stability indicating.

Singh *et al.* (2013) developed an analytical method for the assay of rufinamide using a Phenomenex Luna® C-18 (250 mm × 4.6 mm, 5 µm) column and phosphate buffer-ACN (60:40) mobile phase with 293 nm UV detection. Although the method was isocratic with good retention time and good peak shapes, the long wavelength of detection employed limits its sensitivity.

A stability indicating HPLC method was reported by Annapurna *et al*. (2012) using a C-18 column (250 mm × 4.6 mm, 5 µm) delivered at a rate of 1.0 ml/min and methanol-water (52:48) mobile phase with 210 nm photo-diode array detection. The method was stability indicating with a wide linearity range. However, the total analytical run time is short for the detection of all the related substances.

Patel *et al.* (2014) developed a stability indicating HPLC method for the analysis of rufinamide using a C-18 column (250 mm × 4.6 mm, 5 µm) with ACN-water (60:40 v/v) at pH 7.0 mobile phase. The method is simple and stability indicating but has a short run time (3.043 min) and may not be used for reliable determination of related substances.

### 1.6.3 Spectrophotometric method

Annapurna *et al.* (2013) developed and validated a novel UV spectrophotometric method for the quantitative determination of rufinamide in dosage forms using phosphate buffer pH 8.0 and borate buffer pH 9.0 with a maximum wavelength of absorption observed at 206 nm. However, the method is not stability indicating. Consequently, it does not discriminate between rufinamide related substances and the degradation products.

## 1.7 Study justification

Rufinamide is a very effective drug in the management of seizures related to Lennox-Gastaut Syndrome in children over four years and adults. This drug has been approved for use by the US-FDA for the same condition. However, the drug is only available in the USA and the European Union. In Africa, neither the innovator brand nor any generic of rufinamide is available. This is possibly due to the high cost of the drug. Since rufinamide is not yet registered by the Pharmacy and Poisons Board (PPB), importation is tedious and expensive. Therefore, availability of a good quality and affordable rufinamide generic manufactured locally and registered with PPB, will make it accessible to patients who need it.

As has been reported in literature, the existing methods of analysis of rufinamide suffer various drawbacks. This calls for the development and validation of a relatively cheap, isocratic, selective, specific, sensitive, accurate and stability indicating HPLC method for the analysis of rufinamide and its related substances with UV detection. Such a method should be applicable for routine quality control and market surveillance of rufinamide bulk samples and dosage formulations.

The need for an accurate, reliable, specific and cheap method is necessary to spearhead availability of the drug. This led to the collaboration of the Gesellschaft für International Zusammenarbeit GmbH (GIZ) through the Federation of East Africa Pharmaceutical Manufacturers (FEAPM) with Universal Corporation Limited (Kenya) and the University of Nairobi, to facilitate access to generic rufinamide product(s) in East Africa through local production. This collaboration brought together key partners to create a product development consortium involving industry, academia, pharmaceutical regulator and relevant intellectual property offices through funding from GIZ Pharmaceuticals.

## 1.8 Objectives

### 1.8.1 General Objective

The main aim of this study was to develop a stability indicating high performance liquid chromatographic method for the analysis of rufinamide bulk material and dosage formulations.

### 1.8.2 Specific objectives

1. To develop and optimize a liquid chromatographic method for the analysis of rufinamide.
2. To validate the developed method following the International Conference on Harmonization (ICH) guidelines.
3. To apply the developed and validated method in the assay and stress testing of the rufinamide bulk material.

# CHAPTER TWO

# METHOD DEVELOPMENT

## 2.1 Introduction

Method development is one of the critical areas in pharmaceutical analytical work to ensure quality of active pharmaceutical ingredient (API) and various dosage forms. A good method should be simple, fast, reliable, accurate, precise, robust, reproducible and cost effective utilizing commonly available equipment and reagents.

High performance liquid chromatography is the most commonly applied method in the analysis of pharmaceutical products. Most of the methods highlighted in literature for the analysis of rufinamide API and its dosage forms are based on liquid chromatography. High performance liquid chromatography is usually coupled with different types of detectors, the most widely used being UV. This study involved developing a simple, isocratic, reliable, precise, accurate, reproducible and robust HPLC method for the analysis of rufinamide bulk raw material and dosage forms.

## 2.2 Experimental

### 2.2.1 Chemicals, reagents and solvents

Analytical grade sodium hydroxide pellets, concentrated hydrochloric acid both for degradation studies and monobasic potassium phosphate (Loba Chemie PVt Ltd, Mumbai, India) as a buffer

were used. Analytical grade dibasic potassium phosphate, *tert*-butylammonium hydroxide (TBAOH) 40% w/v solution in water (RFCL Ltd, New Delhi, India) and 85% *ortho*-phosphoric acid (Sigma-Aldrich, St. Louis, MO, USA) were utilized during the study. 1-Octane sulphonic acid sodium salt, 1-hexane sulphonic acid sodium salt, 6% v/v hydrogen peroxide solution (Oxford Lab Chem, Maharashtra, India), disodium hydrogen *ortho*-phosphate (Central Drug House (P) Ltd, New Delhi, India) and potassium bromide (Merck, Darmstadt, Germany) were used during method development. Methanol (Scharlau, Barcelona, Spain) and acetonitrile (Euclid, Mumbai, India) used as organic modifier were of HPLC grade. Purified water was prepared in the laboratory using Aquatron Automatic Water Stills A4000 (Bibby Scientific Ltd, Staffordshire, UK).

### 2.2.2 Rufinamide and related substances

Rufinamide reference standard (potency = 99.7% w/w), rufinamide related compound A (RRCA) and rufinamide related compound B (RRCB) working standards were purchased from United States Pharmacopeial Convention (Rockville, MD, USA).

### 2.2.3 Instrumentation

#### 2.2.3.1 *Melting point apparatus*

The melting points of rufinamide and the related substances were determined using a previously calibrated Stuart melting point apparatus (Barloworld Scientific Ltd, Staffordshire, UK).

### 2.2.3.2 *Infrared spectrophotometer*

A Shimadzu IR Prestige 21 Fourier Transform InfraRed (FTIR) spectrophotometer (Shimadzu Corporation, Kyoto, Japan) making use of IRSolution Software Ver. 1.3 was used to record the infrared spectra of rufinamide and its two related substances. The samples were compressed in potassium bromide discs using a manually operated hydraulic pellet press (Perkin Elmer GmbH, Uberlingen, Germany).

### 2.2.3.3 *Ultraviolet spectrophotometer*

A double beam Genesys 10S UV/VIS spectrophotometer (Thermo Scientific, Massachusetts, MA, USA) and quartz cuvettes of path length 10 mm, were used to obtain the UV spectra of the working standards over the 190 - 390 nm range.

### 2.2.3.4 *pH meter*

A Jenway pH meter (Bibby Scientific Ltd, Stone, Staffs, ST15, OSA, UK) model 3510 (serial number 42630) was used to measure the pH of the buffer and the mobile phase.

### 2.2.3.5 *Liquid chromatographic system*

A Shimadzu Prominence manual HPLC system (Shimadzu Corporation, Kyoto, Japan) was utilized during the study. This system was supported by a CBM 20A (S/N: L20234505098) Prominence communication bus module system controller and a LC Solution software Ver. 1.22, SP1 equipped with a SPD-20A (S/N: L20134506368) Prominence UV/Visible detector which incorporated a deuterium lamp for ultraviolet and a tungsten lamp for visible detection. A LC-20AT (S/N: L2011450625) Prominence solvent delivery system with a dual-plunger tandem-

flow solvent delivery module and a DGU-20$A_3$ (S/N: 20254405376) Prominence degasser were part of the HPLC system. The temperature was controlled using a CTO-10AS VP (S/N: 21044505694) column oven with a block heating thermostatic chamber and a preheater system. All mobile phase preparations were degassed using a bench top Ultrasonic bath cleaner set model WUC-D06H (Daihan Scientific Company Ltd., Seoul, Korea).

## 2.3 Characterization of working standards

Characterization of the working standards was done by means of melting point determination as well as UV and IR spectroscopy.

### 2.3.1 Melting points

The literature melting point ranges for the two working standards are shown in Table 2.1. The experimental melting point values were within the ranges reported in literature (European Medicines Agency, 2011; European Medicines Agency, 2013). This provided preliminary evidence for the identity and purity of the substances.

**Table 2.1: Melting points of working standards**

| | **Melting point** (°C) | |
|---|---|---|
| **Compound** | **Experimental** | **Literature** |
| Rufinamide | 237 - 239 | 237 - 240 |
| Rufinamide related compound A | 198 - 200 | Not reported |
| Rufinamide related compound B | 138 - 139 | 137 – 140 |

### 2.3.2 Ultra-violet absorption spectra

Separately, rufinamide, RRCA and RRCB were dissolved in acetonitrile to yield concentrations of 0.05 mg/ml. The UV absorption spectra were measured in the range 190 - 390 nm using 1 cm quartz cuvettes. The individual spectra obtained are shown in Appendices 1, 2 and 3.

### 2.3.3 Infrared absorption spectra

The FTIR absorption spectra for the working standards were scanned in the range of 4000 - 600 $cm^{-1}$. For this purpose, a 1 cm thick potassium bromide (KBr) disc containing about 1% w/w of each compound was made by triturating the compound with 150 mg of KBr. The spectra obtained are shown in Appendices 4, 5 and 6. These were compared with the corresponding pharmacopoeial (USP, 2015) reference spectra.

### 2.3.4 Purity of the working standards

Rufinamide and the two related substances were used on ‘as is basis’. The percentage purity of rufinamide as per the label claim was 99.7%. The potencies of the two related substances were not given by the manufacturer.

## 2.4 Liquid chromatographic method development

### 2.4.1 Fixed chromatographic parameters

At the onset of method development, some chromatographic parameters were fixed. These parameters include the stationary phase, the detection wavelength, the injection volume and the flow rate.

### 2.4.1.1 *Stationary Phase*

After preliminary investigations, a Phenomenex® Hyperclone base deactivated silanol (BDS) column was selected during the method development as it was found to give the best separation among the evaluated HPLC columns. This was a reversed phase C-18 column measuring 250 mm and 4.6 mm with 5 μm packings. The aperture size of the column packing was 130Å (Phenomenex, Torrance, California, USA).

The choice of this column was based on its potential for wider application. It can be used during liquid chromatographic analysis of a variety of compounds. In addition, being a silica base deactivated column, it may offer greater efficiency and superior peak shapes compared to the conventional silica based reversed C-18 columns.

### 2.4.1.2 *Organic modifier*

At the initial stages of method development, both methanol and acetonitrile were investigated as possible organic modifiers. Acetonitrile achieved separation of most of the compounds under test but it gave long retention times. On the other hand, methanol achieved separation of all the components under analysis with less retention times. Therefore, methanol was chosen as the organic modifier.

### 2.4.1.3 *Detection wavelength*

Each of the working standards except the degradation products were subjected to UV analysis over the 190 – 390 nm range. The UV absorption spectra of these standards were overlaid and they revealed that the maximum wavelength of absorption is below 200 nm. However, the

detection wavelength was set at 210 nm to eliminate absorption instabilities that occur at wavelength below 200 nm.

### 2.4.1.4 *Flow rate and injection volume*

The flow rate tends to influence the column back pressures, analytical time and also the consumption of the mobile phase. The stationary phase packings of most of the analytical columns are designed to work within defined limits of pressures as per the manufacturer's specifications. High mobile phase flow rate may lead to damage of the column as a result of excessive back pressures. Based on the previous studies, the flow rate and injection volume were set at 1.0 mL/min and 20 µL, respectively.

### *2.4.1.5 Reference working solution*

The reference working solutions were prepared by accurately weighing and dissolving the working standards in acetonitrile and water (40:60 % v/v) mixture. The resulting concentrations were as follows: rufinamide 0.8 mg/mL, RRCA 0.2 mg/mL and RRCB 0.2 mg/mL. In addition, about 100 mg of rufinamide was subjected to degradative stress conditions in 1.0 M sodium hydroxide (NaOH, 50 mL) and 3.0% v/v hydrogen peroxide ($H_2O_2$, 50 mL) separately. The resultant mixtures were individually added to the working solution at a volume of 0.1 ml each. Inclusion of the degradation products in the reference working solution was to ensure the development of a stability indicating method.

In order to establish the optimal degradation conditions, rufinamide was subjected to $H_2O_2$ (0.0005%, 0.05%, 1.0%, 2.0% and 3.0% v/v), HCl (0.1 M, 0.5 M and 1.0 M) and NaOH (0.1 M,

0.5 M and 1.0 M). There was no significant degradation of rufinamide in all concentrations of HCl as the degradation products formed were minute in quantity. However, in 3.0% v/v $H_2O_2$ and 1.0 M NaOH, significant degradation products were formed.

### 2.4.2 Chromatographic optimization

During method development, various mobile phases were prepared. In the process organic modifier (methanol), various buffers and ion pairing agents were investigated at varying proportions in the mobile phase composition. The effect of buffer pH was also studied.

