DEVELOPMENT OF A PAEDIATRIC-FRIENDLY FORMULATION INTENDED FOR THE TREATMENT OF MULTI-DRUG RESISTANT TUBERCULOSIS

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DEVELOPMENT OF A PAEDIATRIC-FRIENDLY FORMULATION INTENDED FOR THE TREATMENT OF MULTI-DRUG RESISTANT TUBERCULOSIS

By

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DECLARATION

I, Jethro Nkomo, declare that the work contained in this dissertation is original (except where references have been made) and that neither parts of or the full dissertation has been submitted for another degree in this institute or any other institutes.

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Jethro Nkomo

Signed on the 12th day of April 2017 at the Nelson Mandela University

DEDICATION

This dissertation is dedicated to my mentor, father and teacher – a selfless and visionary man - Mr. C Moyo (Cornelius).

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SUMMARY

Children suffering from multidrug-resistant tuberculosis (MDR-TB) are treated with at least four drugs a day for at least twenty-four months. Approximately 25 000 - 32 000 children worldwide become infected with MDR-TB each year, yet there is a lack of adequate paediatric MDR-TB options for child-friendly dosage forms for the treatment of the condition. The available options are limited to manipulating different dosage forms intended for adults by means of breaking the tablets or otherwise, to deliver the drugs to children. This challenge that is faced by both health care professionals and caregivers subsequently poses drug quality, efficacy, and safety concerns to children being treated for MDR-TB.

The objective of this study was to formulate a paediatric-friendly dosage form for the treatment of MDR-TB in children below the age of eight years. A fixed-dose combination (FDC) in form of a dispersible-tablet that contains two core drugs used in treatment of MDR-TB; levofloxacin and pyrazinamide, was developed.

Quality by design principles was employed in developing the product. The systematic procedure ensures that quality is built into the product throughout the manufacturing process. It allows for identification of the critical quality attributes and modification of critical process parameters to lie within desired ranges. Preformulation studies were conducted on the active ingredients to investigate potential interactions and compatibility. Some of the analytical techniques employed in the process included an HPLC assay method that was developed to simultaneously separate levofloxacin and pyrazinamide, differential scanning calorimetry (DSC), infrared spectroscopy (IR), thermogravimetric analysis (TGA), and powder density studies.

A direct compression tableting process was selected as the method of choice for product formulation. Active ingredients were blended with the excipients and compressed using tableting equipment to successfully produce FDC fast-disintegrating tablets containing 150 mg of levofloxacin and 300 mg pyrazinamide. The product quality was analysed and optimised using mathematical and statistical techniques such as response surface methodology (RSM) and ANOVA, to meet the required standards recommended by the United States Pharmacopoeia. The FDC

dispersible tablet containing levofloxacin and pyrazinamide in the potential treatment of MDR-TB in children was successfully formulated, manufactured and evaluated. The tablet dosage form passed all the relevant quality criteria that governed the scope of this study and disintegrate in approximately 37 seconds when placed in water.

It is generally a sizeable challenge to manufacture fixed-dose combination drug products due to physicochemical differences of various drugs, however, with adequate resources researchers may still find a way to formulate more child-friendly dosage forms for MDR-TB. This may lead to improved drug efficacy, reduced safety risks and decreased burden on caregivers and healthcare workers who must administer the treatment.

Key Words: fixed-dose combination (FDC), levofloxacin, pyrazinamide, dispersible tablet, high performance liquid chromatography (HPLC), quality by design (QbD), response surface methodology (RSM)

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LIST OF CRONYMS

ACN	Acetonitrile
ANOVA	Analysis of Variance
AOR	Angle of Repose
API	Active Pharmaceutical Ingredient
BCS	Biopharmaceutics Classification System
BP	British Pharmacopoeia
CCD	Central Composite Design
CCS	Croscarmellose Sodium
cGMP	Current Good Manufacturing Practice Guide
cMAs	Critical Material Attributes
cPPs	Critical Process Parameters
cQAs	Critical Quality Attributes
CRP	Crospovidone
CSD	Colloidal Silicon Dioxide
DoE	Design of Experiments
DSC	Differential Scanning Calorimetry
FDA	Food and Drug Administration
FDC	Fixed-dose combination
HPLC	High Performance Liquid Chromatography
ID	Internal Diameter
IE	Ion Exchange
ICH	International Conference on Harmonization
IR	Infrared
LOD	Limit of Detection
LOQ	Limit of Quantitation
MCC	Microcrystalline Cellulose
MDR-TB	Multidrug-resistant tuberculosis
MeOH	Methanol
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance
NP	Normal Phase

QbD	Quality by design
QbT	Quality by testing
QTPP	quality target product profile
RH	Relative Humidity
RP	Reverse Phase
RPN	Risk Priority Number
RSD	Relative Standard Deviation
RSM	Response Surface Methodology
SEM	Scanning Electron Microscope
SSG	Sodium Starch Glycolate
TB	Tuberculosis
TGA	Thermogravimetric Analysis
USP	United States Pharmacopoeia
UV	Ultraviolet
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Tuberculosis (TB) is a term that describes an all-inclusive scope of clinical infections caused by *Mycobacterium tuberculosis* (*M*. *tuberculosis*). TB can essentially affect every human organ, and most significantly the lungs (Fitzgerald*, et al.*, 2010). The World Health Organisation (WHO) pronounced TB as a worldwide public health emergency in 1993. The condition has since been a perpetual and major health problem, especially in areas of the global community where multidrug-resistant strains are common (Nachega and Chaisson, 2003). TB is treated with four principal drugs which include isoniazid, rifampicin, pyrazinamide and ethambutol (World Health Organisation, 2017).

The World Health Organisation (2016a) reports that in 2015 alone, approximately 10.4 million new TB incidents occurred and 1.4 million deaths were recorded, hence identifying the infection as one of the top ten causes of death globally. South Africa falls among the six countries that accounted for 60% of these cases. The epidemic is intensified by the existence and spread of multidrug-resistant tuberculosis (MDR-TB) (World Health Organisation, 2008). MDR-TB is defined as a form of TB which is no longer susceptible to isoniazid and rifampicin, the two antibiotics which form the backbone of the first-line drug regimen used in treating pulmonary TB (Pinto and Menzies, 2011) .

A mathematical study model used to estimate the global burden of TB and MDR-TB in children approximated that two million children were suffering from MDR-TB in 2014 and 25 000 of them developed the disease that year (Dodd*, et al.*, 2016). Jenkins and colleagues (2014) had earlier found that about 32 000 children acquire MDR-TB annually. Due to poor reporting, as well as inadequate diagnosis and recording of TB incidences in children, the above-mentioned figures likely underestimate the true existing burden of TB in children (Jenkins*, et al.*, 2014; Seddon and Shingadia, 2014).

At the beginning of 2016, the WHO introduced a new fixed-dose combination (FDC) for the first-line treatment of TB in children (Graham*, et al.*, 2015). Children suffering from MDR-TB, however, still use child-inappropriate formulations whereby tablets need to be broken and crushed and dispersed in a solvent to achieve an appropriate dose. This does not always accomplish the intended outcomes as there are clinical implications around the practice. It may, instead, result in incorrect doses being administered and subsequently place lives in jeopardy (Ivanovska*, et al.*, 2014; Liu*, et al.*, 2014).

Use of non-fixed dosage forms increases the TB burden on the healthcare systems by augmenting challenges in procurement of TB medicines. In addition, use of treatment involving separate individual drugs poses a struggle to the children under treatment, as well as their caregivers (Blomberg*, et al.*, 2001). The awareness of these challenges brings a suggestion of the need for paediatric-friendly formulations for MDR-TB.

The standardised regimen for the treatment of MDR-TB in South Africa is outlined in the standard treatment guidelines and essential medicines list for South Africa. For children below the age of eight years, the regimen is divided into two phases, namely, the intensive and continuation phases. In the intensive phase that stretches over 6 months, children receive the following drugs and doses; oral levofloxacin 15–20 mg/kg/dose once daily, intravenous amikacin 15–22.5 mg/kg daily, oral terizidone 15–20 mg/kg daily, oral ethionamide 15–20 mg/kg daily and oral pyrazinamide 30–40 mg/kg daily. The continuation phase is the same as the intensive phase but without amikacin and extends over 18 months (South African National Department of Health, 2014). Delamanid is a newer drug that is on trial as a possible treatment for MDR-TB in children with and without HIV. High-dose isoniazid is also being investigated for the same condition in children while high-dose rifampicin and levofloxacin might soon find a place in treatment of paediatric TB meningitis (World Health Organisation, 2017).

Liquid dosage forms are the most preferred of the paediatric dosage forms for children under the age of five years. They are easy to swallow and allow for easy dose adjustments (Ali, *et al*., 2014). Oral solids are associated with the risk of choking and present limited dose flexibility (Sam, *et al*., 2012). However, considering that TB is

effectively treated over a long time, stability and long shelf-life are a major priority for the formulation. In that regard, Taneja and colleagues (2015) point out that dispersible tablets offer distinct advantages over liquid formulations in addition to the traditional immediate release oral solid dosage forms. Ivanovska and associates (2014) further elaborate that the advantages are particularly relevant, since liquids are less stable even when refrigerated. Liquid dosage forms are generally bulky and masking taste is difficult, hence liquids pose a challenge regarding product packaging, transportation and storage. Having noted this, the WHO expert forum recommended a paradigm shift towards use of dispersible tablets (World Health Organisation, 2008). It can be concluded that oral solid formulations are the preferred option for chronic conditions (Liu*, et al.*, 2014).

As stated by Hannan colleagues (2016), the introduction of oral dispersible tablets is a novel approach in drug delivery systems that helps build patient therapy compliance levels, as well as reducing the cost. The European Pharmacopoeia (2013) defines dispersible tablets as film-coated or uncoated tablets that can be dispersed in liquid before administration and disintegrate within three minutes. Dispersible tablets transfer to a homogenous dispersion in water or a small amount of breast milk. The formulation helps ease the administration considerably especially for paediatric and geriatric population (Dey and Maiti, 2010).

The ideal solution to the above defined problem would be to prepare a FDC formulation that includes all five drugs that are used in the initial phase of treatment and a second formulation consisting of the four drugs used in the continuation phase. Convenience in terms of administration, patient compliance and potential lower doses awards merit to the concept of FDCs (Desai, 2013). Siew (2015) observes that the major drawback that prevents availability of all necessary FDCs involves manufacturing and product formulation issues. Khan and Ali (2016) detail that the active pharmaceutical ingredients (APIs) in the FDCs must be compatible both chemically and physically amongst themselves as well as with the excipients. New impurities should not be generated by these APIs. Because of these factors, the formulating of an FDC that incorporates all the drugs used for the treatment of MDR-TB is thus a challenge.

The WHO 2016 guidelines for treatment of MDR-TB recommend that patients with MDR-TB should be on a regimen with at least five effective TB medicines during the intensive phase including pyrazinamide and four-core second-line TB medicines. The four-core second-line drugs are chosen as follows; one chosen from Group A, one from Group B, and at least two from Group C. The groups are shown in Table 1.1. Pyrazinamide is the constant drug in this regimen and is added routinely to the treatment, unless noted that the patient is infected with strains strongly suspected of or known to be resistant to pyrazinamide (World Health Organisation, 2016b).

Table 1.1: Recommended drugs used in the treatment of MDR-TB

A. Fluoroquinolones	Levofloxacin Moxifloxacin Gatifloxacin
B. Second-line injectable agents	Amikacin Capreomycin Kanamycin Streptomycin
C. Other core second-line agents	Ethionamide or Prothionamide Cycloserine or Terizidone Linezolid Clofazimine

The WHO confirmed in the 2016 MDR-TB treatment guidelines that fluoroquinolones, specifically high-dose levofloxacin, moxifloxacin, and gatifloxacin, significantly improve therapeutic outcomes in children. This group of drugs is regarded as the integral element of the core MDR-TB regimen. The benefits resulting from their use outweigh the potential known risks. Levofloxacin is the first in line in order of preference for the inclusion of the fluoroquinolones in the regimen. It is followed by moxifloxacin and lastly, gatifloxacin (World Health Organisation, 2016).

Since levofloxacin and pyrazinamide will be required in most patients, these two drugs are consequently going to be the drugs of focus in this study. They are also both

required during the intensive and continuation phases of treatment. In this study, the FDC dispersible tablets containing 150 mg and 300 mg levofloxacin and pyrazinamide respectively per tablet will be formulated following the principles of quality by design (QbD). The strengths represent the lower limits of the normal dose ranges for a weight of 10 kg. It should be noted that with adequate time and resources, a wide range of combinations containing more of the MDR-TB treatment drugs can be formulated.

1.2 APPLICATION OF QUALITY BY DESIGN

The concept of QbD was initiated into the pharmaceutical manufacturing industry by the Food and Drug Administration (2006) as a measure to counter some of the drawbacks of quality by testing (QbT), the traditional system used to warrant quality of drug products. The QbT approach focuses on measuring quality by testing finished products, which are manufactured by following fixed manufacturing processes. The causes of product failure are generally not understood in QbD, resulting in wastage and negative financial implications (Charoo*, et al.*, 2012).

On the other hand, QbD in drug product manufacture is anchored in building quality into the product through design. This current technique is quality risk-management based where critical starting materials as well as parameters involved in processing need to be thoroughly understood and linked to the product's critical quality attributes QbD permits the isolation of variables that bear impact on method performance and it normally follows certain closely linked parameters which are intermediates of each other (Zhang and Mao, 2017). The initial step of QbD involves establishing a quality target product profile (QTPP) of the product, which is a summary of the potential quality characteristics of the final product. This element incorporates dosage form, strength and appearance and route of administration as well as attributes that may have an effect on quality criteria such as purity as well as pharmacokinetic factors such as disintegration (Sangshetti*, et al.*, 2014).

Identifying the product's critical quality attributes (cQAs) should then be accomplished. The cQAs are microbiological, biological and physicochemical characteristics of the final product that arise from the QTPP and should be maintained within suitable ranges as they are employed in directing formulation development. Closely linked to cQAs are

the critical material attributes (cMAs) which should also be within certain standards and limits to guarantee correct quality of the input materials such as drug substances and excipients. Critical process parameters (cPPs) are process characteristics that may influence the cQAs of the product and should be examined before or during process. It is important that cMAs and cPPs be linked to cQAs to meet the product's QTPP (Zhang and Mao, 2017).

Design of experiments (DoE) is then applied to assess the impact of the selected development and manufacturing process variables on cQAs. When the formulation process selected meets the expected and recommended specifications, the product is assessed and optimised according to control strategies. The sequence of elements that shape the principles of design by quality are summarised in the flow diagram below, which was adapted from Zhang and Mao (2017).

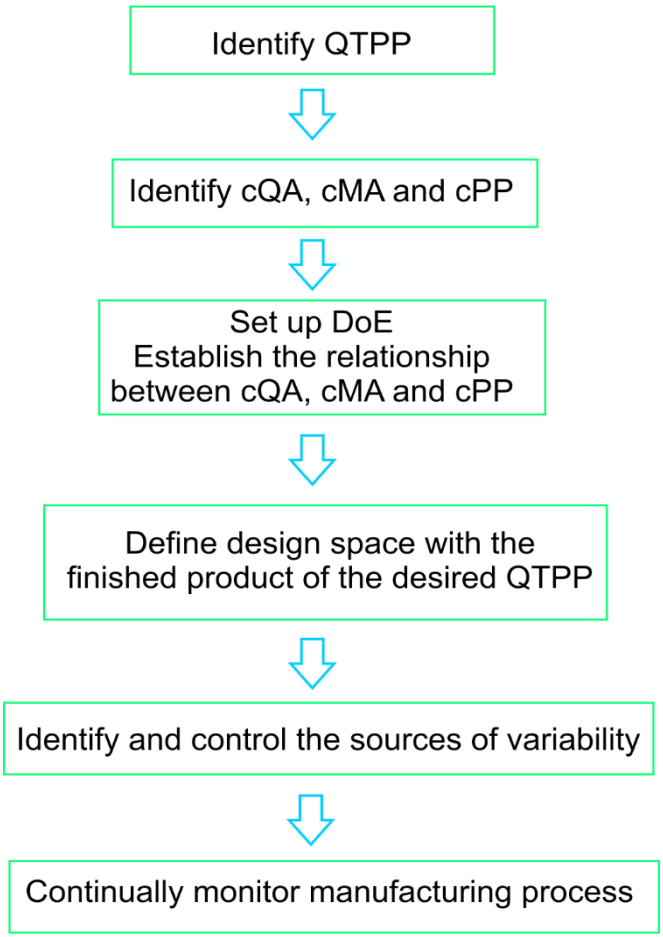


Figure 1.1: Quality management in formulation development using Quality by Design (QbD) (Adapted: Zhang and Mao, 2017).

1.3 PROBLEM STATEMENT

MDR-TB is widespread in both adults and children. Unfortunately, there is currently no approved paediatric-friendly FDC for the five and four drug combinations used in the initial and continuation phases of treatment, respectively. This reduces patient compliance and may result in loss of lives. Formulating and manufacturing a prototype dispersible FDC tablet may therefore prevent challenges that come with using traditional tablets and capsules, lessening pill burden at each dosing period thereby improving adherence and treatment outcomes.

1.4 AIM AND OBJECTIVES

1.4.1 Aim

This study aims to develop an FDC dispersible tablet containing 150 mg of levofloxacin and 300 mg of pyrazinamide for paediatric use.

1.4.2 Objectives

Based on the aim stated above, the objectives of this study are therefore to:

- Determine the preformulation characteristics of both levofloxacin and pyrazinamide;
- Define the Quality Target Product Profile (QTPP) for the FDC formulation;
- Identify the critical quality attributes (cQAs), critical material attributes (cMAs) and critical process parameters (cPPs) for the formulation;
- Determine the relationship between cMAs, cPPs and cQAs through the application of Design of Experiments (DoE);
- Establish, formulate and characterise an optimised FDC dispersible tablet containing levofloxacin and pyrazinamide;

CHAPTER TWO

PROPERTIES OF LEVOFLOXACIN AND PYRAZINAMIDE

2.1 INTRODUCTION

Levofloxacin and pyrazinamide will be the major components of the finished product. It is therefore essential that characteristics of the drug substances be well comprehended to facilitate quality risk management and sound science in product manufacturing. In understanding the physicochemical properties of the drugs, possible factors that affect pharmacokinetic performance of the drug product are be identified, hence in this chapter, the foundations of defining the QTPP are laid (Yu*, et al.*, 2014).

Levofloxacin is a second-generation quinolone. This class of antibiotics was discovered in the early 1960s, first with the identification of nalidixic acid (Andersson and MacGowan, 2003; Andriole, 2005). As much as levofloxacin has a wide range of potential uses, it is mainly focused on the treatment of respiratory conditions (Andersson and MacGowan, 2003). Aldred and colleagues (2014) in agreement with Kabbara and fellow research team (2015) observe and agree that fluoroquinolones are among the most frequently prescribed antibiotic classes globally. Furthermore, a study conducted by Davis and colleagues (2014) indicates that levofloxacin falls within the group of most used antimicrobial agents in its class.

Quinolones compose the mainstay of the treatment regimens for MDR-TB (Thee*, et al.*, 2015). In South Africa, only levofloxacin and moxifloxacin are approved for use in MDR-TB treatment. Levofloxacin is recommended mainly in children below eight years of age whereas the rest of the population uses moxifloxacin (The National Department of Health, 2013). Though a higher dose levofloxacin is needed to achieve similar effectiveness of moxifloxacin, levofloxacin was found to inflict lesser toxicity in its users compared to moxifloxacin, hence it is used in younger children (Johnson*, et al.*, 2006).

Developed in 1952 by Kushner and colleagues while investigating analogs of nicotinamide, pyrazinamide has since been used to treat TB, though it was first considered to be a highly toxic drug (Kushner*, et al.*, 1952; Pretet and Perdrizet, 1980). It is an important sterilizing agent that shortens TB treatment duration in combination with other anti-TB drugs. Pyrazinamide is used in treatment of both TB and MDR-TB.

It is bactericidal and has activity against non-replicating and semi-dormant tubercle bacilli that are not killed by other TB drugs (Zhang*, et al.*, 2003; Zhang*, et al.*, 2014; Pullan*, et al.*, 2016).

Studies were conducted in New Zealand to develop a phospholipid-based pyrazinamide inhalable powder dosage form for treating tuberculosis. Successful development of the dosage form should help deposit pyrazinamide deeper into the lungs and improve drug delivery (Eedara*, et al.*, 2016). FDC formulations available for the treatment of TB in children contain rifampicin, isoniazid and pyrazinamide, then rifampicin and isoniazid, for use during intensive phase and continuation phase respectively (Graham*, et al.*, 2015).

2.2.1 Levofloxacin

2.2.1.1 Description

Levofloxacin is an odourless and bitter to taste, light-yellowish to yellow-white crystalline powder. It is a chiral fluorinated carboxyquinolone, an L-isomer and a pure (S)-enantiomer of the racemic quinolone antimicrobial agent. Chemically, levofloxacin is presented as (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. Levofloxacin's empirical formula is $C_{18}H_{20}FN_3O_4 \cdot ½H_2O$ and the relative molecular weight is 361.37 g/mol (Singh*, et al.*, 2002; World Health Organisation, 2010). The chemical structure of levofloxacin is depicted in Figure 2.1

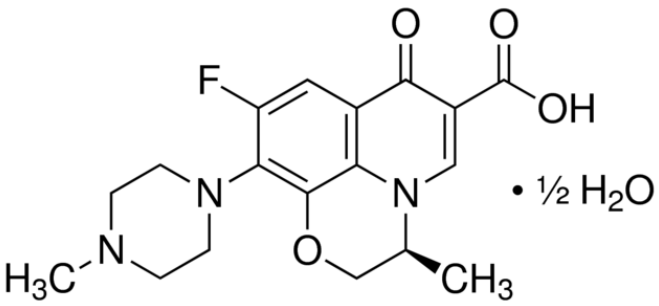


Figure 2.1: Chemical structure of Levofloxacin hemihydrate

2.2.1.2 Solubility

Levofloxacin is sparingly soluble in water. The solubility profile is quite flat, with a range of 73-108 mg/ml between pH 0.56-5.84. Solubility increases proportionally with pH to a maximum of 272 mg/ml at pH 6.74. The minimum pH solubility profile is found within the pH range 7- 8 (Frick, et al., 1998). The solubility of levofloxacin in different solvents is listed in Table 2.1 (Indian Pharmacopoeia Commission, 2007).

Table 2.1: Solubility of levofloxacin in different solvents (Adapted: Indian Pharmacopoeia Commission, 2007)

Solvent	**Solubility**
Dichloromethane	slightly soluble or soluble
Methanol	slightly soluble
Glacial acetic acid	soluble
Acetic acid	sparingly soluble
Chloroform	sparingly soluble
Dilute sodium hydroxide solution	soluble

2.2.1.3 Melting Point and Stability

Melting point of levofloxacin ranges between 224 °C and 226 °C, after which decomposition starts (O'Neil, 2006). Levofloxacin is reported to readily undergo photodegradation and follows first-order degradation kinetics in the initial stages of the reaction. Levofloxacin is more stable around a pH close to 7, which makes this range favourable for formulation purposes (Ahmad*, et al.*, 2013). Gul and team (2015) observe that levofloxacin is not affected by acidic medium when in solution and is inert at room temperature. The drug powder is non-hygroscopic, and therefore stable when exposed to humidity.

2.2.1.3 Crystal Morphology

Levofloxacin exists in three forms of polymorphs, namely crystalline anhydrous α, β and γ. Additionally, the molecule has two forms of pseudopolymorphic or solvated forms which are hemihydrate and monohydrate (Gorman*, et al.*, 2012). In a previous

study that investigated the effect of dehydration on the formation of levofloxacin pseudopolymorphs, Kitaoka and fellow researchers (1995) discovered that application of heat on the hemihydrate eliminates the hydrated water and collapses the crystalline lattice to produce the anhydrous γ form. Further heating produces anhydrous β form which ultimately results in the formation of the α anhydrous form when more heat is applied.

2.2.2 Pyrazinamide

2.2.2.1 Description

Pyrazinamide, a pyrazine analogue of nicotinamide, comes in a white or almost white odourless crystalline powder. Its molecular formula is $C_5H_5N_3O$ and has a relative molecular mass of 123.1g/mol. The chemical names of pyrazinamide are pyrazinecarboxamide and pyrazine-2-carboxamide (Felder and Pitre, 1983). The chemical structure is depicted in the Figure 2.2.

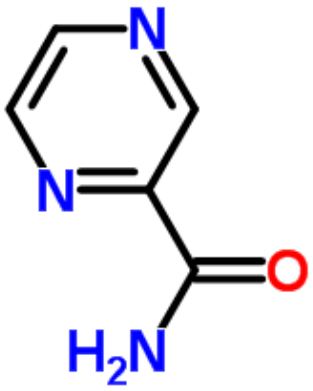


Figure 2.2: Pyrazine-2-carboxylic acid

2.2.2.2 Solubility

The solubility of pyrazinamide in different agents has been adapted from O'Neil (2006) and is summarized in the Table 2.2

Table 2.2: Solubility of pyrazinamide in different solvents

Solvent	**Solubility**
Water	sparingly soluble
Chloroform	sparingly soluble
Ethanol (95 per cent)	slightly soluble
Ether	slightly soluble
Benzene	less soluble

2.2.2.3 Melting Point and Stability

Pyrazinamide powder melts within the temperature ranges of 189 °C and 191 °C (O'Neil, 2006). In powder or solid form, pyrazinamide exhibits good stability. There is no apparent degradation of bulk sample displayed due to exposure to either a dry or wet atmosphere. Pyrazinamide maintains stability when exposed to natural daylight (Felder and Pitre, 1983; Singh*, et al.*, 2002).

2.2.2.4 Crystal Morphology

Pyrazinamide is reported to occur in four distinct polymorphic forms, namely, α-pyrazinamide, β-pyrazinamide, γ-pyrazinamide and δ-pyrazinamide (Takaki*, et al.*, 1960; Tamura*, et al.*, 1961). Furthermore, Takaki and associates (1960) as well as Tamura and fellow partners (1961) agree that α-pyrazinamide is synthesised at room temperature. These findings, however contrast with those compiled by Castro and co-workers (2010) who give an account that the δ-pyrazinamide form is the most stable polymorph at low temperatures. Pyrazinamide polymorphs' relative stability and vibrational spectroscopy have been investigated by Cherukuvada and associates (2010) and they conclude that commercial α-pyrazinamide is the form which is most thermodynamically stable at room temperature.

