FORMULATION DEVELOPMENT OF GENERIC RUFINAMIDE UNCOATED TABLETS

By

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DECLARATION

This dissertation contains my original work which has not been submitted to any university/institution for the award of a degree.

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DEDICATION

This work is dedicated to my loving and caring wife, Diana and our children Russell and Daniella for their love, support and encouragement throughout the duration of this project.

ABBREVIATIONS AND ACRONYMS

AEDs	Antiepileptic drugs
API	Active Pharmaceutical Ingredient
BP	British Pharmacopoeia
FEAPM	Federation of East African Pharmaceutical Manufacturers
FTIR	Fourier Transform Infra-red
GIZ	Gesellschaft für International Zusammenarbeit GmbH
HPMC	Hydroxy propyl methylcellulose
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
ILAE	International League Against Epilepsy
INN	International nonproprietary name
MCC	Microcrystalline cellulose
min	Minutes
O/W	Octanol/Water
°C	degrees Celcius
RSD	Relative standard deviation
SLS	Sodium lauryl sulphate

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ABSTRACT

Introduction

The Lennox-Gastaut syndrome (LGS) is a rare and one of the most severe forms of childhood epilepsy syndrome. The syndrome usually affects children between the ages of 1 and 8 years (typically between 3 and 5 years), but occasionally has its onset in children who are more than 8 years old. A small proportion of the East African population has been found to suffer from poor management of this disease. The disease is usually treated with multiple drug therapy and currently there is no optimal drug combination to manage it effectively. Rufinamide has been found to lead to 50 % reduction in seizure episodes among LGS patients when used as an adjunct therapy in the management of LGS with other Anti-Epileptic Drugs (AEDs). The innovator brand is under patent protection in the United States, Europe and major pharmaceutical markets and therefore the benefit of using Rufinamide, considered an orphan drug, is not available to patients in East Africa. This research project therefore aims to formulate generic Rufinamide tablets that can be scaled up at industry level for use by the East African population that suffers from this condition.

Methodology

Four formulation batches (F1, F2, F3 and F4) with an average tablet weight of 600 mg were developed. F1 comprised of rufinamide, mannitol, hydroxy propyl methyl cellulose (HPMC), lactose monohydrate, maize starch, sodium lauryl sulfate (SLS), sodium carboxy methyl cellulose, anhydrous colloidal silica and magnesium stearate while F2, F3 and F4 had the same composition as F1 but in F2 HPMC was replaced with microcrystalline cellulose intragranularly whereas F3 had the MCC extra granularly. F4 contained both hydroxyl propyl methyl cellulose and microcrystalline cellulose in equal proportions. Wet granulation process with distilled water as the granulating fluid was used and tablet compression was performed using a single punch tablet press (Inweka, India). The tablets were then assessed for quality as per the BP and USP monographs.

Results and Discussion

Microcrystalline cellulose shortened the disintegration times of F2 and F3 while its mode of incorporation (intra or extra granular) had a negligible effect on disintegration and dissolution rate since F2 and F3 had comparable disintegration times, 2.7 and 2.4 minutes respectively. F4

contained half the amount of MCC that was contained in either of F2 or F3 and disintegrated in 11.4 minutes but gave a higher drug release profile than the others. F1 did not contain MCC and required the longest time of 95 minutes to disintegrate.

Conclusion

F2 and F3 were similar to brand as shown by the f_2 values of 61.6 and 58.9 respectively while F4 was different (f_2 = 40.8) but had the highest dissolution profile.

Hence F4 can be developed further by establishing a suitable combination ratio of MCC and HPMC to improve both disintegration and dissolution.

CHAPTER ONE: INTRODUCTION

Epilepsy is the commonest neurological condition affecting people of all ages, race and social class. There are an estimated 50 million people with epilepsy in the world, of whom up to 75% live in resource-poor countries with little or no access to medical services or treatment (Neligan & Sander 1881).

1.1 Lennox - Gastaut Syndrome

Lennox-Gastaut syndrome (LGS) is one of the catastrophic epilepsies of childhood, classified by the International League Against Epilepsy as a symptomatic generalized epilepsy syndrome (Gastaut H et al 1966). The syndrome usually affects children between the ages of 1 and 8 years (typically between 3 and 5 years), but occasionally has its onset in children who are more than 8 years old. LGS begins in childhood but continues to manifest into adulthood in a large number of patients and has a significant morbidity and mortality (Crumrine KP 2011). Among the general population the incidence is estimated at 1:1,000,000 per year with a prevalence of 15 per 100,000 (www.orpha.net). LGS accounts for 5 % of all epilepsies and about 10 % of childhood epilepsies in Europe (Rijckevarsel K 2008).

The syndrome affects boys about 5 times more often than girls (Ferrie CD et al 2009) and has a poor prognosis with regard to both seizures and cognitive outcome. Risk factors for a poor cognitive prognosis include symptomatic etiology, history of nonconvulsive status epilepticus, prior infantile spasms, and early age of seizure onset (Oguni H et al 1996).

1.2 Management of Lennox - Gastaut Syndrome

LGS is notoriously difficult to treat. Many drugs reduce seizures initially, only to lose effectiveness over time (Hancock EC et al 2003).

Drugs used in the treatment and management of LGS include felbamate, lamotrigine, topiramate, valproic acid, rufinamide and benzodiazepines such as clobazam and levetiracetam. Other forms

of treatment may include the use of ketogenic diet, vagus nerve stimulation or corpus callosotomy (Campos-castelló 2004).

1.3 Rufinamide use in management of LGS

Rufinamide is a novel antiepileptic drug chemically known as [1-(2, 6-difluoro-phenyl) methyl-1H-1, 2, 3-triazole-4-carboxamide]. The compound is a triazole derivative structurally unrelated to other anticonvulsants and used for the adjunctive treatment of LGS in children 1 year of age and older. It is also approved for adjunctive treatment of partial seizures in adults and adolescents. Its mechanism of action reportedly involves decreased firing of high frequency sodium-dependent action potentials and prolongation of sodium channel inactivation (Brodie MJ et al 2009).

1.4 Pharmacokinetics of Rufinamide

The compound is well absorbed on oral administration (~85% after an oral dose). It has a slow absorption rate and the extent of absorption decreases as the dose is increased (Perucca EJ et al 2008). After a single 400 mg oral dose in healthy adults, the time to maximum plasma concentration ranges from 1.5 to 10 hours with an average of about 6 hours, with a mean maximum plasma concentration (C_{max}) of 3.03 μg/mL (Deeks ED et al 2006).

The drug has low protein binding (~34%) and the time to maximum plasma concentration is not affected by food (Perucca EJ et al 2008). It has a plasma half-life of 6 to 10 hours and is unaffected by renal disease. Age has no effect on the half-life of the drug.

Rufinamide is eliminated primarily via metabolism, the principal metabolite being a carboxylic acid derivative. This metabolite primarily appears in the urine, and only about 2% of rufinamide occurs in the urine unchanged. The metabolite has no known pharmacological activity. The compound is not metabolized via cytochrome P450 system.

1.5 Dosage regimen of Rufinamide.

1.5.1 Paedriatics

Safety and efficacy of rufinamide has not been established in children less than 1 year old. The initial dose is 10 mg/kg/day orally divided into two equal doses .The dose can be increased by 10

mg/kg every other day. The maintenance dose is 45 mg/kg/day orally divided into two equal doses, and not to exceed 3200 mg/day.

1.5.2 Adults and Geriatrics

The dose is 400-800 mg/day orally divided into two equal doses. The dose may be increased by 400-800 mg every other day up to 3200 mg/day divided into two equal doses. (www:drugs.com/dosage/rufinamide.html).

1.6 Side effects of Rufinamide

Rufinamide appears to have a good safety profile and is well tolerated with minimal expected side effects. Some of the most common side effects such as somnolence and vomiting may be ameliorated by slow titration of the drug. (Hakimian et al 2007)

CHAPTER TWO: LITERATURE REVIEW

2.1 Nomenclature of active pharmaceutical ingredient

The International Non Proprietary Name (INN) of the active pharmaceutical ingredient is Rufinamide. This active substance is chemically known as 1-[(2, 6- difluoro-phenyl) methyl] -1H-1, 2, 3- triazole -4- carboxamide.

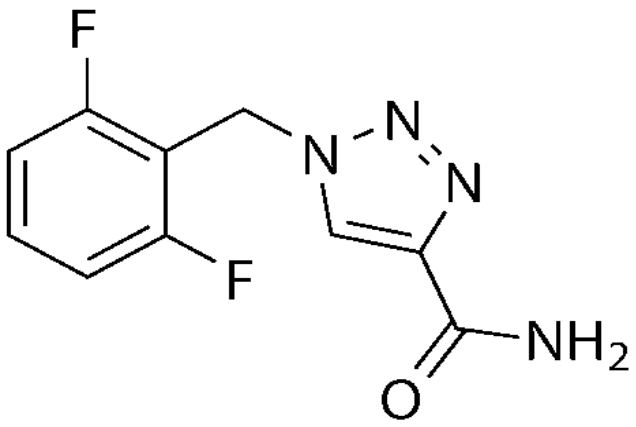

Figure 1: Chemical structure of Rufinamide

2.2 Physicochemical properties

Rufinamide is a fine, white, odorless and slightly bitter, non-hygroscopic powder of needle shaped crystals. The compound has no ionizable functionality and is practically insoluble in water (approximately 0.642 mg/ml). The active substance has a low bulk density, poor flow properties and a strong tendency to agglomerate as a consequence of its needle like crystals.

It has a partition coefficient, log P o/w of 0.65 and melting range of 233 °C to 238 °C. The compound exists in four polymorphic forms, A, A′, B and C with form A being thermodynamically stable. It has a molecular formula of $C_{10}H_8F_2N_4O$ and relative molecular mass of 238.2 (www.ema.europa.eu/).

2.3 Brand product characterization

Rufinamide is marketed as Inovelon®, which is the innovator brand product. Inovelon® is manufactured as film coated tablets in strengths of 100, 200 and 400 mg of the active compound. The tablets are pink in colour, ovaloid in shape, slightly convex and scored on both sides.

2.4 Composition of Brand Rufinamide tablets.

Based on patent literature US 6,740,669 B1 Table 1 lists the composition of brand rufinamide 200 mg tablet.