#### 2.4.2.1 *Neutral conditions*

Initial analysis of the reference working solution was carried out using unbuffered mobile phase containing methanol-water (40:60, % v/v) mixture. A manual injection of 20 µL into the HPLC system was done with the oven temperature initially set at 40 °C. Under these conditions, poor separation of the analyte compounds was observed as illustrated in Figure 2.1. Only RRCB yielded a separated peak at 9.3 min. There was co-elution of rufinamide and RRCA that formed the critical peak pair. The degradation products were also not resolved.

#### 2.4.2.2 *Buffers*

Several phosphate buffers were incorporated in the mobile phase to determine their effects on separation of the analytes. The evaluated buffers included: (1) potassium dihydrogen phosphate, $KH_2PO_4$, (2) sodium dihydrogen phosphate, $NaH_2PO_4$, (3) dipotassium hydrogen phosphate, $K_2HPO_4$ and (4) diammonium hydrogen phosphate, $(NH_4)_2HPO_4$. Phosphate and acetate buffers are the more commonly used buffers for HPLC with UV detection. This is because they can be

utilized at detection wavelengths below 220 nm. To avoid buffer precipitation in the analytical column in the presence of the organic modifier, the effective concentration of the buffer was fixed at 10 mM. Out of the evaluated phosphate buffers, $K_2HPO_4$ offered the best separation of the analytes.

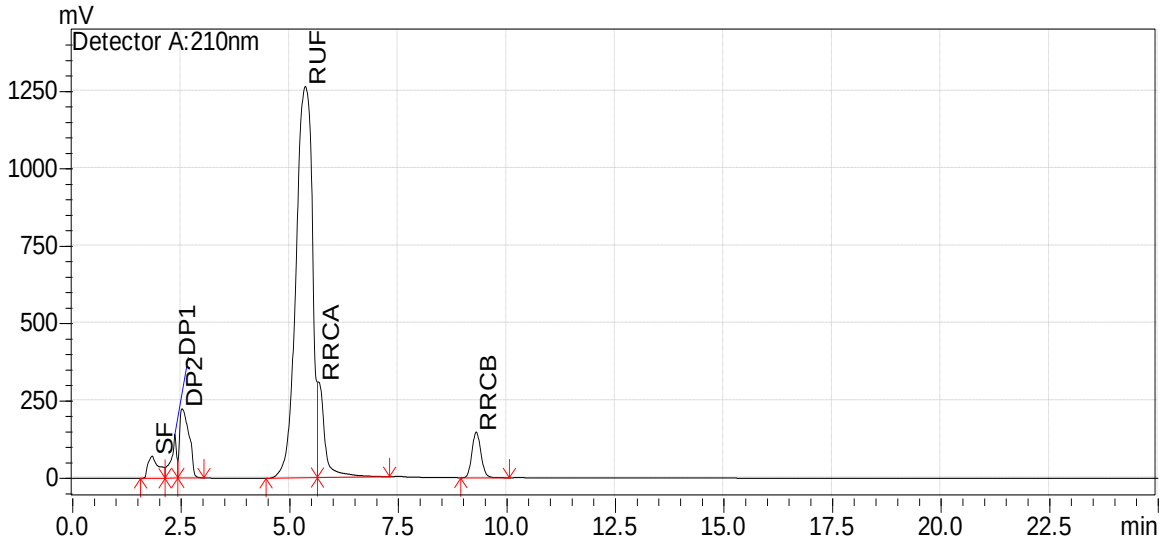



**Figure 2.1: Typical chromatogram of the working mixture using unbuffered mobile phase.** Column: Hyperclone BDS C-18, 250×4.6 mm, 5µm; Column temperature: 40 °C; Mobile phase: methanol-water (40:60, % v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. SF = solvent front; DP1 = degradation product 1; DP2 = degradation product 2; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

A stock solution of 0.1 M of each of the buffer was prepared and incorporated into the mobile phase comprising of water and methanol in the ratio of 10:50:40, % v/v, respectively. The pH of the buffer was not adjusted but nevertheless recorded. The chromatogram obtained using $K_2HPO_4$ pH 10.11 (Figure 2.2) revealed a reduction in the retention times of rufinamide (from 5.4 to 5.1 min) and RRCB (from 9.3 to 8.7 min). However, separation of the critical peak pair of

RUF and RRCA was not achieved. Resolution of degradation products was also not achieved at this point.

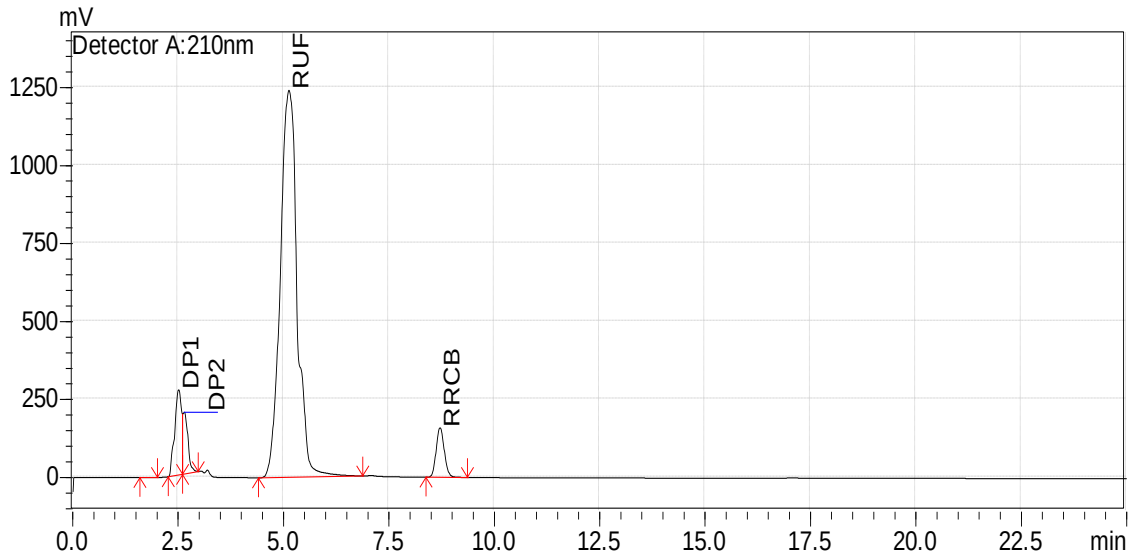



**Figure 2.2: Typical chromatogram of the working mixture using buffered mobile phase**. Column: Hyperclone BDS C18, 250 × 4. 6 mm, 5µm; Column temperature: 40 °C; Mobile phase: Methanol – 0.1M $K_2HPO_4$ - Water (40:10:50, % v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. SF = solvent front; DP1 = degradation product 1; DP2 = degradation product 2; RUF = rufinamide; and RRCB = rufinamide related compound B.

### *2.4.2.3 Buffer pH*

The phosphate buffer pH was controlled using equimolar *ortho*-phosphoric acid. The influence of adjusting the buffer pH on the separation of the peaks of the analytes and other chromatographic parameters were investigated. For this purpose, mobile phases were prepared adjusting buffer pH to 5.0, 5.5, 6.0, 6.5 and 7.0. The results obtained are summarized in Table 2.2 and graphically illustrated in Figure 2.3.

**Table 2.2: Effect of buffer pH on chromatographic parameters of the analytes**

| Mobile phase composition | Analyte component | Retention time (min) | Resolution | Capacity factor | Peak symmetry |
|---|---|---|---|---|---|
| MeOH-0.1 M $K_2HPO_4$ pH 5.0-$H_2O$ (40:10:50, % v/v/v) | DP | 2.55 | 0.08 | 0.04 | - |
| | RUF | 5.32 | 5.02 | 1.17 | - |
| | RRCA | 5.64 | - | 1.30 | - |
| | RRCB | 9.24 | 6.38 | 2.77 | 1.14 |
| MeOH-0.1 M $K_2HPO_4$ pH 5.5-$H_2O$ (40:10:50, % v/v/v) | DP | 2.53 | 0.13 | 0.04 | - |
| | RUF | 4.96 | 4.26 | 1.04 | 1.22 |
| | RRCA | 5.02 | - | - | - |
| | RRCB | 8.27 | 6.19 | 2.41 | 1.15 |
| MeOH-0.1 M $K_2HPO_4$ pH 6.0-$H_2O$ (40:10:50, % v/v/v) | DP | 2.47 | 0.00 | 0.00 | 1.65 |
| | RUF | 4.96 | 4.78 | 1.00 | 1.17 |
| | RRCA | 5.00 | 0.48 | 1.01 | - |
| | RRCB | 8.27 | 8.05 | 2.34 | 1.15 |
| MeOH-0.1 M $K_2HPO_4$ pH 6.5-$H_2O$ (40:10:50, % v/v/v) | DP | 2.53 | 0.00 | 0.00 | 1.24 |
| | RUF | 4.98 | 5.07 | 1.10 | 1.14 |
| | RRCA | 5.01 | 0.47 | 1.41 | - |
| | RRCB | 8.32 | 7.80 | 2.27 | 1.14 |
| MeOH-0.1 M $K_2HPO_4$ pH 7.0-$H_2O$ (40:10:50, % v/v/v) | DP | 2.48 | 1.37 | 0.18 | - |
| | RUF | 4.99 | 5.62 | 1.37 | 1.01 |
| | RRCA | 5.01 | - | 1.37 | - |
| | RRCB | 8.32 | 7.61 | 2.95 | 1.14 |

Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Column temperature: 40 °C; Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. DP = degradation product; RUF = rufinamide; RRCA = rufinamide related compound A; RRCB = rufinamide related compound B.

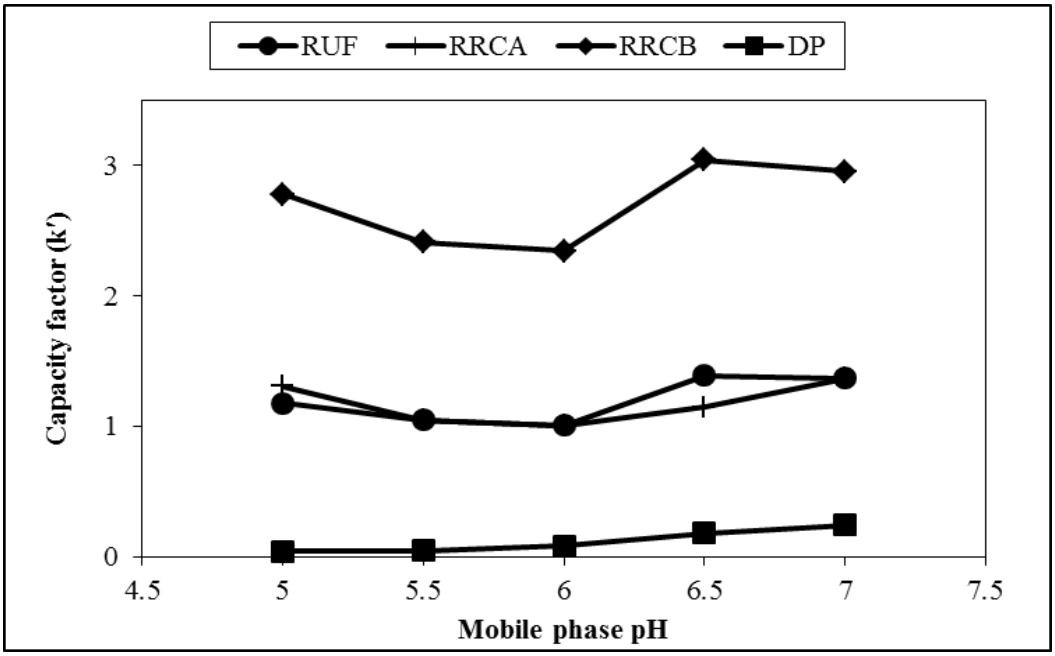



**Figure 2.3: Effect of $K_2HPO_4$ buffer pH on separation of analytes.** Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Column temperature: 40 °C; Mobile phase: Methanol – 0.1M $K_2HPO_4$ - Water (40:10:50, % v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. DP = degradation product; RUF = rufinamide; RRCA = rufinamide related compound A; RRCB = rufinamide related compound B.

From Table 2.2, it can be observed that the retention times of the analytes was high at pH 5.0 and decreased as the pH increased upto pH 6.0 beyond which a marginal rise was observed. While there was separation and resolution of degradation products and RRCB peaks at all pH values investigated, the separation of the critical peak pair of RUF and RRCA was only observed at pH 5.0 and 6.5 as illustrated in Figure 2.3. However, the resolution at pH 6.5 was better than at pH 5.0 and therefore buffer pH of 6.5 was chosen for subsequent method development.

### 2.4.2.4 *Buffer concentration*

In order to improve separation of RUF and RRCA as well as the peak symmetry of all the peaks, the effect of 0.1M $K_2HPO_4$ buffer concentration in the mobile phase was investigated. For this

purpose, effective concentration of 5 mM, 10 mM and 15 mM $K_2HPO_4$ were incorporated in the mobile phase at pH 6.5. The results obtained are summarized in Figure 2.4.