2.3 CLINICAL PHARMACOLOGY OF LEVOFLOXACIN AND PYRAZINAMIDE

2.3.1 Levofloxacin

2.3.1.1 Mode of action

All antibiotic agents under the chemical class of fluoroquinolones have the same mechanism of action against bacteria. Normal transcription and replication requires relaxation of positively supercoiled DNA. DNA gyrase is responsible for this relaxation process. Topoisomerase IV is responsible for the separation of the replicated daughter cell chromosomal DNA (Deck and Winston, 2015). Levofloxacin targets and blocks the bacterial topoisomerase II (DNA gyrase) and topoisomerase IV enzymes, thereby inhibiting bacterial DNA synthesis (Hooper and Strahilevitz, 2010).

2.3.1.2 Spectrum of activity and resistance

Levofloxacin has been known to possess moderate to good activity against Gram-positive bacteria, including *Streptococcus pneumoniae* (*S. Pneumoniae*). On the other hand, it displays excellent activity against gram-negative aerobic bacteria (Deck and Winston, 2015). *In vitro* investigations conducted by Akcali and co-researchers (2005) to test the activity of ciprofloxacin, ofloxacin and levofloxacin against *M. tuberculosis* revealed these agents as being highly potent against the bacteria. As observed by Oethinger and collegues (2000) as well as Hooper (2002), resistance of bacteria to levofloxacin develops due to mutations in the bacterial chromosomal genes that encode topoisomerase IV DNA gyrase. Bacteria may also develop a pump-mediated efflux mechanism through which the drug is actively transported out of the bacteria (Aldred*, et al.*, 2014).

Gold and Moellering (1996) reported that there is no quinolone-modifying or inactivation activities identified in bacteria. This theory was nullified after a decade by further studies performed and reported by Robicsek and collegues (2006). The findings maintain that some bacteria reduce activity of quinolones through a mechanism that modifies the piperazinyl substituent by *N*-acetylation of the amino nitrogen. The latter idea was to be later echoed by Hernández and colleagues (2011)

who detail the compromise of enzymatic potency of quinolones by specific gene possessing bacteria.

2.3.1.3 Clinical use

Levofloxacin is reported to have increased activity against Gram-positive organisms when weighed against ciprofloxacin or ofloxacin. It is clinically indicated for the treatment of lower respiratory tract, sinus, urinary tract and soft tissue infections (Langtry and Lamb, 1998; Rossiter, 2014). Relative to other quinolones, levofloxacin has enhanced activity against aerobic Gram-positive bacteria and is effective in the treatment of severe infections caused by *S. pneumoniae*, including those strains that are penicillin resistant. Though not quite as active against aerobic Gram-negative bacteria, levofloxacin is effective against *Pseudomonas aeruginosa* (*P. aeruginosa*) and most infections caused by these bacteria (Hauser, 2015).

Levofloxacin has also been established as a possible prophylaxis agent against *Aeromonas* infections during leech therapy (Bauters*, et al.*, 2014). The revision of the South African DR-TB treatment guidelines in 2012 recommended the use of levofloxacin for prevention or treatment of DR-TB in children below eight years of age (Thee*, et al.*, 2014).

2.3.1.4 Side effects and interactions

As with all the fluoroquinolones, levofloxacin is generally well tolerated. Pseudomembranous colitis remains the main potential adverse effect, just like virtually all the antimicrobial agents (Wimer*, et al.*, 1998). Gastrointestinal symptoms, including diarrhoea, are the most common side effects (Deck and Winston, 2015). They are experienced by 5% to 10% of individuals taking levofloxacin. It is documented that about 5% of patients on levofloxacin experience adverse effects involving the central nervous system such as headache and dizziness. Rashes occur in approximately 1% to 2% of the patients (Hauser, 2015; Liu, 2010; Anderson and Perry, 2008).

It has been observed that fluoroquinolones have a potential of causing reversible arthropathy whereby growing cartilage is damaged. Levofloxacin is no exception in

this regard (Liu, 2010; Deck and Winston, 2015). Deck and Winston (2015) further elaborate that tendonitis and tendon rupture may occur in adults where the tendons are fully developed. However, this is a rare complication resulting from levofloxacin use. Recent studies conducted by Lee and associates (2015) point out that fluoroquinolones are associated with aortic aneurysm and dissection resulting from collagen degradation.

Reports of levofloxacin risk in prolongation of the QTc interval have been recorded. To a lesser extent than other quinolones, levofloxacin can block potassium channels. This delays repolarization in cardiac tissue, subsequently resulting in ventricular arrhythmias such as torsades de pointes (Hooper and Strahilevitz, 2010; Deck and Winston, 2015; Patel*, et al.*, 2010). Studies have revealed that hypoglycaemia may be precipitated using levofloxacin, especially in the geriatric population (Kanbay*, et al.*, 2006; El Ghandour and Azar, 2015).

Co-administration of levofloxacin with magnesium, calcium and aluminum containing antacids markedly reduces oral bioavailability. This is presumably due to the formation of poorly absorbed cation-quinolone complexes (Radandt*, et al.*, 1992). Multivitamin-mineral supplements that contain both iron and zinc or zinc alone, have also been found to reduce quinolone absorption (Hooper and Strahilevitz, 2010; Radandt*, et al.*, 1992). Levofloxacin shows weak inhibition of the cytochrome P450 isoform CYP2C9 enzymes (Zhang*, et al.*, 2008). Consequently, levofloxacin displays insignificant potential to alter CYP2C9 substrate drug pharmacokinetics (Fish and Chow, 1997). The clearance of levofloxacin is predominantly via the renal route. The rate of elimination is decreased by co-administration with cimetidine and probenecid, resulting in increased levofloxacin half-life (Baxter, 2010).

2.3.1.5 Substantial risk groups

As fluoroquinolones possess a similar core structure, individuals who are hypersensitive to any fluoroquinolone should also avoid using levofloxacin due to cross sensitivity (Anovadiya*, et al.*, 2011). Researchers found that athletes and sporting individuals are at risk of developing tendinopathy and potential tendon rupture with levofloxacin and other fluoroquinolone usage (Ganske and Horning, 2012; Lewis

and Cook, 2014). Several cases in literature reveal that diabetes mellitus patients are a high-risk group with regards to use of levofloxacin and some fluoroquinolones. Life-threatening metabolic coma, severe hypoglycemia, induced crystal nephropathy and increased hepatotoxicity are some of the reported cases which researchers conclude are linked to use of levofloxacin in diabetics (Bansal*, et al.*, 2015; Micheli*, et al.*, 2012; Liu*, et al.*, 2015; Coelho*, et al.*, 2011).

The geriatric population appears to be more susceptible to the potential risks of levofloxacin use. Cases of levofloxacin associated hypoglycemia, as well as Stevens - Johnson syndrome in the elderly have been reported (Kanbay*, et al.*, 2006; Burgos Arguijo*, et al.*, 2010). Due to the potential risk of adverse musculoskeletal effects following fluoroquinolone treatment, fluoroquinolones are generally not recommended for use in children below the age of 18. Since the resulting arthropathy is reversible, research supports fluoroquinolone use in children in cases where standard antibiotic therapy is not responding well (Choi*, et al.*, 2013; Deck and Winston, 2015).

With fear that they could be teratogenic, fluoroquinolones have been labelled as unsafe during pregnancy. However, there is no clinically supported evidence to solidify these assumptions (Loebstein*, et al.*, 1998). The growing body of data suggests that the drugs may in fact be safe for use during pregnancy (Bar-Oz*, et al.*, 2009; Yefet*, et al.*, 2015).

2.3.2 Pyrazinamide

2.3.2.1 Mode of action

Pyrazinoic acid, the active metabolite of pyrazinamide, disrupts membrane transport function in *Mycobacterium tuberculosis* (Zhang*, et al.*, 2003). Bacterial energy production, coenzyme A functionality and trans-translation mechanism in proteins synthesis are also inhibited (Zhang*, et al.*, 2014). Pyrazinamide is more effective against old non-replicating bacilli due to their low membrane potential. The highest rate of activity of the drug is displayed in acidic environment (Zhang*, et al.*, 2003). Figure 2.3 shows the possible mechanism of action which pyrazinamide adopts to damage bacterial cell membrane.

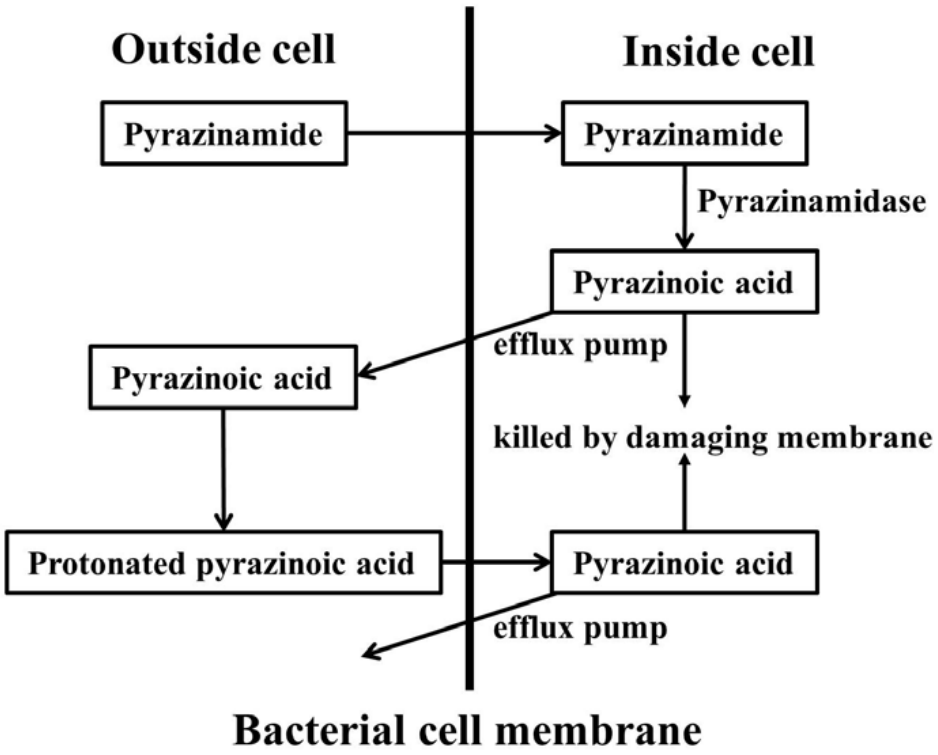


Figure 2.3: The proposed mode of action of pyrazinamide (Adapted: Zhang, *et al*., 2003)

2.3.2.2 Spectrum of activity and Resistance

Pyrazinamide has a relatively narrow antimicrobial spectrum. Its clinical activity is significant only against *Mycobacterium africanum* and *M. tuberculosis* (Drew, 2016). As mentioned earlier, the active form of pyrazinamide is pyrazinoic acid. Pyrazinamidase converts the prodrug into the active form. According to several findings, resistance to pyrazinamide is mostly as a result of mutations in the gene encoding pyrazinamidase, which prevents formation of the active form of the drug (Zhang*, et al.*, 2014).

2.3.2.3 Clinical uses

Investigations performed by Crowle in 1986 yielded results with the conclusion that 12.5 µg/mL of pyrazinamide is bactericidal for tubercle bacilli. In South Africa, the drug forms part of the multidrug standardised regimen for treatment of both TB and MDR-TB in adults and children (The National Department of Health, 2012; The National

Department of Health, 2013). Co-administering pyrazinamide with rifampicin or isoniazid leads to a significant reduction in the anti-TB therapy duration, while also lowering the likelihood of TB relapse (Gumbo, 2011).

2.3.2.4 Side effects and interactions

Adverse effects of pyrazinamide include elevated uric acid levels in the serum which may lead to gout. Hepatotoxicity has been reported from approximately 15% of patients and jaundice in about 2-3% of users (Felder and Pitre, 1983; Chang*, et al.*, 2008). In rare instances, death due to hepatic necrosis has also been reported (Chang*, et al.*, 2008). Gastrointestinal-related adverse manifestations are common, whereas dermatological reactions due to pyrazinamide use are rare (Khayyam*, et al.*, 2010).

According to a study done to determine the pharmacokinetics of pyrazinamide under fasting conditions, antacids were found to cause no clinically significant changes in pyrazinamide pharmacokinetic parameters (Peloquin*, et al.*, 1998). Upon administration with antacids, the absorption peak time of pyrazinamide was reduced by approximately 17%, nevertheless, this effect is not clinically important (Peloquin*, et al.*, 1998). As highlighted earlier, it is common for pyrazinamide to cause hyperuricaemia and as a result, uricosuric effects of anti-gout drugs may be antagonised (Cullen*, et al.*, 1957; Pham*, et al.*, 2014).

2.3.2.5 Substantial risk groups

In their review of the data with regards to TB treatment in pregnant women, Loto and Awowole (2012) discovered that use of pyrazinamide in pregnancy is now recommended by most international health organisations. Physicians had initially avoided its use on grounds of unavailability of concrete information on its teratogenicity. However, despite the inclusion of pyrazinamide in the treatment of tuberculosis in pregnant women, there have been no reports of significant adverse events (Anderson, 1997; Bothamley, 2001).

The analysis of the published data to review occurrences of anti-TB drug-induced hepatotoxicity in children was carried out by Donald (2011). Results approximate that 0.06% of children treated with pyrazinamide in combination with isoniazid and rifampicin developed jaundice (n=12 708), while 8% of the children studied displayed abnormal liver function test results (n=1 225). In another study conducted on Japanese paediatric patients, it was reported that eight in 99 children receiving pyrazinamide developed severe hepatotoxicity. All the eight were below the age of five years (Ohkawa*, et al.*, 2002). Age is one of the major risk factors of drug-induced hepatotoxicity. Close monitoring of liver function should be carried out on younger children and the elderly receiving pyrazinamide for tuberculosis chemotherapy (Ohkawa*, et al.*, 2002; Tanizaki*, et al.*, 2013).

Individuals known or suspected to have liver injury should avoid use of pyrazinamide. Similarly, patients suffering from gout or who are hypersensitive to pyrazinamide or its components should not be re-challenged (Saukkonen*, et al.*, 2006). Dosing frequency should be reduced to thrice a week in patients with chronic renal failure (Stamatakis*, et al.*, 1988).

2.4 PHARMACOKINETICS OF LEVOFLOXACIN AND PYRAZINAMIDE

2.4.1 Levofloxacin

2.4.1.1 Dosage

With relevance to this study, the standardised treatment guidelines for tuberculosis in children below eight years old suffering from MDR-TB in South Africa exists (The National Department of Health, 2013). A dose of between 15 mg and 20 mg/kg/dose once daily of oral levofloxacin is recommended. The maximum dose of levofloxacin is 1 000 mg per day.

2.4.1.2 Absorption and Distribution

Following oral administration, levofloxacin's absorption from the gastrointestinal tract is rapid and approximately 100%, with the absolute bioavailability also approaching 100% (Chien*, et al.*, 1997; North*, et al.*, 1998). The plasma-concentration time profiles

following oral or intravenous infusion over an hour have insignificant differences. Time to reach peak plasma concentrations (t_{max}) ranges from 0.8 to 2.4 hours and the peak concentration (C_{max}) ranges from 0.6 to 9.4 mg/L upon administration of 50 to 1000 mg (Fish and Chow, 1997).

Levofloxacin is widely distributed throughout the body, in tissues and fluids (Zhu*, et al.*, 2016). In one research, it was found that the mean volume of distribution is 1.1 L/kg with relatively poor penetration into the cerebrospinal fluid. It is further approximated that 24 to 38% of the drug is bound to albumin serum proteins (Fish and Chow, 1997).

2.4.1.3 Metabolism and Elimination

Studies show that levofloxacin undergoes limited metabolism in humans. This is confirmed by results concluding that approximately 80% of the drug is excreted unchanged in the urine, with formation of no metabolites (Fish and Chow, 1997). The majority of the elimination of levofloxacin is accounted for by renal metabolism (Hooper and Strahilevitz, 2010).

2.4.2 Pyrazinamide

2.4.2.1 Dosage

The paediatric standard treatment guidelines and essential medicines list for South Africa (The National Department of Health, 2013) spells out the recommended drug doses for treatment of various conditions. For the treatment of MDR-TB in children under the age of eight years, oral pyrazinamide 30 to 40 mg/kg daily is recommended. The dose is maintained through both the intensive and continuation phases.

2.4.2.2 Absorption and Distribution

Through evaluations made in both healthy adult volunteers and patients, outcomes show that pyrazinamide is rapidly absorbed, with more than 90% bioavailability. T_{max} is reached within an hour or two, with a half-life of eight to 11 hours (Ellard, 1969; Lacroix*, et al.*, 1989; Roy*, et al.*, 1999). About 15 to 40% of the drug is protein bound

(Woo*, et al.*, 1996). In patients with tuberculous meningitis, pyrazinamide was found to penetrate well into the cerebrospinal fluid (Nau*, et al.*, 2010; Donald, 2010).

2.4.2.3 Metabolism and Elimination

Pyrazinamide is metabolized by microsomal deamidase into metabolites which include pyrazinoic acid, 5-hydroxy-pyrazinamide and 5-hydroxy-pyrazinoic acid (Lacroix*, et al.*, 1989). These compounds are excreted by the kidneys. Pyrazinuric acid has also been identified in minor quantities in human urine (Beretta*, et al.*, 1987). In cases of renal failure, clearance of pyrazinamide is reduced, and dosing frequency must be reduced accordingly, normally to either thrice or twice weekly instead of the standard once daily doing. Re-dosing after a hemodialysis session should be considered since the process eliminates pyrazinamide from the body (Stamatakis*, et al.*, 1988; Malhotra, 2003). Figure 2.4 summarises the different metabolism pathways followed by pyrazinamide after being ingested.

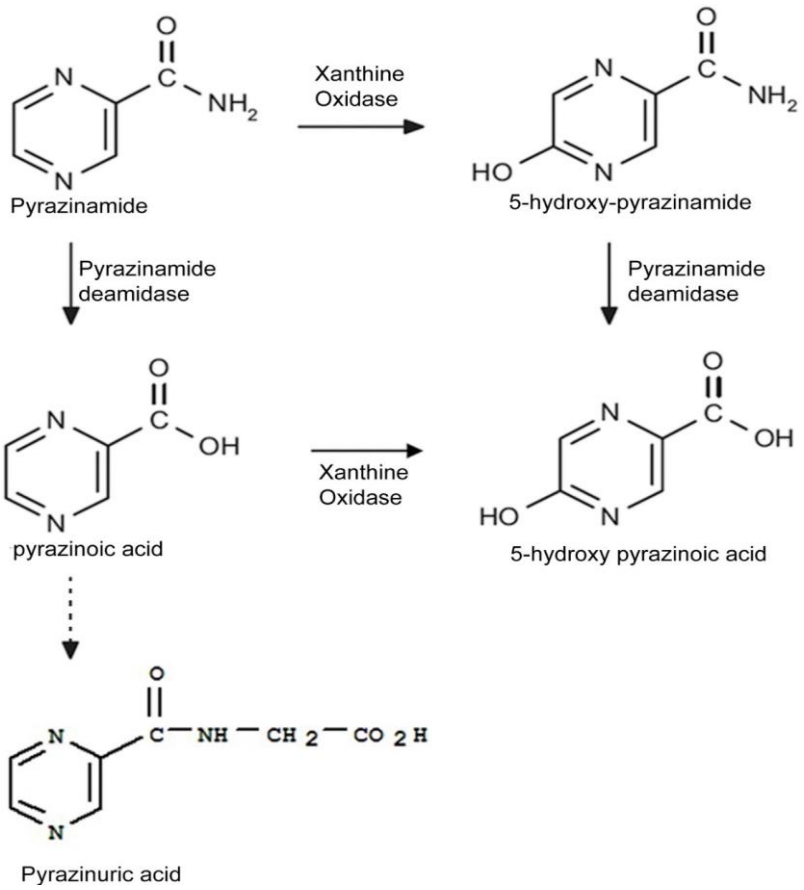


Figure 2.4: Metabolic pathways of pyrazinamide (Redrawn from Lacroix, *et al*. 1989)

2.5 CONCLUSION

Levofloxacin and pyrazinamide share some common physical properties. They both exhibit high melting and are stable at room temperatures while also being non-hygroscopic at ambient conditions. These properties facilitate easy handling in the laboratory, as well as during tablet manufacturing.

The similarities allow for the compounding of an FDC formulation containing levofloxacin and pyrazinamide. The adverse effects of the two drugs are clearly detrimental to the target children population. These drugs have, however, been used for decades and it has been concluded that the benefits outweigh their risk. Pharmaceutical care remains essential in ensuring that the side effects do not go unnoticed and are managed accordingly.

CHAPTER THREE

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE DETERMINATION OF LEVOFLOXACIN AND PYRAZINAMIDE

3.1 INTRODUCTION

3.1.1 Overview

Several chemical and spectroscopic analytical techniques exist in this technological era. Miller (2005) reports that in a study conducted in America, it was found that approximately 5% of all laboratory-based researchers in 2013 used chromatographic analysis in one form or another. It is tradition to acknowledge Mikhail Semenovich Tswett as the first to describe the chromatographic analysis technique, shortly after 1900 (Zolotov, 2003; Rouessac and Rouessac, 2007). After their literature survey, Siddiqui and colleagues (2013) drew the conclusion that high-performance liquid chromatography (HPLC) is the most popular of the chromatographic tools.

HPLC is a modern form of preparative liquid chromatography improved from the earlier forms. The technique is highly enhanced with regards to resolution and selectivity (Rouessac and Rouessac, 2007). Amidst the analytical techniques and technology advancements, HPLC remains the preferred analytical tool for analysing API. It is the principal method endorsed by most official compendia (Shabir, 2003; Misiuk, 2010; Siddiqui*, et al.*, 2013).

Levofloxacin and pyrazinamide have previously been analysed in biological fluids and pharmaceutical dosage forms. Most of the analytical methods, however, report the simultaneous analysis of these compounds in a sample matrix containing both levofloxacin and pyrazinamide with other chemicals at a time. The objective of this study was to develop and validate an HPLC method for the simultaneous determination of levofloxacin and pyrazinamide in their pharmaceutical solid dosage forms.

3.1.2 Classification of HPLC

There are many advantages of HPLC over other separation techniques. The primary benefit of HPLC lies in its ability to perform its functions with great precision, speed and sensitivity, yielding high resolution outcomes (Ahuja, 2006). There are four main types of HPLC techniques, namely; normal-phase (NP-), reversed-phase (RP-), ion-exchange (IE-) and size-exclusion (SE-) HPLC (Braithwaite and Smith, 1999). The techniques' identities are defined by the differences in dominant types of molecular interactions that prevail during the separation process (Kazekevich and LoBrutto, 2007).

3.1.2.1 Normal-Phase HPLC (NP-HPLC)

NP-HPLC is a technique whose separation mode utilises polar stationary phases and organic solvents as mobile phases (Jandera, 2011). It exploits the polar interaction strength differences that exist between each analyte within a matrix and the stationary phase. The relatively less polar analyte elutes quicker along the chromatography column as a result of weak analyte-stationary phase interaction and *vice versa* (Kazekevich and LoBrutto, 2007).

Benefits of NP-HPLC, amongst others, are its usability for samples that decompose in aqueous solutions and the stability of columns. NP-HPLC is however not without unfavourable conditions, these include, but not limited to, limitations in types of sample mixtures that can be separated, limitation in range of solvents and high cost (Snyder*, et al.*, 1997c). The shortcomings of NP-HPLC gave rise to the development of RP-HPLC (Chinou, 2010).

3.1.2.2 Reversed-phase HPLC (RP-HPLC)

Undoubtedly the most popular technique of chromatography, RP-HPLC is used in up to 90% of all HPLC separation modes (Kumar and Kumar, 2012). RP-HPLC employs mainly van der Waals or hydrophobic forces (dispersive forces) for the analysis of low molecular weight compounds. Popularity of RP-HPLC lies in its ability to identify and quantify closely related substances with favourable retention and selectivity (Kazekevich and LoBrutto, 2007). As opposed to NP-HPLC method, RP-HPLC makes

effective use of non-polar stationary phase in collaboration with polar mobile phases (Kenkel, 2014).

As described in literature, the backbone of the non-polar stationary phase is a structure framed with silica particles. Chemically bonded to the structure are carbon chains of varying lengths. Classic representations of the workhorse include octyl-(C_8) and octadecyl-(C_{18}) groups (Engelhardt, 2011; Kenkel, 2014). A dynamic array of stationary phases that comprise silica support bonded to other functional groups exist commercially. Phases containing phenyl groups, aminopropyl, cyano, nitrile, ethers and diols, among others, show pronounced selectivity and retention towards specific analytes under certain conditions (Engelhardt, 2011). Mobile phases ordinarily consist of water-based solutions with varying concentrations of miscible organics. Methanol (MeOH), acetonitrile (ACN) and tetrahydrofuran are the most commonly used eluents (Snyder and Kirkland, 1979; Snyder*, et al.*, 1997c).

The mechanisms surrounding separation techniques of RP-HPLC have been studied over years. Consequently, research has shown that pH of the mobile phase plays a significant role in sample separation. It has been established that the recommended pH range is between 2 and 8 (Snyder*, et al.*, 1997c).

3.1.2.3 Ion-exchange HPLC (IE-HPLC)

High-resolution IE-HPLC concept was routinely used for amino acid analysis since its introduction in the early 1960s. With time, the procedure found its functionality being recruited in analysing physiological fluids such as urine and serum compounds (Snyder and Kirkland, 1979). IE-HPLC exploits the difference in affinities of analyte ions for the oppositely charged groups of the hydrophobic stationary phase packing in the column. The mechanism of separation can be described using Equation 3.1, where an exchange between two positively charged ions, X^+ and Y^+ in a solution and exchange resin R^- occurs (Snyder*, et al.*, 1997c; Kazekevich and LoBrutto, 2007).

$$X{\cdot}R + Y^+ \leftrightarrow Y{\cdot}R + X^+ \qquad \textbf{Equation 3.1}$$

3.1.2.4 Size-exclusion HPLC (SE-HPLC)

As the name suggests, size-exclusion chromatography technique separates molecules in relation to their relative sizes. Through the use of well-defined and distributed porous spaces in the packing material, molecules in the analyte are excluded depending on their molecule hydrodynamic radius (Kazekevich and LoBrutto, 2007; Trojer*, et al.*, 2011).

Contrary to other HPLC modes, bigger molecules are eluted faster than the smaller ones. This is because small sized molecules flow through the pore spaces where they are distributed throughout the entire column volume and larger particles only move around the adsorbent particles (Barth and Boyes, 1992; Ricker and Sandoval, 1996). SE-HPLC is the only chromatographic separation technique where the analyte and the stationary phase should not have any positive interaction (Kazekevich and LoBrutto, 2007).

3.2 Analytical methods for the analysis of levofloxacin and pyrazinamide

To derive the most suitable conditions for the development of a method for the simultaneous analysis of levofloxacin and pyrazinamide, related published methods were examined and compared. Close attention was paid to HPLC methods evolved to analyse the single drug compounds. This information was used to compose a method that allows for analysis of the two compounds simultaneously. Table 3.1 exhibits a summary of some of the observations reported by different researchers.