Table 1: Composition of brand rufinamide tablet 200 mg

Core material	Quantity (mg)	% Composition (w/w)
Rufinamide	200	53.5
Anhydrous colloidal silica	1.78	0.5
Microcrystalline cellulose	73.25	19.6
Hydroxy propyl methyl cellulose	10.00	2.7
Lactose monohydrate	40.00	10.7
Magnesium stearate	4.00	1.1
Maize starch	20.00	5.3
Sodium carboxy methyl cellulose	10.00	2.7
Sodium lauryl sulfate	1.00	0.3
Film coat		
Hydroxy propyl methyl cellulose	6.43	1.7
Red iron oxide	0.09	0.02
Polyethylene glycol 8000	1.16	0.3
Talc	4.66	1.2
Titanium dioxide	1.66	0.4
Total tablet weight	374	100

2.5 Biopharmaceutical Classification System of Rufinamide.

Rufinamide is classified by the Biopharmaceutics Classification System (BCS) as a class II active pharmaceutical ingredient because of its low water solubility and high intestinal permeability. The dissolution of poorly water soluble drugs is the rate limiting factor for absorption. It is therefore important to increase the solubility or the dissolution rate in order to enhance absorption and bioavailability (Douroumis et al. 2007)

2.6 Solubility enhancement of poor water soluble drugs

A number of approaches have been carried out in order to increase the solubility of poorly water soluble drugs. These include:

(i) Physical modifications techniques such as particle size reduction like micronization and nano suspension, modification of the crystal habit like polymorphs, amorphous form and crystallization, drug dispersion in carriers like eutectic mixtures, solid dispersions, solid solutions and cryogenic techniques.

(ii) Chemical modifications techniques such as change of pH, use of buffer, derivatization, complexation, and salt formation.

(iii) Miscellaneous methods: Supercritical fluid process, use of adjuvant like surfactant, solubilizers, solvency, hydrotrophy, and novel excipients (Savjani et al. 2012).

However, all these techniques have potential limitations. In the present study solid dispersion by solvent evaporation method was used to improve the poor water solubility of rufinamide.

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, in amorphous particles (clusters) or in crystalline particles (Ahire et al. 2015).

2.7 Problem statement

The Lennox-Gastaut syndrome (LGS) is a rare and one of the most severe forms of childhood epilepsy syndrome. In East Africa, empirical evidence indicates up to 1000 paediatric patients suffer from poorly managed LGS at any one time in each partner state (Aga Khan University Hospital neurology clinic, 2011)

The disease is usually treated with multiple drug therapy and currently there is no optimal drug combination to manage it effectively. Rufinamide has been found to lead to 50 % reduction in seizure episodes among LGS patients when used as an adjunct therapy in the management of LGS with other AEDs. The innovator brand is widely available in the developed world and is under patent protection in the United States, Europe and major pharmaceutical markets (www.orpha.net).

2.8 Study justification

The benefit of using rufinamide for management of LGS in patients in East Africa is limited by cost and intellectual property. This research project therefore aims to formulate generic rufinamide tablets that can be scaled up at industry level for use by the East African population that suffers from this condition.

2.9 Objectives

2.9.1 General objective

The main objective of this study was to formulate generic rufinamide uncoated tablets of 200 mg strength

2.9.2 Specific objectives

The specific objectives of this study were to:

1. Carry out pre-formulation studies to aid the development of generic rufinamide uncoated tablets
2. Carry out formulation optimization studies to aid the development of generic rufinamide uncoated tablets
3. Test the quality of the formulated generic rufinamide uncoated tablets including carrying out comparative dissolution studies of generic rufinamide uncoated tablets with respect to the innovator brand tablets

CHAPTER THREE: METHODOLOGY

3.1 Study location

The study was carried out in the Pharmaceutics laboratory at the Department of Pharmaceutics and Pharmacy Practice, School of Pharmacy.

3.2 Materials

Rufinamide Polymorph A was purchased from Amino Chemicals Limited, Malta. Anhydrous colloidal silica, microcrystalline cellulose (Avicel -101), hydroxyl propyl methyl cellulose, lactose monohydrate, magnesium stearate, maize starch, sodium carboxyl methyl cellulose, sodium lauryl sulfate, red iron oxide, polyethylene glycol 8000, talc and titanium dioxide were obtained from Universal Corporation Ltd, Kenya. All excipients used were of pharmaceutical grade while reagents and solvents used were of analytical grade

3.3 Equipment

A Single station table press type iEP-1 (Inweka, India), Fourier Transform Infrared Spectrophotometer (Shimadzu IR Prestige 2.1,Tokyo Japan), Stability Chamber (Binder APT.lineTM KBF, Germany), High Perfomance Liquid Chromatography (Shimadzu ,Tokyo Japan), Oven drier (Memmert, Germany), Weighing balance (Satorius, England), Disintegration Apparatus (GmbH, Heusenstamn, Germany), Dissolution Apparatus (Erweka, Germany), Electronic Tablet Hardness Tester (Schleuniger & Co, Germany), Friability Tester (Erweka, Germany) and an Electronic light microscope (Olympus, Tokyo Japan) were employed for this study.

3.4 Pre - formulation studies

3.4.1 Identification of Active Pharmaceutical ingredient

The identity of active pharmaceutical ingredient was established using Fourier transform infra-red spectroscopy whereby the peaks of the rufinamide were matched for concordance with those published in literature. A KBr disk of rufinamide raw material was prepared and used to obtain an IR spectrum of rufinamide in the range of 4000 – 600 cm^{-1} using a Shimadzu IR Prestige 2.1

Fourier Transform Infra-red (FTIR) spectrophotometer (Shimadzu Corp., Kyoto, Japan) operating on IR solution software Ver. 1.3.

3.4.2 Drug-Excipient Compatibility Studies

The excipients used in formulating generic Rufinamide tablets were selected based on the excipients used for the innovator product. Individual samples of drug and excipients as well as physical mixtures of drug and excipients in equal proportions were stored at 40 °C ± 2 ˚C and 75 % ± 5% RH in open and closed containers for 1 month. The stability chamber used was Binder APT.line^{TM} KBF, Germany.

Common excipients functioning as filler, disintegrant, and lubricant were evaluated in the excipient compatibility study. Infra- red spectra of drug, excipients and physical mixtures of drug and excipients were recorded on an FTIR spectrophotometer in the range of 4000-600cm^{-1} using potassium bromide discs. A Shimadzu IR Prestige 2.1 Fourier Transform Infra-red (FTIR) spectrophotometer (Shimadzu Corp., Kyoto, Japan) operating on IR Solution software Ver. 1.3 was used for this purpose.

3.4.3 Evaluation of powder properties.

The particle size of Rufinamide powder was established to be 28 µm according to the certificate of analysis from Amino Chemicals Ltd, Malta. The particle shape appeared needle like when observed under an electronic light microscope (Olympus Corp., Tokyo, Japan) using × 40 lens.

It was not possible to carry out the angle of repose, the bulk and tapped densities, the hausner's ratio and the compressibility index due to the very cohesive nature of rufinamide powder,.

3.4.4 Solubility profile of Rufinamide

Solubility studies were performed by dissolving 50 mg of active pharmaceutical ingredient in six different solvents to make up to 100 ml solution and the solutions were observed for clarity. The solvents used were water, isopropyl alcohol, ethanol, methanol, chloroform and dimethyl sulfoxide (DMSO). Rufinamide was found to have the lowest solubility in water and the highest solubility in DMSO. The increasing order of solubility was as follows: Water < isopropyl alcohol < ethanol <methanol < chloroform < DMSO. This correlates with previous studies reported in

literature whereby the solubility of Rufinamide is 0.642 mg/ml and 48 mg/ml in water and DMSO respectively.

3.5 Formulation Studies

Rufinamide is classified as a Biopharmaceutical Classification System class II compound (Low solubility, High permeability).The compound also exists in four polymorphic forms namely A, A', B and C with form A being thermostable. The polymorph A was used in the formulation studies.

Different formulation approaches were carried out to enhance the solubility of the drug. These were:

(i) Rufinamide / HPMC solid dispersion in ethanol, methanol, isopropanol, chloroform and chloroform : methanol solvents

(ii) Rufinamide / Mannitol solid dispersion in DMSO.

The solid dispersion of Rufinamide / mannitol in DMSO provided the best solubility and hence was adopted for formulation optimization studies. Four formulation batches were constituted as shown in Table 2.

Table 2: Tablet composition of formulation batches

Ingredient	Weight (mg)			
	F 1	**F 2**	**F 3**	**F 4**
Rufinamide	200	200	200	200
Mannitol	200	200	200	200
Hydroxy propyl methyl cellulose (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 carboxy methyl cellulose	24	24	24	24
Magnesium stearate	6	6	6	6
Anhydrous colloidal silica	3	3	3	3
Total	**600**	**600**	**600**	**600**

***MCC incorporated intragranularly in F2 while F 3 was extra granularly.**

3.5.1 Preparation of Solid dispersions

Fifteen grams of rufinamide was added to 200 ml of Dimethyl sulfoxide analytical grade. The mixture was then sonicated for 30 minutes to obtain a clear solution followed by addition of 15 g of mannitol and the mixture sonicated for another 10 minutes to obtain a clear solution.

The solution was then transferred into an oven and the temperature set at 40 ˚C and left to evaporate for 10 days to obtain a white powder. The powder was reweighed and transferred into a crucible in preparation for granulation.

3.5.2 Wet granulation process

3.5.2.1 Formulation 1

Hydroxy propyl methyl cellulose, lactose monohydrate, maize starch and sodium lauryl sulfate were added to the powder containing the solid dispersion and mixed for 5 minutes followed by granulation with distilled water. The granules were placed on the bench to dry and sized. This

was followed by addition of sodium carboxy methyl cellulose, magnesium stearate and anhydrous colloidal silica which were mixed for 2 minutes and then compressed into tablets.

3.5.2.2 Formulation 2

Microcrystalline cellulose, lactose monohydrate, maize starch and sodium lauryl sulphate were added to the powder containing the solid dispersion and mixed for 5 minutes followed by granulation with distilled water. The granules were placed on the bench to dry and sized. This was followed by addition of sodium carboxy methyl cellulose, magnesium stearate and anhydrous colloidal silica which were mixed for 2 minutes and then compressed into tablets.