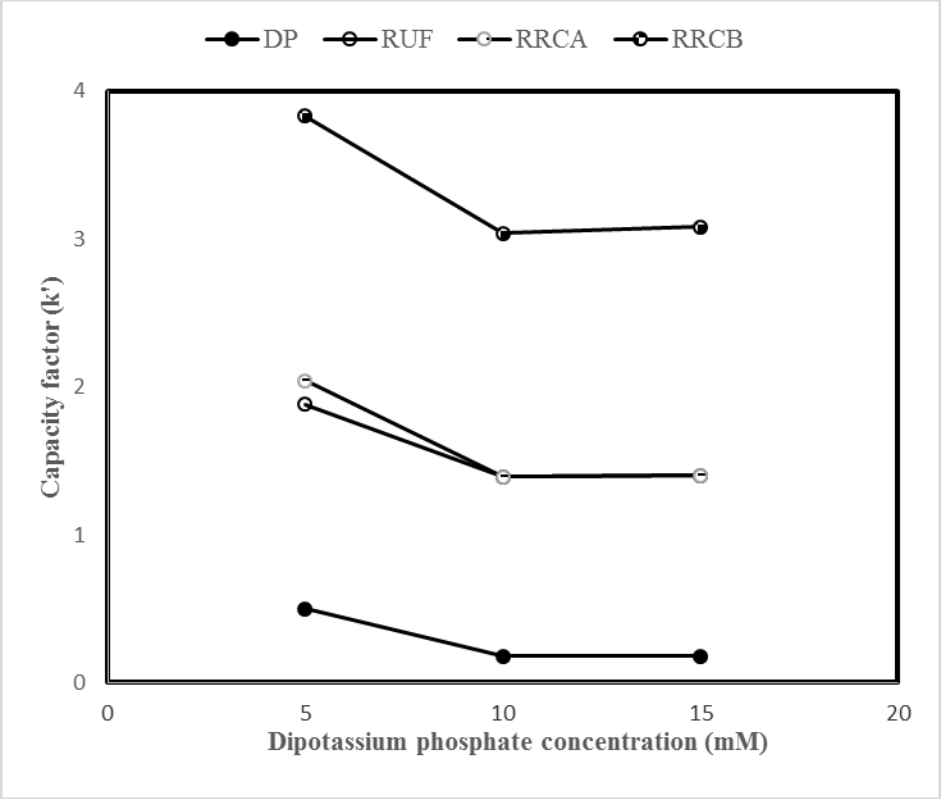



**Figure 2.4: Effect of 0.1M $K_2HPO_4$ buffer concentration on separation of analytes.** Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Column temperature: 40 °C; Mobile phase: methanol – 0.1M $K_2HPO_4$ - water (40:X:50±X, % v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. DP = degradation product; RUF = rufinamide; RRCA = rufinamide related compound A; RRCB = rufinamide related compound B.

The buffer concentration had an influence on the separation of RUF and RRCA peak pair. At buffer concentration of 10 mM and 15 mM, there was co-elution of the critical peak pair as well as all the degradation products. As the buffer concentration decreased, the separation and resolution of the peaks of RUF and RRCA improved. However the retention times of all the components were not significantly affected. For rufinamide, the retention times were 5.0, 5.1 and 5.2 min at effective buffer concentration of 5 mM, 10 mM and 15 mM, respectively.

From the observations on Figure 2.4, an effective buffer concentration of 5 mM was therefore chosen for the subsequent investigations. The chromatogram obtained with 5 mM effective buffer concentration incorporated in the mobile phase at pH 6.5 is shown in Figure 2.5.

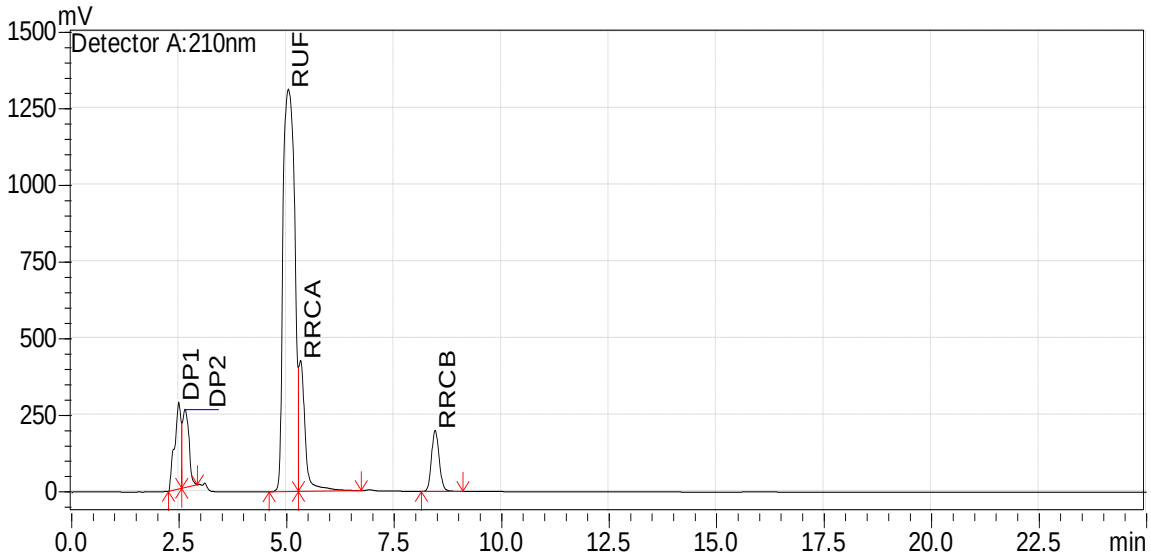



**Figure 2.5: Typical chromatogram of the working mixture using 5 mM of 0.1M $K_2HPO_4$ pH 6.5 in mobile phase.** Column: Hyperclone BDS C18, 250 mm × 4.6 mm, 5 µm; Column temperature: 40 °C; Mobile phase: methanol – 0.1M $K_2HPO_4$ pH 6.5 - water (40:5:55, % v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. SF = solvent front; DP = degradation product 1 and 2; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

### 2.4.2.5 *Methanol concentration*

The influence of methanol concentration in the mobile on the separation of RUF and RRCA peak pair as well as the capacity and resolution factors was studied. The influence of methanol concentration was investigated at four levels, 25% to 40% v/v. The results obtained are illustrated in Figure 2.6 and summarized in Table 2.3.

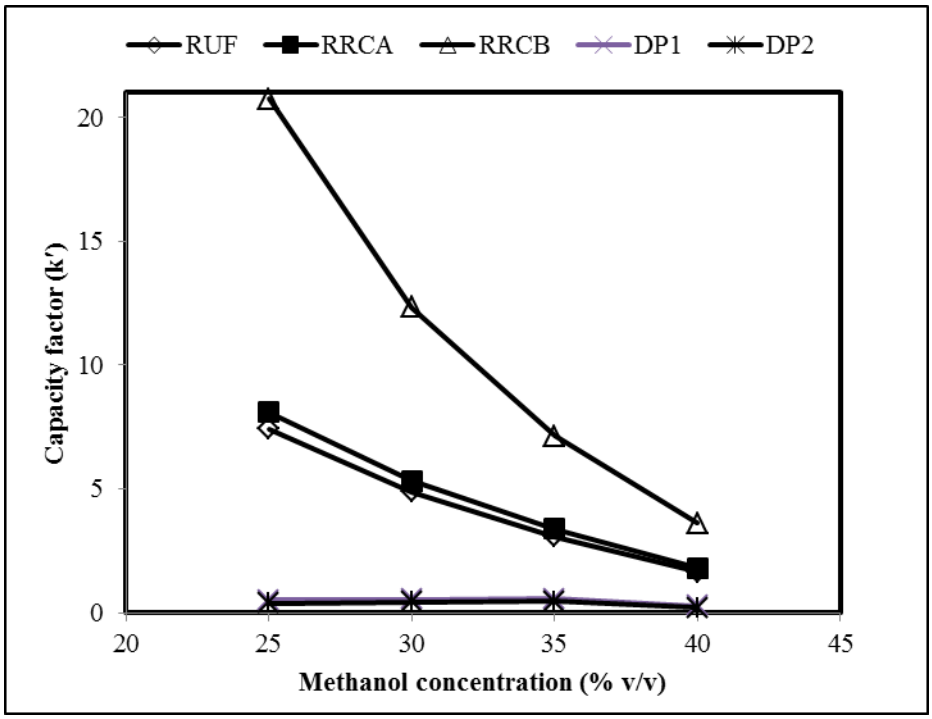



**Figure 2.6: Influence of methanol concentration on separation of analytes.** DP1 = degradation product 1 and 2; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

Methanol concentration had a great influence on the retention times of RUF, RRCA and RRCB. As expected, decreasing methanol concentration increased the retention of these three components with the greatest effect being on RRCB. This is because of the reduction in elution strength as methanol concentration in the mobile phase decreases. The retention times of the degradation products were not significantly affected.

Capacity factors and resolution also increased as methanol concentration was decreased. For the critical peak pair, separation improved with decrease in methanol concentration. The resolution of the critical peak of RUF and RRCA increased from 0.16 to 1.24 while that of RUF with respect to degradation products increased from 3.40 to 19.00. However, at 25% v/v methanol concentration, the run time was more than 20 min thus limiting application of this parameter to

routine analytical work. At 30% methanol concentration, separation of the critical peak pair improved significantly with a capacity factor of 5.32 and resolution of 1.06.

**Table 2.3: Effect of methanol concentration on chromatographic parameters of analytes**

| % v/v methanol concentration | Analyte component | Retention time (min) | Resolution | Capacity factor | Peak symmetry |
|---|---|---|---|---|---|
| 40 | DP1 | 2.07 | 0.00 | 0.00 | - |
| | DP2 | 2.49 | 0.62 | 0.20 | - |
| | DP3 | 2.62 | 0.13 | 0.26 | - |
| | RUF | 5.45 | 3.40 | 1.63 | - |
| | RRCA | 5.77 | 0.16 | 1.78 | - |
| | RRCB | 9.56 | 2.05 | 3.61 | 1.12 |
| 35 | DP1 | 2.37 | 1.88 | 0.36 | - |
| | DP2 | 2.52 | 0.40 | 0.45 | - |
| | DP3 | 2.70 | 0.16 | 0.55 | - |
| | RUF | 7.07 | 3.78 | 3.08 | - |
| | RRCA | 7.57 | 0.79 | 3.36 | - |
| | RRCB | 14.10 | 10.91 | 7.13 | 1.09 |
| 30 | DP1 | 2.39 | 2.78 | 0.43 | - |
| | DP2 | 2.56 | 0.52 | 0.53 | - |
| | DP3 | - | - | - | - |
| | RUF | 9.78 | 13.05 | 4.87 | - |
| | RRCA | 10.54 | 1.06 | 5.32 | - |
| | RRCB | 22.23 | 15.60 | 12.34 | 1.07 |
| 25 | DP1 | 2.39 | 3.51 | 0.39 | - |
| | DP2 | 2.62 | 0.83 | 0.52 | - |
| | DP3 | - | - | - | - |
| | RUF | 14.51 | 19.00 | 7.41 | 1.01 |
| | RRCA | 15.66 | 1.24 | 8.08 | - |
| | RRCB | 37.51 | 19.98 | 20.75 | 1.06 |

Column: Hyperclone BDS C18, 250 × 4.6 mm 5µm; Column temperature: 40 °C; Mobile phase: methanol – 0.1M $K_2HPO_4$ pH 6.5 - water (30:5:65, % v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. DP = degradation product 1, 2 and 3; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

Increasing methanol concentration reduced the retention times of RUF, RRCA and RRCB but significantly affected separation and resolution of the critical peak pair. Thus, a methanol concentration of 30% v/v in the mobile phase was chosen for the subsequent method development as it provided sufficient resolution of the critical peak pair with retention time of 22.2 min for the last eluting peak of RRCB.

### 2.4.2.6 *Ion-pairing agents*

Ion-pairing agents are substances added in the mobile phase to modulate the retention of the ionic components present in a mixture and improve the separation and peak symmetry. The most commonly used ion-pairing agents are alkyl sulfonates and tetra-alkylammonium salts. Other ion-pairing agents are tertiary amine compounds and alkyl sulphates. Usually, ion-pairing agents are incorporated in the mobile phase at very low concentrations of less than 10 mM. These agents act by interacting with acidic and basic components in a mixture. They also interact with the stationary phase to improve on separation of the analyte molecules in the column.

The ion-pairing agents evaluated included triethylamine, *tert*-butyl ammonium hydroxide, triethanolamine, sodium hexane sulphonate and sodium octane sulphonate. Stock solutions containing 0.1 M of each ion-pairing agent were prepared and incorporated in 0.1 M $K_2HPO_4$ buffer before the pH was adjusted to 6.5. A suitable aliquot of ion-pairing agent solution was added to the mobile phase so as to produce a final effective concentration of 5 mM. Only sodium octane sulphonate improved the resolution of the critical peak pair as well as separation of the other analyte components.

Introduction of sodium octane sulphonate reduced the retention times of RUF from 9.78 to 8.97 min, RRCA from 10.54 to 9.80 min and RRCB from 22.23 to 19.13 min, thus reducing the run time to less than 20 min. The resolution of RUF and RRCA peak pair also improved from 1.06 to 1.12.

To further optimize the resolution, the effective concentration of octane sulphonic acid (OSA) was investigated. Octane sulphonic acid sodium salt was incorporated in the buffer at effective concentrations of 5 mM, 10 mM and 15 mM before adjusting the pH. The results obtained are illustrated in Figure 2.7 and summarized in Table 2.4.