Table 3.1 HPLC system and conditions for the analysis of levofloxacin and pyrazinamide

Analyte	Sample matrix	Column	Mobile phase	Flow rate	UV Detector	Retention time	Reference
Levofloxacin	Pharmaceutical Injectable Formulation	Phenomenex® C_{18}, 150mm x 4.6mm, 4µm	H_2O: ACN: 0.025M phosphoric acid, (60:20:20, v/v/v) pH 3.0	1 ml/min	294 nm	3.52 min	(Hurtado, *et al.*, 2007)
	Human plasma	Inertsil® C_{18} (ODS) 250mm x 4.6mm, 5µm	20Mm Monopotassium phosphate (KH_2PO_4): ACN (80:20, v/v) pH 2.5	1 ml/min	235nm	5.9±0.05 min	(Kumar, *et al.*, 2011)
	Raw material, Dosage forms, Serum	Purospher STAR® C_{18} 250mm x 4.6mm, 5 µm	MeOH: H_2O (70:30, v/v)	1 ml/min	294 nm	2.1 min	(Naveed, *et al.*, 2014)
	Tablet formulation	Shim-pack® CLC-ODS 250mm x 4.6mm, 5µm	Buffer (840 ml of 0.05M Citric acid monohydrate and 10 ml of 1M ammonium acetate): ACN (850:150 v/v) pH 2.9	1 ml/min	293 nm	15-17 min	(Bivha, *et al.*, 2014)
Pyrazinamide	Human plasma	Supelco® LC-18 (DB) 150mm x 4.6 mm, 5µm	0.02M phosphate buffer: MeOH (96.8: 3.2, v/v) pH 7.4	1.5ml/min	268 nm	5.2 min	(Revankar, *et al.*, 1994)
	Human Plasma, Bronchoalveolar Fluid	Axxiom® ODS 250mm x 4.6mm, 5µm	2.0% ACN in 0.02M KH_2PO_4, phosphoric acid buffer, pH 2.6	1 ml/min	268 nm	8.4 min	(Conte, *et al.*, 2000)
	Human plasma	Phenomenex® C_{18}, 150mm x 4.6mm, 5µm	MeOH: 10mM KH_2PO_4 (15:85 v/v) pH 7.4	1 ml/min	268 nm	6.80 min	(Siddhartha, *et al.*, 2013)
	Pharmaceutical Formulations	Zodiac® C_{18} column 250mm x 4.6 mm, 5µm	MeOH: H_2O (80:20 v/v) pH 4.8	1 ml/min	240 nm	5.2 min	(Lakshmi, *et al.*, 2015)

HPLC analyses for quantification of levofloxacin have been performed on a wide spectrum of diverse types of samples including biological fluids, raw materials and pharmaceutical formulations (Hurtado*, et al.*, 2007; Kumar*, et al.*, 2011; Naveed*, et al.*, 2014). On the contrary, there is dearth of reports that show that there is analysis of the drugs in their pure forms and pharmaceutical dosage forms (Revankar*, et al.*, 1994; Conte*, et al.*, 2000).

Generality, as displayed by the facts compiled in the Table 3.1 suggests that previous methods have derived benefit from RP-HPLC with C_{18} or C_8 non-polar stationary phases for compound separation. Mobile phases were composed of organic modifiers and buffer components such as ACN, MeOH and KH_2PO_4. In cases where ACN was not included in the mobile phase mixture, MeOH was employed instead (Siddhartha*, et al.*, 2013; Naveed*, et al.*, 2014; Lakshmi*, et al.*, 2015). The amount of ACN range was 15-20% of the mixture composition, whilst MeOH was 3.2-85% (Table 3.1).

Although both drugs exhibit retention times of 15 minutes or less, retention times of up to 17 minutes have been reported in analysis of levofloxacin (Bivha*, et al.*, 2014). Pyrazinamide is a weakly acidic drug (Gu*, et al.*, 2008) and on the other end, levofloxacin is amphoteric (Kumar*, et al.*, 2012). Buffer systems were used to suppress ionisation of the compounds during separation, although non-buffered methods have also proved to yield satisfactory results (Naveed*, et al.*, 2014; Lakshmi*, et al.*, 2015). Consequently, pH was modified and ranged between 2.5 and 7.4. The methods employed ultraviolet (UV) absorption spectroscopy, where detector wavelength was set between 235-294nm for levofloxacin and 240-268nm for pyrazinamide. Three of the four instances (Table 3.1) show the wavelength of 268 nm in detection of pyrazinamide.

The published data, as referenced in Table 3.1, on conditions for analysis of the separate compounds in various sample matrices was manipulated to develop a suitable HPLC method for simultaneous detection of levofloxacin and pyrazinamide in raw materials and tablet dosage form.

3.3 EXPERIMENTAL

3.3.1 Materials and reagents

Levofloxacin was purchased from Sgonek® Biological Technology Co, Ltd (Xian Shi, Shaanxi Sheng, China) and pyrazinamide was kindly sponsored by Aspen Pharmacare® (Eastern Cape, South Africa). ACN, orthophosphoric acid and tri-ethylamine were procured from Merck KGaA (Darmstadt, Germany).

HPLC grade water for preparation of samples and buffers was purified using a Purite® water purification system (Suez Water Purification Systems Ltd, Thames, Oxford shire). Water was then filtered through 0.2 µm GVS Cellulose acetate filters (GVS Life Sciences, Massachusetts, USA) before use.

3.3.2 HPLC System

A Shimadzu® Prominence LC-20A Modular HPLC system (Shimadzu Corporation, Tokyo, Japan) consisting of separate modules was used. The unit included a model LC-20AT solvent delivery unit, absorbance detector model SPD-20A fitted with UV-VIS dual detectors and SPD-M20A diode array detector component, model CTO-20AC column oven as well as a model SIL-20AC HT auto-sampler module. Degassing of all the liquids was done using DGU-20A5 degasser unit. LCSolution® version 1.25 operation software was used to read and capture all HPLC data. Successful separation was reached through a Luna® C_{18}, 150 mm x 4.6 mm, 5 µm column (Phenomenex, Milford, Massachusetts, USA). Temperatures were maintained at 25 °C throughout the separation process.

3.3.2.1 Column selection

Selecting a suitable column for the RP-HPLC system analysis depends on a range of factors. Markedly, the inherent physicochemical properties of the drug compound to be analysed determine the type and extent of forces of interaction that occur between the analyte and stationary phase during separation (Braithwaite and Smith, 1999; Ahuja, 2006; Engelhardt, 2011).

As discussed earlier in this chapter (Section 3.2), pyrazinamide is a weak acid whereas levofloxacin is amphoteric. Acidic compounds are known to protonate in environments where the pH values are higher than the pKa of the organic acid and the opposite conditions cause basic compounds to dissociate. Mixing the two compounds alters the pH values of the individual solutions which necessitates the use of a buffer to maintain a suitable environment with a desired retention during separation. The final mixture formed plays a role in the selection process of a stationary phase (Snyder*, et al.*, 1997c). Following review of literature as compiled in Table 3.1 and evaluation of physicochemical properties of the drug compounds, a silica based reverse phase C_{18} column was selected for use in the analysis.

Particle size and mean diameter of the packing material of the stationary phase significantly influence the concomitant peak-height and resolution of HPLC. There is evidence to support that columns whose particle size ranges between 3 - 10 µm display favorable performance responses in the selection of an HPLC method. The best compromise of reproducibility, efficiency and reliability have been observed when employing stationary phases with the packing of adsorbing materials which are 5 µm in size (Snyder*, et al.*, 1997a). Although presenting with higher clogging risks, columns that contain 3.5 µm sized particles offer outstanding compromise between good performance and column durability compared to those of 5 µm particles (Kirkland*, et al.*, 1994). However, the benefits of the latter column outweigh those of the former, which led to a column packed with 5 µm particles being selected for this study.

Column length and internal diameter (I.D) are selected as per specifications of the analysis. For conventional analytical applications, a column length of 150 mm with I.D ranging between 3 - 4.6 mm is acceptable. Columns with I.D of 8 - 50mm are generally preferred for pilot, preparative and semi-preparative assays of analytical samples (Snyder*, et al.*, 1997a). The theoretical plate number of a 3 mm I.D column diminishes by 10-20% in comparison with a 4.6 mm I.D column packed with the same particles due to the elevated proportion of column dead volume (Trojer*, et al.*, 2011). The 4.6 mm I.D column is more economic and for the above stated reasons, a column with 4.6 mm I.D and 150 mm length was employed in this analysis.

3.3.2.2 Preparation of stock solutions

For the preparation of stock solutions, 60±0.05 mg of levofloxacin and 120±0.05 mg pyrazinamide powders were weighed accurately using a Model XP205 Mettler Toledo® analytical precision balance (Mettler Instruments, Zurich, Switzerland). The measured powders were each quantitatively transferred into clean, separate 100 ml A-grade volumetric flasks. The drug powders were dissolved in mobile phase and stirred for two minutes to fashion 600 µg/ml levofloxacin and 1200 µg/ml pyrazinamide stock solutions using a Model STR-MH-180 magnetic hotplate stirrer (FMH Instruments®, Cape Town, South Africa). These concentrations represented 300% of the target concentrations. Stock samples were kept in the fridge at a temperature of ±4 °C for the duration of the experimental study.

Aliquots were extracted from the stock solutions and transferred into clean 50 ml A-grade volumetric flasks to create solutions containing levofloxacin and pyrazinamide. The resultant separate drug solutions were serially diluted using the mobile phase mixture. These solutions were mixed to produce two sets of solutions in which each set had one constant drug concentration while the other was varied. The first set contained levofloxacin in concentrations of 300, 250, 200, 150, 100 and 50 µg/ml while pyrazinamide was kept constant at 200 µg/ml. The second set carried solutions with pyrazinamide in concentrations of 600, 500, 400, 300, 200 and 100 µg/ml while levofloxacin was maintained at 100 µg/ml.

3.3.2.3 Preparation of buffers

The conditions for the separation were optimized by employing a 0.025 M orthophosphoric acid buffer. In preparation of the buffer, 2 ml of orthophosphoric acid (85%) was slowly added to about 230 ml of HPLC grade water, then altered to a final volume of 1169 ml with the water. The pH was adjusted to 3.7 using triethylamine and the pH was monitored using Model 744 Metrohm pH meter (Metrohm SA (Pty) Ltd, Cape Town, South Africa).

3.3.2.4 Preparation of mobile phase

The mobile phase consisted of water, 0.025 M orthophosphoric acid buffer and ACN in a ratio of 68:20:12, respectively. The water and buffer were filtered through 0.2 µm

GVS Cellulose acetate filters then degassed on the Model DGU-20A5 degasser unit before use. The constituents of the mobile phase were mixed online. The mobile phase was pumped through the HPLC system for about an hour to equilibrate the system to the conditions before injecting samples.

3.4 RESULTS AND DISCUSSION

3.4.1 Effect of organic solvent composition

The retention time (R_t) of levofloxacin, as well as the peak resolution (R_s) of both levofloxacin and pyrazinamide were significantly influenced by the content of ACN in the mobile phase as displayed in Figure 3.1 and Table 3.2 respectively.

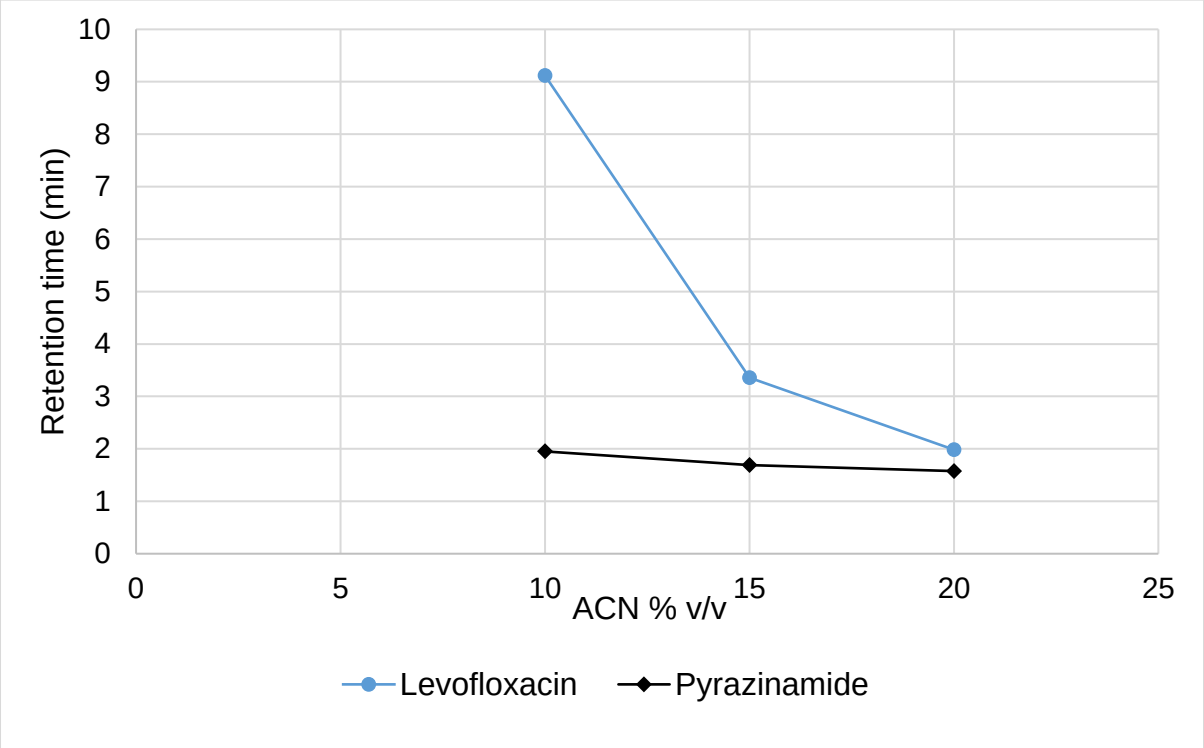


Figure 3.1: The effect of ACN content on R_t of levofloxacin and pyrazinamide

Trivial changes in R_t were observed for pyrazinamide with respect to change in ACN content in the mobile phase, where R_t is 1.95 and 1.57 minutes when ACN content in mobile phase was 10 and 20% v/v, respectively. The increase in the amount of ACN in the mobile phase from 10 to 20% v/v also resulted in a decreased R_s as observed in Table 3.2. The increase in the amount of ACN in the mobile phase from 10 to 20% v/v also resulted in a decrease in R_s as observed in Table 3.2.

The mobile phase containing12 % v/v ACN produced results with the most acceptable R_t for both drug compounds, that is, 1.836 minutes for pyrazinamide and 5.751 minutes for levofloxacin, in addition to a good R_s of 7.9. Although the R_s delivered by ACN content of 10% is the highest, at 9.11, the resultant peak tailing factor of levofloxacin is above 2, therefore the mobile phase containing 10% ACN did not produce fully acceptable chromatograph results (Figure 3.1 and Table 3.2).

Table 3.2: The effect of ACN content on R_s and Peak tailing factor of levofloxacin

ACN content (% v/v)	Peak resolution (R_s)	Peak tailing factor
10	9.11	3.82
12	7.90	1.26
15	2.43	1.57
20	1.13	1.62

3.4.2 Effect of buffer concentration

The R_t of solutes may be affected by buffer concentration in reverse-phase chromatographic technique due to effects of interactions between the ionised acidic residual silanols on the silica stationary phase support surface and the protonated basic solutes (Snyder*, et al.*, 1997c). Table 3.3 exhibits how pyrazinamide R_t is almost constant through the changes of buffer concentration due to incomplete dissociation of the compound. Similar results have been documented in a previous study (Thoithi*, et al.*, 2002). In contrast, the zwitterionic levofloxacin R_t increase with increase in buffer molarity presumably due to non-hydrophobic interactions as well as silanophilic interactions (Pistos*, et al.*, 2005).

Manipulating buffer concentration may lead to improvement of peak shape and symmetry because the cation of the buffer represses the ion exchange interactions occurring between the ionised residual silanols on the silica stationary phase support surface and the protonated base. Buffers of concentration in the range of 0.010 – 0.050 M are generally adequate for use in most reverse-phase HPLC and seldom concentrations bigger than 0.100 M (LoBrutto, 2007). Using the buffer

concentration of 0.010 M resulted in an unacceptable tailing of the levofloxacin peak, as shown in Table 3.4. Food and Drug Administration (1994) suggests that a peak tailing factor of ≤ 2 is considered desirable for general HPLC quantitative analysis.

Table 3.3: The effect of buffer concentration on and retention time

Buffer Concentration (M)	Retention time (min)	
	Levofloxacin	Pyrazinamide
0.010	4.791	1.847
0.025	5.751	1.836
0.100	6.157	1.823

Table 3.4: The effect of buffer concentration on peak tailing

Buffer concentration (M)	Peak tailing factor	
	Levofloxacin	Pyrazinamide
0.010	3.61	1.10
0.025	1.46	1.06
0.100	1.13	1.04

3.4.3 Effect of buffer pH

Change in buffer pH had effect on R_t of levofloxacin and almost no effect on the R_t of pyrazinamide as displayed in Table 3.5. Pyrazinamide is a weak acid with minor interactions with the residual silanols of the silica stationary phase. Levofloxacin's retention is due to non-hydrophobic and silanophilic interactions (Pistos*, et al.*, 2005; LoBrutto, 2007).

Table 3.5: The effect of buffer pH on retention time

Buffer pH	Retention time	
	Levofloxacin	Pyrazinamide
1.7	4.884	1.828
3.7	5.751	1.836
6.7	10.530	1.832

When a high pH of 6.7 was used, analytical run time had to be increased beyond 10 minutes. This is shown in Table 3.4. At low pH of 1.7, there is coelution of pyrazinamide and the solvent front that is detected as impurity at 1.63 minutes.

3.4.4 Effect of flow rate

Mobile phase flow rate through the column has a significant effect on the retention time. A change in flow rate gives an opposite effect on the analytical run time, that is, increasing the rate decreases the analytical run time as shown in Table 3.6. In general terms, run time can be significantly reduced with no detrimental effect on column efficiency and quality of separation. However, lower flow rates are preferred for their lower flow resistance and lower backpressure (Kazekevich and LoBrutto, 2007). To maintain a reasonable run time of 10 minutes and a good resolution, a rate of 1.0 ml/min was used.

Table 3.6: The effect of Flow rate on retention time

Flow rate (ml/min)	Analytical run time (min)	
	Levofloxacin	**Pyrazinamide**
0.5	11.166	3.622
1.0	5.751	1.836
1.5	3.835	1.229
2.0	2.899	0.931

3.4.5 Chromatographic conditions

Considering the conditions examined above, the following set of conditions were used for successful simultaneous analysis of levofloxacin and pyrazinamide as displayed in Figure 3.2 and outlined in Table 3.6.

Table 3.7: Chromatographic conditions

Column	Luna® C_{18}, 150mm x 4.6mm, 5µm
Flow rate	1 ml/min
Injection volume	20 µL
UV Detector	294 nm
Temperature	25 °C
Mobile phase	Water (68% v/v), 0.025 M orthophosphoric acid buffer of pH 3.7 (20%v/v) and ACN (12%v/v)
Pump mode	Low pressure gradient
Run time	10 mins

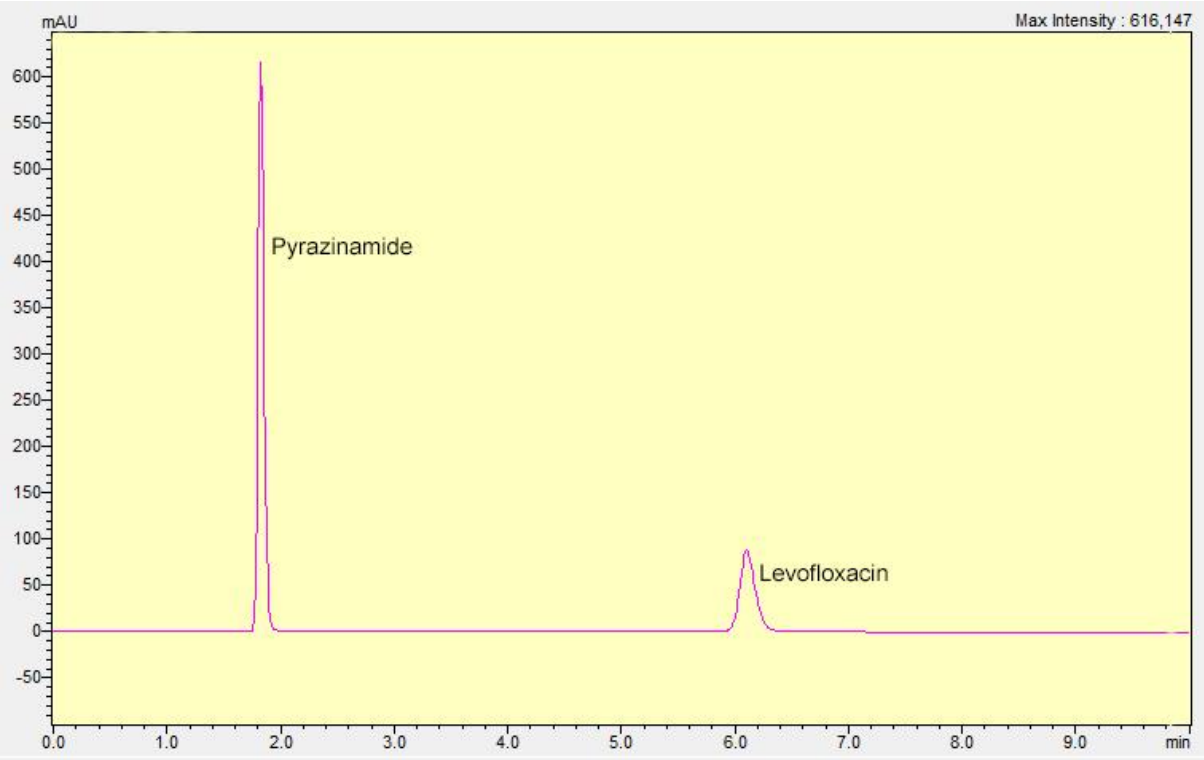


Figure 3.2: Chromatogram of pyrazinamide and levofloxacin obtained under the conditions described in Table 3.7

3.5 METHOD VALIDATION

3.5.1 Introduction

Method validation is a process performed to verify that a chosen analytical tool design is suitable for a specific intended purpose before its implementation (Green, 1996). For reasons of quality, safety and efficacy, it is of paramount importance to prove that the analytical methods are able to quantify pharmaceutical products during development and manufacture (Rozet*, et al.*, 2007). The International Conference on Harmonisation (ICH) and the United States Pharmacopoeia (USP), in addition to other regulatory bodies, have set in place guidelines with protocols for validating pharmaceutical analytical methods (Shabir, 2003).

It is a general principle to outline the scope of applicability of an analytical method. Some methods may be validated to be usable on a single specific type of equipment and a particular range of environmental conditions (Singh, 2013). There are various validation parameters of different criteria that should be established in the process of method testing. These are laid down to ensure consistent, accurate and reliable data. Depending on the nature of the scientific investigation, they include, but are not limited to: linearity, precision, limits of quantitation (LOQ), limits of detection (LOD) and selectivity or specificity (Green, 1996; Shabir, 2003; Singh, 2013).

As alluded to earlier in the text, accessible literature is indigent in terms of information pointing towards previous HPLC methods for simultaneous analysis of levofloxacin and pyrazinamide. According to the Medicines Control Council (2010), this warrants further HPLC separation method validation.

3.5.2 Linearity and range

Linearity describes the ability to produce analytical results that display direct proportionality to the chemical concentration of a given analyte in a matrix, within a specific range (Araujo, 2009). In assayed studies, without regard to exceptions, standard solutions are prepared in at least five reporting levels to allow for detection of curvature in plotted graphs. The analyte concentration range is normally between

50 and 150% of the target concentration (Green, 1996). In this study, the range was between 25 and 150%.

As mentioned by Green (1996), it is common practice to examine the correlation coefficient (R^2) to qualify linearity of data, where $R^2 > 0.999$ is regarded as sufficient proof of an acceptable quality of fit for data presented by the regression line. Some analytical validation sources further encourage the use of statistical methods in testing for linearity (Analytical Methods Committee, 1988; Araujo, 2009; Miller and Miller, 2010).

The term range represents the lower and upper analyte concentration intervals within which appropriate linearity, precision and accuracy are found. Accuracy and linearity studies enable determination of the range (Green, 1996; Araujo, 2009). Linearity and range for the analysis of levofloxacin and pyrazinamide were determined through plotting the average peak area of the drugs against their concentrations of calibration samples, as summarized in Table 3.8.

Table 3.8: The resultant mean-peak area as a function of concentration for levofloxacin and pyrazinamide

Levofloxacin			**Pyrazinamide**		
Concentration (µg/ml) (n=6)	**Mean peak area**	**% RSD**	**Concentration (µg/ml) (n=6)**	**Mean peak area**	**% RSD**
50	131972	0.21	100	384439	0.03
100	270934	0.04	200	769677	0.14
150	232081	0.54	300	1116750	0.02
200	550056	0.40	400	1505460	0.09
250	701583	0.18	500	1843320	0.08
300	836290	0.99	600	2193510	0.02

The resultant calibration curves of levofloxacin (Figure 3.3) and pyrazinamide (Figure 3.4) showed the regression line equation and subsequent R^2 values as follows:
Levofloxacin: $y = 2\,818x - 59\,989$ with R^2 value of 0.999
Pyrazinamide: $y = 3\,615.7x + 36\,693$ with R^2 value of 0.9996

Both R^2 values indicate that the method was linear over the selected concentration ranges for the two drugs.