3.5.2.3 Formulation 3

Lactose monohydrate, maize starch and sodium lauryl sulphate were added to the powder containing the solid dispersion and mixed for 5 minutes followed by granulation with distilled water. The granules were placed on the bench to dry and sized. This was followed by addition of microcrystalline cellulose, sodium carboxy methyl cellulose, magnesium stearate and anhydrous colloidal silica which were mixed for 2 minutes and then compressed into tablets.

3.5.2.4 Formulation 4

Hydroxy propyl methyl cellulose, microcrystalline cellulose lactose monohydrate, maize starch and sodium lauryl sulphate were added to the powder containing the solid dispersion and mixed for 5 minutes followed by granulation with distilled water. The granules were placed on the bench to dry and sized. This was followed by addition of sodium carboxy methyl cellulose, magnesium stearate and anhydrous colloidal silica which were mixed for 2 minutes then compressed into tablets.

3.6 Tablet compression process

The die fill volume of the tablet press was manually adjusted to give a tablet weight of 600 mg. This was done by weighing powder equivalent to 600 mg and transferring it into the die, then adjusting the lower punch of the die such that the powder was at the same level with the die table. Similarly the force was also adjusted to produce hard shiny tablets on compression. The tablet press was operated manually both filling of the die with powder and compression to produce one tablet at a time until the required batch size was achieved. The formulation batches were then taken through quality testing to check for compliance with quality standards.

3.7 Quality Assessment

3.7.1 Weight Uniformity Test

Twenty tablets from each batch were picked at random and individually weighed using an analytical balance and presented as a mean with a percentage relative standard deviation (%RSD) limit of ≤ 5%.

3.7.2 Resistance to Crushing of Tablets

Ten tablets from each batch were picked at random and placed in a reproducible manner between the jaws of an electronic tablet hardness tester (Schleuniger & Co., Germany). The pressure was applied and the force measured at tablet break and recorded in Newtons.

3.7.3 Resistance to Abrasion

Twenty tablets from each batch were selected at random de dusted, weighed (W_0) and placed in Friabilator apparatus (Erweka, Germany) and rotated 100 times for 4 minutes. The tablets were removed from the Friabilator apparatus, de dusted and reweighed (W)

The percentage friability was calculated using equation 4.

$$\% F = \{[W_0 - W]/ W_0\} \times 100 \qquad \text{.....Equation 4}$$

3.7.4 Disintegration Test

Six tablets of each formulation batch were picked at random and placed individually in tubes of a disintegration apparatus (Erweka, Germany). The tubes were vertically raised and lowered through a distance of 55 ± 2mm at an agitation speed of 29 to 32 cycles per minute in immersion fluid of distilled water contained in a one liter low form beaker held in a water bath at 37°C ± 2˚C. The disintegration time was recorded as the time taken for all the tablets to go into solution completely through the sieve and no particle remained on the basket of the system.

3.7.5 Assay of Tablets

A High performance liquid chromatography method was used for assay of the drug content using a validated method (Patel & Nageswara Rao 2011).

3.7.5.1 Preparation of mobile phase

The mobile phase was made up of a solvent mixture of acetonitrile: water (60:40%, v/v). Six hundred milliliters of acetonitrile HPLC grade and 400 ml of distilled water were mixed in a 2 liter bottle and degassed with helium gas.

3.7.5.2 Preparation of the standard

The standard stock solution was prepared by accurately weighing 25 mg of USP RS (Rufinamide, CAT No.1606401) in a 25 ml volumetric flask and making up to volume with the mobile phase to give a concentration of 1 mg/ml. One milliliter of the standard stock solution was transferred into a 50 ml volumetric flask and made up to volume with the mobile phase. The solution was sonicated for 10 minutes and filtered through 0.45µm membrane filters. The working concentration was 20 µg/ml.

3.7.5.3 Preparation of sample

Five tablets from each formulation batch were weighed and pulverized into fine powder with the aid of a pestle and mortar. Weight equivalent to 25 mg rufinamide was accurately weighed into a 25 ml volumetric flask and made up to volume with the mobile phase. The contents of the volumetric flask were sonicated for 10 minutes to give a concentration of 1mg/ml. One milliliter of the assay preparation was transferred into a 50 ml volumetric flask and made up to volume with the mobile phase. The solution was sonicated for 10 minutes and filtered through 0.45µm membrane filters. The working concentration was 20 µg/ml.

3.7.5.4 Liquid Chromatographic System

The chromatograph comprised of a Shimadzu Model CBM-20A HPLC system equipped with a UV detector set at 215 nm and separation was achieved from a Luna® C 18 column (250mm ×4.6mm, 5µm particle size) maintained at 25˚C in a thermostat oven. Isocratic elution was performed using acetonitrile and water (60:40%, v/v) with a flow rate of 0.8 ml/minute.

3.7.5.5 Procedure

Equal volumes of 20 µl of the standard and sample solutions were injected into the HPLC system at 215 nm wavelength detection and the responses of the major peaks measured. The percentage content of rufinamide in each of the formulated batches was calculated.

3.7.5.6 Determination of percentage drug content

The percentage assay content was obtained using equation 5.

% Assay = AT/AS × WS/WT × DT/DS × P/100 × ATW/ LC × 100 ……..Equation 5

Where: AT – Peak area of test sample, AS – Peak area of standard, WS – Weight of standard in mg, WT – Weight of test sample in mg, DS – Dilution of standard solution, DT – Dilution of test sample solution, P - Percentage potency of standard, ATW – Average tablet weight and LC – Label claim.

3.7.6 Dissolution Test

The dissolution test was undertaken using USP dissolution apparatus II paddle type (Erweka, Germany). The dissolution medium was 6.8 phosphate buffer + 2% w/w SLS maintained at 37 ˚C ± 2 ˚C and the paddle rotated at 100 rpm. The dissolution vessels were filled with 900 ml of dissolution medium. Sample aliquots of 5 ml were withdrawn at 15, 30, 45, 60, 90 and 120 min and estimated for drug content through HPLC. Equal volumes of fresh dissolution medium were replaced immediately to maintain sink conditions.

The 6.8 phosphate buffer was prepared by addition of 40.8 g of KH_2PO_4, 5.4g of NaOH and 0.9 g of SLS to 6000 ml of distilled water. The pH was adjusted using 0.2M NaOH solution.

The sample aliquots were filtered and assayed using a HPLC method. One milliliter of each sample was pipetted into a 10 ml volumetric flask and made up to volume with the dissolution medium. The solutions were sonicated for 10 minutes to obtain a concentration of 0.022 mg/ml.
Rufinamide standard stock solution was prepared by accurately weighing 2.2 mg of USP RS (Rufinamide, CAT No.1606401) in a 10 ml volumetric flask and making up to volume with the dissolution medium to give a concentration of 0.22 mg/ml.
The solutions were filtered using 0.45µm membrane filters and 20µl of these solutions were injected into the HPLC system and the peak areas recorded from the respective chromatograms at 215 nm.

The percentage drug released was obtained using equation 6 as shown:

% Dissolution = AT / AS × DS × P/100 × VDM / LC × 100 …….……Equation 6

Where:

AT – Peak area of test sample, AS – Peak area of standard, DS – Dilution of standard solution, P - Percentage potency of standard, VDM – Volume of Dissolution Medium and LC – Label claim.

CHAPTER FOUR: RESULTS, DISCUSSION AND CONCLUSION

4.1 Identification of active pharmaceutical ingredient

The initial identification test is necessary to ensure that the required active ingredient is used in the formulation of the product. The identity of rufinamide was confirmed by FTIR spectrum as shown in Figure 2. The FTIR spectrum of rufinamide showed 6 major peaks corresponding to four functional groups as shown in Table 3.

Table 3: Functional groups and wave numbers of rufinamide FTIR spectrum

Functional Group	Wave number (cm^{-1})
N - H	1396.46; 1473.62 (bend)
C = O	1631.78 (str)
= C - H	3095.75; 3184.48 (str)
N – H	3412.08 (str)

4.2 Drug – Excipient Compatibility Studies

The interaction between drugs and excipients can alter stability and bioavailability of drugs, thereby affecting their safety and/or efficacy. The successful formulation of a stable and effective solid dosage form depends on the careful choice of the excipients. Therefore pharmaceutical development of solid dosage forms should involve pre formulation studies of the drug and excipients (Bozda'-Pehlivan et al. 2011).

A number of experimental techniques such as Differential Scanning Calorimetry, FT-IR spectroscopy, X-ray powder diffraction, Scanning Electron Microscopy, High Performance Liquid Chromatography can be used for these studies. In the present study FT-IR spectroscopy was used. Samples of drug and excipients as well as physical mixtures of drug and excipients in equal proportions were stored under accelerated stability conditions for 1 month to fasten drug ageing and interactions of drug and excipients. The effect of moisture was also evaluated by storing samples in open and closed containers.

It was observed that there were no changes in the IR spectra of drug and excipients blend, which show there were no physical interactions as a result of some bond formation between drug and excipients. The IR spectra of drug and excipients blend are shown in appendices 1 to 9.