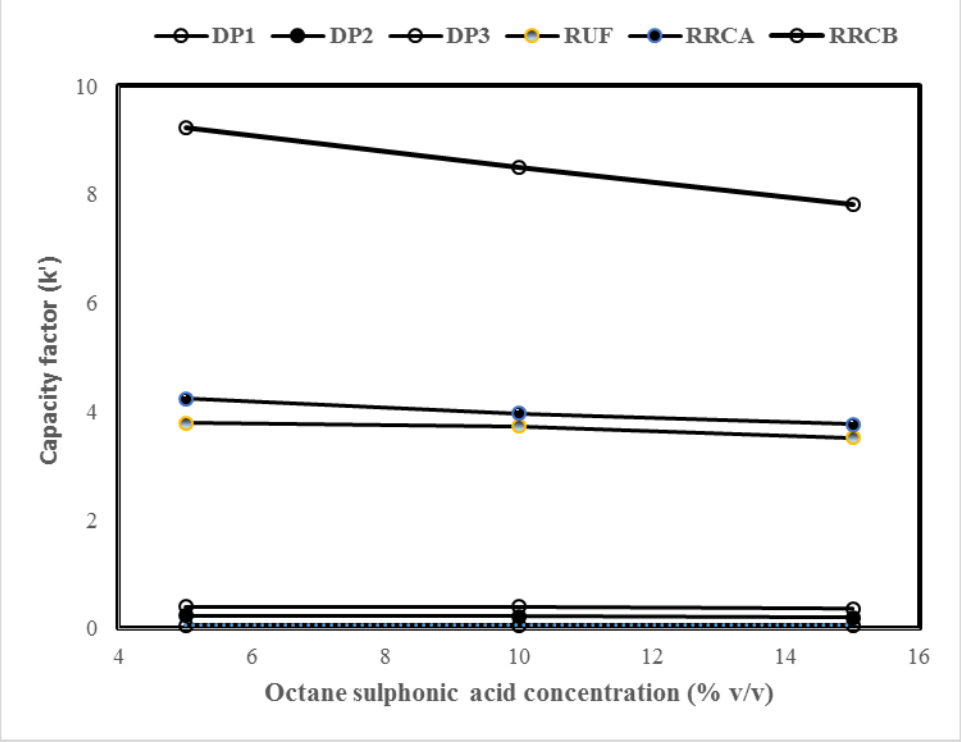



**Figure 2.7: Influence of octane sulphonic acid concentration on separation of analytes.** DP = degradation product 1, 2 and 3; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

**Table 2.4: Effect of octane sulphonate concentration on chromatographic parameters of analytes**

| Effective OSA concentration (mM) | Analyte component | Retention time (min) | Resolution | Capacity factor | Peak symmetry |
|---|---|---|---|---|---|
| 5 | DP1 | 1.99 | 1.12 | 0.06 | 1.63 |
| | DP2 | 2.33 | 1.69 | 0.24 | 0.00 |
| | DP3 | 2.63 | 0.93 | 0.40 | 0.00 |
| | RUF | 8.97 | 10.63 | 3.79 | 0.00 |
| | RRCA | 9.80 | 1.11 | 4.24 | 0.00 |
| | RRCB | 19.13 | 13.66 | 9.23 | 1.09 |
| 10 | DP1 | 1.99 | 0.54 | 0.06 | 1.75 |
| | DP2 | 2.29 | 1.62 | 0.22 | 1.50 |
| | DP3 | 2.64 | 1.44 | 0.40 | 1.21 |
| | RUF | 8.88 | 16.77 | 3.73 | 0.74 |
| | RRCA | 9.32 | 1.16 | 3.97 | 1.10 |
| | RRCB | 17.79 | 18.73 | 8.49 | 1.09 |
| 15 | DP1 | 2.00 | 0.63 | 0.06 | 1.67 |
| | DP2 | 2.28 | 1.54 | 0.20 | 0.00 |
| | DP3 | 2.59 | 1.21 | 0.37 | 1.50 |
| | RUF | 8.56 | 22.56 | 3.52 | 0.99 |
| | RRCA | 9.02 | 1.68 | 3.76 | 0.00 |
| | RRCB | 16.68 | 17.25 | 7.81 | 1.09 |

Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Column temperature: 40 °C; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5 - water (30:X:5:65-X, % v/v/v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. OSA = octane sulphonic acid sodium salt; DP = degradation product 1, 2 and 3; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

The introduction of ion-pairing agent in the mobile phase led to overall reduction of retention times of the analyte molecules in the working solution. Varying the effective concentration of sodium octane sulphonate from 5 mM to 15 mM resulted in reduction of retention times of almost all the components under analysis. The reduction was least with the degradation products. The resolution of RUF and RRCA peak pair also increased from 1.11 to 1.68 as the concentration of the ion-pairing agent was increased. Therefore, a concentration of 10 mM of

sodium octane sulphonate was chosen as the optimum for subsequent method development. At this concentration, symmetry of the critical peaks was 0.74 and 1.10 for RUF and RRCA, respectively. The chromatogram obtained at a concentration of 10 mM of OSA is illustrated in Figure 2.8.

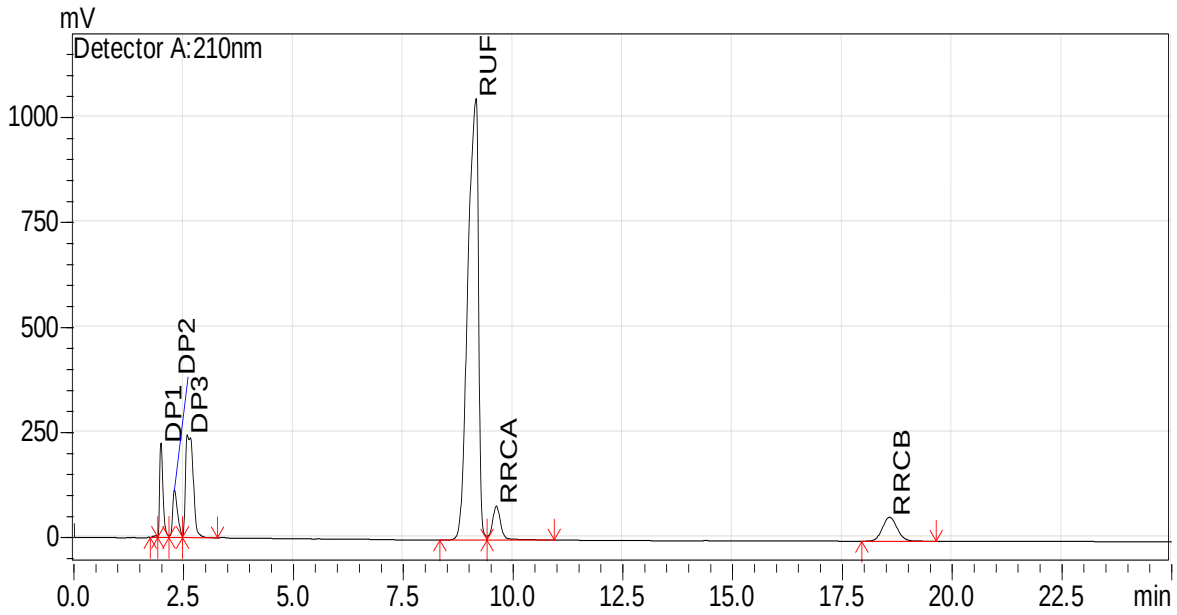



**Figure 2.8: Typical chromatogram of the working mixture using 10 mM of 0.1 M OSA.** Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Column temperature: 40 °C; Mobile phase: methanol – 0.1 M OSA - 0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. OSA = octane sulphonic acid sodium salt; DP = degradation product 1, 2 and 3; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

### 2.4.2.7 *Column temperature*

Temperature is very critical in liquid chromatographic separation process since it affects the viscosity and density of the mobile phase, and consequently, column back pressures. At high temperatures, many liquid mobile phases tend to have lower density and viscosity resulting in lower column back pressures. As the temperatures are increased, the degree of mass transfer of the components through the column increases. This results in reduced retention of the analytes in the column with an overall decrease in the analysis run time. Selectivity of the components may

also be affected by change in the column temperatures. Further, temperature influences separative efficiency of silica-based columns. Above 60 ℃, the packing of the silica based columns exhibit instability dependent on the mobile phase pH. In addition, majority of analytes may be degraded during chromatographic separation.

The influence of temperature on the separation of degradation products, RUF, RRCA and RRCB was investigated while maintaining the already optimized mobile phase composition of methanol - 0.1 M dipotassium hydrogen phosphate pH 6.5 - 0.1 M octane sulphonic acid - water (30:5:10:55, % v/v/v/v). Temperature was investigated in the 35 - 50 ℃ range at 5 ℃ intervals. The results obtained are outlined in Table 2.5.

It can be observed that as the temperature was increased there was systematic reduction of retention times of RUF, RRCA and RRCB. Retention times of degradation products were affected marginally. The total run time was also influenced such that as temperature increased from 35 ℃ to 50 ℃, the run time reduced by almost 3 min from about 20 min to 17 min. Increasing the temperature affected resolution of the components variably. For RUF, resolution increased from 13.18 to 18.18 as temperature increased from 35 ℃ to 40 ℃, thereafter decreasing as temperature rose to 50 ℃. In the case of RRCA, resolution decreased from 2.16 at 35 ℃ to 0.98 at 45 ℃ and then increased to 1.08 at 50 ℃. However, resolution of RRCB increased from 18.3 at 35 ℃ to 19.2 at 50 ℃. The resolution of RUF and RRCA peak pair was greatest (2.16) at 35 ℃. The effect of raising temperature of the column was found to negligibly improve peak symmetry.

**Table 2.5: Effect of column temperature on chromatographic parameters of analytes**

| Column temperature (°C) | Analyte component | Retention time (min) | Resolution | Capacity factor | Peak symmetry |
|---|---|---|---|---|---|
| 35 | DP1 | 1.98 | 0.50 | 0.06 | 1.71 |
| | DP2 | 2.29 | 1.63 | 0.22 | 0.00 |
| | DP3 | 2.65 | 0.97 | 0.41 | 0.00 |
| | RUF | 9.45 | 13.18 | 4.05 | 1.04 |
| | RRCA | 10.41 | 2.16 | 4.42 | 0.00 |
| | RRCB | 19.59 | 18.34 | 9.47 | 1.09 |
| 40 | DP1 | 1.97 | 0.56 | 0.06 | 1.76 |
| | DP2 | 2.28 | 1.66 | 0.22 | 1.41 |
| | DP3 | 2.57 | 1.15 | 0.38 | 2.02 |
| | RUF | 9.15 | 18.18 | 3.91 | 0.69 |
| | RRCA | 9.61 | 1.21 | 4.16 | 0.00 |
| | RRCB | 18.57 | 18.84 | 8.98 | 1.08 |
| 45 | DP1 | 1.98 | 0.60 | 0.06 | 1.73 |
| | DP2 | 2.27 | 1.62 | 0.22 | 1.47 |
| | DP3 | 2.62 | 1.39 | 0.40 | 1.20 |
| | RUF | 8.82 | 15.87 | 3.72 | 0.70 |
| | RRCA | 9.21 | 0.98 | 3.93 | 0.00 |
| | RRCB | 17.63 | 18.94 | 8.44 | 1.09 |
| 50 | DP1 | 1.98 | 0.58 | 0.06 | 1.75 |
| | DP2 | 2.27 | 1.58 | 0.21 | 0.00 |
| | DP3 | 2.62 | 1.43 | 0.40 | 1.15 |
| | RUF | 8.30 | 13.52 | 3.43 | 0.95 |
| | RRCA | 8.76 | 1.08 | 3.68 | 0.00 |
| | RRCB | 16.62 | 19.21 | 7.8 | 1.10 |

Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. OSA = octane sulphonic acid sodium salt; DP = degradation product 1, 2 and 3; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

Based on the observations made on the effect of column temperature, particularly on the resolution of RUF and RRCA peak pair, 35 °C was found to be the optimum column temperature as illustrated in Figure 2.9. Lower temperatures resulted in higher resolution of the critical peak pair but lengthened the total analytical time, a situation not ideal in analytical work, and increased the mobile phase consumption. This would push up the overall cost of analysis.