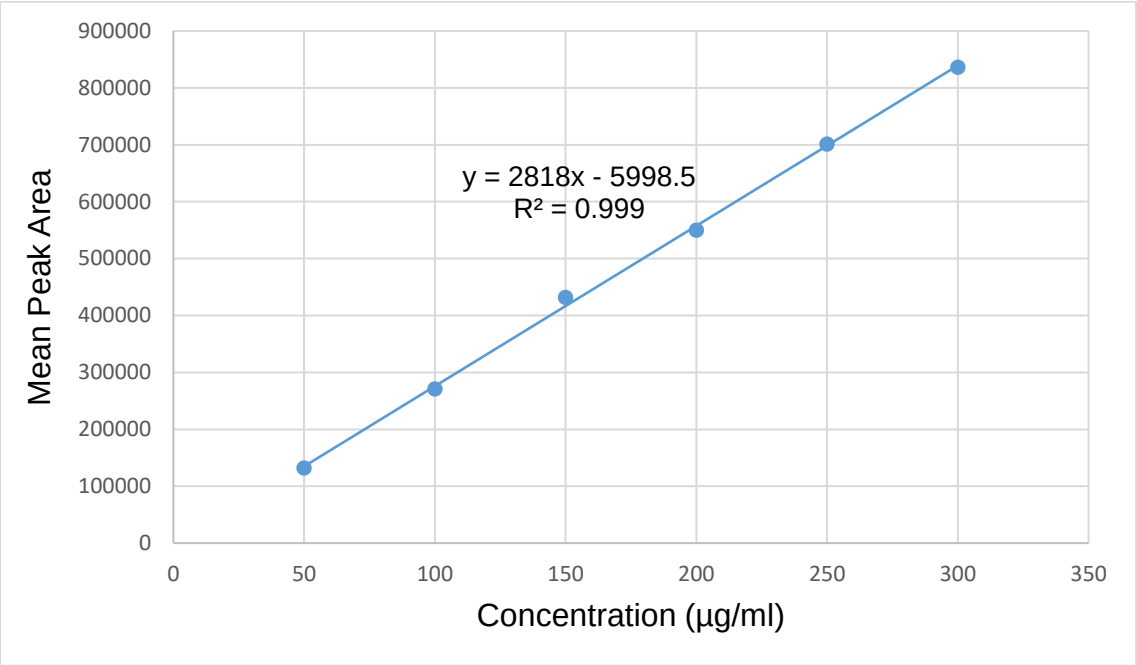


Figure 3.3: Linearity and range curve for Levofloxacin plotted using the data displayed in Table 3.8

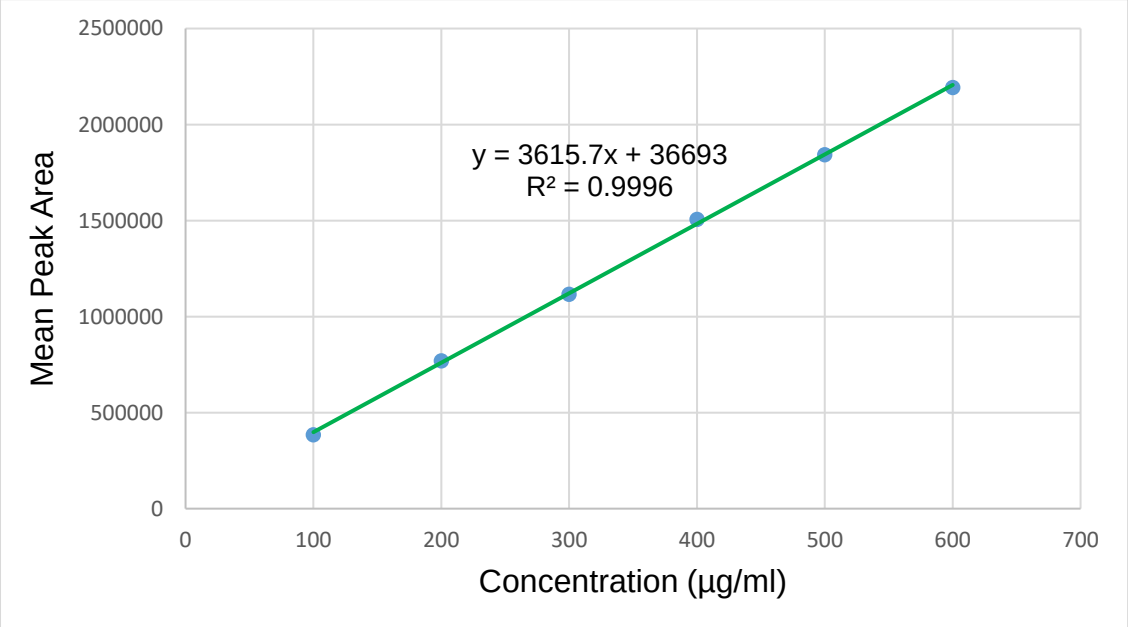


Figure 3.4: Linearity and range curve for pyrazinamide plotted using the data displayed in Table 3.8

3.5.3 Precision

The ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) (2004) defines precision as the degree of agreement among individual quantity results of replicate multiple tests of a quantity, under constant conditions. Rosing and colleagues (2000) further elaborate that precision indicates the random error in terms of the degree of scatter.

In 1995, a more concise definition was proposed by the ICH. Precision is categorised into three levels, namely, repeatability, intermediate precision and reproducibility. The precision of an analytical analysis is conventionally expressed numerically as measurement of percent relative standard deviation (%RSD) for a significant number of tests (International Organisation for Standardization, 2004). In this study, tolerance limit for %RSD was set at < 5%.

3.5.3.1 Repeatability

Repeatability, also recognised as intra-assay precision, represents the outcomes of a measurement procedure, employing the same measuring system over a short-time interval under constant conditions carried out by the same analyst (Shabir*, et al.*, 2007). Repeatability should be determined using at least nine determinations that cover the specified concentration range for the analysis. Normally, three replicates of three concentration levels are acceptable. Alternatively, a minimum of six samples of 100% concentration may be determined (International Conference on Harmonisation, 1995; Snyder*, et al.*, 1997b).

In assessing repeatability, triplicate injections and assessment of each sample of the three concentration levels, 25, 75 and 150% were performed and they represented low, medium and high concentrations. Three days were set aside for precision parameter testing. The repeatability data for the predetermined calibration range was generated on Day 1. The statistical representation of the information summarising repeatability and intermediate precision data is shown in Table 3.9. The % RSD values were below 5%. These calculations satisfy the limit set in our laboratory and therefore demonstrate acceptable repeatability of the method.

Table 3.9: Data representing repeatability and intermediate precision for the simultaneous analysis of levofloxacin and pyrazinamide

	Levofloxacin			Pyrazinamide		
Day	**Concentration (µg/ml)**	**Mean peak area**	**% RSD**	**Concentration (µg/ml)**	**Mean peak area**	**% RSD**
1	50	131972	0.21	100	384439	0.03
	150	232081	0.54	300	1116750	0.02
	300	836290	0.99	600	2193510	0.02
3	50	131821	0.60	100	385475	0.13
	150	231962	0.18	300	1126852	0.14
	300	839667	0.19	600	2184594	0.33
5	50	131412	0.32	100	386231	0.10
	150	231827	0.11	300	1120654	0.07
	300	836798	0.27	600	2200513	0.18

3.5.3.2 Intermediate precision

International Conference on Harmonisation (1995) in agreement with Snyder and colleagues (1997b) interpret intermediate precision as the agreement of all performed measurement results, standards included, where the method is utilised a number of times in the same laboratory but on various days, non-identical equipment and different analysts. Intermediate precision tests the reliability of the method when employed under various conditions outside the initial environment where the method was developed (Segall*, et al.*, 2000).

Precision parameter testing were used to establish the intermediate precision of the method on day 1, 3 and 5. The analysis was carried out using the three predetermined samples of low, medium and high concentration levels in triplicate. The intermediate precision data for analysis is exhibited in Table 3.9.

3.5.3.3 Reproducibility

Reproducibility of a method examines the analytical method precision when analysis is run using the method in question, in different laboratories (Food and Drug Administration, 2013). This type of precision is necessary when methodology

standardisation is required across laboratories, due to laboratory transfer, or in cases where there are changes of software platforms, instrumentation, or critical reagents (Liao*, et al.*, 2006). In this study, however, analytical methodology standardisation needed not be determined, since the same laboratory was utilised and the service of the same equipment was employed by the same analyst throughout the duration of the study.

3.5.4 Accuracy

Accuracy expresses the extent of how close the resultant measurement value is, in relation to the accepted nominal or reference true value, under a set of specific conditions (Shah*, et al.*, 2000). Coupled with precision, accuracy plays an integral role in determining the error of the analysis. In that effect, accuracy is one of the significant criteria in analytical method evaluation (Rosing*, et al.*, 2000).

There are numerous ways to ascertain accuracy. In one of their articles, Shabir (2003) outline at least three techniques of assessing the criterion. The first suggested pathway involves comparing test measurements with an alternative, existing, accurate and well characterised approach. The second and third approaches, respectively, as also stated by Rosing and colleagues (2000), encompass comparing measurements of a sample of known concentration against the true reference value and spiking measured amounts of analyte into blank matrices then calculating percent recovery.

In a manner conforming to the Food and Drug Administration recommendations, accuracy studies data collection was done from nine determinations (Food and Drug Administration, 1994). Analysis was run in triplicate on three concentration levels (80%, 100% and 120%) of the target product concentration in the interest of covering the required range of concentrations under investigation. Accuracy results were displayed as % RSD and percent bias.

Bias refers to the average deviation of the determined value for the analyte under scrutiny from the accepted true value and serves to express the systematic extent of trueness in numerical values (Taverniers*, et al.*, 2004; Johnson, 2008). The accuracy test results had a 5% tolerance set for the % RSD while <5% was allowed for the % bias. A summary of the assessment is shown in the following table (Table 3.10). The

analysis was run in triplicate on three concentration levels (80%, 100% and 120%) of the target product concentration (conc).

Table 3.10: Accuracy and bias results for the simultaneous analysis of levofloxacin and pyrazinamide

Levofloxacin				Pyrazinamide			
Conc (µg/ml)	Mean conc determined (µg/ml) (n=3)	Precision (% RSD)	% Bias	Conc (µg/ml)	Mean conc determined (µg/ml) (n=3)	Precision (% RSD)	% Bias
50	49.91	0.38	1.63	100	100.14	0.10	- 0.09
150	150.83	0.08	-1.25	300	298.67	0.03	3.31
300	299.27	0.17	2.14	600	601.35	0.06	- 2.47

3.5.5 Limit of quantitation (LOQ) and limit of detection (LOD)

The limit of quantitation (LOQ) defines the least possible concentration of an analyte in a sample that can be quantitatively measured with acceptable precision and accuracy under prescribed operational conditions of an analytical procedure (International Conference on Harmonisation, 1995; LoBrutto and Patel, 2007). In contrast, limit of detection (LOD) refers to the lowest analyte amount that can be identified in a sample although cannot necessarily be precisely measurable under the given conditions of the experiment (United States Pharmacopoeia - National Formulary [USP30 - NF25], 2007c).

The USP30 - NF25 (2007c) further recommends application of signals-to-ratio procedure in evaluating LOQ and LOD parameters in analytical analysis. LoBrutto and Patel (2007) clarify that in determining the LOQ and LOD, the low concentration analyte sample signals are measured and compared to signals of blank samples. The minimum amount at which the analyte in the sample is reliably quantified or detected is indicated.

Typically, the acceptable peaks for LOQ and LOD produce signal-to-noise ratios of approximately 10:1 and 2:1 or 3:1 respectively. However, the Food and Drug Administration (1994) expresses an opinion that the practicality of the concept of the noise level is not necessarily feasible. The noise levels determined during method development phase may differ with the assayed sample noise levels due to use of different detectors. On that effect, the Food and Drug Administration proposes that the assurance of observing and quantifying analyte can be placed in utilising analyst projected LOQ and LOD reference standard in test method. Caution should be taken not to interpret baseline noise as extraneous peaks. Other approaches, as discussed by Carr and Wahlich (1990) and later on by LoBrutto and Patel (2007), entail the establishment of the calibration curve and % RSD of the response, as well as using the precision values. The parameters can be expressed in terms of equations 3.2 and 3.3.

$$\text{LOQ} = \frac{10\sigma}{S} \qquad \textbf{Equation 3.2}$$

$$\text{LOD} = \frac{3.3\sigma}{S} \qquad \textbf{Equation 3.3}$$

Where,

σ represents the standard deviation of the response

S represents the slope of the calibration curve

Regardless of the approach used, the limits of quantitation and detection should thereafter be validated by analysing several samples known to be of acceptable limits (International Conference on Harmonisation, 1995; United States Pharmacopoeia - National Formulary [USP30 - NF25], 2007c). The signal-to-noise ratio method was employed to establish LOQ and LOD. Baseline noise was established through repeat analysis of six blank injections of samples with decreasing analyte amount. The resultant LOQ and LOD values for both levofloxacin and pyrazinamide were similar and had values of 0.10 µg/ml and 0.05 µg/ml, respectively.

3.5.6 Specificity and selectivity

An analytical technique is considered specific if it is capable of accurately quantifying analyte response in the presence of interference in the sample matrix under prescribed assay conditions. Interference may present as potential components such as impurities and excipients (Green, 1996). The degree to which a technique is specific is termed selectivity (Gustavo and Ángeles, 2007). It is vital to establish the specificity of the method since parameters such as linearity, accuracy and precision are, to a significant extent, dependent on specificity (Snyder*, et al.*, 1997b).

In determining the specificity of the method, resolution of levofloxacin and pyrazinamide was assessed in samples containing potential excipients to be included in the tablet formulation. Excipient amounts predicted to be used in the product were included in the samples. Commercially available levofloxacin and pyrazinamide tablets purchased from a pharmacy were exploited for the specificity studies. Both chemicals were well resolved from the assayed samples, concluding that the method was specific, thereby suggesting a positive specificity outcome for the simultaneous analysis of the two compounds.

3.5.7 Forced Degradation Studies

The ICH stability testing of new drug substances and products guidelines (2003) has been adopted by most chemical regulatory authorities, including the South African Medicines Control Council. Stability testing guidelines require that the analytical method used for compound separation be capable of discriminating between analyte under scrutiny and their subsequent decomposition products that may occur during study (Bakshi and Singh, 2002).

Forced degradation studies serve to reinforce the appropriateness of the analytical method for its intended purpose. Assay methods intended for examining stability should be performed on forced degradation substances under various conditions, for example temperature, oxidation, pH and light (Bakshi and Singh, 2002). Both quantitative and qualitative aspects of individual degradation substances are to be evaluated (International Conference on Harmonisation, 2003). Nonetheless, only the qualitative analysis of forced degradation products was applied in this research.

Chromatograms generated from the forced degradation studies were measured against the ones generated from the standard samples.

3.5.7.1 Sample Preparation

For all of the forced degradation studies except oxidative studies, a milligram of levofloxacin and pyrazinamide each were weighed and quantitatively transferred into separate 100 ml volumetric flasks. The drug powders were dissolved and made up to volume to supply solutions of concentration of 100 µg/ml using medium defined for the individual studies.

3.5.7.2 Oxidative degradation

Hydrogen peroxide has been extensively exploited in forced degradation studies as an oxidative agent of drug substances at concentrations between 0.1 - 3%. Temperatures are normally maintained at 25 °C, whereas the pH is allowed to remain neutral (Blessy*, et al.*, 2014). Other oxidizing agents include oxygen, metal ions and radical initiators (Lalitha Devi and Chandrasekhar, 2009). About 1 mg of each drug substance was dissolved in 9 ml of mobile phase in 10 ml A-grade volumetric flasks. Solutions were made up to volume with 30% v/v hydrogen peroxide and mixed well after which they were incubated at 25 °C for seven days. Small amounts of samples were drawn and analysed with HPLC on day 1, 3 and 5.

3.5.7.3 Hydrolytic degradation

It is a customary practice to use 0.1 - 1.0 M hydrochloric acid or sulphuric acid for acid stress testing and 0.1 - 1.0 M sodium hydroxide or potassium hydroxide for alkaline stress testing. Each drug powder was weighed and quantitatively transferred into two 10 ml A-grade volumetric flasks. The contents of one volumetric flask was dissolved and made up to 10 ml volume with 0.1 M hydrochloric acid and the second was dissolved and made up to 10 ml volume with 0.1 M sodium hydroxide. This was done for both ingredients. The samples were incubated at 40 °C for 5 days in a Series 2000 incubator (India). Aliquots were drawn out from the samples and analysed in day 1, 3 and 5.

3.5.7.4 Photolytic degradation

The drug powders were dissolved in 10ml of HPLC grade water and exposed to the sunlight for 5 days. Sampling on HPLC was done on with HPLC on day 1, 3 and 5.

3.5.7.5 Results and Discussion

3.5.7.5.1 Oxidative degradation in aqueous medium

Both drug substances exhibited considerable degradation after incubation in 3% v/v H_2O_2 solution at 25 °C. The peak height of levofloxacin remains almost constant through the seven days of incubation (Figure 3.5), while the concentration of pyrazinamide seems to be depleting (Figure 3.6). In a study conducted by Hamdi El Najjar and colleagues (2013), remarkable depletion of levofloxacin to undetectable levels was observed when exposed to higher concentrations of hydrogen peroxide. The unchanging concentration of levofloxacin in this study may have been due to use of lower hydrogen peroxide concentration. On the other hand, as once substantiated by Asai (1961), the diminishing concentration of pyrazinamide may have been due to its 1- or 4-oxide derivative whose peak is seen increasing with incubation time as Figure 3.6 displays.

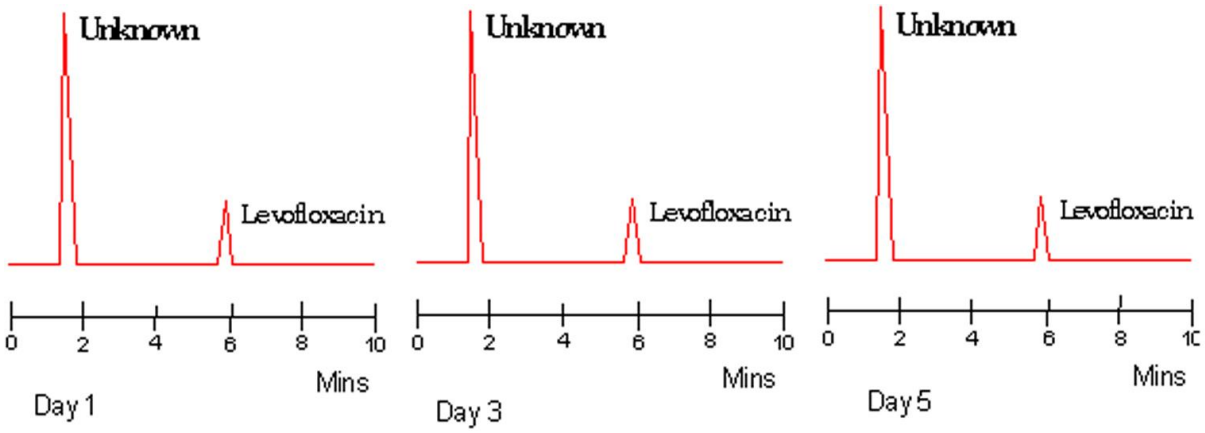


Figure 3.5: Chromatograms of levofloxacin in 3% v/v hydrogen peroxide solution

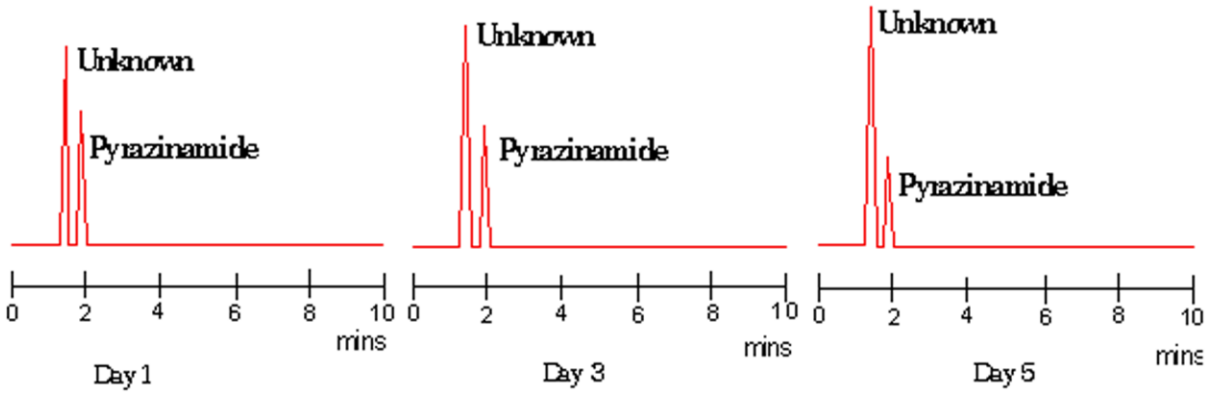


Figure 3.6: Chromatograms of pyrazinamide in 3% v/v hydrogen peroxide solution

3.5.7.5.2 Acid degradation and alkali degradation

Results of analyses performed following forced hydrolysis of the drug substances by 0.1 M hydrochloric acid and 0.1 M sodium hydroxide are displayed in Figures 3.7 - 3.10. Observations conducted after exposure of levofloxacin to acidic conditions unveil by-products of levofloxacin that appear as two peaks between 1.8 and 2 minutes (Figure 3.7). The peak heights of the by-products decrease with length of exposure time. Different observations following treatment of levofloxacin using 0.1 M hydrochloric acid and 0.1 M sodium hydroxide have been reported, where levofloxacin was concluded to be stable under these conditions (Gul*, et al.*, 2015).

An impurity peak is observed at 6 minutes in the analysis of pyrazinamide in hydrochloric acid chromatogram (Figure 3.8). The peak height increases with the number of days, probably due to the increase in the presence of a product of hydrolysis. As noted earlier in the report, converting pyrazinamide compound into a pharmacologically active form involves its hydrolysis in an acidic medium. A bacterial enzyme known as pyrazinamide amidohydrolase catalyses the hydrolysis of pyrazinamide into pyrazinoic acid and ammonium (Zhang*, et al.*, 2003). Remarkable stability is observed during the five-day exposure of pyrazinamide to 0.1 M sodium hydroxide solution (Figure 3.10), as no unknown compounds are detected in the 10-minute analysis of the sample.

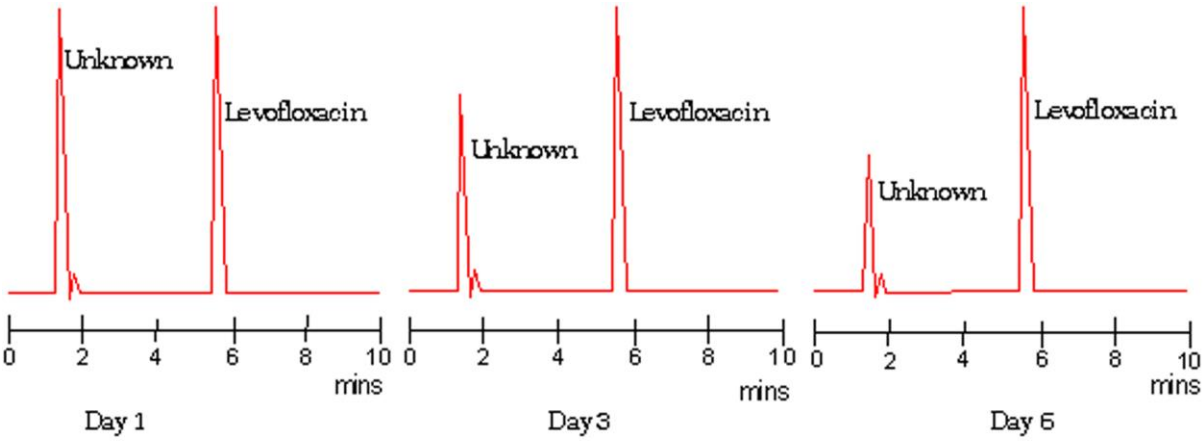


Figure 3.7: Chromatograms of levofloxacin in 0.1 M hydrochloric acid

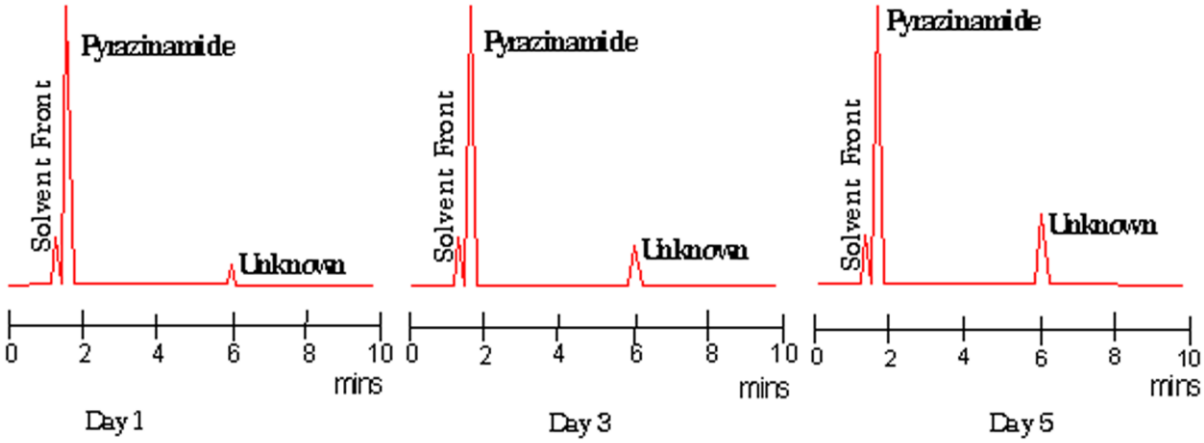


Figure 3.8: Chromatograms of pyrazinamide in 0.1 M hydrochloric acid

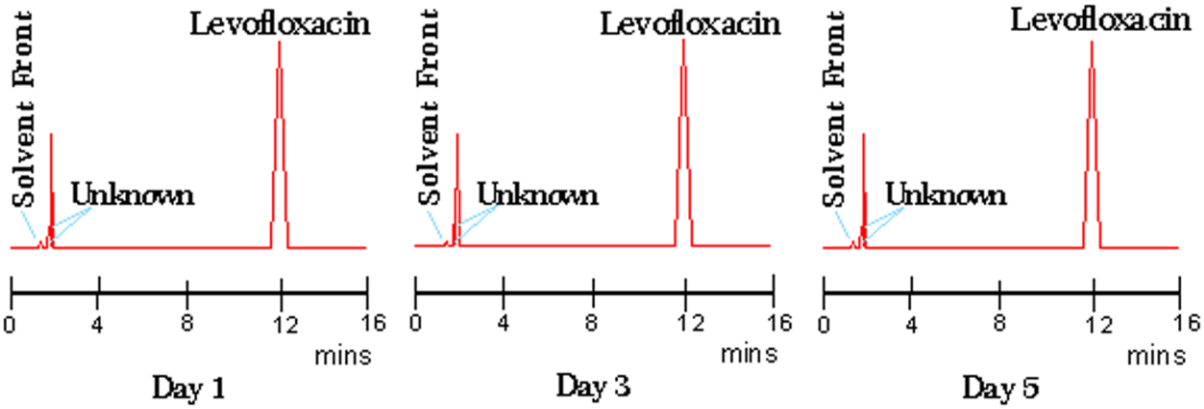


Figure 3.9: Chromatograms of levofloxacin in 0.1 M sodium hydroxide solution

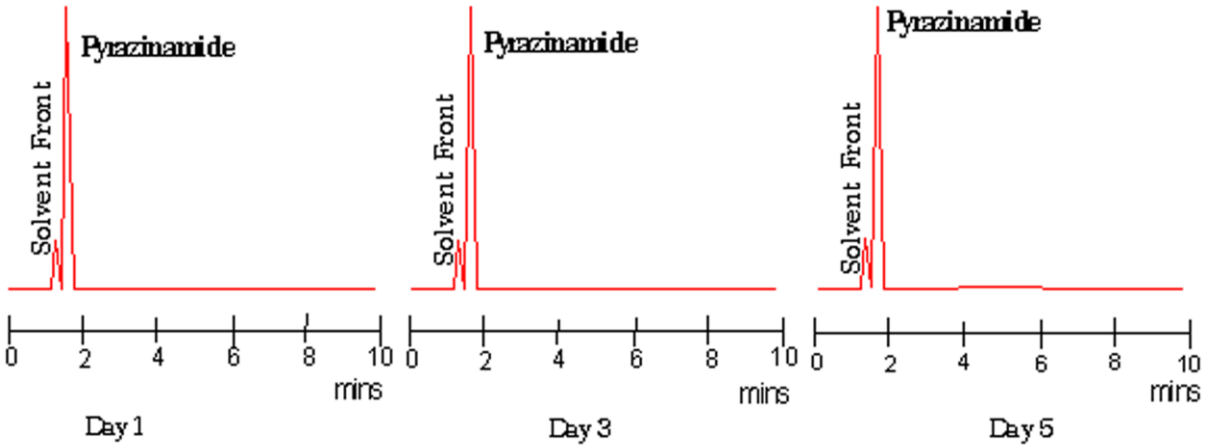


Figure 3.10: Chromatograms of pyrazinamide in 0.1 M sodium hydroxide solution

3.5.7.5.4 Photo degradation in aqueous medium

Analysis of levofloxacin in water after exposure to sunlight showed four distinct degradation products (Figure 3.11). In another study performed to determine photodegradation products of levofloxacin in aqueous solutions using near ultraviolet light, it was discovered that nine decomposition products of levofloxacin were produced (Yoshida*, et al.*, 1993). In contrast, no decomposition products were isolated during the analysis of pyrazinamide that was treated with similar conditions. This confirms the statement that pyrazinamide is stable when exposed to natural daylight (Felder and Pitre, 1983).