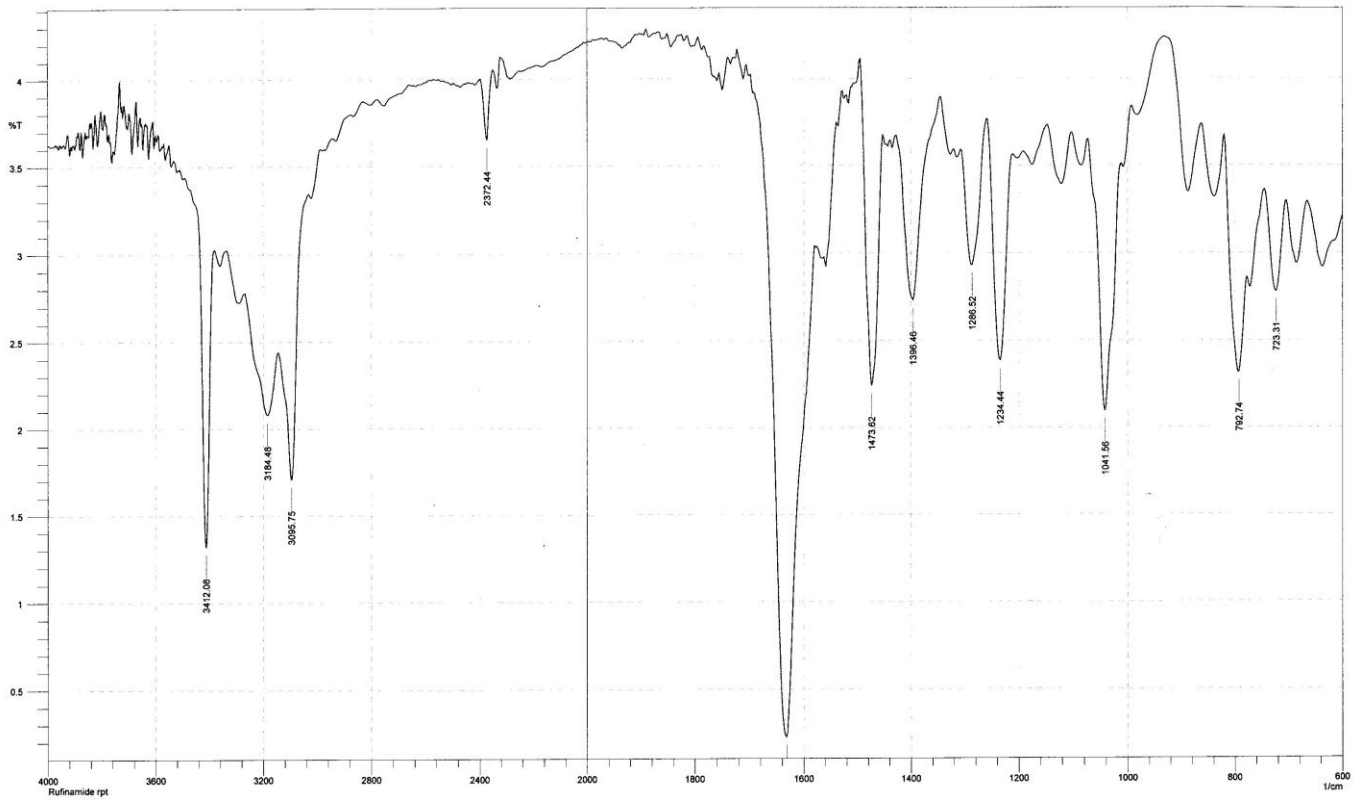

Figure 2: FTIR spectrum of rufinamide polymorph A

4.3 Weight Uniformity Test

Quality control tests such as uniformity of weight are routinely undertaken on specifically selected portions of manufactured batches of dosage forms during formulation development and large-scale batch manufacture in the pharmaceutical industry. Weight uniformity testing is used as an indicator to determine potential areas of difficulty in a manufacturing process, such as incomplete or inefficient mixing which may also affect content uniformity**.** The results for uniformity of weight are summarized in Table 4.

Table 4: Mean tablet weight of formulation batches

Batch No.	Weight (mg)	% RSD	% deviation of tablet with min weight	% deviation of tablet with max weight
F 1	591.0 ± 7.18	1.2	1.86	3.21
F 2	601.0 ± 9.68	1.6	1.83	3.61
F 3	605.0 ± 7.61	1.3	2.48	0.83
F 4	605.5 ± 9.99	1.7	2.56	2.39

The target weight per tablet was set at 600 mg. All batches complied with the requirements for weight uniformity as described in the BP which recommends a deviation of ≤ 5% from the average tablet weight for not more than 2 tablets and none of the tablets should deviate by > 10% for tablets weighing more than 250 mg.

4.4 Resistance to Crushing of Tablets

Hardness is a non-compendial test. The hardness or crushing strength assess the ability of tablets to withstand handling without breaking or chipping. It can also influence other parameters such as friability and disintegration. The results for tablet hardness test are as summarized in table 5.

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Table 5: Mean tablet hardness of formulation batches

Batch No.	Hardness (N)
F 1	141 ± 11.61
F 2	102 ± 14.26
F 3	153 ± 37.41
F 4	60 ± 13.12

F 3 required the highest amount of force (153 N) while F 4 required the least amount of force (60 N) to break. All formulation batches were above the lower limit of tablet hardness set at 40 N in house.

4.5 Resistance to Abrasion

Resistance to abrasion is also a useful indicator of the ability of tablets to withstand mechanical stress especially during handling and transportation. Friability has also a direct co relation with weight and content uniformity since substantial amount of drug content may be lost through abrasion. The BP specifies a percentage friability of < 1 % for tablets to comply. The results of the percentage friability of the formulation batches are presented in table 6.

Table 6: Percentage friability of formulation batches

Batch No.	Friability (%)
F 1	0.3
F 2	2.3
F 3	1.2
F 4	1.6

Only batch F1 complied with the BP specifications for friability test of < 1%. Both F2 and F3 did not comply with the BP specification for hardness test. This could be as a result of omitting HPMC in the formulation that provides good binding properties. F4 also failed to comply with the hardness test which may also be attributed to the small amount of HPMC used in the formulation (10% w/w) compared to F1 which had twice the same amount.

4.6 Disintegration Test

Different formulation factors are known to affect results of disintegration test. The disintegration test measures the time required for a tablet to disintegrate into particles when in contact with gastro intestinal fluids. This is a necessary condition and could be the rate – determining step in the process of drug absorption. The type and amount of excipients used in tablet formulation as well as the manufacturing process may also affect both disintegration and dissolution. The results of the mean disintegration time of the formulation batches are summarized in Table 7.

Table 7: Mean tablet disintegration time of formulation batches

Batch No.	Disintegration time (min) (n = 6)
F 1	94.5
F 2	2.7
F 3	2.4
F 4	11.6

n is the number of disintegration tests.

F2 and F3 disintegrated in less than 3 minutes. Both formulations contained 20% w/w of MCC. F2 had intragranular MCC while F3 had extra granular MCC. F4 contained 10% w/w of intragranular MCC and took approximately 4 times longer than F2 and F3 to disintegrate. F1 did not contain MCC and took the longest time to disintegrate, approximately 95 mins. This shows that MCC has the effect of accelerating tablet disintegration though the mode of incorporation has negligible effect as observed with F2 and F3. Further the amount of MCC incorporated in the tablet formulation may have an effect on the disintegration time as observed with F4. Three formulations: F2, F3 and F4 disintegrated in less than 15 minutes hence complied with the BP specifications for disintegration of uncoated tablets.

4.7 Assay of Tablets

The aim of the assay was to ascertain the presence of the required amount of active ingredient in the formulated batches. Significant variations in drug content could lead to ineffective therapeutic drug levels or overdosing that may lead to toxicity. Rufinamide tablets should contain not less than 95% and not more than 105% of the stated amount (USP, 2015). The mean percentage assay content of three formulation batches is presented in Table 8.

Table 8: Percentage assay content of formulation batches

Batch No.	Content (%) (n = 3)	% RSD
F 2	104.2	0.1
F 3	101.5	1.0
F 4	102.7	0.5

n is the number of replicate sample injections

Three formulations: F2, F3 and F4 complied with the USP requirement for content assay of rufinamide tablets. F2 had the highest percentage content (104.2%), while F3 had the least (101.5%). The chromatogram for the assay of F2 is shown in appendix 10. F1 was not assayed for content since it did not comply with the BP requirement for disintegration and therefore was ruled out for further development.

4.8 Dissolution Test

Dissolution of drug is the rate determining step for oral absorption of poorly water soluble drugs and solubility is the basic requirement for the absorption of the drug from gastrointestinal tract (GIT). Drug products with different formulations and excipients may have different dissolution profiles or release characteristics and therefore different bioavailability. In the present study comparative dissolution studies of three formulation batches of generic rufinamide tablets with respect to innovator brand were conducted according to the method described in the Indian patent number WO 2014013511 A2 for rufinamide solid dispersion preparation.

The similarity factor (f_2) test was used to compare the dissolution profiles of the formulated products and the innovator brand. The f_2 test measures the similarity in the percent dissolution between two dissolution curves. It is inversely proportional to the average squared difference between the two profiles and is a logarithmic reciprocal square root transformation of the sum of squared error given by equation 7.

$$f_2 = 50 \times \log \{[1+ (1/n) \Sigma_{t=1}^{n} (R_t-T_t)^2]^{-0.5} \times 100\} \quad \ldots\ldots\ldots\ldots\ldots\ldots\text{Equation 7}$$

where, n is the number of testing time points; R_t is the average dissolution value of the reference product units at time t and T_t is the average dissolution value of the test product units at time t (Hasan et al 2007 and Shah 2001). An f_2 value > 50 indicates similarity of test and reference dissolution profiles.

The dissolution profiles of F2 and F3 were similar to the innovator brand product as depicted by their f_2 values of 61.6 and 58.9 respectively. F4 had an f_2 value of 40.8 hence was different from the brand product in dissolution but gave a higher drug release profile than the others. The chromatogram for the dissolution of rufinamide in F4 at 45 minutes is shown in appendix 11.

The brand product was film coated whereas the formulated products were uncoated and therefore took a longer time to disintegrate which could have resulted in a lower dissolution profile when compared to the formulated products.

Withdrawal of sample aliquots and replacement of the dissolution medium at various time points was performed manually using graduated plastic syringes and this could have affected the accuracy of the dissolution results.

The mode of incorporation of MCC in the formulation had a negligible effect on the dissolution rate of F2 and F3 despite being reported in literature that the dissolution rate may be increased by the use of extra granular MCC in tablet formulations (Li, Jason Z., et al, 1996).

The results of the comparative dissolution studies are summarized in Table 9 and graphically presented in Figure 3.