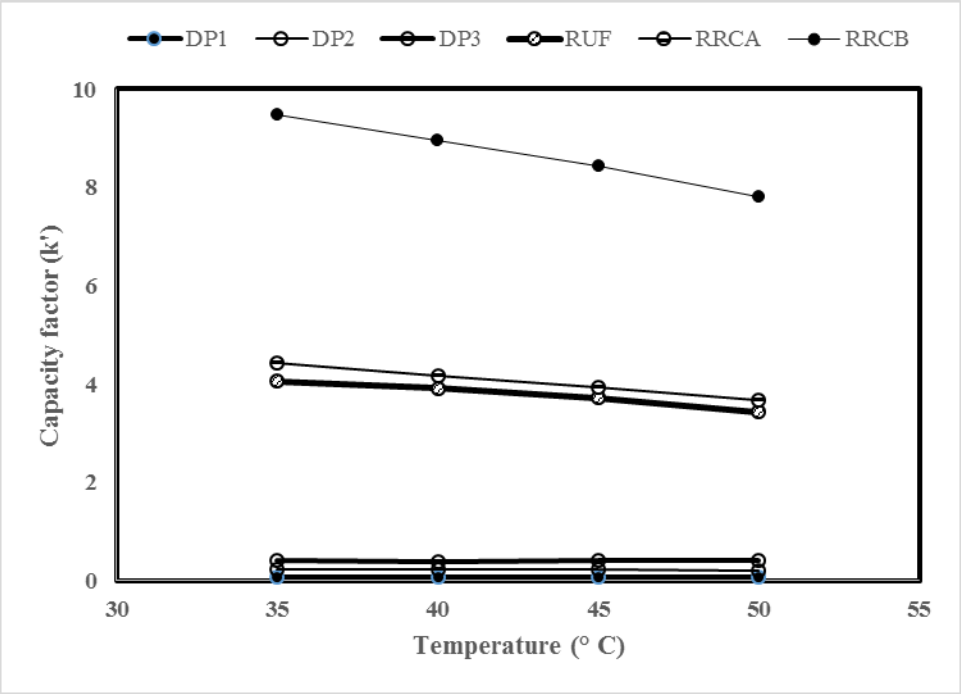



**Figure 2.9: Influence of column temperature on separation of analytes.** Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. OSA = octane sulphonic acid sodium salt; DP = degradation product 1, 2 and 3; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

## 2.4.3 Optimized chromatographic conditions

The optimum chromatographic conditions for the separation were established as a mobile phase comprising of methanol - 0.1 M octane sulphonic acid - 0.1 M dipotassium hydrogen phosphate pH 6.5 - water (30:10:5:55 % v/v/v/v) delivered at a rate of 1.00 mL/min and the stationary

phase comprising of a reversed phase Phenomenex® Hyperclone BDS C-18 chromatographic column of dimensions 250 mm × 4.6 mm with particle size of 5 µm maintained at 35 °C with detection wavelength set at 210 nm.

The representative chromatogram illustrating the separation of the analytes contained in the working mixture solution analyzed under the established optimum conditions is shown in Figure 2.9. According to the USP 2015, RRCB is used for identification purposes only. This implies that the total analysis run time could be reduced to around 12 min since the main RUF peak elutes at 9.4 ± 0.2 min.

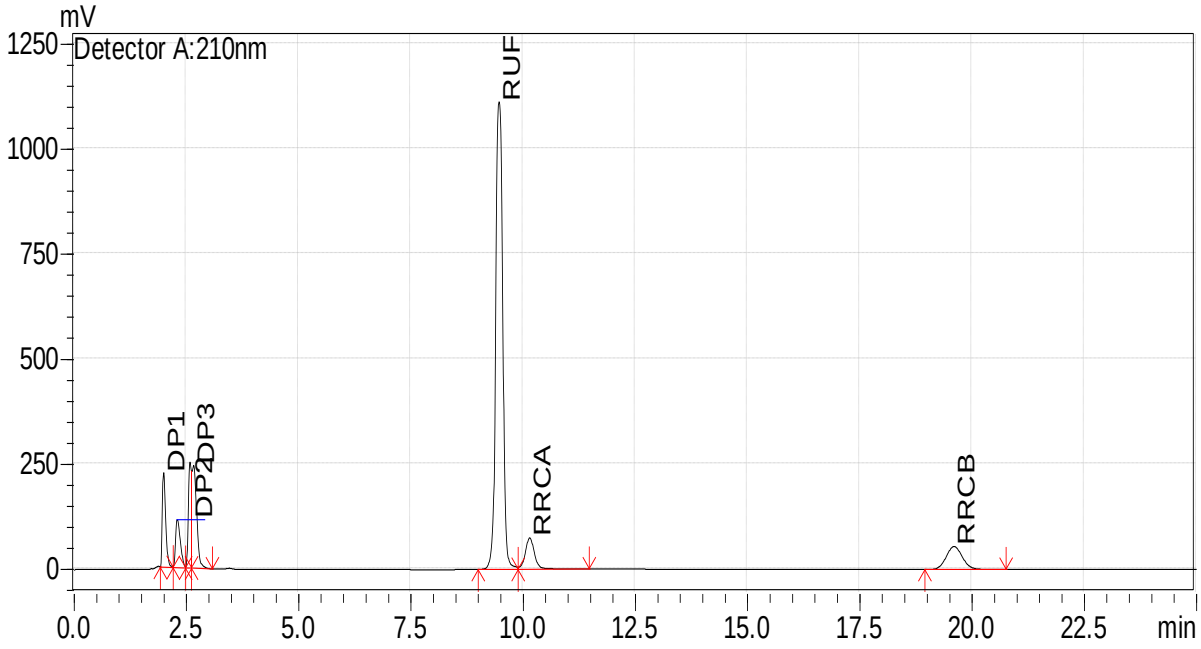



**Figure 2.10: Typical chromatogram of the working mixture under optimized conditions.** Column: Phenomenex® Hyperclone BDS C18, 250 mm × 4.6 mm, 5µm; Column temperature: 35 °C; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. OSA = octane sulphonic acid sodium salt; DP = degradation product 1, 2 and 3; RUF = rufinamide; RRCA = rufinamide related compound A; and RRCB = rufinamide related compound B.

# CHAPTER THREE

# METHOD VALIDATION

## 3.1 Introduction

The main aim of validating an analytical procedure is to illustrate that it is suitable for its particular use as per ICH guidelines (ICH Q2B, R1, 2005). An analytical method validation is the process of determining that the attributes of a method meet the essentials for its intended purpose in analysis. During method development the attributes that are studied include precision, specificity, accuracy, linearity, range, sensitivity and robustness. These are investigated through laboratory studies as underlined by ICH guidelines.

## 3.2 Linearity and range

The linearity of a procedure is its capacity to obtain results that are proportional to the analyte concentration in the sample within a specified range. The ICH guidelines recommend linearity to be demonstrated with at least five concentrations of the analytical working concentration over the range of 80% to 120% (ICH Q2B R1, 2005). Linearity is normally described by the coefficient of determination ($r^2$) of the regression line slope. Linearity acceptance criteria is that $r^2$ should be greater than 0.99 for the least squares method of regression line analysis (ICH, 2005). The range of a method is the span between the upper and the lower analyte concentration in the sample for which it has been shown that the method has a satisfactory measure of

accuracy, precision and linearity (ICH Q2B, R1, 2005). The range of a method is usually derived from linearity studies depending on the intended procedure application.

Linearity of the developed method was determined by preparing a solution of rufinamide working standard (1.5 mg/mL) from which working solutions were prepared by diluting appropriately using acetonitrile and water (40:60 % v/v) to yield solutions containing 50%, 75%, 100%, 125% and 150% of 0.8 mg/mL of rufinamide. Each of the solution was then analyzed at the optimum chromatographic conditions in triplicate. The normalized peak areas were plotted against the concentration. The data obtained was further subjected to linear regression analysis as illustrated in Figure 3.1. The developed method was found to exhibit good linearity over the 0.2 – 1.2 mg/mL (corresponding to 50% - 150%, respectively) range of the analytical concentration with a linear regression coefficient, $r^2$, value of 0.9997.

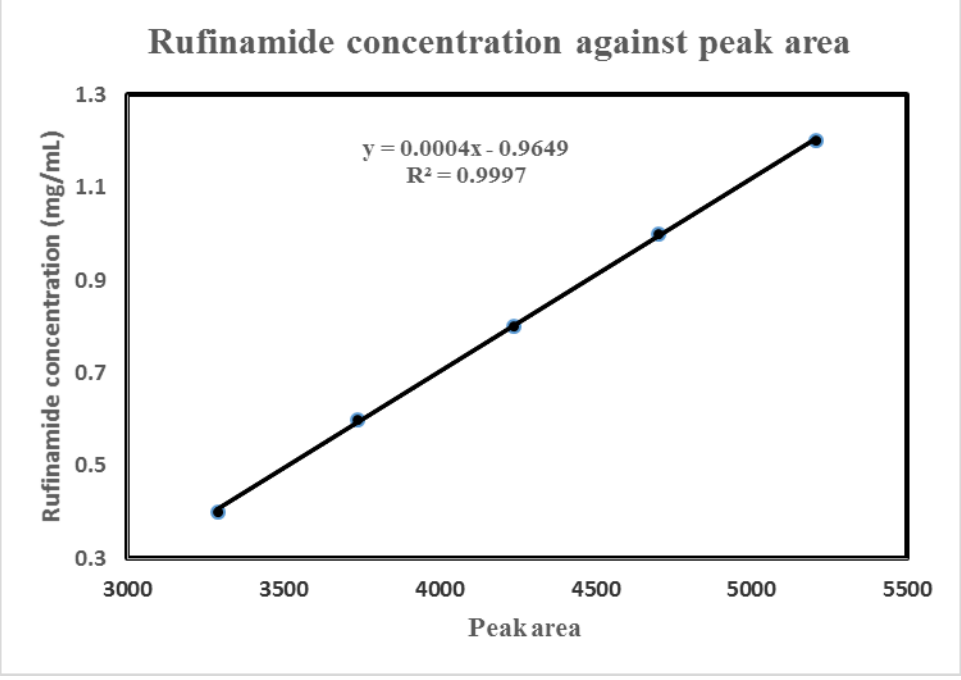



**Figure 3.1: Linear regression showing rufinamide concentration against normalized peak area.**

## 3.3 Precision

The precision of a method is the extent of scatter to which each test result from replicate analysis of the same sample after multiple sampling are similar to one another under the prescribed conditions (ICH Q2B, R1, 2005). Precision is expressed by the variance, relative standard deviation (RSD) and confidence interval of the results obtained from a number of similar determinations of a homogenous authentic sample. Precision is considered at three levels: repeatability, intermediate precision and reproducibility (ICH Q2B, R1, 2005, ICH, 2005).

### 3.3.1 Repeatability

Repeatability expresses precision under similar working conditions over a short time (ICH Q2B, R1, 2005). Repeatability is assessed by at least nine tests within a range of the method. The coefficient of variation (CV) should not be more than 2.0% for the replicate injections (FDA, 2014; Shabir and Bradshaw, 2011).

In this study, repeatability was determined by preparing three different standard concentration of rufinamide containing 0.8 mg/mL on the same day and subsequently injecting each solution into the chromatographic system six times under optimized conditions. The CV of the peak areas was calculated and found to be 0.96%. This was within the ICH recommended limit (FDA, 2014; Shabir and Bradshaw, 2011).

### 3.3.2 Intermediate precision

Intermediate precision or inter-day precision, expresses precision within laboratory variations such as different analysts, days and equipment (ICH Q2B R1, 2005). In this study, inter-day

precision was shown by running five replicate injections of a freshly prepared rufinamide standard solution (0.8 mg/mL) on different days over a three day period. For each day of analysis, fresh mobile phase was prepared. The coefficient of variation of the normalized peak areas obtained was calculated and found to be 0.64% for the three days as illustrated in Table 3.1. The CV was less than 2% for all the determinations, indicating that the developed method was precise (FDA, 2014; Shabir and Bradshaw, 2011).

**Table 3.1: Precision results**

| | **Repeatability** | **Intermediate precision** |
|---|---|---|
| Coefficient of variation (CV) | 0.96 | 0.64 |

Column: Hyperclone BDS C18, 250 mm × 4.6 mm, 5µm; Mobile phase: methanol – 0.1 M OSA - 0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. OSA = octane sulphonic acid sodium salt.

## 3.4 Specificity

Specificity of a procedure is the capacity to evaluate the analyte in the presence of components likely to be present, for example, degradation products, excipients and impurities (ICH Q2B R1, 2005; Green, 1996). Specificity is therefore the extent of the degree of interference of the main peak from other substances that are likely to be present in the sample. In HPLC, the chromatographic parameters commonly used to express specificity are resolution, efficiency and tailing factor. In this study, resolution between rufinamide and all the other components was greater than 1.5. The efficiency of the stationary phase or column was more than 1500 theoretical plates for rufinamide peak. The symmetry factor was less than 2 as per ICH guidelines (ICH, 2005; Green, 1996). The developed method was therefore found to be specific for rufinamide.

## 3.5 Sensitivity

The detection limit (LOD) is the lowest concentration or quantity of an analyte that can be detected but not necessarily quantified as a precise amount under specified experimental conditions (ICH Q2B R1, 2005; ICH, 2005). The LOD was determined by preparing serial dilutions of rufinamide 1 mg/mL stock solution. The solutions were injected in triplicate and the signal to noise (S/N) ratio values of the rufinamide height determined with reference to a blank injection of the diluent solution run under the same chromatographic conditions. The LOD was obtained from the lowest rufinamide concentration that yielded a defined peak at S/N ratio of 2 - 3. Limit of detection was found to be 7.81 μg/mL.

The quantitation limit (LOQ) is the lowest concentration of an analyte in a given sample that can be quantified with suitable precision and accuracy under specified experimental conditions (ICH Q2 R1, 2005; ICH, 2005). The acceptance criteria of LOQ is that the degree of precision from peak areas of replicate injections (n=3) is RSD of less than 10 – 20% and at a S/N ratio of 10 (ICH, 2005). Limit of quantitation of the developed method was determined in a similar procedure for LOD and was found to be 15.53 μg/mL with RSD of 0.78%.

Both LOD and LOQ values demonstrated that the developed method is sensitive.