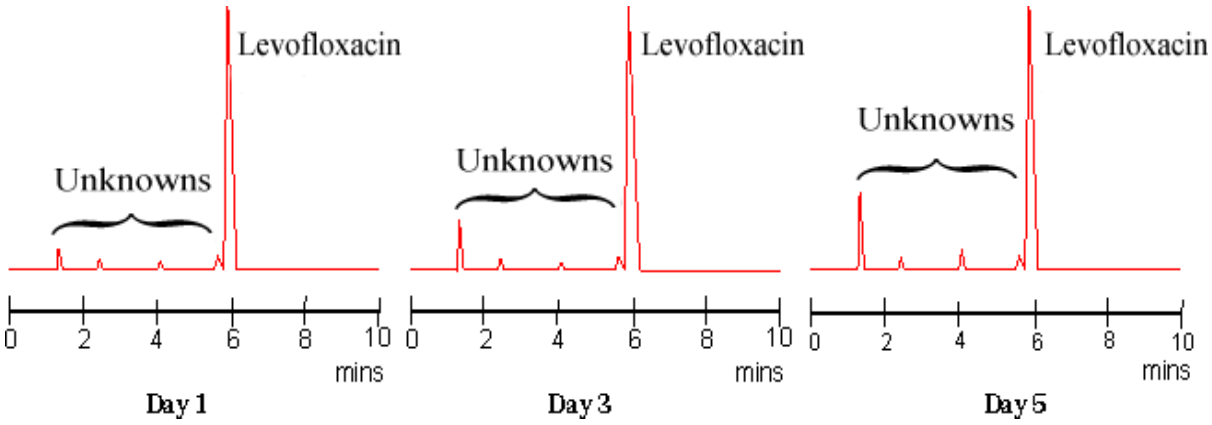


Figure 3.11: Chromatograms of levofloxacin in water after exposure to sunlight

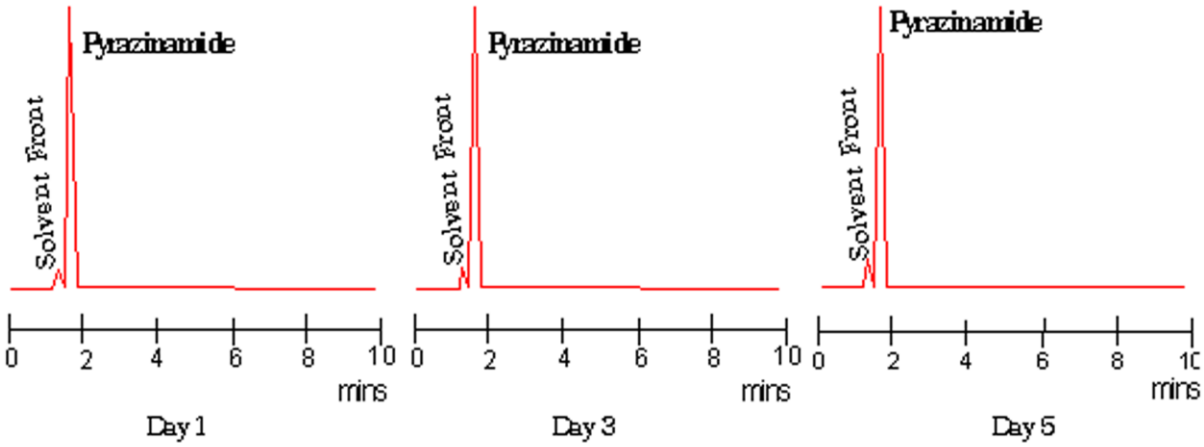


Figure 3.12: Chromatograms of pyrazinamide in after exposure to sunlight

3.6 CONCLUSIONS

Method development and analytical validation processes for HPLC in drug development assist in ensuring that the proposed methods measure the required parameters accurately and are therefore reliable to produce a drug product that conforms to acceptable standards. A reliable analytical method ascertains that drug substances involved are well resolved and can be identified and quantified within a mixture of other substances or excipients included in the formulation (LoBrutto and Patel, 2007). In developing a reliable chromatographic method, a suitable column must be chosen, as well as mobile phase constituents in correct amounts. Mobile phase flow rate, buffer molarity, temperature and pH play an important role in creating a method with successful results.

An HPLC method with chromatographic conditions summarized in Table 3.7 was successfully developed for the simultaneous separation of levofloxacin and pyrazinamide drug substances. The method demonstrated accepted linearity and range, precision, accuracy and selectivity. Moreover, the method can be used to resolve these drug compounds in other individual dosage forms as well as in isolating degradation products. Additional analytical tools such as a photodiode array and a mass spectrometer may, however, be useful in identification and quantification of more products resulting from decomposition of drug substances.

CHAPTER FOUR

PREFORMULATION AND POWDER ASSESSMENT

4.1 INTRODUCTION

A pharmaceutical dosage form consists of API(s) and different excipients that help warrant efficient drug delivery to the respective biological target, in addition to facilitating the drug fabrication process. Prior to selecting the final ingredients of a dosage form, the compounds undergo a phase known as preformulation. This stage was aptly described by Akers (1976) as test activities imposed on a new drug compound to fashion beneficial facts for development of a drug dosage form that is stable and displays optimum bioavailability. Furthermore, Lau (2001) in agreement with Vilegave and associates (2013) have clarified preformulation testing as characterisation of the physicochemical properties of the drug compound(s) for the subsequent formulation process. The aim is to produce ideal conditions for manufacturing the dosage form.

Since the 1950s, preformulation studies have proven useful in minimising experimental errors in drug product development (Vilegave*, et al.*, 2013). As an exhortation to pharmaceutical scientists and a conclusion to a book on the same matter, Wells (1988) emphasises that preformulation studies erect foundations which predestine the drug manufacturing process for favourable outcomes. Myriads of possible tests can be executed as part of preformulation analysis. Due to the diverse nature of tests encompassing preformulation activity, the investigation has taken a multidisciplinary study form that involves different branches of expertise. It should thus be regarded as paramount by all involved parties (Lau, 2001).

Preformulation studies can be performed on different scales. The scope is contingent on the nature of drug substance, the formulation scientist's expertise and preference as well as the availability of resources. Some of the drug compound properties that can be examined include, amongst others, salt formation, pH-dependent solubility profile, dissociation constant (pKa), polymorphism or crystallinity and particle size (Steele, 2004; Allen and Ansel, 2014).

There is a wide range of commercially available excipients to choose from in formulating a pharmaceutical dosage form. The excipients to be included in the

formulation are picked based on their compatibility with the API and their integral role in the dosage form to be created (Chaurasia, 2016). If accomplished effectively, preformulation studies establish compatibility properties of all the ingredients involved in the drug development process (Vilegave*, et al.*, 2013).

4.1.1 Physiochemical properties

4.1.1.1 Particle size and shape

The particle size of the API and other powders in the formulation has an impact on the physical activity of the dosage form, subsequently influencing the performance of the final drug product, including content uniformity, bioavailability and dissolution (Staniforth and Taylor, 2013). Moreover, both API and excipient particle size have effects on the manufacturing process with regards to blend uniformity and powder flowability. These factors ultimately have consequences on medicine quality, safety and efficacy (Gaisford, 2013).

In pharmaceutical solid dosage form development, improving the efficiency of the physical properties of ingredient powder flowability is of critical significance. Powder flow properties affect drug product manufacturing processes such as tablet compressibility, granulation, blending, powder density and conveyance (Sarraguça*, et al.*, 2010). Both the particle size and shape attributes are pivotal in characterisation of powder flowability (Guo*, et al.*, 1985; Yu*, et al.*, 2011). Expressions which qualitatively define particle shapes have been coined and adopted into the pharmaceutical industry. The shapes comprise of fibrous, spherical, crystalline and flaky categories (Prasad and Wan, 1987; Abdullah and Geldart, 1999).

It has been observed that, generally, more spherical particles contribute to better powder flow properties and hence, blend homogeneity compared to irregularly shaped particles. The more spherical the particles, the less weight variance is shown in tablets during compression (Gaisford, 2013). However, uniformity is not entirely a function of particle shape, as size, density and morphology factors reportedly have equally significant effects. It has been documented that achieving blend homogeneity during formulation of low dose drugs can prove to be a daunting task owing to challenges

associated with physical instability and content segregation (Leuenberger, 1982; Sun, 2006; Le*, et al.*, 2012).

Crystallinity of drug substance and potential excipient compounds can either remain unchanged during manipulation of particle size or polymorphic conversion may occur, and influence the final dosage form in more ways than one. Selecting an appropriate manufacturing process, nevertheless, is critical to avoid drastic alterations of the molecular and physiochemical properties (Gaisford, 2013). Table4.1 displays the typical steps involved in manufacture of pharmaceutical solid dosage forms. The possible physicochemical modifications on particles and the resultant implications on the product are also summarised (Cavatur, *et al*., 2008).

Table 4.1: Impact of manufacturing process on molecular and physiochemical properties of the drug substance (Cavatur, *et al*., 2008).

Processing step	Purpose of step	Possible solid-state transformation	Implication on tablet formulation
Milling	Size reduction; to improve powder flow and content uniformity	Polymorphic conversion, dehydration, amorphous phase transformation	Chemical stability, dissolution rate and bioavailability.
Roller compaction	Dry granulation, size increase; to improve powder flow and content uniformity	Polymorphic conversion, dehydration and amorphous phase transformation	Chemical stability, dissolution rate and bioavailability.
Wet massing	Wet granulation, size increase to improve powder flow and content uniformity	Polymorphic conversion, hydrate formation, salt to free acid/base conversion; amorphous phase transformation	Chemical and physical stability and dissolution rate
Granule drying	Solvent removal	Polymorphic conversion, hydration	Chemical stability; dissolution rate

The influence of particle size on dissolution rate, solubility and bioavailability has been recorded by a number of researchers (Liversidge and Cundy, 1995; Mosharraf and Nyström, 1995; Jinno*, et al.*, 2006). The dissolution rate of pharmaceutical particles as a function of particle surface area and other factors can be described mathematically using Equation 4.1, which is known as the Noyes–Whitney equation (Aulton, 2013).

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{k_1 A(C_s - C)}{h} \qquad \textbf{Equation 4.1}$$

Where,

$\frac{\mathrm{d}m}{\mathrm{d}t}$ = Solute dissolution rate

k_1 = Dissolution rate constant

A = Surface area of the solute particles

C_s= Equilibrium solubility

C = Solute concentration in solution

h = Thickness of boundary layer

As documented by Jinno and colleagues (2006), and supported by equation 4.1, reducing particle size significantly increases rate of dissolution and oral absorption of drugs that exhibit poor solubility in water because of the subsequent increase in interfacial surface area. Notwithstanding the evidence, Aulton (2013) argues that increasing surface area of particles augments interparticle static charge, resulting in agglomeration complications which can modify material crystallinity.

Micronisation of drug powder potentiates acquirement of particle interface energy and may ultimately lead to unstable dosage form. This theory is reiterated and clarified by Khadka and colleagues (2014), as they highlight that the impediment is more visible if the particles are reduced to the ultrafine range (5 microns or less), hence decreasing particle size indefinitely can be counterproductive. Gaisford (2013) points out that segregation and poor uniformity in powder blends is normally a consequence of dissimilarities in particle size of different formulation components.

4.1.1.2 Powder density

Individual drug(s) and excipient component densities may have a sizeable impact on the overall powder blend behavioural characteristics during the formulation process. In a study conducted by Murakami and colleagues (2001), densities of individual materials were found to have a close relationship with flowability, compressibility and compatibility of the powder blend. During powder characterisation in the development of solid dosage forms, powdered drug substances and excipients are commonly characterised based on their true, bulk and tapped densities (Hancock*, et al.*, 2003).

4.1.1.2.1 True density

The true density of a matter, also known as absolute density, is defined as the average mass per unit volume of the solid substance, completely disregarding all pores and voids that do not compose the fundamental part of the molecular packing (Amidon*, et al.*, 2009; Ma and Hadzija, 2013). In solid dosage form development, the true density parameter occupies a significant role. It has been used in the evaluation of porosity and mechanical behaviour (Sun, 2005; Deepika*, et al.*, 2013) as well as powder fluidization aspects of pharmaceutical powders (Hancock*, et al.*, 2006).

Various systems that are suitable for distinct types of substances are employed in calculating true density for materials. All the methods may present with their own relative beneficial factors, challenges and limitations (Cao*, et al.*, 2008). However, gas pycnometry is the most commonly used method to measure true density when a small amount of material is used. The technique is reliable, non-destructive and simple to reproduce (Amidon*, et al.*, 2009). Conversely, gas pycnometry has its flaws when employed in systems that comprise loosely packed solvents where gas may adsorb onto powder or when the channel hydrates with volatile contaminants. In such cases, measurement errors may be introduced (Sun, 2004; Amidon*, et al.*, 2009). The true density, ρ, can be expressed numerically using Equation 4.2.

$$\rho = \frac{\omega}{Vp} \qquad \textbf{Equation 4.2}$$

Where,

ω = Absolute weight of the sample

Vp = Powder volume

4.1.1.2.2 Bulk density

Bulk density denotes the mass per unit volume of untapped powder sample, including interparticle voids as well as envelope spaces of the particles. It also refers to mass of powder that can be filled into a defined volume. The bulk density is often the powder density as passively poured into a measuring volume. (Abdullah and Geldart, 1999; Amidon*, et al.*, 2009).

Two methods of measuring the effective bulk density are outlined in the United States Pharmacopoeia - National Formulary [USP30 - NF25] (2007a). The bulk density is typically determined by gently allowing a sample mass of powder into a graduated cylinder or volumeter, then leveling off excess powder carefully to avoid compaction. Mathematically, bulk density can be calculated using Equation 4.3:

$$\text{Bulk density (g/mL)} = \frac{M}{V_0} \qquad \textbf{Equation 4.3}$$

Where,

M = mass

V_0 = untapped volume

Bulk density of powder for the same material may vary from one analyst to another, depending on the milling process and process used to pack the material into the volume (Abdullah and Geldart, 1999; Amidon*, et al.*, 2009). Bulk density values of powders are contingent on factors such as particle size distribution, compaction and consolidation. Consequently, a constant value of a given powder is not guaranteed between manufacturers (Abdullah and Geldart, 1999).

4.1.1.2.3 Tapped density

Tapped density is the ratio of mass to volume of a powder sample after the vessel in which the powder is contained has been mechanically tapped for a defined time period at a constant velocity (United States Pharmacopoeia - National Formulary [USP30 - NF25], 2007a; Aulton, 2009). The tapped density values show the random dense packing of a powder once it has consolidated. They are, in general, numerically lower for particles of irregular shape such as flakes in contrast to regularly shaped particles such as spherical particles (Aulton, 2009). Equation 4.4 shows how tapped density can be calculated:

$$\text{Bulk density (g/mL)} = \frac{M}{V_f} \qquad \textbf{Equation 4.4}$$

Where,

M = mass

V_f = final tapped volume

The forces that exist among particles of powder have an impact on both the bulking and flow properties of the powder (United States Pharmacopoeia - National Formulary [USP30 - NF25], 2007a). The World Health Organisation (2012) points out that comparing the bulk and tapped powder densities gives an accurate measure of interactions for a specific powder. Such quantification is expressed as an indication of powder flowability, for example, the Carr's index (compressibility index) and the Hausner ratio.

Carr's index is the percentage compressibility of a powder and represents a direct measure of the possible arch or bridge strength of the powder. Hausner ratio, on the other hand, is associated with interparticulate interaction and is expressed as the ratio of the tapped density to the bulk density (Aulton, 2009). Equations 4.5 and 4.6 express the mathematical calculations of the Carr's index and Hausner ratio respectively:

$$\text{Carr's Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Bulk Density}} \times 100 \qquad \textbf{Equation 4.5}$$

$$\text{Hausner ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}} \qquad \textbf{Equation 4.6}$$

The interpretations of Carr's index and the Hausner ratio values are displayed in Table 4.2.

Table 4.2: Powder flowability in relationship to Carr's index and Hausner ratio

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

4.1.2 Drug-excipient compatibility

Excipients are commonly regarded as ingredients that are included in a formulation along with the API(s) to serve various purposes. Some of their functions, amongst many others, are to facilitate manufacture of a drug dosage form, secure the stability of the formulation, improve the bioavailability of the API(s) as well as to enhance patient acceptability (Bhattacharyya*, et al.*, 2006). Ideally, excipients should not exert any pharmacological effect but should assist to build a robust and reliable drug product that delivers precise amounts of API to the site of action (Moreton, 2006).

In the past, excipients were assumed to be inert ingredients, which has been proven to be inaccurate through various studies (Bayomi*, et al.*, 2001; Bozdağ-Pehlivan*, et al.*, 2011). Instead, excipients have shown to have the potential of interacting with each other or the API in the formulation and may be responsible for causing adverse effects and hypersensitivity reactions in final product users. The excipient-excipient and drug-excipient interactions result in alteration of drug safety and efficacy (Haywood and Glass, 2011).

Excipient interactions are found in physical, chemical and biopharmaceutical or physiological categories. They may either be beneficial or detrimental. In physical

interactions, molecular structures of the components are retained. Chemical interactions result in formation of new compounds whereas physiological interactions are fundamentally physical in nature whereby excipient interacts with body fluids after administration (Moreton, 2006).

4.1.2.1. Beneficial and detrimental drug-excipient interactions

Favourable drug-excipient interactions have been exploited to facilitate drug manufacturing processes. Many beneficial drug-excipient interactions are known. An example include lubrication benefits as an outcome of adding magnesium stearate to a formulation (Steele, 2004). Magnesium stearate has hydrophobic properties that enable it to reduce adhesion of ingredient powder blend to equipment during tableting process. Therefore, magnesium stearate ultimately improves powder flow. However, excessive blending time has proved to result in magnesium stearate particle abrasion, leading to their increased surface area and consequently formation of poor tablet dissolution and disintegration. If excessive blending with magnesium stearate occurs, it results in poor disintegration. The tablet is said to be ‘waterproofed’, as a result of excessive hydrophobic material present, which prevents sufficient water entry for disintegration to occur (Moreton, 2006; Adeyeye, 2008). Some more examples of beneficial drug-excipient interactions are summarised in Table 4.3.

A drug- excipient interaction known as the Maillard reaction occurs between amines and reducing sugars of drugs and excipients. When the primary amine and the glycosidic hydroxyl terminal on the reducing sugar react, an imine is formed. The imine disintegrates into yellow-brown coloured molecules called Amadori compounds. The reaction is catalyzed by magnesium ions, for example magnesium stearate, in high humidity environment (Moreton, 2006).

A study conducted by Adeyeye (2008) concluded that the chemical drug-excipient interaction through the Maillard reaction is promoted by the availability of amorphous compounds in the powder blend. Furthermore, increasing drug and excipient surface areas by milling or other means spurs on the reaction. Table 4.3 displays some of the excipients commonly used in manufacturing tablets as well as their beneficial interactions.

Table 4.3: Examples of excipients used in tablets manufacture and their functions (modified from Haywood and Glass (2011).

Excipient	Beneficial Interaction	Examples
Diluents	Provide bulk and enable accurate dosing of API	Sugar compounds - lactose, dextrin and glucose. Inorganic compounds - silicates, calcium and magnesium salts
Binders, compression agents	Bind the tablet ingredients together, giving form and mechanical strength	natural or synthetic polymers - starches, sugars, sugar alcohols and cellulose derivatives
Disintegrants	Aid dispersion of the tablet, releasing the active ingredient and increasing the surface area for dissolution	Compounds which swell or dissolve in water - starch, cellulose derivatives and alginates, crospovidone (CRP)
Glidants	Improve the flow of powders by reducing interparticle friction and adhesion. Can be used as anti-caking agents.	Colloidal anhydrous silicon and other silica compounds
Lubricants	Reduce interaction between particles and equipment	Stearic acid and its salts - magnesium stearate
Tablet coatings and films	Protect tablet from the environment, increase mechanical strength, modify release of the API	Polymers that are insoluble in acid - are used for enteric coatings to delay release of the active ingredient.
Colouring agents	Improve acceptability to patients, aid identification and prevent counterfeiting. Increase stability of light-sensitive drugs	Mainly synthetic dyes and natural colours

4.2 METHODS

4.2.1 Powder density

About 10g each of levofloxacin and pyrazinamide powders were gently and separately passed through 841 µm sieve to break any agglomerates that could have been formed prior to powder use. The sieved powders were filled into respective tared 100ml graduated measuring cylinders, in which both bulk volume (V_b) and tapped volume (V_t) were determined for each material. The values sequentially enabled establishment of the bulk densities and tapped densities of the powders as, per Equations 4.3 and 4.4 respectively.

Tapped densities were determined with the aid of a tapped volumeter, Erweka® model SVM 203 (GmbH, Heidenstam, Germany) which was set at 200 taps per minute for 3 minutes. Tests were completed in triplicate for each substance under investigation. The Carr's index, Hausner ratio and powder porosity (ε) were assessed using Equations 4.5, 4.6, and 4.7, respectively.

$$\varepsilon = 100\left(1 - \frac{\rho_t}{\rho}\right) \qquad \textbf{Equation 4.7}$$

Where;

ρ_t = tapped density

ρ = true density

4.2.2 Infrared (IR) spectroscopy

A Spectrum 100 FT-IR ATR Spectrophotometer (Perkin Elmer® Ltd, Beaconsfield, England) was used to generate the IR spectra of individual API substances as well as the binary mixture. Preparation of the mixture was achieved with the use of a mortar and pestle, where both the samples were included in equal amounts. Four samples of small amounts of the blend were then extracted and analysed (n=15) across the wavenumber range 4000 to 450 cm^{-1} and resolution of 4 cm^{-1}.

4.2.3 Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC)

TGA and DSC analysis for the APIs was completed with the aid of Model SDT 2960 simultaneous DSC - TGA thermal analyser (TA Instruments Inc, New Castle, USA) with calorimetric accuracy or precision of ± 2%. Approximately 4 mg of levofloxacin and pyrazinamide powder sample were separately spread on the instrument platinum pan and evaluated in a dry nitrogen environment. The purge rate was set to 20 ml per minute and temperature was from 25 °C to 600 °C, at a heating rate of 10 °C per minute. A binary mixture sample of the two compounds mixed in the ratio 1:2 for levofloxacin and pyrazinamide, respectively, was also analysed under the same conditions. The resultant data graphs of the analysis were deciphered using the Universal V4.5A TA Instruments software. The peak and onset temperatures, in addition to the enthalpy of transition (ΔH) were generated for the peaks.

4.3 RESULTS AND DISCUSSION

4.3.1 Powder density

4.3.1.1 True density

True densities of the APIs supply practical guidance into knowledge that can be used in characterisation of properties of powders for process development (Aulton, 2009).

Table 4.4: True density values for levofloxacin and pyrazinamide

Drug Substance	True density (g/cm^3)
Levofloxacin	1.480
Pyrazinamide	1.496

True density values summarised in Table 4.4 were taken from the drug substance reports of analysis and were used to calculate the statistical values of powder porosity and subsequently determine possible powder compressibility.

4.3.1.2 Bulk and tapped density

The determined bulk and tapped density values for levofloxacin and pyrazinamide are summarised in Table 4.4, which also included the Carr's indices and Hausner ratios.