Table 9: Percentage cumulative drug release of brand and formulation batches

Time in minutes	**% Cumulative Dissolution (n = 3)**			
	Brand	**F2**	**F3**	**F4**
15	22.6 (2.1)	29.3 (14.8)	25.8 (10.2)	29.1 (11.2)
30	26.4 (7.3)	29.1 (8.3)	33.8 (12.1)	40.6 (29.8)
45	24.8 (2.8)	27.1 (14.5)	30.1 (23.0)	45.9 (28.6)
60	25.7 (16.6)	29.5 (19.2)	32.2 (33.4)	46.1 (15.5)
90	33.5 (29.8)	26.7 (7.8)	27.9 (0.5)	49.1 (48.7)
120	40.8 (32.0)	31.7 (2.9)	31.2 (15.9)	34.9 (19.3)

% RSD in parentheses

n is the number of replicate sample injections

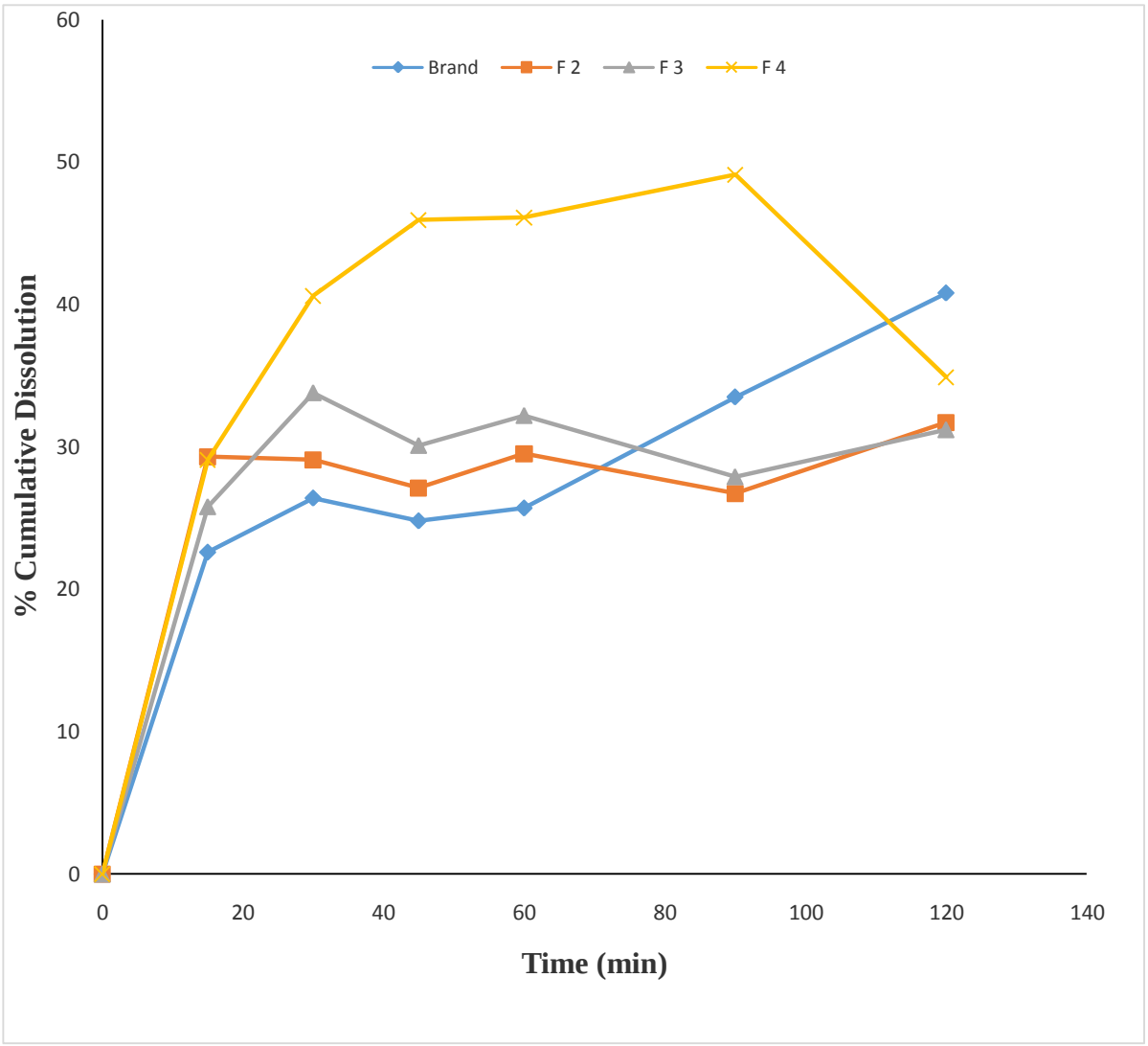

Figure 3: Dissolution profile of innovator brand and generic rufinamide tablets

4.9 Conclusion and Recommendations

Microcrystalline cellulose reduced the tablet disintegration time and dissolution rate at a concentration of 20% w/w in F2 and F3 while it increased the disintegration time and dissolution rate at a concentration of 10% w/w in F4. However the mode of incorporation of MCC in the tablet formulation (i.e. intra or extra granular) had negligible effect on the disintegration and dissolution rate on the two formulations.

F2 and F3 were similar to brand as shown by the similarity factor (f_2) values of 61.6 and 58.9 respectively while F4 was different (f_2 = 40.8).

F4 had the highest dissolution profile and therefore can be developed further by establishing an optimum combination ratio of MCC and HPMC in order to reduce the disintegration time and increase the dissolution rate. Film coating may be necessary in order to mask the bitter taste of the drug substance rufinamide.

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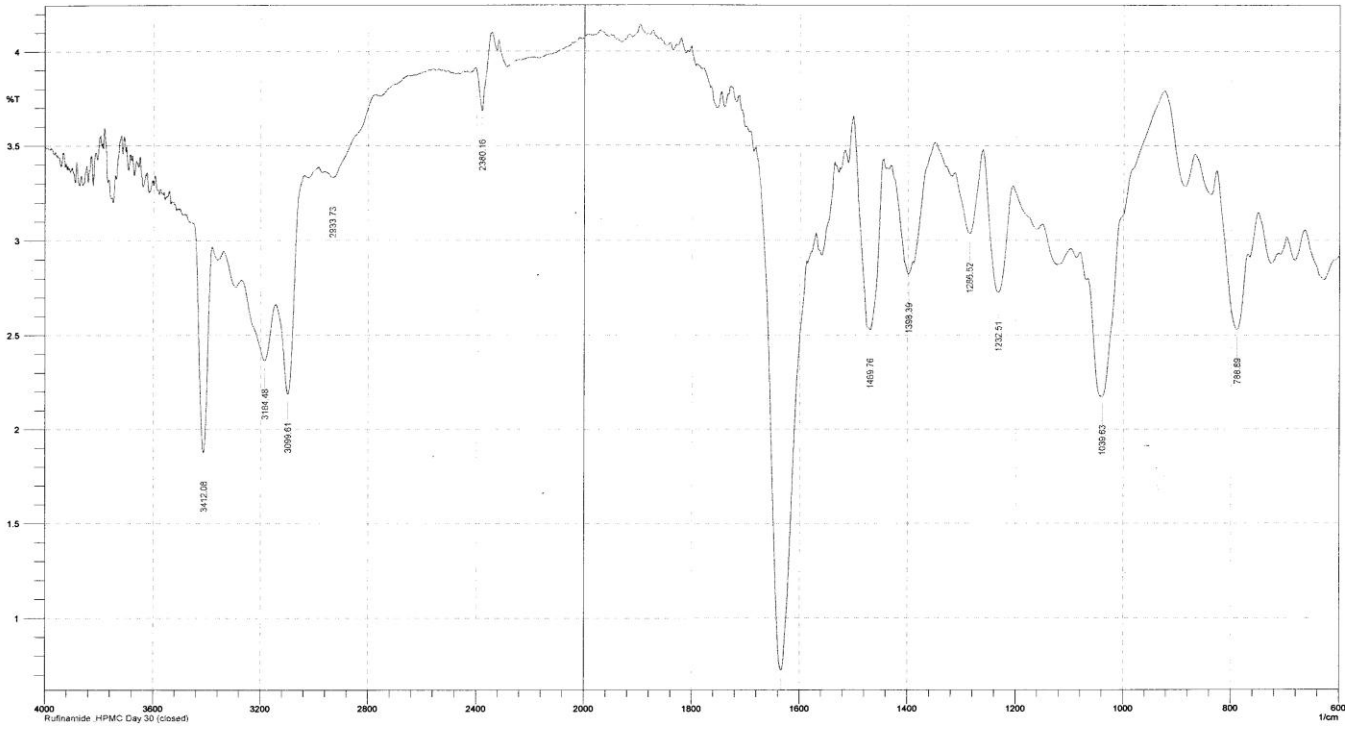
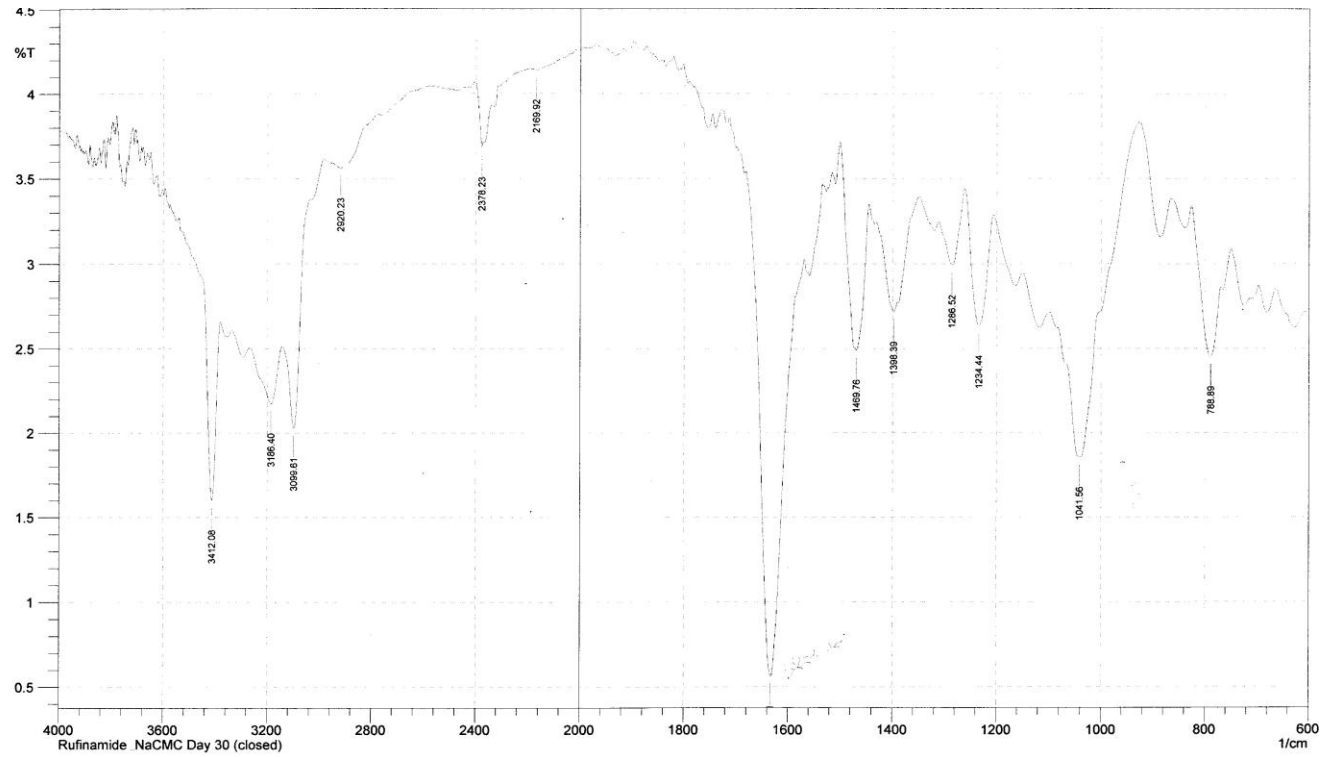
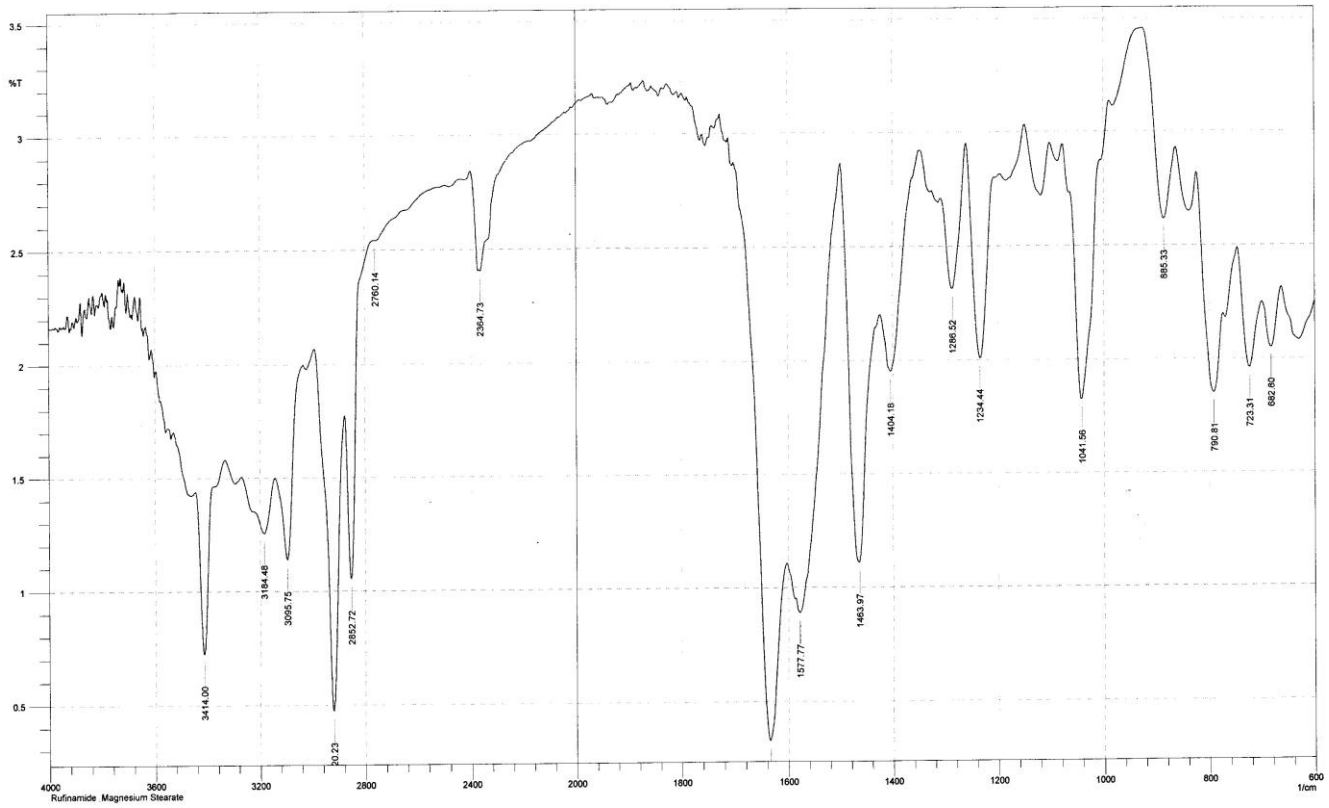
APPENDICES