## 3.6 Robustness

The robustness of a method is the ability to remain unaffected by small intentional variations in the method characteristics (ICH, 2005; Green, 1996). Normally, experimental designs are utilized in testing robustness. This involves use of special computer software like Statgraphics®

Centurion XVI that determines the number of experiments and factors to be adjusted in addition to evaluating the data obtained.

In absence of such a tool for comprehensive robustness experimental design, simple robustness of the developed method was evaluated from the degree of variation observed in peak areas and retention times from the same rufinamide standard solution analyzed while adjusting each of the following liquid chromatographic factors: (1) methanol concentration; (2) pH of the buffer; and (3) the column temperature. Having adjusted a particular chromatographic factor, six similar injections of the same rufinamide standard solution were run and the RSD of both peak areas and retention times of rufinamide peak calculated. The degree of variation observed was then used to infer the robustness of the method.

The effect of altering each of the three chromatographic factors was tested at three levels as illustrated in Table 3.2. The results obtained are summarized in Table 3.3.

**Table 3.2: Levels of robustness**

| **Parameter** | **Levels** | | |
|---|---|---|---|
| | **-1** | **0** | **+1** |
| Methanol concentration (% v/v) | 25 | 30 | 35 |
| Buffer pH | 6.0 | 6.5 | 7.0 |
| Column temperature (° C) | 35 | 40 | 45 |

Column: Hyperclone BDS C18, 250×4.6 mm, 5µm); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL.

From Table 3.3, it can be observed that the method was not significantly affected by changes in the three chromatographic factors namely: methanol concentration, column temperature and pH of the buffer system. By altering all the three chromatographic factors, quantification of the rufinamide peak was not largely affected.

**Table 3.3: Influence of mobile phase methanol concentration, buffer pH and column temperature on peak area and retention time of rufinamide**

| Chromatographic factor changed | Factor level | Peak area RSD (%) | Retention time RSD (%) |
|---|---|---|---|
| Column temperature (°C) | 35 | 0.36 | 0.06 |
| | 40 | 0.11 | 0.11 |
| | 45 | 0.36 | 0.16 |
| Mobile phase methanol concentration (% v/v) | 25 | 0.53 | 0.18 |
| | 30 | 0.55 | 0.09 |
| | 35 | 0.53 | 0.09 |
| Mobile phase buffer pH | 6.0 | 0.66 | 0.05 |
| | 6.5 | 0.86 | 0.09 |
| | 7.0 | 0.65 | 0.06 |

Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. RSD = relative standard deviation.

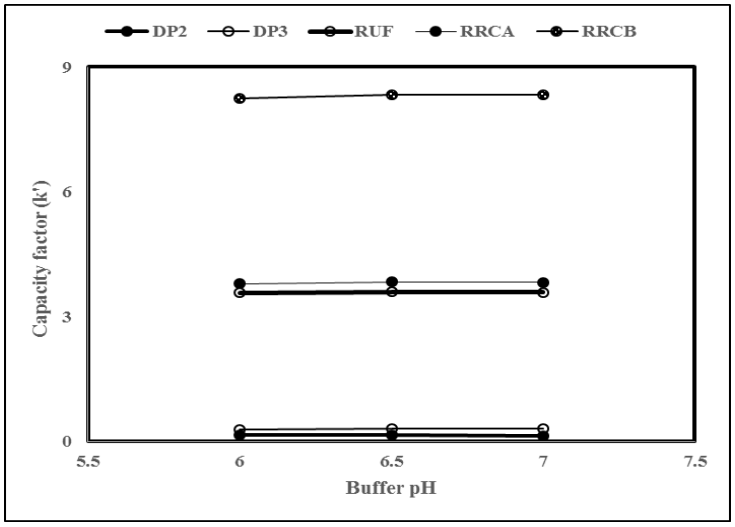
Figure 3.2 demonstrates the effects of changing buffer pH, methanol concentration and column temperature on the capacity factors of the component peaks. Resolution was determined with reference to the immediate preceding peak. For DP2, resolution was determined with respect to the DP1 peak.

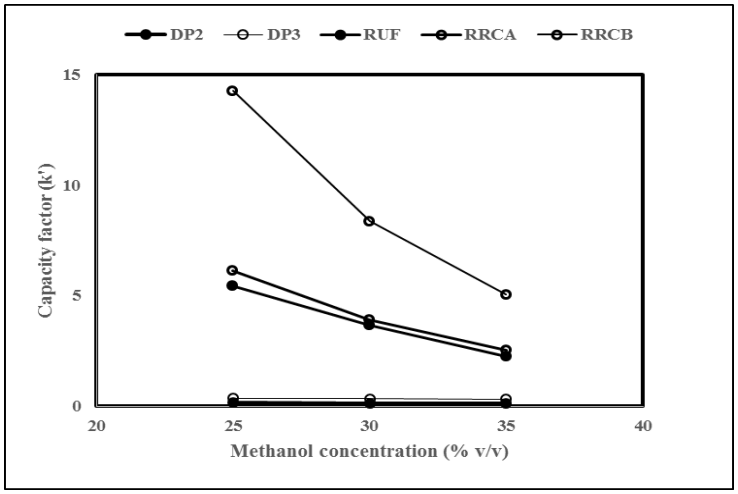
The greatest effect on the chromatographic factors was observed to be with methanol concentration. Both the capacity factor and resolution were greatly influenced implying the need for accurate measurement of methanol concentration during the mobile phase preparation to ensure the analysis time is not interfered with. However, the resolution between RUF and RRCA was greater than 2.0 indicating the robustness of the method.

The consequence of buffer pH and column temperature on the capacity and resolution factors was less significant compared to that of altering the methanol concentration. In both cases, the resolution of RUF and RRCA peak pair was within the acceptable limits.

## 3.7 Accuracy

The accuracy of a method is the measure of closeness of consistency between the standard value, which is either accepted as a conventional true value or an acceptable reference value, and the experimentally determined value. Accuracy should be determined across the range of the method (ICH Q2 R1, 2005; ICH, 1994). Accuracy is reported as a percentage of analyte recoverable by assay by spiking placebo samples (ICH, 2005).



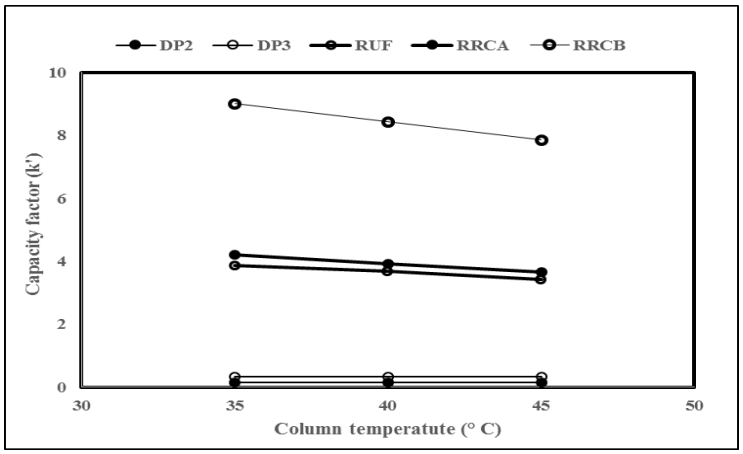




**Figure 3.2: Influence of buffer pH, mobile phase methanol concentration and column temperature on capacity factors.** Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5-water (30:10:5:55, % v/v/v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL.

In this study, accuracy was demonstrated by a placebo mixture consisting of hydroxypropyl methylcellulose (HPMC) and microcrystalline cellulose (MCC) spiked with known amounts of rufinamide. Accuracy is normally assessed with at least nine determinations at three concentration levels covering a certain range. The three concentration levels are injected into the developed system in triplicate. The mean recovery of the assay should be within 100 ± 2.0% at each concentration level over the range of 80 - 120% of the nominal concentration. The results obtained are summarized in Table 3.4. The developed method was found to be accurate because the mean recovery determined was 99.5% which is within the limits specified in the ICH guidelines (ICH, 2005).

**Table 3.4: Recovery of rufinamide from spiked samples**

| Target concentration (%) | Amount added (mg) | Amount recovered (mg) | Recovery (%) | Recovery (CV) | Mean recovery |
|---|---|---|---|---|---|
| 80 | 0.9 | 0.892 | 99.1 | 0.23 | 99.5 |
| 100 | 1.1 | 1.092 | 99.3 | 0.11 | |
| 120 | 1.3 | 1.302 | 100.2 | 0.42 | |

Column: Hyperclone BDS C18, 250 mm × 4.6 mm, 5µm; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.0 mL/min; Detection: 210 nm; Injection volume: 20µL. CV= coefficient of variation.

# CHAPTER FOUR

# ANALYSIS OF SAMPLES

## 4.1 Introduction

The main purpose of development and validation of any analytical method is to create a reliable procedure that may be utilized in the analysis of commercial products. The method developed and validated in this study was intended for analyzing generic rufinamide for use in the East African region as well as Africa as a whole. Generic rufinamide will be manufactured by Universal Corporation Limited in collaboration with FEAPM through GIZ funding. The generic formulation studies took place in the Department of Pharmaceutics and Pharmacy Practice of the University of Nairobi.

## 4.2 Acquisition of samples

Test samples were tablets and included the rufinamide brand product (Inovelon®) 200 mg tablets purchased from United Kingdom and formulated generic rufinamide 200 mg tablets. The formulated tablet batches were coded as F1, F2, F3 and F4, and had composition illustrated in Table 4.1.

**Table 4.1: Composition of formulated generic rufinamide tablets**

| Ingredient | Weight (mg) | | | |
|---|---|---|---|---|
| | **F1** | **F2** | **F3** | **F4** |
| Active ingredient (rufinamide) | 200 | 200 | 200 | 200 |
| Mannitol | 200 | 200 | 200 | 200 |
| Hydroxypropyl methylcellulose (HPMC) | 122 | - | - | 61 |
| Microcrystalline cellulose (MCC) | - | 122* | 122* | 61 |
| Lactose monohydrate | 20 | 20 | 20 | 20 |
| Maize starch | 22 | 22 | 22 | 22 |
| Sodium lauryl sulphate (SLS) | 3 | 3 | 3 | 3 |
| Sodium carboxymethylcellulose | 24 | 24 | 24 | 24 |
| Magnesium stearate | 6 | 6 | 6 | 6 |
| Anhydrous colloidal silica | 3 | 3 | 3 | 3 |
| **Total** | **600** | **600** | **600** | **600** |

*MCC was incorporated intragranularly and extragranularly in F2 and F3, respectively.

## 4.3 Sample preparation

Rufinamide standard (25 mg) was accurately weighed, transferred to a 100 mL volumetric flask and dissolved in acetonitrile to the mark. The mixture was sonicated for about 5 min to ensure complete dissolution. Two millilitres of the resulting solution were transferred into a 50 mL volumetric flask and diluted to the mark using ACN - water (60:40, % v/v) mixture. The solution was sonicated for 15 min, filtered and 20 µL injected into chromatographic system for analysis using the developed HPLC method.

Sample solutions of the brand and generic products were prepared by separately pulverizing 20 tablets to a fine powder using mortar and pestle. A weight of the powder equivalent to 25 mg of rufinamide was weighed and transferred into a 100 mL volumetric flask. Acetonitrile was added to dissolve the powder and the mixture sonicated for about 5 min, then made to the volume. Two millilitres of the resulting solution were transferred into a 50 mL volumetric flask and diluted to volume using the diluent to get a concentration of about 10 μg/mL. The resultant solution was sonicated for 15 min, filtered and 20 μL injected for analysis using the developed HPLC method.

## 4.4 Assay results

The assay results obtained from the analysis carried out on the samples are summarized in Table 4.2. The typical chromatograms obtained on the analysis of the samples and rufinamide bulk material are illustrated in Figure 4.1, 4.2 and 4.3. The average percentage assay values for Inovelon®, F1, F2, F3 and F4 were 99.7, 97.1, 104.2, 98.1 and 98.2%, respectively. According to the official USP monograph, rufinamide tablets contain an amount of rufinamide equivalent to not less than 95.0% and not more than 105.0% of the labeled amount of rufinamide (USP, 2015). Therefore, all the tested samples complied with the assay of rufinamide tablets according to USP (2015) specifications.