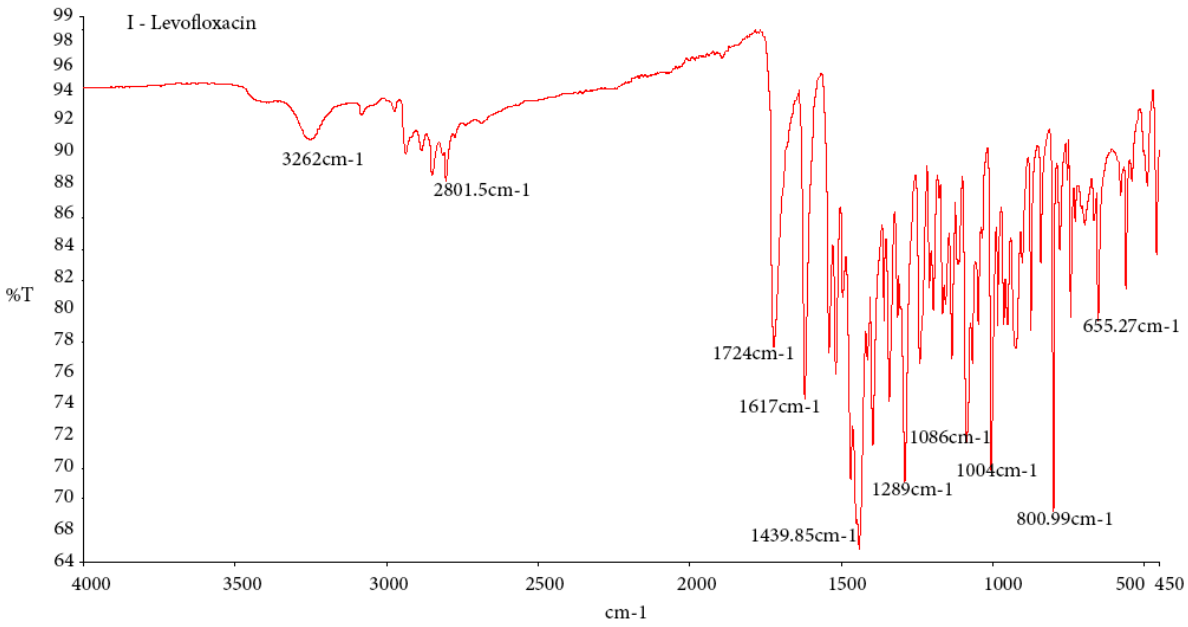
Table 4.5: True density values for levofloxacin and pyrazinamide

Drug Substance	Bulk density	Tapped density	Carr's Index	Hausner Ratio
Levofloxacin	0.537	0.557	3.59	1.04
Pyrazinamide	0.630	0.760	17.1	1.21

The values for Carr's index and Hausner ratio suggest that levofloxacin possesses excellent powder compressibility and flowability properties while pyrazinamide displays fair characteristics. These results are indicative that powder blends consisting of the drug substances may positively impact the tableting process. Granulation may therefore not be entirely necessary, and thus direct compression can be used as the tableting method.

4.3.2 IR spectroscopy

The generated IR spectra of levofloxacin, pyrazinamide and their binary mixture are depicted in Figure 4.1.



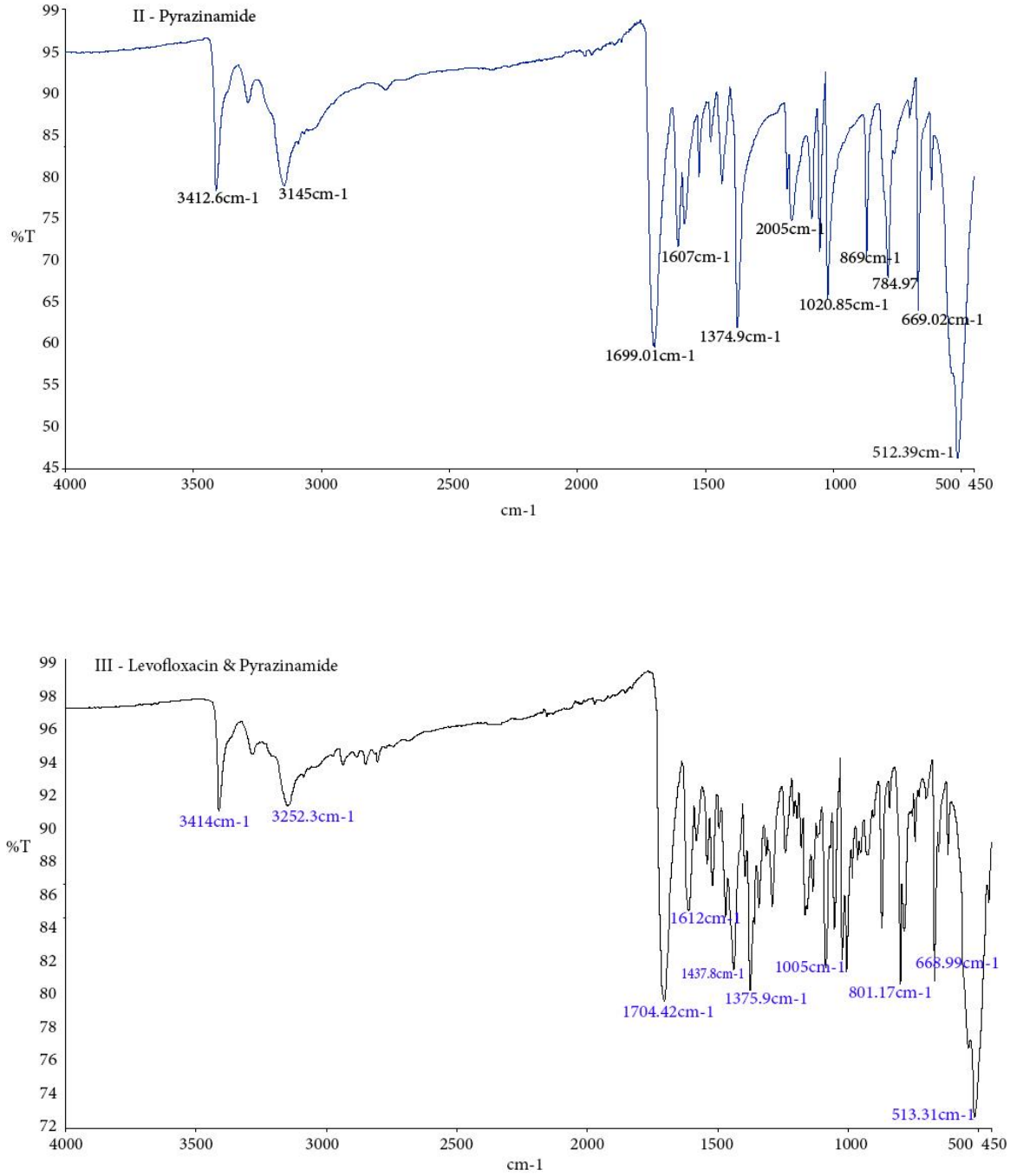


Figure 4.1: The experimental infrared spectrum of levofloxacin (I), pyrazinamide (II) and their binary mixture (III) generated at 4 scans and 4 cm^{-1} resolution

The absorption band frequencies and group assignments for the interpretation of IR spectra are displayed in Table 4.5 as reported and modified (Felder and Pitre, 1983; Gunasekaran*, et al.*, 2013).

Table 4.6: Typical IR absorption band assignments

Wave number (cm^{-1})	Assignment
3425, 3290, 3160	NH, OH of –COOH stretching
2900-3000	CH stretch aliphatic
2848	CH_2
1716	C=O
1614	NH^+, CN
1585, 1528	C=C and C=N (rings)
1380 - 1396	OH bending
1382	vibration of rings
1208	CN stretching
1183-782	CH out of plane, NH_2 rock

The IR spectrum of the binary mixture of levofloxacin and pyrazinamide displays the presence of band properties mostly retained from the individual compound spectra. The IR spectrum of the binary mixture in Figure 4.1 (III) reveals the levofloxacin hydroxyl out-plane and in-of-plane bending vibrations at the wave numbers of 1380 cm^{-1} and 1396 cm^{-1}, respectively. The increase in values is due to increased bending vibrations owing to the presence of hydrogen bonding through carbonyl groups (Gunasekaran*, et al.*, 2013). The C=O stretch observed at 1716 cm^{-1} is ascribable to pyrazinamide primary amide as also shown in Figure 4.1 (II). The secondary amide NH+ absorption band is represented by the peak at wave number 1614 cm^{-1} (II). The possible reason is that intensity of the aliphatic band is decreased in the IR spectrum of the binary mixture in comparison to the spectra of the individual drug components because of the decreased density of the particles of each substance per surface area.

4.3.3 Thermogravimetric analysis

The thermogram generated following TGA of levofloxacin and pyrazinamide are given in Figure 4.2 and Figure 4.3, respectively. The TGA plot for levofloxacin shows a mass loss of 2.46% when heated to 47.87°C due to loss of half a mole of water per mole of the drug. The temperature coincides with the onset of the peak of levofloxacin DSC

thermogram in Figure 4.4, thereby confirming the presence of the hemihydrate. An article for a study of the same compound in a previous study, however, reported a 2.59% mass loss at 70°C (Gorman*, et al.*, 2012). The differences in these results could be accredited to the possible dissimilarities in the methods used to synthesise the compound, as well as the differences in instrumentation used for analysis. Subsequently, there is negligible weight loss until the temperature of 231.18°C, where the compound rapidly melts.

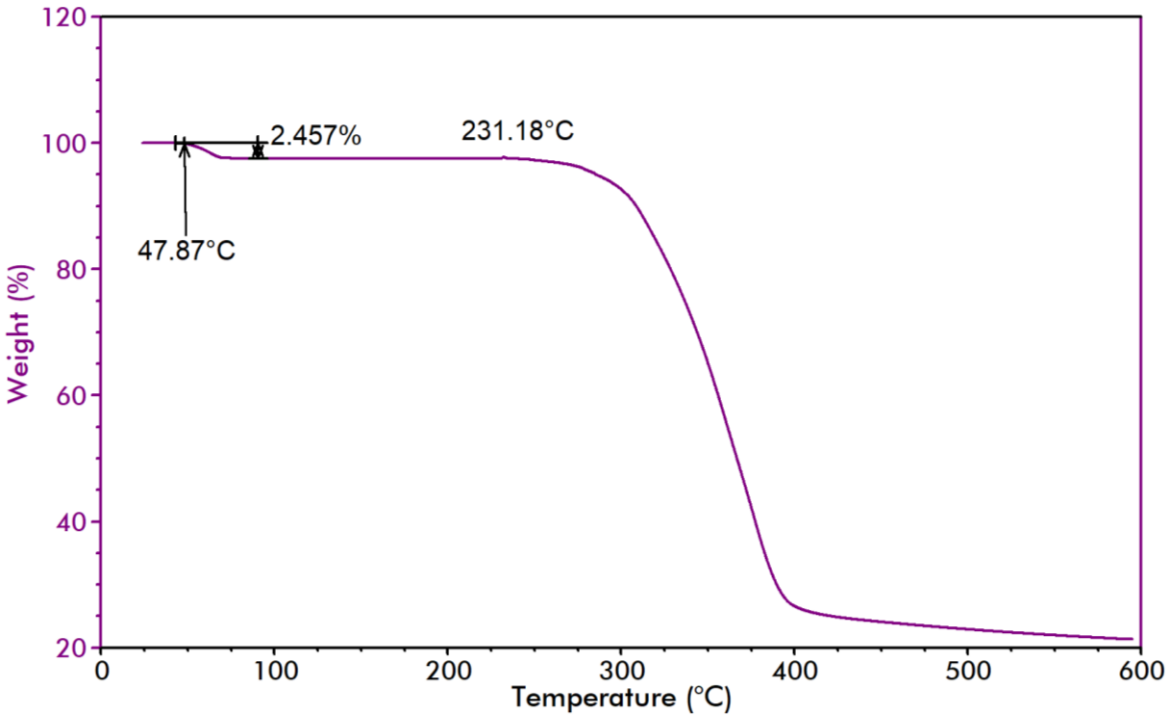


Figure 4.2: Typical TGA plot of levofloxacin at 10°C/min heating rate

Figure 4.3 displays a rapid loss in mass from the temperature of 134.2°C to 206°C due to decomposition of pyrazinamide. The thermogravimetric curve depicts thermostability of pyrazinamide in the heating range of 25°C to approximately 134°C, after which degradation occurs. The graph shows a decrease of only 1.2% in weight of the drug as a probable consequence of loss of moisture. Similar behaviour of pyrazinamide has been previously observed (Liu*, et al.*, 2016). A total of 100% of pyrazinamide is lost to decomposition when the temperature reaches 206°C. The two compounds display distinct thermodynamics at ambient temperature, with no temperature induced interactions.

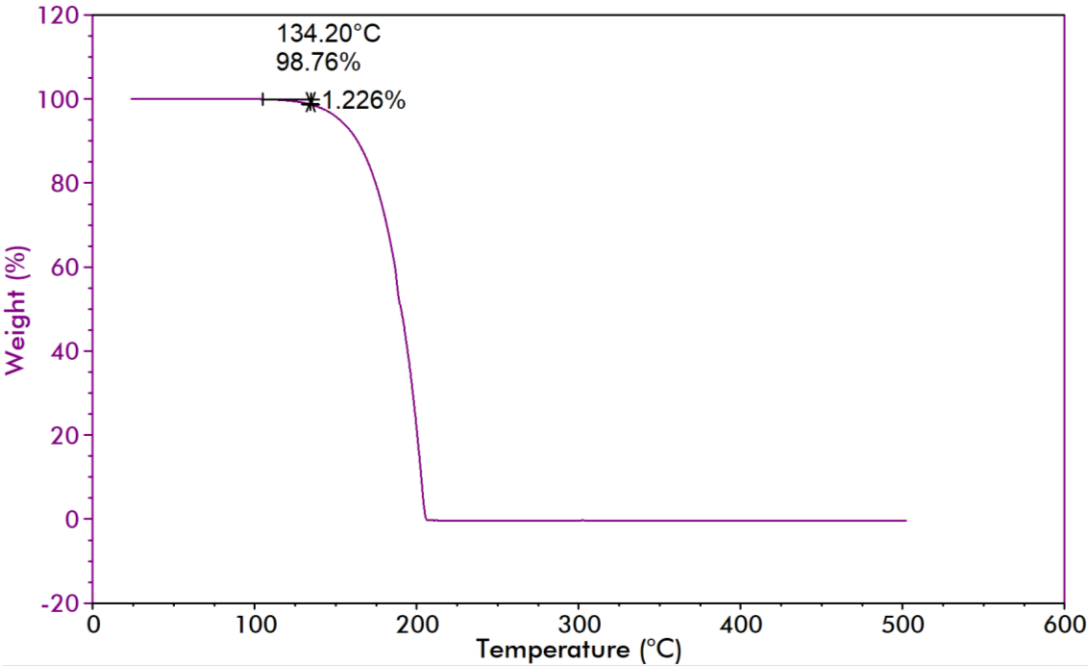


Figure 4.3: Typical TGA plot of pyrazinamide at 10°C/min heating rate

4.3.4 Differential scanning calorimetry

Four distinct peaks are depicted in Figure 4.4 that shows the DSC thermogram for levofloxacin hemihydrate at a heating rate of 10°C/min.

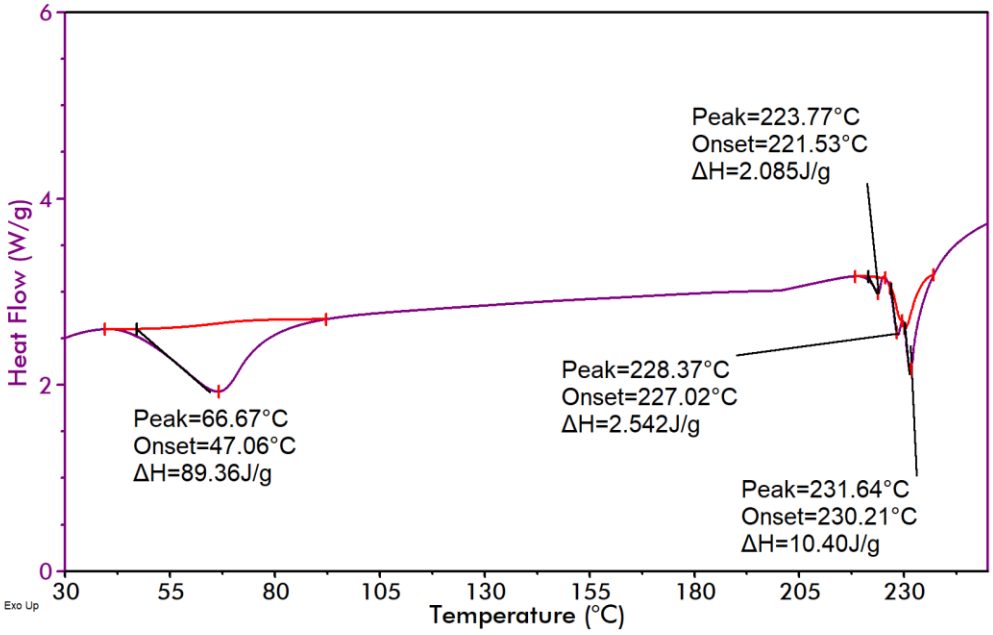


Figure 4.4: Typical DSC thermogram for levofloxacin hemihydrate at a heating rate of 10°C/min

The curve that depicts the hemihydrate shows an onset of an endothermic peak at 47.06°C and climaxes at 66.7°C, with enthalpy change of 89.36 J/g. This is due to the loss of the hemihydrate water and the formation of anhydrate γ-polymorph form of levofloxacin. The loss in mass was observed in the TGA curve of the hemihydrate.

The γ-form begins to melt at the temperature of 221.53°C and peaks at 223.77°C, producing a β-form of the drug, with a resultant of 2.085 J/g of enthalpy change, as depicted by the thermogram. The partially crystallised new solid is the β-polymorph, which melts when the temperature is increased to 228.37°C, resulting in consumption of 2.542 J/g of heat energy and the formation of α-polymorph. This crystal then melts at 231.64°C with energy change of 10.40 J/g. These values slightly correspond to those that were reported by a group of researchers two decades ago (Kitaoka*, et al.*, 1995). The γ-polymorph form of levofloxacin was observed to be stable across a wider range of temperature, between about 67 - 224°C.

As the crystal morphology of pyrazinamide was discussed earlier (section 2.2.2.4), pyrazinamide is known to exist in four forms of polymorphs. It was also highlighted that the commercially available conformation is in the α-polymorph. Figure 4.4 gives the DSC thermogram for pyrazinamide that exhibit three endothermic peaks.

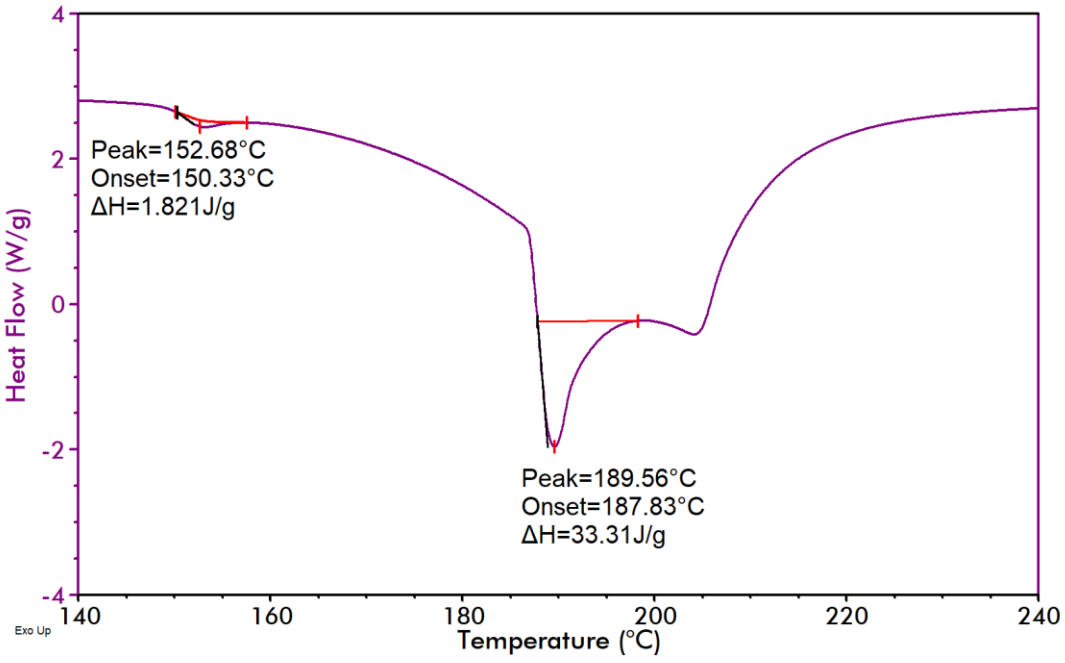


Figure 4.5: Typical DSC thermogram for pyrazinamide at a heating rate of 10°C/min

The first peak at 152.68°C with ΔH of 1.821 J/g indicates a non-reversible solid-solid phase transformation from α-form to the γ-form. The second peak at 189.56°C with ΔH of 33.31 J/g indicates the melting phase of the γ-form. Both values are fairly similar to those observed by Pharm and research team (2013). The endothermic peak that evolves after the melting phase at 204°C corresponds to the sublimation of the γ-polymorph, as shown by the TGA plot in Figure 4.3 and confirmed in an article by Cherukuvada and colleagues (2010). This suggests that the compound purely consists of the α-polymorph.

The thermogram for the binary mixture of levofloxacin and pyrazinamide at a heating rate of 10°C/min is depicted in Figure 4.6. The curve at 62.21°C is indicative of the presence of levofloxacin, which would have been expected to peak at approximately 66°C as in the individual drug compound. However, the peak onset values do not differ. The three peaks at 160.85°C, 164°C and 179.13°C do not coincide with any of the endotherms observed from the two separate thermograms, therefore are non-reflective of any of the individual compounds. This implies that levofloxacin and pyrazinamide interact at higher temperatures, possibly with the formation of a eutectic mixture.

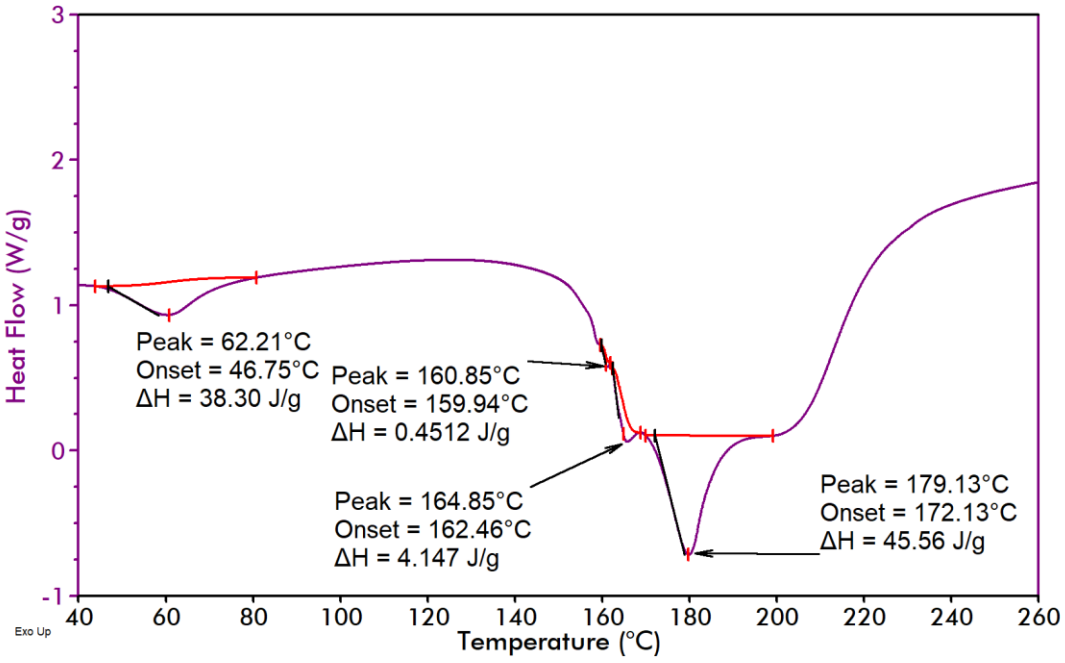


Figure 4.6: Typical DSC thermogram for binary mixture of levofloxacin and pyrazinamide at a heating rate of 10 °C/min

4.4 CONCLUSIONS

Preformulation studies minimise trial-and-error attempts in optimising dosage form manufacture conditions during formulation development. The studies help predict important characteristics of the pharmaceutical product in question. Preformulation studies may not necessarily offer product certainty, but the data forms crucial foundation on which quality may be constructed into the product (Lau, 2001).

Data from powder density studies indicates that both drug compounds exhibit potentially satisfactory flow and compressibility properties. This prediction is according to the respective numerical values of Carr's Index, Hausner ratio and powder porosity. Furthermore, since both powders have good flowability, the individual powders are highly likely to be physically compatible with each other in the resultant blend during mixing and direct compression process.

The IR spectroscopy results (Figure 4.1) suggest that there is no significant interaction of levofloxacin and pyrazinamide in this formulation. The IR spectrum of the binary compound mixture (Figure 4.1 (III)) showed that the components remained intact and maintained their individual properties after being mixed. The major peaks that define the separate substances could still be identified in the spectrum of the mixture. This is because in an IR spectrometer, samples are examined under ambient temperatures.

Thermogravimetric analysis of the compounds concluded that levofloxacin and pyrazinamide are stable under the conditions of formulation. TGA and DSC plots of the separate compounds display different thermogravimetric degradation profiles for the two drug materials, with no apparent cross over of pathways. However, the DSC thermogram for binary mixture of the drugs (Figure 4.6) suggests that interactions may occur between the two substances at high temperatures. This evidence also suggests that long term stability testing of the dispersible tablet may be necessary in formulations that contain both levofloxacin and pyrazinamide.

The preformulation studies have been useful in providing data that is essential for laying the foundation of the dosage form formulation and point a favourable direction

towards the more suitable methods to subsequently follow in accomplishing a successful manufacturing process. The studies helped to eliminate possible active drug-drug interaction speculations.

CHAPTER FIVE

FORMULATION DEVELOPMENT AND MANUFACTURE OF FIXED-DOSE COMBINATION DISPERSIBLE TABLETS

5.1 INTRODUCTION

Drug administration via the oral route has maintained its status as the most prevalent and favoured method of drug delivery for decades, for both local and systemic pharmacological effects in adults and children. For reasons that include ease of administration and accuracy of dosage, solid dosage forms have wide acceptance in the pharmaceutical field (Dey and Maiti, 2010). Moore (1998) establishes that children are by no means fractions of adults. Children form an exceptional group of patients, with distinct pharmacotherapeutic requirements and those needs should be investigated and satisfied. Children are a heterogenous group that exhibit distinct aspects in terms of drug pharmacokinetics and pharmacodynamics.

In conjunction with oral liquid preparations, dispersible tablets have become part of the solution to the challenges involving paediatric-unfriendly dosage forms, and are now recommended worldwide (Ivanovska, *et al*., 2014). The advent of child-friendly FDC formulations for the management of drug-sensitive TB in 2016 has been a game-changing approach so far. Development of similar child-friendly formulations for the treatment of MDR-TB would be a milestone.

Manufacturers need to overcome several hurdles in their quest to develop child-friendly dosage forms for MDR-TB. One basic challenge lies with the unavailability of an approved paediatric dosing guideline for FDC dispersible tablet formulation intended for the treatment of MDR-TB. The lack of child-friendly dosage forms is aggravated by the fact that the worldwide drug-regulatory policies decelerating the pace of innovative formulations towards the market. The process for registration of is tedious and bureaucratic, especially for medicines intended for children (Taneja, *et al*., 2015). Regarding the challenge of attaining bioequivalence between the independent drug products and the FDC product, oftentimes, the possibility of combining multiple APIs in a single preparation may alter the expected biopharmaceutical and pharmacokinetic outcomes (Mitra and Wu, 2012).