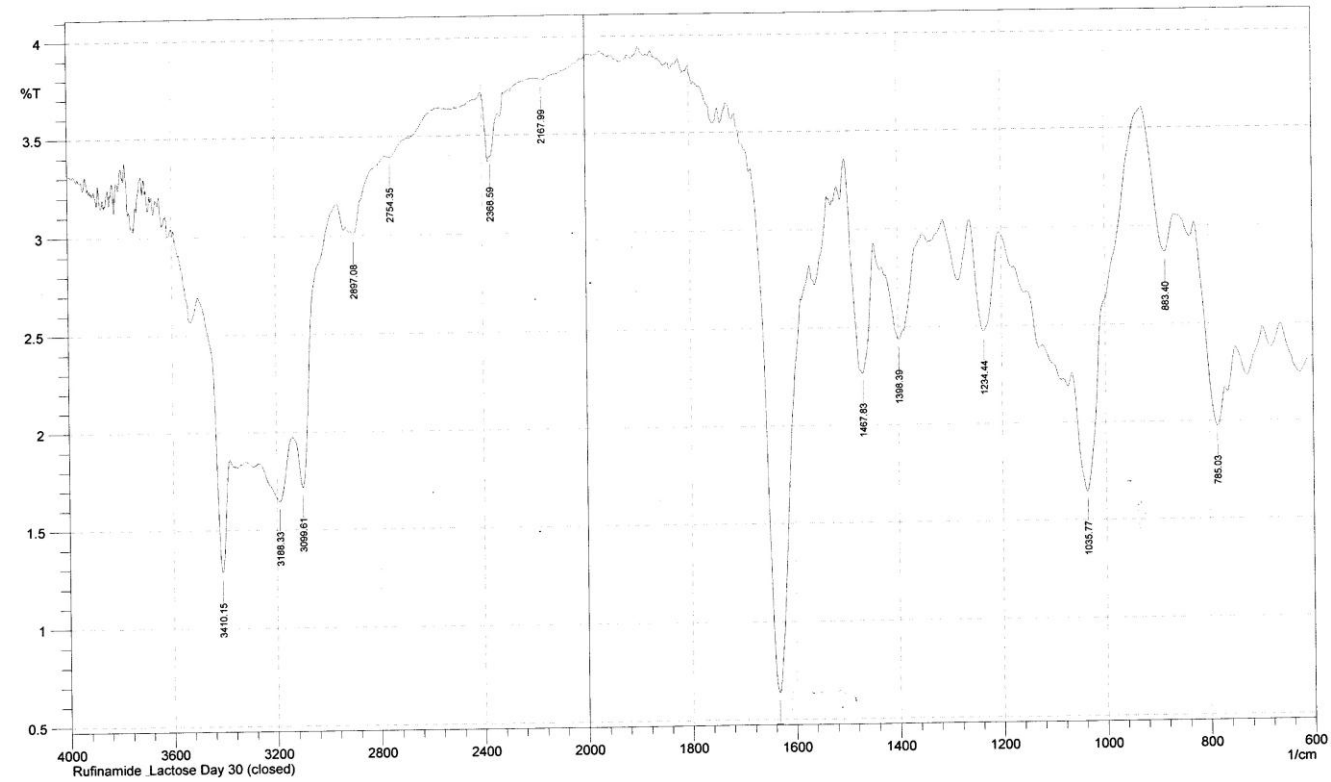
Appendix 1: FTIR spectrum of rufinamide and hydroxy propyl methyl cellulose



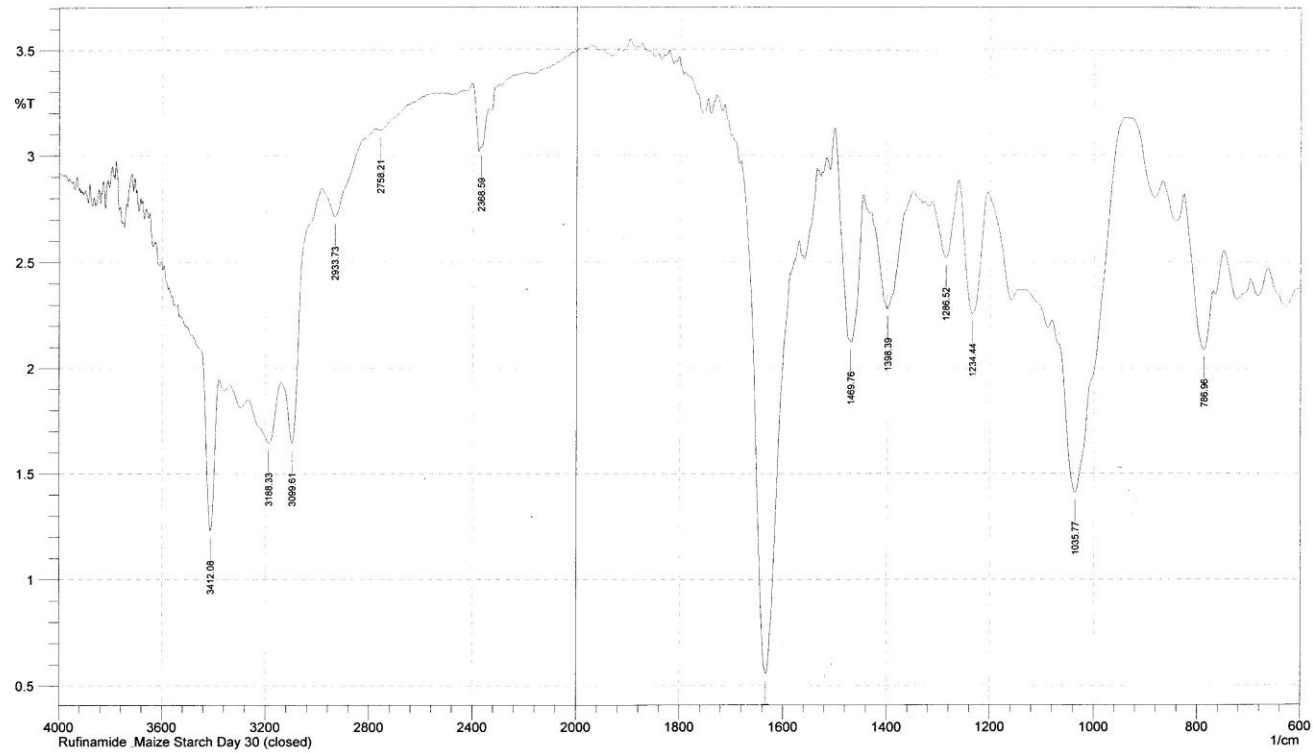
Appendix 2: FTIR spectrum of rufinamide and sodium carboxy methyl cellulose