**Table 4.2: Assay of rufinamide in Inovelon® and formulated generic rufinamide tablets expressed as percentages of labeled content**

| Product | Percentage assay content* | | | |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 | Average |
| Inovelon® | 99.38 (0.6) | 99.40 (0.1) | 100.20 (0.2) | 99.7 |
| F1 | 95.47 (0.2) | 98.19 (0.1) | 97.69 (0.2) | 97.1 |
| F2 | 104.24 (0.1) | 103.61 (0.5) | 104.80 (0.3) | 104.2 |
| F3 | 96.39 (0.4) | 97.81 (0.4) | 100.06 (0.4) | 98.1 |
| F4 | 98.39 (0.1) | 98.54 (0.6) | 97.64 (0.6) | 98.2 |

*Figures in parenthesis represent the percentage relative standard deviation, n = 3

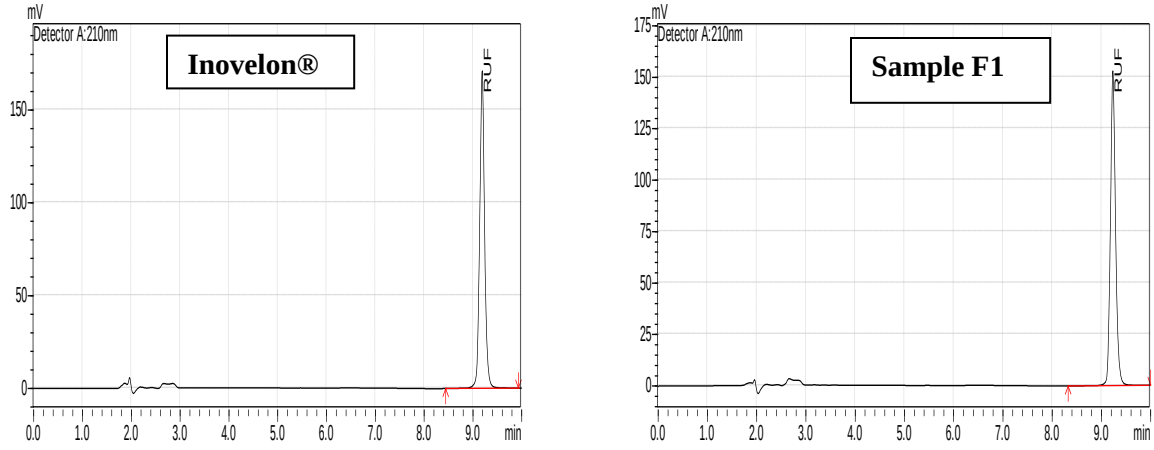



**Figure 4.1: Typical chromatograms of Inovelon® and Sample F1.** Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Column temperature 35 °C; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.00 mL/min; Detection: 210 nm; Injection volume: 20µL; Sample concentration: 10 µg/mL. OSA = octane sulphonic acid sodium salt and RUF = rufinamide.

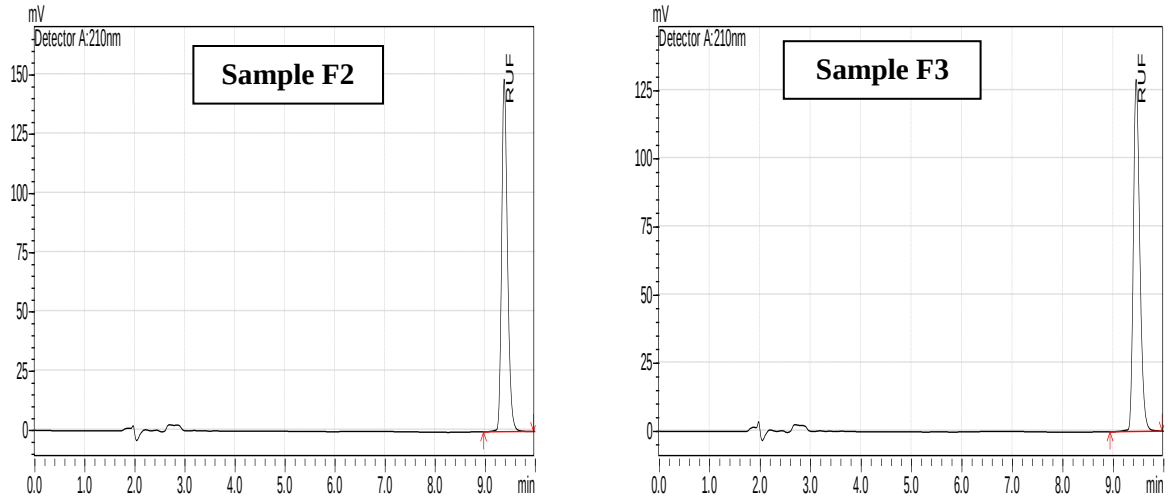



**Figure 4.2: Typical chromatograms of sample F2 and F3.** Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Column temperature 35 °C; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.00 mL/min; Detection: 210 nm; Injection volume: 20µL; Sample concentration: 10 µg/mL. OSA = octane sulphonic acid sodium salt and RUF = rufinamide.

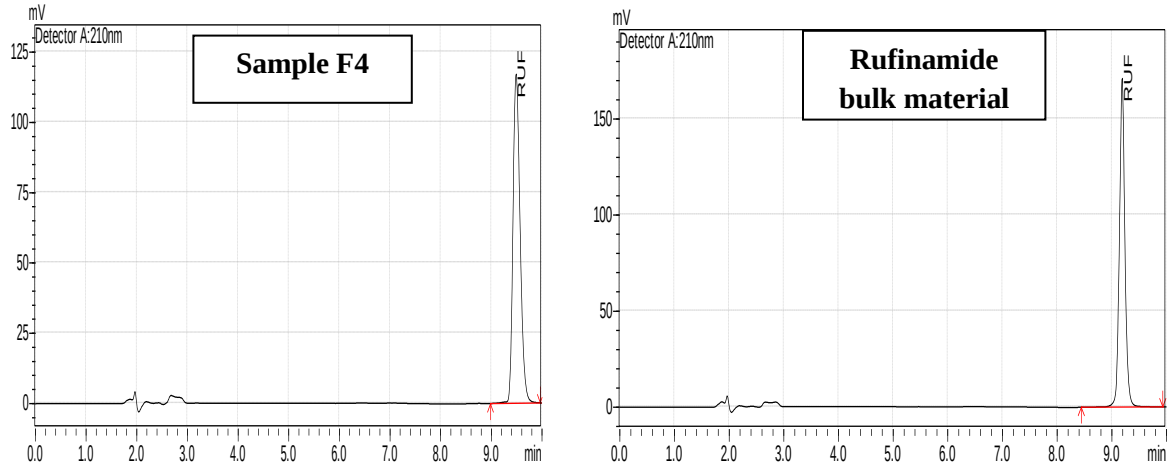



**Figure 4.3: Typical chromatograms of sample F4 and rufinamide bulk material.** Column: Hyperclone BDS C18, 250 × 4.6 mm, 5µm; Column temperature 35 °C; Mobile phase: methanol –0.1 M OSA-0.1M $K_2HPO_4$ pH 6.5 - water (30:10:5:55, % v/v/v/v); Flow rate: 1.00 mL/min; Detection: 210 nm; Injection volume: 20µL; Sample concentration: 10 µg/mL. OSA = octane sulphonic acid sodium salt and RUF = rufinamide.

# CHAPTER FIVE

# GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 5.1 General discussion

The development and validation of a HPLC method for the analysis of rufinamide provides an opportunity for the commercial production of generic rufinamide in East Africa for the first time. This will go a long way to ensure that the manufactured rufinamide tablets meet expected quality standards to ensure effectiveness and safety in clinical use.

The developed method utilizes readily available and affordable reagents making the method applicable both in pharmaceutical manufacturing industries and regulatory authority laboratories. In addition, the method uses UV detection which is very common in many HPLC methods and thus fixed wavelength detectors may be incorporated.

The validation of the developed liquid chromatographic method illustrated that the method is linear, specific, precise, sensitive and accurate. Robustness data showed that the method is not affected greatly by small deliberate alterations in critical chromatographic factors of organic modifier concentration, buffer pH and column temperature. The method is also fast hence can find routine application during extensive analytical work.

Although rufinamide was observed to be resistant to common degradation conditions, degradative decomposition was achieved in 3% v/v hydrogen peroxide and 1.0 M sodium hydroxide at 80 ℃. Since the developed method achieved separation of the main rufinamide peak from the degradation products as well as commercial related substances, it is stability-indicating. Therefore can be utilized in stability studies.

## 5.2 Conclusion

A RP-HPLC method was developed for the analysis of rufinamide bulk material and dosage formulations. The optimum chromatographic conditions established for the developed method are summarized as follows; Analytical column: A Phenomenex® hyperclone BDS C18 chromatography column of dimensions 250 mm × 4.6 mm with particle size of 5 μm maintained at a temperature of 35 °C. Mobile phase composition: methanol - 0.1 M octane sulphonic acid - 0.1 M dipotassium hydrogen phosphate pH 6.5 - water (30:10:5:55, % v/v/v/v) delivered at a rate of 1.0 mL/min and 210 nm UV detection. Validation of the analytical method indicated that it exhibited good linearity over a specified range of concentrations. The linear equation was $y = 0.0004 x – 0.9649$ with regression coefficient $R^2 = 0.9997$. Simple robustness studies showed that the quantitative accuracy of the method was not largely affected by small deliberate alterations in key liquid chromatographic factors of mobile phase methanol concentration, column temperature and buffer pH.

In addition, the method was established to be stability indicating because it enabled identification and separation of degradative products arising from alkaline and oxidative degradation. Rufinamide was observed to be relatively stable in acidic environment as it formed

minute quantities of degradation products not easily detectable. The acids used in degradation were hydrochloric acid and sulphuric acid at concentrations of 0.1 M, 0.5 M and 1.0 M.

The developed procedure was effectively employed in the analysis of innovator product Inovelon® and formulated tablets. Analysis of the Inovelon® brand and four batches of formulated rufinamide tablets using the developed method indicated that all complied with the official pharmacopoeial specifications (USP, 2015) for rufinamide content.

## 5.3 Recommendations

There is need to characterize and quantify the degradation products formed from rufinamide and if possible make them available for inclusion in the working mixture. Characterization may involve coupling the LC method with mass spectrometry and nuclear magnetic resonance spectroscopy. This would broaden the applicability of the method. Further, it is proposed that robustness of the developed analytical procedure be evaluated through collaborative studies with other analytical laboratories. This will ensure the method becomes universal in its application. There is also need to derive correction factors for applicability in impurity profiling.