Mitra and Wu (2012) discuss the challenge of attaining bioequivalence between the independent drug products and the FDC product. Oftentimes, the possibility of combining multiple APIs in a single preparation may alter the expected biopharmaceutical and pharmacokinetic outcomes.

5.1.1 Dispersible tablets overview

Dispersible tablet systems were first developed approximately three decades ago to resolve dysphagia problems related to conventional oral dosage forms for psychotic, paediatric and geriatric patients (Dey and Maiti, 2010). Dispersible tablets contain API substances and disintegrate rapidly, normally within a minute when in contact with a liquid (Mali*, et al.*, 2014).

Dispersible tablets are ordinarily designed to be dispersed in liquid to form a homogeneous solution prior to administration (World Health Organisation, 2008). Dispersible tablets have also been referred to as fast-melting tablets (Fu, *et al*., 2005), fast-dissolving tablets (Nagendrakumar, *et al*., 2009), fast-disintegrating tablets (Parkash, *et al*., 2011), and orodispersible tablets for the tablets that dissolve rapidly in the mouth without the aid of water or chewing (Dey and Maiti, 2010) among other terms. Dispersible tablets take the form of the typical solid dosage preparations, except that dispersible tablets have superdisintegrants with resultant near-instantaneous dispersal (Parkash, *et al*., 2011).

An ideal dispersible tablet must possess certain qualities. Essentially, the preparation should be able to disintegrate in saliva or small amount of liquid in a short space of time. The formulation should present with high drug loading capacity while preserving stability in environmental conditions such as humidity and temperature, satisfactory taste-masking properties with pleasurable mouthfeel as well as have no residue remaining in the mouth after administration (Mali*, et al.*, 2014; Ivanovska*, et al.*, 2014). Due to its numerous benefits, dispersible tablet system is gaining even wider preference. There are several methods of manufacturing dispersible tablets. However, product properties may differ subject to the method of preparation followed (Parkash, *et al*., 2011).

5.1.1.1 Advantages of dispersible tablets

Dispersible tablets provide unique features of combined dual benefits of solid and liquid dosage forms (Garg and Gupta, 2013). As alluded to earlier (Section 1.1), dispersible tablets allow for easy administration to younger children, the elderly, psychiatric patients and patients who experience swallowing challenges. Risks of choking or suffocation normally accredited to solid dosage forms are reduced. As opposed to conventional dosage forms, dispersible formulations are convenient for use and hence enhance compliance and effective therapy (Mali*, et al.*, 2014).

As a virtue of being solid dosage forms, disperse tablets offer accurate dosing and ease of transportation. Owing to easy, rapid disintegration and fast dissolution, absorption and drug bioavailability are improved (Patil*, et al.*, 2014). Manufacturing techniques are based on use of conventional tableting procedures, yet enabling high drug loading capacity, thereby producing the tablets is cost effective. There are opportunities in innovative formulation differentiation for a large number of legacy and novel drug products which provide new business avenues for manufacturers (Garg and Gupta, 2013; Roy, 2016).

5.1.1.2 Limitations of dispersible tablets

The major drawback of dispersible tablets is associated with tablet mechanical strength. Roy (2016) and Masih*, et al.* (2017) assert that the porous and soft compressed structure render dispersible tablets friable and brittle, consequently causing them to require careful handling. Mali*, et al.* (2014) and Roy (2016) agree that it is difficult to formulate large doses of drugs into dispersible tablets and another challenge arises when taste-masking of certain drugs is required. The hygroscopic nature of the product necessitates a need for special packaging to keep the dispersible tablets stable in wet climates.

5.1.2 Manufacturing methods

A broad variety of technologies for manufacture of dispersible tablets have been developed. These include direct compression, amongst many others (Table 5.1; Roy 2016). The molding and lyophilisation technologies are some of the most exploited methods. They have been found to yield dispersible tablets which disintegrate in less

than 30 seconds, although the tablets are highly friable and have low mechanical strength (Abdelbary*, et al.*, 2004). On the other hand, although dispersible tablets produced through direct compression may take longer than a minute to disintegrate, they display relatively low friability.

5.1.3 Manufacturing method of choice

Direct compression involves a limited number of process steps and is a cost-effective and rapid process that employs the widely used excipients and equipment. Moreover, the technique can accommodate high doses of API and larger final weight of the product (Dobetti, 2001). Direct compression was therefore the preferred method in this study.

5.1.3 Excipients

Excipients are ingredients that are included in pharmaceutical formulations to serve various purposes, such as diluents, protective agents or materials used to improve bioavailability of active ingredients. Excipients also facilitate smooth manufacture of the drug products (Haywood and Glass, 2011). Some materials possess multi-functionality properties, where the same ingredient can be employed for a different purpose, depending on the concentration at which they are added (Jivraj*, et al.*, 2000). The following section covers some of the most commonly used excipients.

Table 5.1: Methods most commonly used in dispersible tablet manufacturing

Technique	Characteristics of the Method
Direct compression	Most popular method where addition of superdisintegrants at concentrations of between 2-5% is the basic principle. It is the simplest of the techniques and may be carried out using conventional tableting equipment (Patel*, et al.*, 2014).
Freeze drying or Lyophilization	Normally light weight and porous tablets are formed through this method, where the solvent is extracted out of the frozen drug solution. The resultant product shows enhanced dissolution profile. The method is suitable for drugs that are affected by elevated temperature (Pahwa*, et al.*, 2010).
Molding	Also termed solid dispersion, produce tablets that disintegrate within 5-15 seconds. Two approaches exist. 1. Compression molding - moistened powder blend is compressed into mold plates and solvent is then removed by an air-drying process (Dobetti, 2001). 2. Heat molding - molten matrix carrying dispersed or dissolved drug is poured into blister packaging then solidified (Patel*, et al.*, 2014).
Cotton candy process	A process of rapid and simultaneous melting and spinning of polysaccharide matrix is used. The drug substance and other excipients are added to the recrystallised and milled candy floss matrix, then compressed to produce dispersible tablets with high mechanical strength (Garg and Gupta, 2013).

Continuation of Table 5.1

Melt granulation	The process requires that the binder be molten liquid or solid that can melt. The molten binder is used to agglomerate the powder blends during granulation. polyethylene glycol (PEG) is the most widely used melt binder (Seo and Schæfer, 2001)
Mass extrusion	Powder blend of drug substance(s) and excipients are mixed with solvent mixture that contains water soluble PEG and methanol. The resultant softened powder mass is extruded through a syringe or extruder to produce cylindrical product that is divided into tablets (Parkash*, et al.*, 2011).
Phase transition	This method revolves around phase transition of sugar alcohol to produce tablets with adequate hardness. The tablets that contain two sugar alcohols of high and low melting points, are initially prepared using the conventional compression method followed by heating. The heating subsequently increases bond strength between tablet particles, leading to formation of improved tablet hardness (Kuno*, et al.*, 2008).
Nanonisation	A recent technology that involves reducing drug particle size milling it into nanoparticle size. The technology produces rapidly disintegrating tablets that have an improved drug absorption profile and bioavailability. It also comes in handy during the formulation of drugs that show poor water solubility (Hannan*, et al.*, 2016).

5.1.3.1 Superdisintegrants

A superdisintegrant is a type of excipient that is added in lower concentrations than conventional disintegrants to the formulation to accelerate break up and dispersion of the solid tablet matrix rapidly. The disintegration of the tablet containing superdisintegrants occurs once the tablet encounters water or a fluid environment (Preethi*, et al.*, 2013). There are several mechanisms through which tablet disintegration occurs. These include, amongst others, swelling, water wicking, heat of wetting and strain recovery (Augsburger*, et al.*, 2007).

Depending on the characteristics of the API and the required drug release profile, superdisintegrants are normally included at 1-10 %, and in other cases, up to 20% of the total weight of the dosage unit. Inclusion of effective superdisintegrants may improve ingredient compatibility and allow compressibility without any negative effect on physical strength of tablets carrying a high-dose drug (Dobetti, 2001). Sodium starch glycolate (SSG), croscarmellose sodium (CCS) and CRP are the most commonly used superdisintegrants (thereafter in the text simply referred to as disintegrants). SSG is spherical in shape and therefore improves powder flow. It also possesses strong swelling properties. CRP, on the other hand, is fibrous in nature and highly compactable (Augsburger*, et al.*, 2007). Alginic acid, soy polysaccharides, calcium silicate, xanthan gum, and gellan gum have also been listed as some of the commercially available disintegrants employed in rapid disintegrating formulations (Mando and Hasan, 2015).

5.1.3.2 Binders

Binders are also known as adhesives and are normally added to impart cohesive qualities to the powdered mass. Binding agents may be used in liquid (solution binder) or dry (dry binder) form to facilitate agglomeration and granule formation during direct compression. The binder form used is contingent on the type of process employed in producing material for compression. In direct compression, the binding agent is added to powder mixture in dry form while it may be dissolved in granulating fluid during wet granulation processes (Mahato and Narang, 2012).

Widely used binding agents in direct compression processes include microcrystalline cellulose (MCC) and silicified MCC. MCC, in particular, has been ranked as one of the

most effective binding agents due to its exceptional dry binding properties (Thoorens*, et al.*, 2014). Binders utilised in wet granulation are mostly polymeric in nature and include polyvinylpyrrolidone (PVP), saccharides, gelatin, cellulose derivatives (such as methylcellulose), as well as alginic acid derivatives (such as sodium alginate) (Jivraj*, et al.*, 2000). The type and concentration of binding agent has an impact on the ultimate friability and physical strength of the tablet (Mahato and Narang, 2012).

5.1.3.3 Diluents

Diluents are also termed fillers or bulking agents. A tablet must be of reasonable weight for practical compressible size and improved physical strength, especially in tablets that contain a low dose of API. Diluents are included in the tablet formulation to make up the bulk volume or weight of tablet unit if the weight of the drug is less than 50 mg. For easy patient handling purposes, a tablet must have a weight of at least 50 mg. Examples of diluents are MCC, mannitol, sodium chloride, dicalcium phosphate dehydrate, sorbitol, lactose, starches, dextrose and sucrose (Mahato and Narang, 2012).

5.1.3.4 Anti-frictional agents

5.1.3.4.1 Lubricants

Lubricants are ingredients that prevent tablet material from sticking to the punch and die surfaces during the compression stage, thereby aiding smooth ejection of tablet units from the dies. Directly after compression, tablets may expand and bind to the die surfaces. Lubricants improve powder flow characteristics and reduce friction following compression. High friction during compression may cause capping and fragmentation of tablets. Lubricants are mostly used in concentration of less or equal to 1% by weight, however, talc is ordinarily used in higher quantities (Mahato and Narang, 2012; Sakr and Alanazi, 2013).

Frequently used lubricants are hydrophobic materials and include stearic acid, magnesium stearate, calcium stearate, hydrogenated vegetable oils, PEG, talc as well as glyceryl behanate. Magnesium stearate is generally used most often, except in instances where it is chemically incompatible. In such cases, stearic acid or talc are used as substitutes (Sakr and Alanazi, 2013). Some lubricants are water-soluble and

are utilised in the formulation of water-soluble tablets. Sodium benzoate, carbowax 4000, sodium chloride, as well as a mixture of sodium acetate and sodium benzoate are typical examples of such lubricants (Mahato and Narang, 2012).

5.1.3.4.2 Glidants

Glidants are included in tablet preparations to decrease inter-particulate friction and improve powder mixture flow characteristics. On all occasions, glidants are added in dry form immediately before compression. Fumed (colloidal) silica is the most exploited glidant, whereas asbestos-free talc is used as both lubricant and glidant (Sakr and Alanazi, 2013). Starch may also be used as a glidant (Mahato and Narang, 2012).

5.1.3.4.3 Anti-adherents

Anti-adherents act to reduce the sticking of powder mixture particles to the punch faces. This prevents occurrence of a phenomenon known as picking or sticking. Sticking occurs especially on punch surfaces that have markings such as symbols or engravings, where thin layers of powder may build up, leading to uneven tablet surfaces (Alderborn, 2013)

5.2 METHODS

5.2.1 Materials

Levofloxacin was procured from Sgonek® Biological Technology Co, Ltd (Xian Shi, Shaanxi Sheng, China) and pyrazinamide was sponsored by Aspen Pharmacare® (Eastern Cape, South Africa). Mannitol, SSG, CRP and CCS were purchased from Aspen Pharmacare (Eastern Cape, South Africa), whereas MCC, colloidal silicon dioxide (CSD), talc and magnesium stearate were purchased from JRS Pharma (Johannesburg, Gauteng, South Africa).

5.2.1.1 Microcrystalline cellulose (MCC)

MCC is one material with various excipient functions. Thoorens and colleagues (2014) summarise MCC as the most widely used binder in direct compression tableting processes. It offers broad compatibility with many APIs in addition to exhibiting

physiological inertness. Furthermore, it is considered as an important diluent with lubricant and disintegrant properties both in wet granulation and direct compression. However, Bala and fellow research team (2013) agree with Mastafa and colleagues (2013) that these characteristics should not replace the necessity of including true lubricants and disintegrants when MCC has been used. Instead, MCC can be used in collaboration with superdisintegrants to promote more rapid disintegration.

5.2.1.2 Crospovidone (CRP)

CRP is a highly hydrophilic yet insoluble form of PVP that possesses rapid moisture sorption properties along with good swelling characteristics. On the other hand, the excipient has been documented to have good binding properties because of its excellent flow and plastic deformability characteristics (Barabas and Adeyey, 1996). It is therefore a good tablet disintegrant and binder. Studies suggest that CRP may improve solubility of steroid APIs and is compatible with a wide range of organic and inorganic tableting ingredients. It is normally used at concentrations of 2–5% by weight (Rowe*, et al.*, 2009).

5.2.1.3 Croscarmellose sodium (CCS)

CCS is an example of crosslinked polymer of carboxymethyl cellulose sodium. It is used as a disintegrant at concentrations of up to 5% w/w, although normally, 2% w/w is sufficient for use in direct compression tablet formation process. Though a hygroscopic material, CCS is stable at ambient conditions (Rowe*, et al.*, 2009).

5.2.1.4 Sodium starch glycolate (SSG)

SSG is a sodium salt of cross-linked or non-cross-linked carboxymethyl ether of starch that is commonly employed in tablet and capsule formulation as a disintegrant (United States Pharmacopoeia - National Formulary [USP30 - NF25], 2007b). As summarised by Rowe and colleagues (2009), SSG is usually used in both wet granulation and direct compression at optimum concentrations of about 4% w/w, though concentrations of up to 8% w/w are sometimes added. In most cases, 2% w/w of SSG in a formulation is normally sufficient. In another study, it was found that the inclusion of SSG as a disintegrant by direct compression method improved the photo-stability

of norfloxacin in a tablet formulation due to a phenomenon known as barrier effect of the starch granules (Córdoba-Borrego*, et al.*, 1999).

5.2.1.5 Colloidal silicon dioxide (CSD)

CSD is a fumed silica manufactured by vapor hydrolysis of chlorosilanes has has various excipient functions, although it has been primarily employed in pharmaceutical formulations as a glidant at concentrations between 0.1–1% w/w. It has been categorised as an anti-caking agent, adsorbent, viscosity-increasing agent, suspending agent, disintegrant, as well as an emulsion stabiliser (Rowe*, et al.*, 2009). It may be necessary to add extra lubricant when including CSD in a powder mixture to circumvent sticking and picking complications (Mahalingam*, et al.*, 2008).

5.2.1.6 Magnesium stearate

The chief function of magnesium stearate in pharmaceutical formulations is to provide lubrication in capsule and tablet manufacture. The usual recommended concentrations range from 0.25% to 5.0 % w/w. Recent studies have confirmed that lubrication by magnesium stearate in high concentrations may lead to a decrease in tensile strength and an increase in the brittleness of tablets (Paul and Sun, 2017). As mentioned earlier (section 4.1.3.1), magnesium stearate improves powder flow at low concentrations and sufficient blending time. Excessive blending time results in magnesium stearate particle abrasion, and ultimately, poor tablet dissolution and disintegration (Moreton, 2006). It can be concluded that the amount of lubricant added, and the time of blending needs to be closely monitored for optimal tableting results.

5.2.2 Manufacturing equipment

All the raw materials were weighed using a Model XP205 Mettler Toledo® analytical precision balance (Mettler Instruments, Zurich, Switzerland). Prior to use, the materials were sieved through stainless steel mesh cloths that conform to the requirements of the International Standards (ISO 565). Powder blending was accomplished using a cube mixer attached to Model AR 403 All-purpose Erweka drive unit (Erweka® GmbH, Heusenstamm, Germany). The direct compression process was achieved on a Version-2 single punch eccentric tablet press EP-1 (Erweka® GmbH, Heusenstamm, Germany).

5.2.3 Method of manufacture

As detailed in chapter one of this report, the direct compression method was employed to supply dispersible tablets that each contain 150 mg of levofloxacin and 300 mg of pyrazinamide. The doses were chosen following evaluation of typical individual drug doses, as well as reviewing relevant literature. Six different formulae were developed. The first three (F1 – F3) were based on the typical formulation approach, with use of three different superdisintegrants at 5% w/w. The next three (F4 – F6) had the concentration of the different disintegrants increased to 8% w/w. A summary of the different formulation designs is displayed in Table 5.2 and a schematic summary of the manufacturing method is outlined in Figure 5.1. The figure is explained in section 5.2.3.1.

Table 5.2: Preliminary formulation design with varied disintegrants but constant total mass per tablet of 900 mg

Excipients	**F1 (mg)**	**F2 (mg)**	**F3 (mg)**	**F4 (mg)**	**F5 (mg)**	**F6 (mg)**
Levofloxacin	150	150	150	150	150	150
Pyrazinamide	300	300	300	300	300	300
Croscarmellose sodium	45	-	-	72	-	-
Sodium starch glycolate	-	45		-	72	
Crospovidone	-	-	45	-	-	72
Colloidal silicon dioxide	15	15	15	15	15	15
Microcrystalline cellulose	385	385	385	358	358	358
Mg Stearate	5	5	5	5	5	5
Total (mg)	900	900	900	900	900	900

5.2.3.1 Direct compression procedure

Levofloxacin, pyrazinamide and MCC were passed through a sieve screen of aperture size 841 µm and blended with 50% of the total superdisintegrant at a rotor speed of 100 rpm for five minutes. CSD and the other 50% of superdisintegrant were sieved through a sieve screen of aperture size 780 µm, while magnesium stearate was sieved through a sieve screen of aperture size 315 µm and added to the blended powder

mixture. Further mixing of the subsequent powder blend was performed for three to five minutes. The powder blends were compressed in the rotatory tablet press equipped with 12 mm flat-faced punches, where tablets weighing approximately 900 mg each were produced.

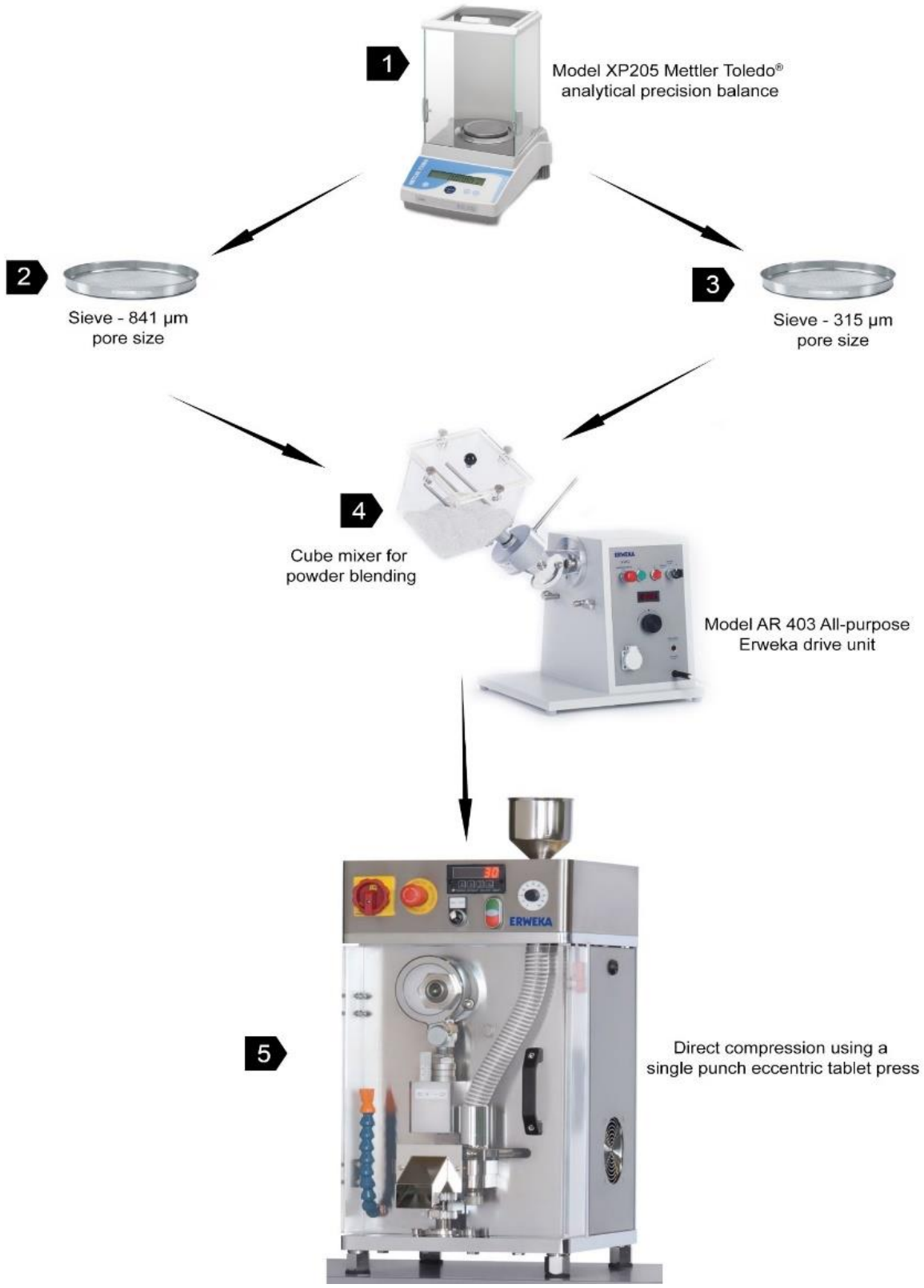


Figure 5.1: Dispersible tablet manufacturing method

5.2.3.2 Physical characterization of powder blends

The powder blends were subjected to pre-compression tests such as bulk density, tapped density, Hausner ratio and Carr's index analyses. The results of the tests were calculated as described in Section 4.1.1.2.

5.2.4 Physical characterization of dispersible tablets

5.2.4.1 Physical Appearance

Dosage form products, especially those formulated for children should be physically appealing and acceptable to the patient to maximise adherence (Ruiz*, et al.*, 2017). The tablets were physically and closely inspected and colour, shape, feel, size, ease of handling as well as odour were noted.

5.2.4.2 Weight Variation

Weight variation is determined to establish drug substance consistency in the tablet units and maintain an insignificant range around the desired concentration. The weight variation of tablets should be within the limits recommended by the USP as displayed in Table 5.3. The average weight of 10 randomly selected tablets weighed individually and accurately using a Model XP205 Mettler Toledo® analytical balance (Mettler Instruments, Zurich, Switzerland) was determined. The % RSD was then established from the results.

Table 5.3: Weight variation limits as per recommended by the USP

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

5.2.4.3 Breaking Force

Tablet breaking force is referred to in literature as hardness or crushing strength. It defines the amount of force a tablet requires to fracture it in a specific plane. Tablets should have sufficient mechanical strength to withstand operations such as

processing, packaging, transportation and handling without breaking, yet still be able to disintegrate and liberate the active ingredient at the proper site (Mahato and Narang, 2012). The breaking force and diameter parameters of tablets were simultaneously established using Model TBH125 series tablet hardness tester (Erweka® GmbH, Heusenstamm, Germany).

5.2.4.4 Friability

Friability describes the propensity of a tablet to crumble or shed powder due to falling or from mechanical stress. The extent of tablet fragility depends on factors such as compressed powder blend, hardness and shape (Allen and Ansel, 2014). As recommended by the USP, the mean weight loss from samples should not exceed 1%. A sample of 10 tablets was tested for friability using a Model TA3R friabilator (Erweka® GmbH, Heusenstamm, Germany). The tablets were dedusted and weighed accurately using a Model XP205 Mettler Toledo® analytical balance (Mettler Instruments, Zurich, Switzerland). They were then placed in the friabilator and the device was set to rotate at 25 rpm for four minutes, allowing the tablets to tumble 100 times. The tablets were dedusted once more and accurately weighed. The friability was evaluated using Equation 5.1:

$$Fr = \left(\frac{w_1 - w_2}{w_1}\right) \text{x}100 \qquad \textbf{Equation 5.1}$$

Where,

Fr = friability

w_1 = weight before rotation

w_2 = weight after rotation

5.2.4.5 Disintegration test

Disintegration must occur for drug to be released and for dissolution and absorption to take place. Disintegration is mainly a function of mechanical hardness and a disintegration test is performed to measure the time it takes for tablets to completely break up under specified conditions (Allen and Ansel, 2014). The test was performed on all prepared batches using a model ZT 320 Series Disintegration Tester equipped with an external temperature sensor and two beakers (Erweka®, Germany). The apparatus beakers were filled with 900 ml of distilled water maintained at 25 ± 0.2 °C,

then six, randomly selected tablets were placed in the separate tubes of the basket-rack and covered with a disk. The basket was set to oscillate vertically at a constant frequency rate of 30 cycles per minute. The time for disintegration for each tablet was recorded.

5.2.4.6 Tablet assay

To ensure that the tablets contain the prescribed dose of drug substance, tablets must be assayed. Twenty randomly selected tablets were ground into fine powder using a mortar and pestle. An amount equivalent to one tablet (900 mg) of the powder was accurately weighed and quantitatively transferred into a 100 ml A-grade volumetric flask. The drug substances were extracted by dissolving the powder using a mobile phase solution consisting of distilled water, 0.025 M orthophosphoric acid buffer and ACN in a ratio of 68:20:12, respectively, then made up to volume with the same solution. The resultant solution was stirred using a Model STR-MH-180 magnetic hotplate stirrer (FMH Instruments®, Cape Town, South Africa) then passed through a 12 µm pore sized filter paper. Exactly 1 ml was transferred into a 100 ml A-grade volumetric flask and made up to volume with the mobile phase solution. This was filtered once more again using a 12 µm pore sized filter paper. Samples of the resultant solution were analysed in triplicate using the validated HPLC method detailed in Chapter Three.