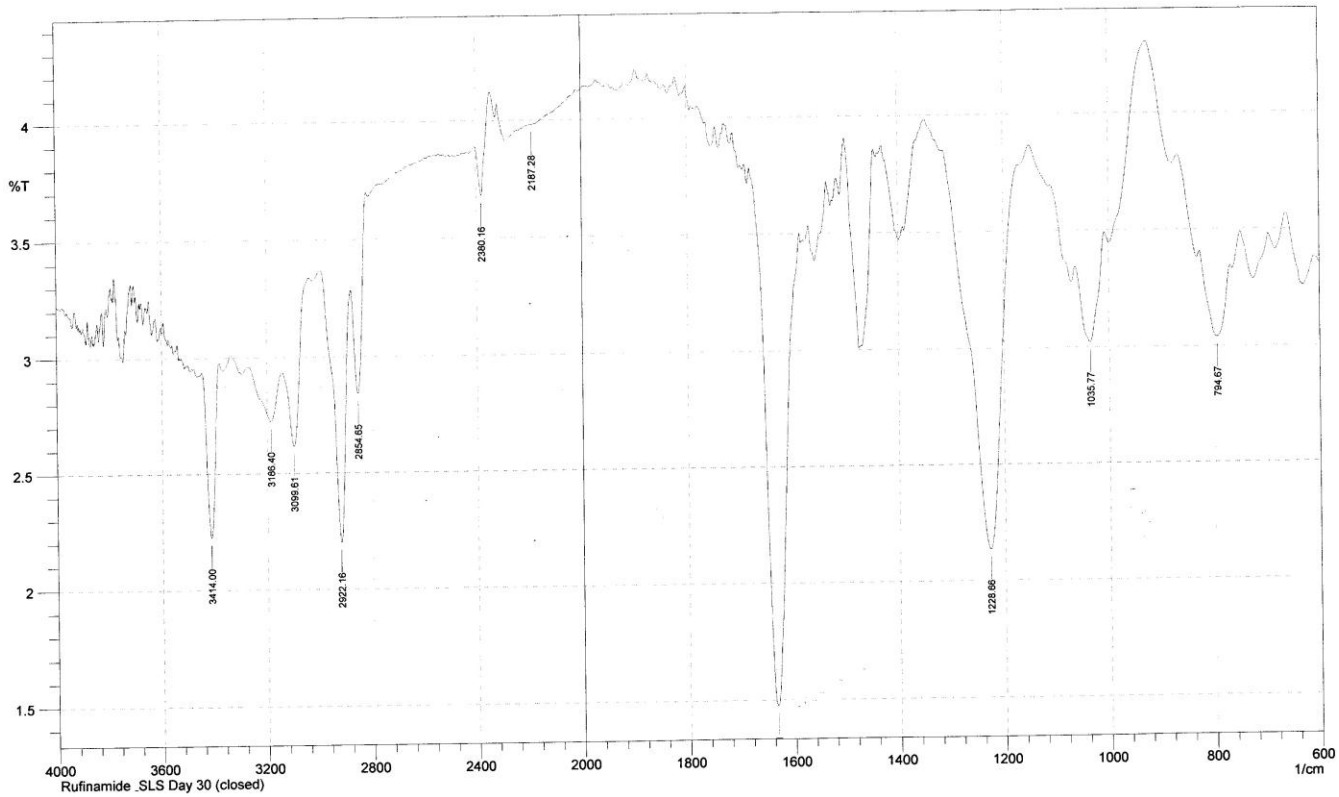
Appendix 3: FTIR spectrum of rufinamide and magnesium stearate



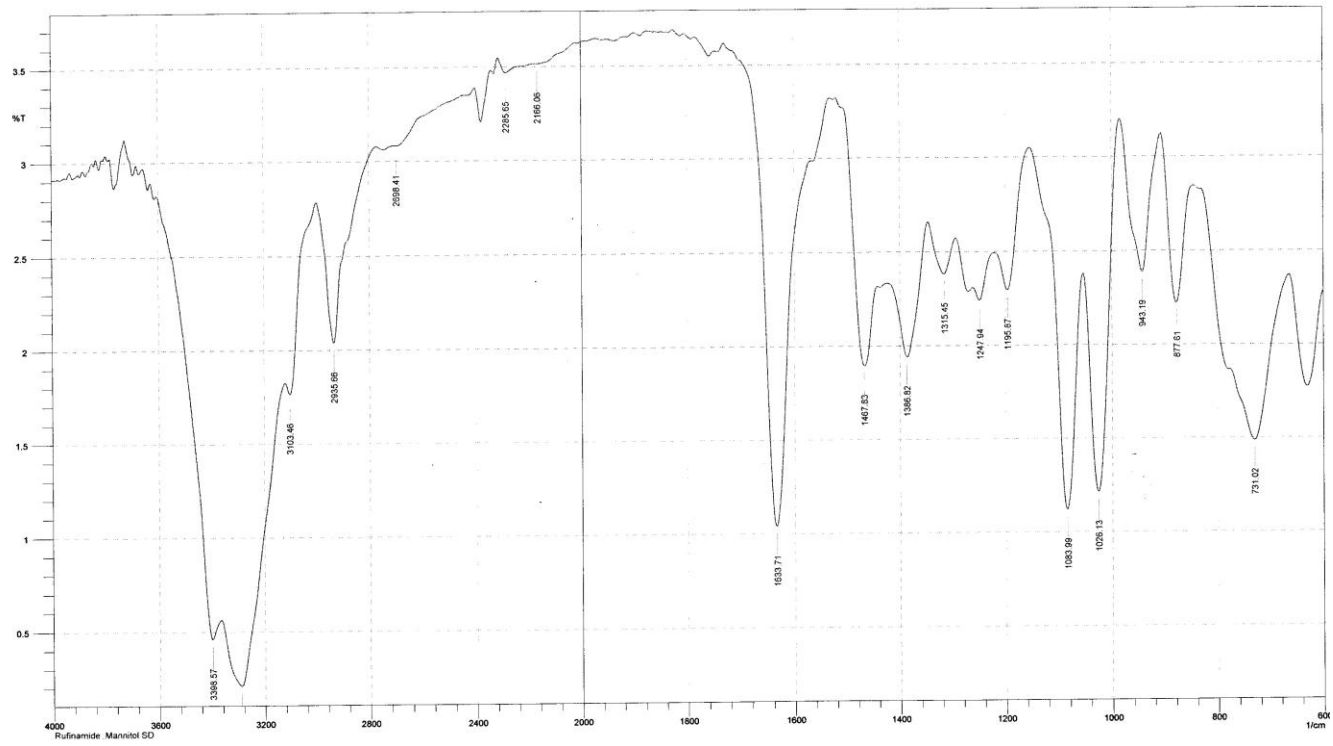
Appendix 4: FTIR spectrum of rufinamide and lactose monohydrate



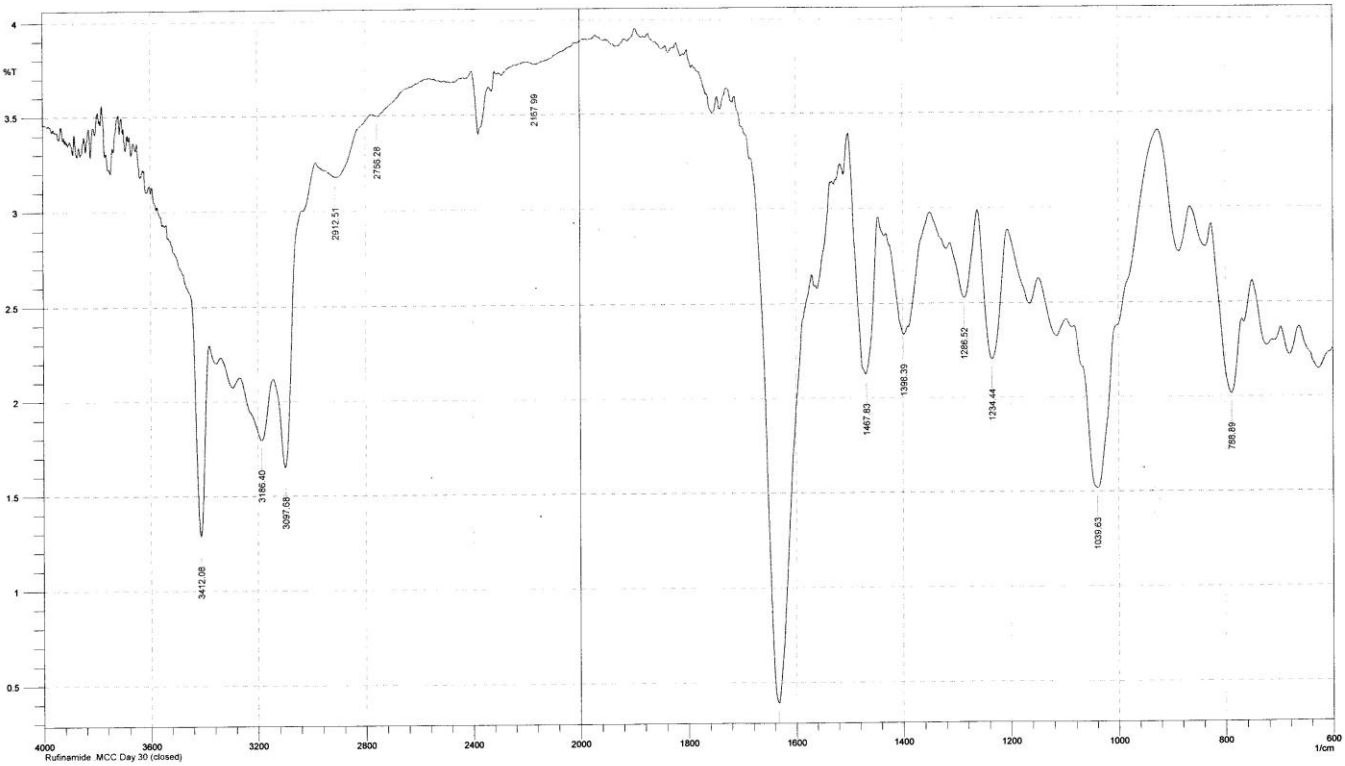
Appendix 5: FTIR spectrum of rufinamide and maize starch