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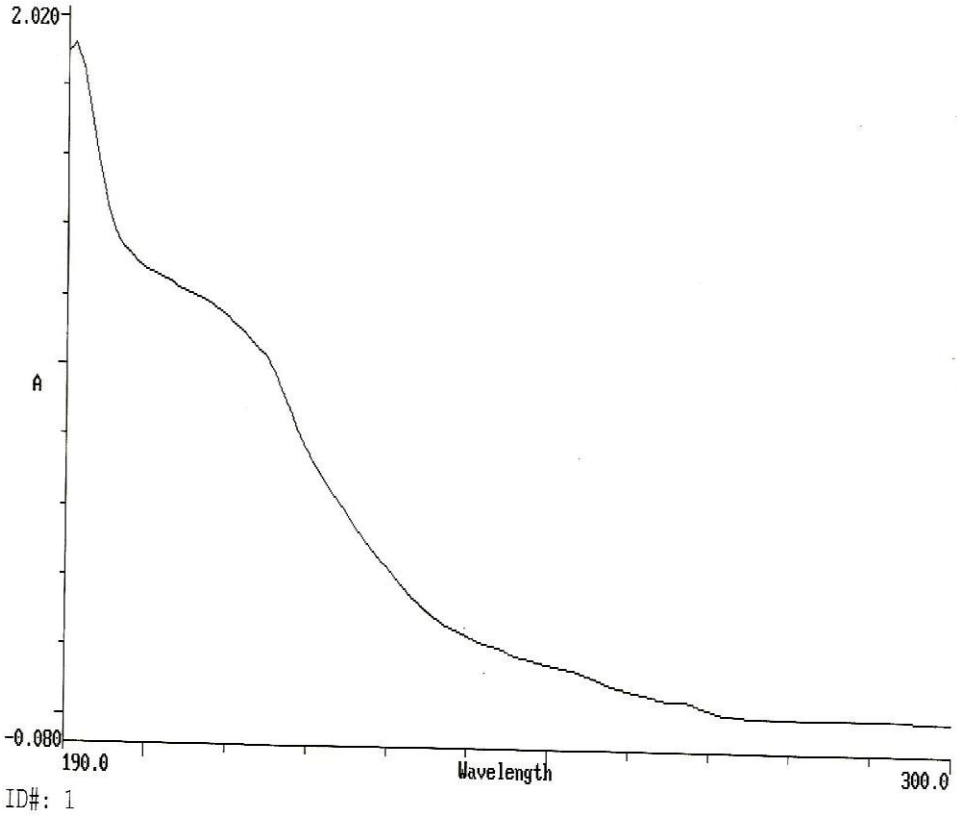
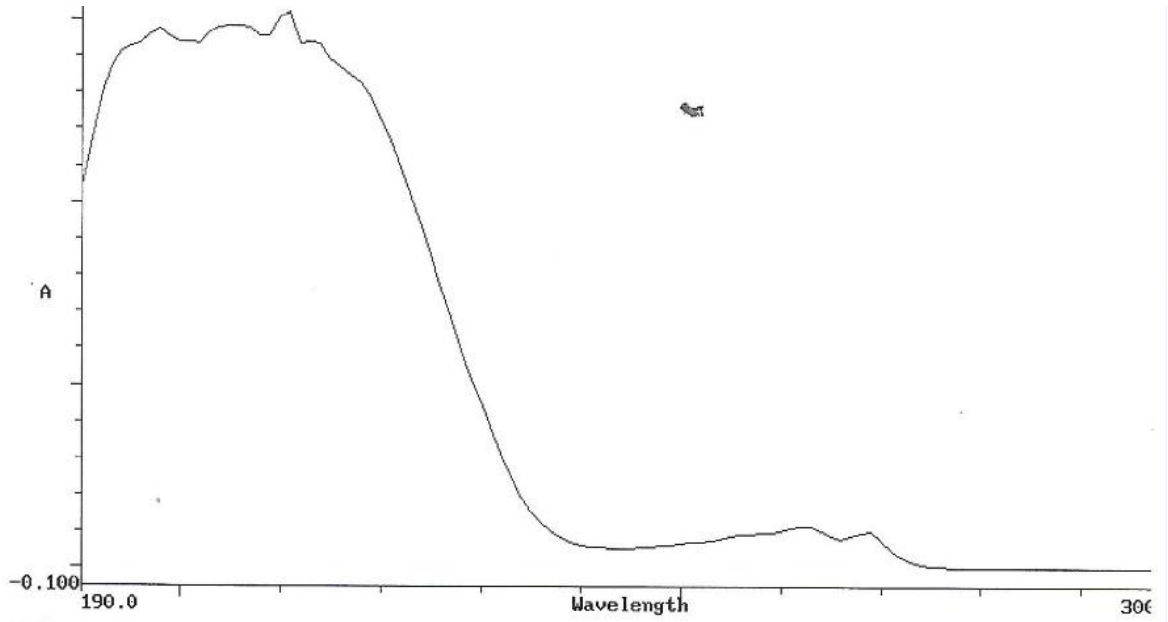
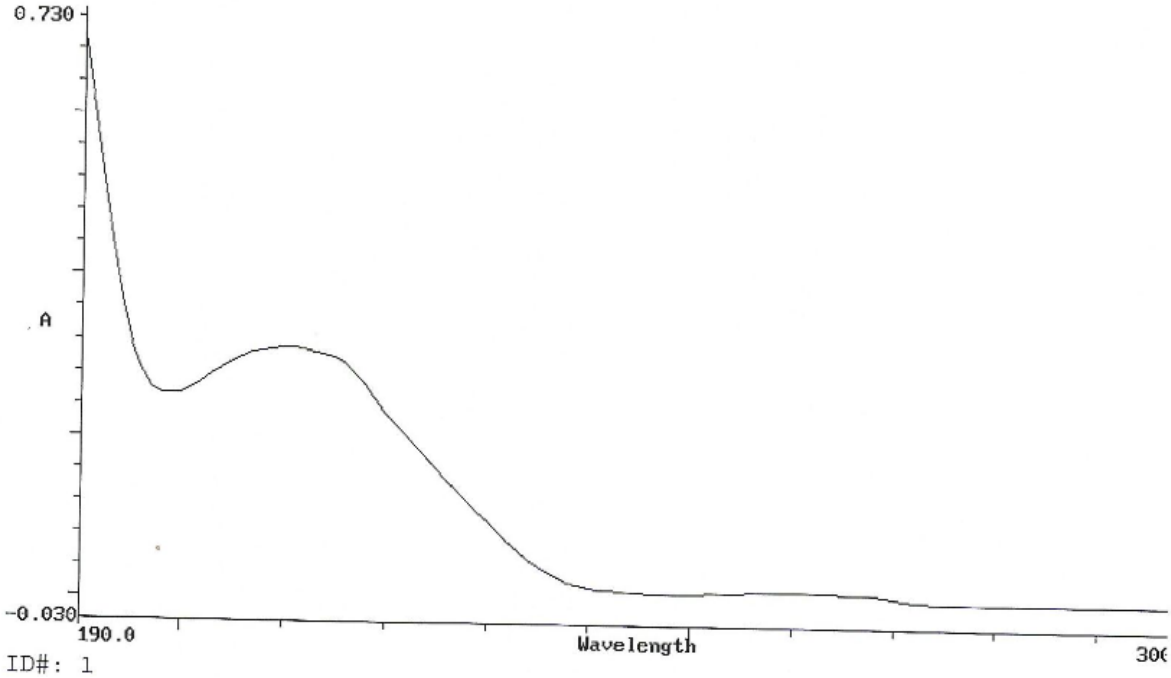
# APPENDICES



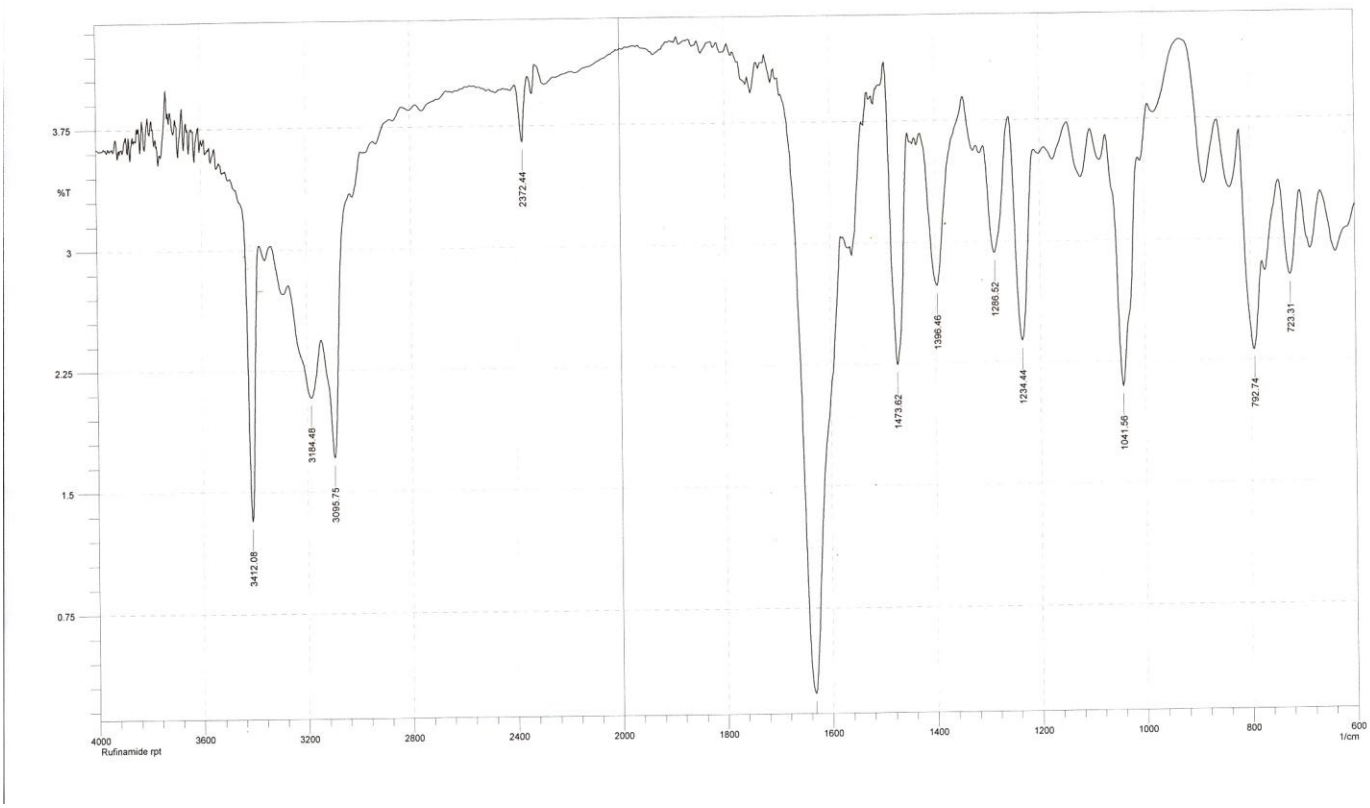

**Appendix 1: Ultraviolet absorption spectrum of rufinamide.**



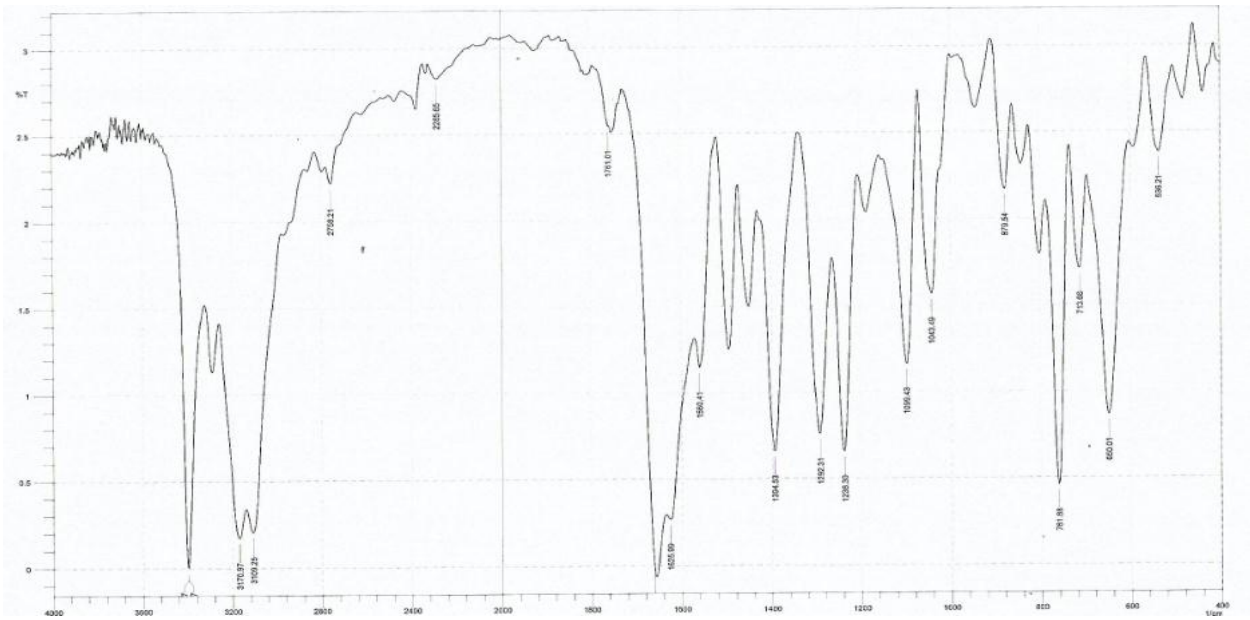

**Appendix 2: Ultraviolet absorption spectrum of rufinamide related compound A.**



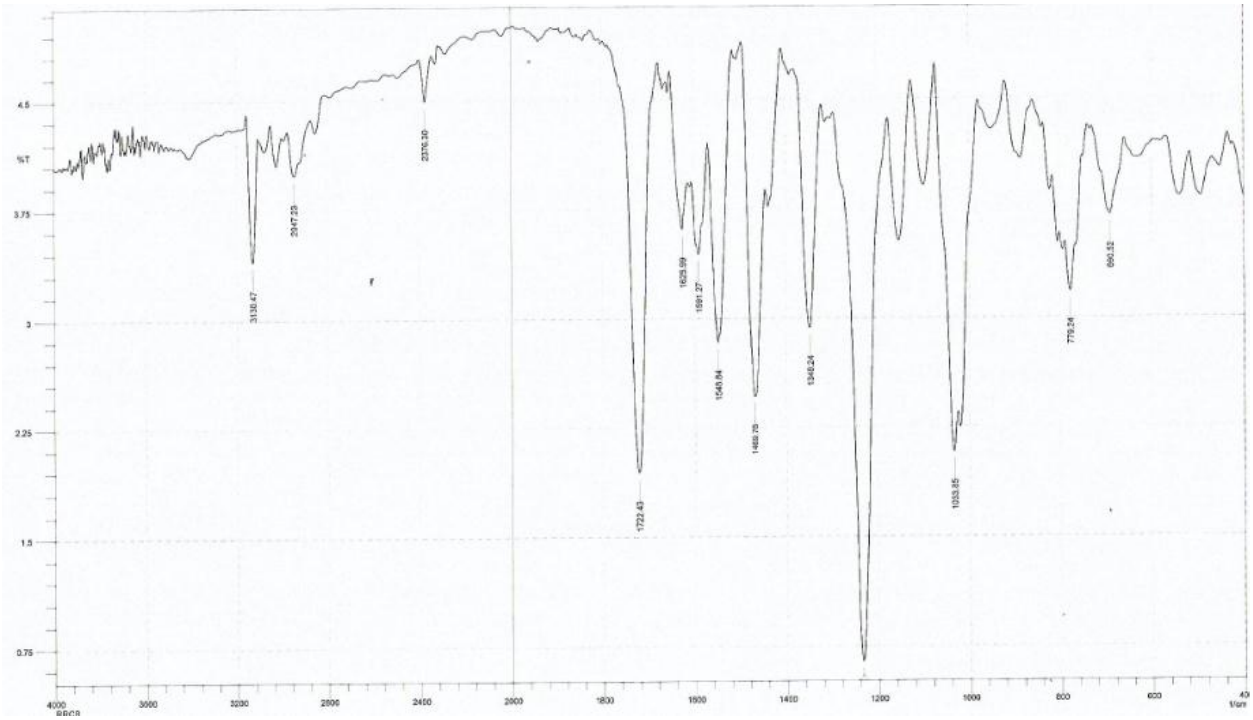

**Appendix 3: Ultraviolet absorption spectrum of rufinamide related compound B.**




**Appendix 4: Infrared absorption spectrum of rufinamide.**




**Appendix 5: Infrared absorption spectrum of related compound A.**




**Appendix 6: Infrared absorption spectrum of rufinamide related compound B.**