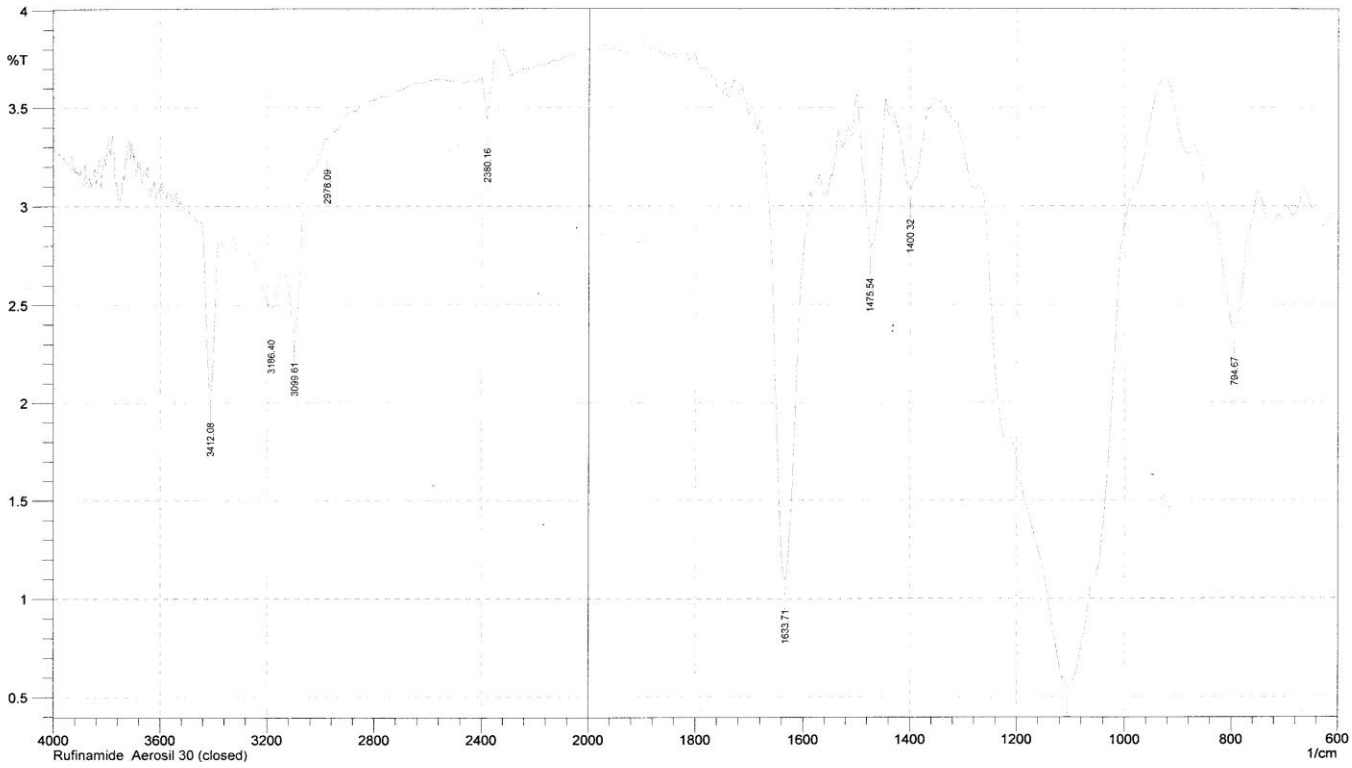
Appendix 6: FTIR spectrum of rufinamide and sodium lauryl sulfate



Appendix 7: FTIR spectrum of rufinamide and mannitol



Appendix 8: FTIR spectrum of rufinamide and microcrystalline cellulose



Appendix 9: FTIR spectrum of rufinamide and anhydrous colloidal silica

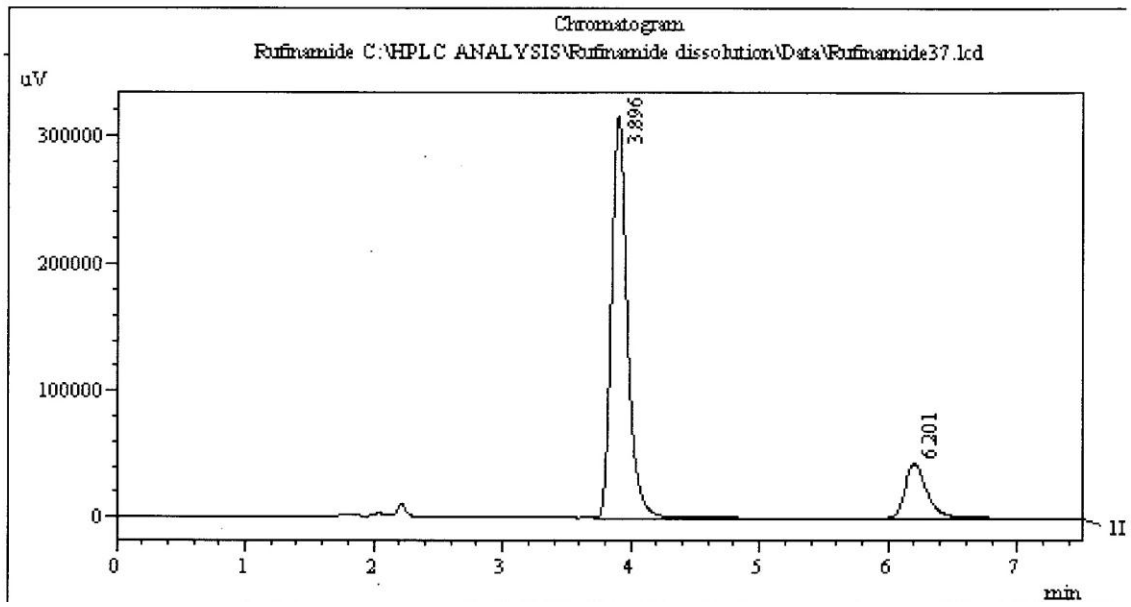
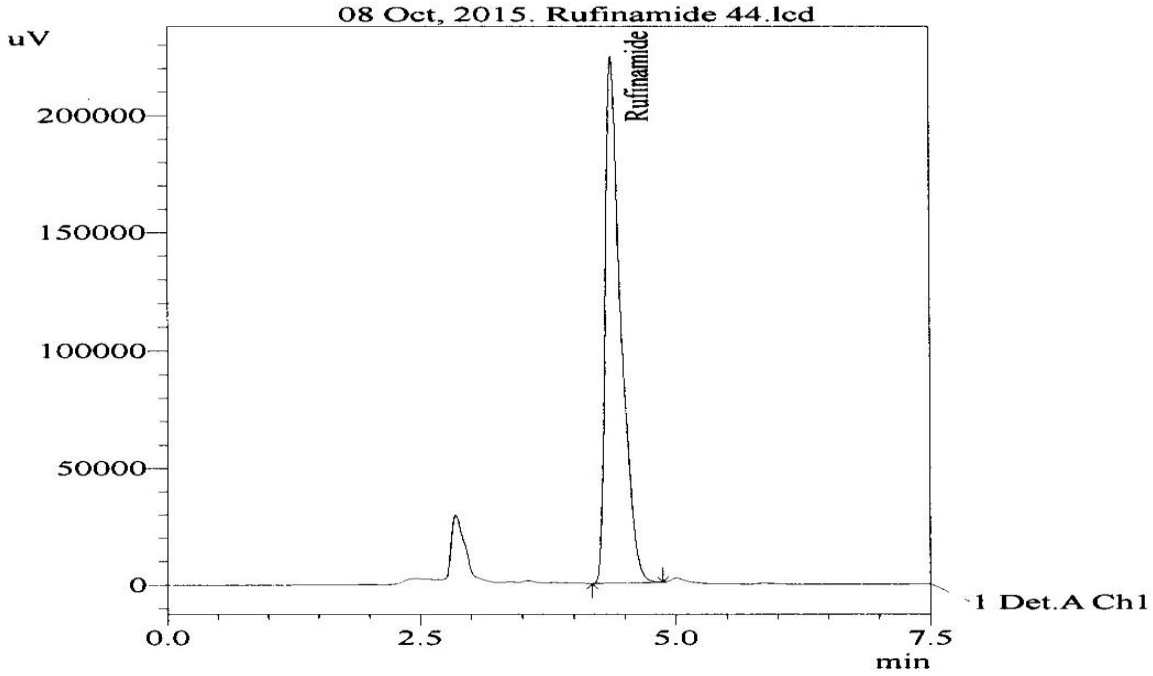


Figure Appendix 10: Chromatogram of assay of rufinamide content in formulation 2



Appendix 11: Chromatogram of dissolution of rufinamide in formulation 4 at 45 minutes.