5.3. APPLICATION OF QUALITY BY DESIGN

5.3.1 Defining the quality target product profile (QTPP) for the formulation

As defined in Section 1.2, QTPP is a summary of ideal attributes or characteristics of a drug product that ensure its efficacy and patient safety. These attributes need to be achieved as they constitute the basis of a successful drug development design (Sangshetti, et al., 2014). The QTPP prospectively sets the boundaries and goals of the drug product and may be reviewed and improved as useful data is gathered at the various steps of the development. The established QTPP helps to ensure that the product is suitable for its intended use as the attributes are designed into the formula. The identified QTPP are expressed as targets which form the basis for the establishment of the cQAs (Food and Drug Administration, 2006).

Table 5.4 lays out the QTPP for the levofloxacin-pyrazinamide dispersible tablet. Notwithstanding the fact that significant elements such as product stability and factors of packaging may be identified as part of QTPP (Charoo*, et al.*, 2012). This can provide a scope for future studies to explore.

5.3.2 Identification of the critical quality attributes (cQAs)

The cQAs were derived from the identified targets of the QTPP. The cQAs express the possible attributes that impact quality, safety and efficacy of the product. These attributes are evaluated and monitored to determine the ones whose impact on drug product is of high risk. The predicted risks are evaluated and prevented through quality risk management (Charoo*, et al.*, 2012). Table 5.5 displays the identified cQAs for the formulation, and the table indicates that tablet disintegration is the most likely cQAs that can be influenced by formulation and process variables. Subsequently, risk assessment on tablet disintegration time attribute was performed and prioritised as displayed in Tables 5.6 and 5.7, respectively.

Table 5.4: QTPP for the FDC dispersible tablets

QTPP Elements		Target	Justification
Dosage form		Dispersible tablet	A novel combination of the nature of dosage form
Dosage design		Uncoated tablet, fast disintegrating	
Strength		150 mg pyrazinamide and 300 mg levofloxacin	
Route of administration		Oral, palatable dosage form	
Proposed Indication		Treatment of MDR-TB	Combination of the drugs is already being used for the stated indication
Drug product quality attributes	Physical attributes	Round, cream coloured, shallow concave, bevelled edged tablets	Observed from the initial trial run phases of tablet development
	Identification	Set against pharmacopoeial standards	
	Assay (±5% of target)	150 mg pyrazinamide (142.5 - 157.5 mg) 300 mg levofloxacin (285 - 315 mg)	According to the MDR-TB treatment guidelines
	Disintegration	Disintegration time should be three minutes in distilled water at the temperature of 25 ± 0.2 °C	Limits are specified in the pharmacopoeia
	Residual solvents	Not applicable	
	Degradation products (HPLC)	HPLC analysis compared to forced degradation studies (3.5.7). Product should contain minimal degradation products	Minimal degradation products expected
	Content uniformity	Weight variation limits as specified in the USP	Content uniformity limits as specified by the USP

Table 5.5: Critical quality attributes (cQAs) for the FDC dispersible tablets

Quality Attributes		Target	cQA?	Justification
Physical Impression	Appearance	Round, white shallow concave, bevelled edged tablets	No	Tablet colour and shape are for patient acceptability and are not directly linked to safety and efficacy.
	Size	Diameter and thickness to be established	No	Since the dispersible tablet will be dissolved in water before swallowing, size is not a crucial factor
	Odour	No unpleasant odour	No	Noticeable odour affects patient acceptability and not directly linked to safety and efficacy.
Uniformity of dosage units		Conforms to the USP	No	Weight variation test was conducted for control of drug substance uniformity in dosage units.
Breaking force		To be established	No	Hardness may affect disintegration time, but is not critical relating to safety and efficacy
Friability		<1.0% w/w after 25 rpm for four minutes	No	As per compendial requirements for tablets, friability of less than 1.0% w/w of mean weight loss is acceptable for low impact on patient safety and efficacy.
Disintegration		Must be less than three minutes	Yes	Tablets should completely disintegrate rapidly in a small amount of liquid and facilitate swallowing.
Assay		Positive for levofloxacin and pyrazinamide. API doses to fall within the ±5% allowance	Yes	Preformulation studies showed that assay for the drugs is within specification, therefore, will not discussed as a cQA

5.3.3 Quality Risk Assessment

The risk assessment of the effects of active ingredients, excipients and process attributes on drug product quality was performed to evaluate the impact of variables that affect quality of drug product and the impact of factors on quality will be discussed in relation to the effect they have on the disintegration process. The preliminary hazard analysis method of evaluation was employed in the risk assessment to identify the variables that impact quality of drug product. Table 5.6 exhibits that the risks are identified as low (-), medium (+) or high (++) depending on the criticality of the potential risk factor to the quality of the product. The risk factors are dealt with according to priority.

Table 5.6: Risk assessment of attributes that affect drug product quality

	Critical material attributes (cMAs)						**Critical process parameters (cPPs)**	
Drug Product cQAs	Effect of API attribute on drug product quality		Effect of excipients on drug product quality				Effect of operations on drug product quality	
	Particle size	Solubility	CCS	SSG	CRP	Mg stearate	Blending	Compression
Disintegration	+	-	++	++	++	++	++	+

Particle size may influence compressibility during processing. Savjani and colleagues (2012) discussed that particle size impacts the rate of dissolution, where a smaller particle size leads to an increased surface area, resulting in an improved dissolution profile . Since the APIs already have good solubility profiles and the major attribute to be achieved in this dosage form is disintegration of the tablets, particle size is therefore identified as medium-risk factor while solubility is not a risk.

All the superdisintegrants are ranked as high-risk excipients because they determine the rate of dispersion of the active ingredients into fluids. The superdisintegrants promote moisture penetration and cause the matrix in the dispersible tablet formulation to disperse into the primary particles of the dosage form (Augsburger*, et al.*, 2007).

Magnesium stearate is used as a lubricant and mainly influences the bonding strength of particles within the tablets. Larger amounts of magnesium stearate have been shown to cause tablet brittleness (Paul and Sun, 2017). Effects of magnesium stearate are therefore classified as high-risk effects due to its possible high impact on tablet disintegration. Research has found that powder blending time for the lubricant as well as compression force applied in tableting during the direct compression stage may have potential to affect the rate of tablet disintegration (Marais*, et al.*, 2003).

5.3.4 Failure Mode and Effects Analysis

Failure mode and effects analysis (FMEA) has been implemented in diverse production activities as a quality management tool to improve product quality. A FMEA is a methodical system of predicting the likelihood of occurrence of process and product failures and eliminating or ameliorating their consequences before they materialise. It is normally conducted during the stages of product development and ensures production of products that are without defect and safe for use (McDermott, et al., 2009). The FMEA identifies the cMAs and the cPPs that tilt the cQAs of the product outside the criteria described by the QTPP. Moreover, FMEA exposes and prioritises possible effects of specific failure modes due to various materials and manufacturing processes within.

Failure modes are possible ways in which a formulation process or product can fail (Davis, et al., 2008). Potential effects of failure modes are determined by factors of severity, occurrence and detection. Severity is the impact of failure, should it arise, whereas occurrence estimates the probability of occurrence of failure and detection is the probability that failure can be detected before the effects are displayed (McDermott, et al., 2009). With the use of preformulation data, failure modes were identified and rated according to estimated severity, occurrence and detection, on a scale of one to ten, with ten being the highest. A risk priority number (RPN) was then generated by determining the numerical product of the ranking for the three factors, as shown in Table 5.7.

Table 5.7: FMEA analysis of FDC dispersible tablets depicting RPN of the failure modes

Attributes	Item	Process Step/Input	Potential Failure Mode	Potential Effect of Failure	Severity	Potential Cause for Failure	Occurrence	Current Risk Control	Detection	RPN
APIs	Particle size	Disintegration	Low disintegration in water	Failed disintegration	8	Chemical property of APIs	2	Control disintegration	1	16
Excipients	Type & percentage of disintegrant composition	Disintegration	Undesirable disintegration time	Failed disintegration	8	Incorrect disintegrant or disintegrant quantity	7	Quality control	3	168
	Mg Stearate Effects	Disintegration	Long disintegration time	Failed disintegration	7	Over or under lubrication	3	Quality control	1	21
Manufacturing process	Blending	Disintegration	Non-homogenous powder blend	Failed disintegration	7	Powders not well mixed	7	Control blending time	3	174
	Compression	Disintegration	Tablet too hard to disintegrate	Failed disintegration	5	Compression force	4	Establish best compression force	2	40

5.4 RESULTS AND DISCUSSION

5.4.1 Micromeritic analysis of powder

The micromeritic analysis outcomes of powder blends are displayed in Table 5.8. The results suggest that all the powder blends have good compressibility and flowability, and therefore all the formulae were used to produce batches of tablets. The quantities of the APIs remain consistent throughout all the powder blends and they form 50% w/w of each formulation. MCC composes about 43% w/w of the mixture in the first three formulations and approximately 40% w/w in the rest.

Variation is applied to the type and amount of disintegrants used in each mixture. For this reason, the Carr's index and Hausner ratio values for the separate powder blends do not show much differences from each other. Increasing the amount of disintegrants (F4-6) did not have a significant impact on the flow properties of the powder blends. Formula 1 (F1) of the powder blend was employed in manufacturing the tablets in batch 1 (B1) and so forth, that is, the six batches were each made from a different formula.

Table 5.8: Pre-compression properties of different formula blends

Formula	F1	F2	F3	F4	F5	F6
Bulk density (gm/cm^{-1})	0.54	0.56	0.52	0.55	0.57	0.53
Tapped Density (gm/cm^{-1})	0.62	0.64	0.60	0.63	0.65	0.61
Carr's index (%)	14.8	14.2	15.1	14.7	14.0	15.1
Hausner ratio	1.15	1.14	1.15	1.15	1.14	1.15

5.4.2 Physico-mechanical properties of the tablets

The physico-mechanical properties of the manufactured batches of the dispersible tablets were assessed and recorded in Table 5.9. All the batches passed the weight variation test and the assay evaluation. Upon assessing tablets friability, the results of all batches were found to be within the acceptable limits, where tablets lost less than 1% of their weight after being exposed to the standard conditions of friability test.

The disintegration test times were acceptable for all batches. However, the batches that were formulated with 5% w/w of disintegrants were found to be, on average, about twice slower to disintegrate in comparison to those formulated with 8% w/w of the disintegrant. The batch that contained SSG displayed the best disintegration performance at 44 seconds, thereby selected as the most ideal batch. These results coincide with those found in a research conducted by Desai and colleagues (2014) where SSG was optimal at 8% w/w in aspirin and ibuprofen orodispersible tablets.

Table 5.9: Physico-mechanical evaluation of the tablets

Batch	B1	B2	B3	B4	B5	B6
Weight variation (mg)	897±0.91	905±1.13	855±0.97	912±0.97	909±1.18	879±1.17
Breaking Force (N)	44.9±1.12	44.2±1.1	45.5±1.21	42.0±1.42	43.1±1.77	42.7±1.50
Friability (% w/w)	0.81±0.07	0.75±0.10	0.69±0.09	0.65±0.06	0.64±0.04	0.70±0.07
Disintegration (sec)	96.12±1.3	93.68±1.2	99.21±1.3	49.87±1.4	44.82±1.0	52.36±1.2
Levofloxacin content (%)	101±0.89	99±1.2	99±1.8	98±2.13	98±1.21	101±0.91
Pyrazinamide content (%)	99±1.05	98±2.01	102±0.66	97±1.14	99±1.11	101±1.01

* All values are presented as mean ± SD

5.4.3 Quality by design

As detailed in Chapter one and highlighted again in this chapter, the QbD method gathers data already known about components and processes of manufacturing and establishes the most suitable pathway to produce a quality product. After the QTPP was defined and the cQAs identified, characteristics and parameters of the desired drug product were set. This enabled prediction of possible problems that could potentially upset the acceptable limits, hence quality risk assessment, as well as the FMEA tool were utilised. The RPN then ranks the urgency for corrective action to avoid or minimise potential failure mode.

Disintegration time due to the disintegrant used and its percentage composition (% w/w) in formulation as well as blending time after addition of magnesium stearate were the potential failure modes with RPN higher above 100 (Table 5.7), and therefore called for further investigations, which were accomplished through DoE. All the other modes were within the acceptable levels. The extent of impact of these variables were investigated in the subsequent step of QbD in the next chapter.

5.5 CONCLUSIONS

Following a literature review, a suitable formulation design was developed, with six different formulae from which six batches of the dispersible tablets were successfully manufactured using direct compression method. Physical assessment of the tablets showed that the rate of disintegration improved with use of 8% w/w of any of the disintegrants compared to the 5% w/w composition, an indication that varying the quantity of disintegrants has an impact on the rate of disintegration.

The variation test for all the batches was found to be within the ±5 limit as recommended by the USP for uncoated tablets weighing more than 250 mg. In addition, the batches passed the drug content assay test for both drug substances, evidence that the blending time was adequate (Poux*, et al.*, 1991). The batch containing 5% w/w CSP (K90) displayed the slowest disintegration profile while the batch formulated with 8% w/w of SSG showed the best performance from all the formulations. Attributes that potentially affect quality of the dosage form were identified and controlled using QbD. These variables will be used to pool data that will facilitate optimisation of conditions to manufacture an improved version of the drug product in the next chapter.

CHAPTER SIX

OPTIMISATION OF A DISPERSIBLE TABLET FORMULATION

6.1 INTRODUCTION

6.1.1 Optimisation

The term optimisation defines improving the performance of an existing process or system so that it can deliver the maximum possible value. In pharmaceutical product development relevance, optimisation refers to discovering the most suitable conditions or proportions of excipients that allow a procedure or formulation to produce the best response (Araujo and Brereton, 1996). The conventional optimisation techniques monitored the influence of a single variable at a time while all the other parameters were kept constant. As observed by Lundstedt (1998) and colleagues, the consequent drawback of the method is that the entire effects of the factor on the response are not shown. The other downside includes the use of an increased number of resources as more experiments must be conducted.

The use of multivariate statistical techniques have been employed to overcome the problems of conventional methods of pharmaceutical formulation optimisation. Among the applicable multivariate methods used in analytical optimisation is a tool known as statistical design of experiment (DoE) or experimental design (Tye, 2004). In DoE, experiments are drawn up in a matrix manner and used to predict the possible coefficients in an empirical mathematical model. The model subsequently predicts the output of the formulation within limits (Lewis, 2007). Together with other statistical and mathematical models, DoE is applied in a technique termed response surface methodology (RSM), that maps the next experimentation phase (Tye, 2004).

6.1.2 Response surface methodology

Researchers agree that RSM is a set of carefully selected statistical strategies coupled with mathematical techniques that are used to simultaneously establish a practical relationship between various independent variables and a response from an experimental design (Bezerra*, et al.*, 2008; Khuri and Mukhopadhyay, 2010). In their publication on the experimental attainment of optimum conditions, Box and Wilson

(1951) outline the details of their development of the RSM technique. The experimental methodology creates a mathematical model that can be represented graphically by a three-dimensional (3D) image or contour plot. The relationship between the control (independent) variables and the (dependent) response is expressed as shown by Equation 6.1 (Baş and Boyacı, 2007).

$$y = f(x_1\ ,\ x_2 \ldots, x_n) + \varepsilon \qquad \textbf{Equation 6.1}$$

Where,

y = Response

$x_1\ ,\ x_2 \ldots, x_n$ = Control Variables

n = Number of control variables

ε = Error observed in the response

As per recommendations by Khuri (2017), a RSM study should always start with specifying the problem and planning the experimental variables. The study can be accomplished by following the steps outlined below.

i. Setting up the selected experimental design to adequately approximate response values for given control variables.
ii. Determining the significance of data obtained from the selected design through fitting the hypothesised empirical model.
iii. Establishing the optimal composition of the model's control variables that produce the most favourable response.

6.2 EXPERIMENTAL

6.2.1 Proposed evaluation design

After the potential high-risk factors that affect the cQAs had been identified, an experimental design had to be performed to determine the significance of the effects of these risks. The risk assessment segment of QbD, coupled with literature search, pre-formulation studies and preliminary tablet formulations completed in the previous chapter identified the major parameters that have important influence on cQAs. These were found to be blending time, in addition to disintegrant quantity in the formulation.

The aim of experimental screening phase is to discern the extent that each of the factors have on the overall formulation performance in respect to the cQAs. Due to the disintegration performance of the batch (B5) formulated using formula (F5) as displayed in Table 5.2, the formula was adopted and employed to work as a basic reference. The disintegrant quantity in the formulation as well as blending time after addition of magnesium stearate were varied. Table 6.1 shows the basic formula for experimental batches.

Table 6.1: The basic formula of the experimental batches

Excipients	Quantity
Levofloxacin	150 mg
Pyrazinamide	300 mg
Sodium starch glycolate (SSG)	5-8%
Colloidal silicon dioxide (CSD)	15 mg
MCC (Avicel® PH-102)	q.s.
Mg Stearate	5 mg
Total	900 mg

6.2.2 Materials and equipment

The materials and equipment described in Sections 5.2.1 and 5.2.2 respectively were the same as utilised during the optimisation phase of this study.

6.2.3 Design of Experiments

6.2.3.1 Central composite design (CCD)

The initial protocol of optimising dispersible tablets formulation involved experimental design which was accomplished by screening the batches using response surface method in a nature of central composite design. The CCD with three levels and two factors was selected as the method of choice and applied to in the optimisation of parameters of manufacturing the dispersible tablets.

The CCD was described by Box and Wilson and the design can be represented by a cube with different marking points as exhibited in Figure 6.1, where (o) is the center

point, (●) is factorial design and (×) denotes axial or star points (Lundstedt*, et al.*, 1998). There are six axial or star points that lie in the center of each face of the cube which represent experimental runs and when projected, they cross each other at the center point of the cube.

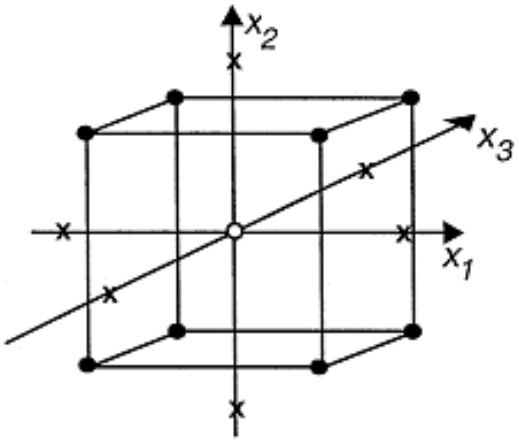


Figure 6.1: Basic central composite design with three variables (Adapted: Lundstedt, *et al*., 1998)

The independent factors are investigated at five levels which are signified by -α, -1, 0, +1 and +α coded values. The space that separates star points from the center point of a uniformly routable CCD is determined by the number of variable factors and may be calculated using Equation 6.2 (Bezerra*, et al.*, 2008).

$$a = \frac{k}{2^4} \qquad \textbf{Equation 6.2}$$

Where,

α = axial point

k = factor number

Several experiments are required for the design to be successful, and the number of experiments can be determined by Equation 6.3. The experiment number provides an indication of the resources required to produce adequate data for the study.

$$\mathrm{N} = k^2 + 2k + \mathrm{C}_0 \qquad \textbf{Equation 6.3}$$

Where,

N = experiment number

k = factor number

C_0 = replicate number of the central point

The advantages of CCD include the fact that the system efficiently generates adequate data on effects of experimental variables from a minimum number of runs, even when the experimental plan involves only two variables. Other designs such as Box-Behnken require three or more input variables. There is a good range of CCD forms which permits a flexible choice of different types of experimental conditions. The design also allows for sequential analysis, where the first set analyses two-factor and linear interaction effects, followed by estimation of curvature effects. Should the initial set of experiments indicate insignificant curvature effects, the subsequent runs need not be carried out (Montgomery, 2013).

In a study where CCD was eventually ranked as the best method of choice for accomplishing DoE within that study, CCD was found to have the ability to combine more extreme factors and include borderline regions compared to other designs (Rakic´*, et al.*, 2014). Drawbacks of CCD were revealed in another study that was conducted to compare CCD and Taguchi method in an optimisation method. The study concluded that CCD method required more experimental runs, showed low efficiency in the product manufactured under the suggested conditions and quantification of contribution of some factors was not possible (Asghar*, et al.*, 2014).

6.2.3.2 Central composite design model

The set-up of experiments and evaluation of effects of the variable factors and responses were performed using RSM based on CCD with center and axial points to fit quadratic models. This was achieved with the aid of a statistical data analysis software, Design-Expert® version 7.0.0 software (Stat-Ease Inc., Minneapolis, USA). According to risk assessment of QbD, the most important parameters that affect disintegration time of the dispersible tablets are disintegrant quantity in the formulation and blending time after addition of a glidant. These factors are the control variables to be investigated and optimised as shown in Table 6.2. The table also reveals the levels within which the factors were investigated.

Table 6.2: High-risk factors and levels for experimental variables

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded	Mean	Std. Dev.
A (x_1)	Disintegrant Quantity	% w/w	Numeric	5.00	8.00	-1.000	1.000	6.500	1.177
B (x_2)	Blending Time	Minutes	Numeric	3.00	5.00	-1.000	1.000	4.000	0.784

The design produced 13 experimental runs, five of which were the replicates at the central point, according to Equation 6.3.

Table 6.3: Experimental design matrix with observed disintegration time for dispersible tablets formulations using central composite design

		Independent variables		Disintegration Time (y)
Experiment	Sequence	x_1 (%w/w)	x_2 (mins)	Observed Values (sec)
2	1	8.00	3.00	40.12
4	2	8.00	5.00	44.34
12	3	6.50	4.00	43.01
6	4	8.62	4.00	51.09
7	5	6.50	2.59	52.76
10	6	6.50	4.00	43.43
9	7	6.50	4.00	42.91
1	8	5.00	3.00	88.23
11	9	6.50	4.00	44.00
5	10	4.38	4.00	95.21
13	11	6.50	4.00	43.73
8	12	6.50	5.41	35.81
3	13	5.00	5.00	60.55

Replicates were included to enhance the statistical power of the design. The series of experimental runs was generated in a randomized order as a list of variable parameter combinations and the response of the actual runs according to Table 6.3.

6.2.3.3 Evaluation of model

The significance of the RSM quadratic model and its terms were evaluated through analysis of design matrix that showed the model residuals as well as the predicted standard errors of the model terms. The values were all generated using Design-Expert® version 7.0.0 software (Stat-Ease Inc., Minneapolis, USA). The degrees of freedom for lack of fit and pure error are three and four respectively and these are indicative of a valid lack of fit test.

The standard errors for all the model terms were found to be less than one and therefore acceptable. Variance inflation factors (VIFs) were also close to one, the ideal value in all the types of coefficients, indicating that coefficients are well estimated and the estimated values are not inflated by multicollinearity (Katrutsa and Strijov, 2017). The factors did not correlate to each as proved by the Ri^2 which is also close to the ideal value which is zero. This set of evidence summarised in Table 6.4 depicts that the RSM quadratic design model for optimisation of the dispersible tablets fits the data and may be used for this purpose.

Table 6.4: Design matrix evaluation for RSM quadratic model for the optimisation of dispersible tablets formulation

Terms	Std. Error	VIF	Ri^2
A	0.35	1.00	0.0000
B	0.35	1.00	0.0000
AB	0.50	1.00	0.0000
A^2	0.38	1.02	0.0170
B^2	0.38	1.02	0.0170

In addition to the design matrix, the standard error of the design was depicted using a fraction of design space (FDS) and a three-dimensional (3D) plot in Figure 6.2 and 6.23 respectively.

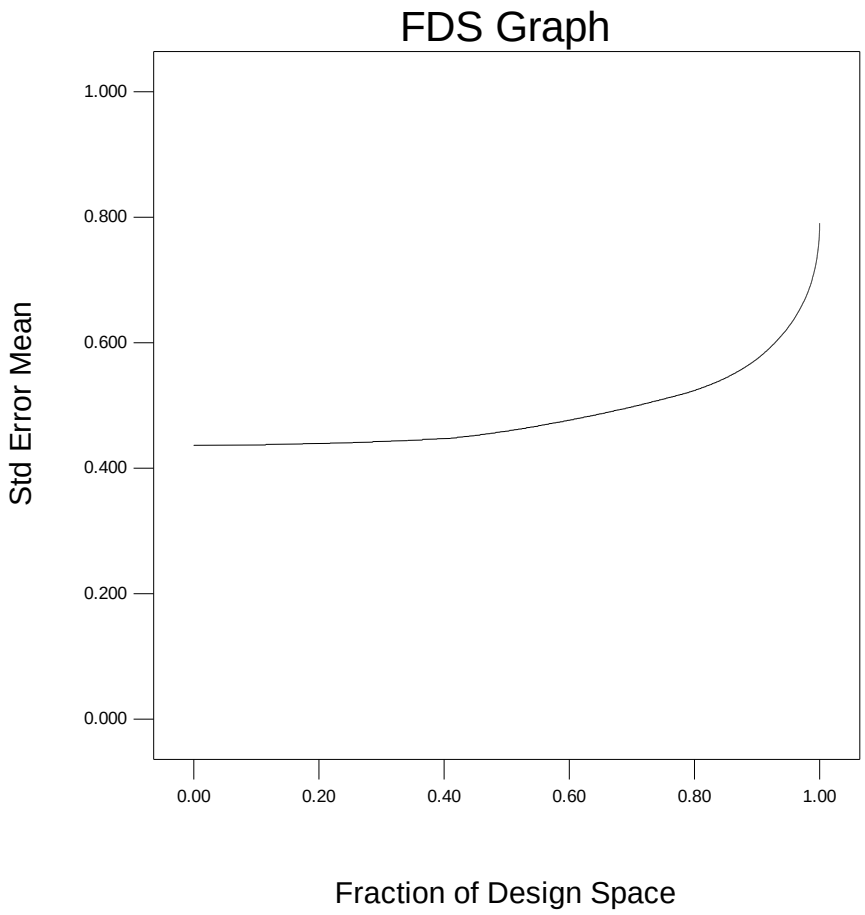


Figure 6.2: FDS plot for the RSM quadratic model showing the standard error within most of the design space

Evaluation of the model using FDS was conducted to determine error in prediction within a specific volume of the design space. The FDS graph displays low error in predictions of the model where high degree of linearity exists. There is however slight rise in prediction error at higher design space fractions, and this is expected (Zahran*, et al.*, 2003).

The 3D plot of standard error shown in Figure 6.3 shows that all design space points with equal distance from the center point also have equal variance. Apart from an indication of rotatability of the design, is this is also evidence of linearity of the larger part of the design space and low errors in value predictions. However, the edges of the plot are slightly elevated above the surface, another sign that at higher fractions of the design space, insignificant increase in error values may occur. The graph, in

collaboration with the outcomes of design matrix, support the fitness of the method in optimisation of levofloxacin-pyrazinamide dispersible tablets.

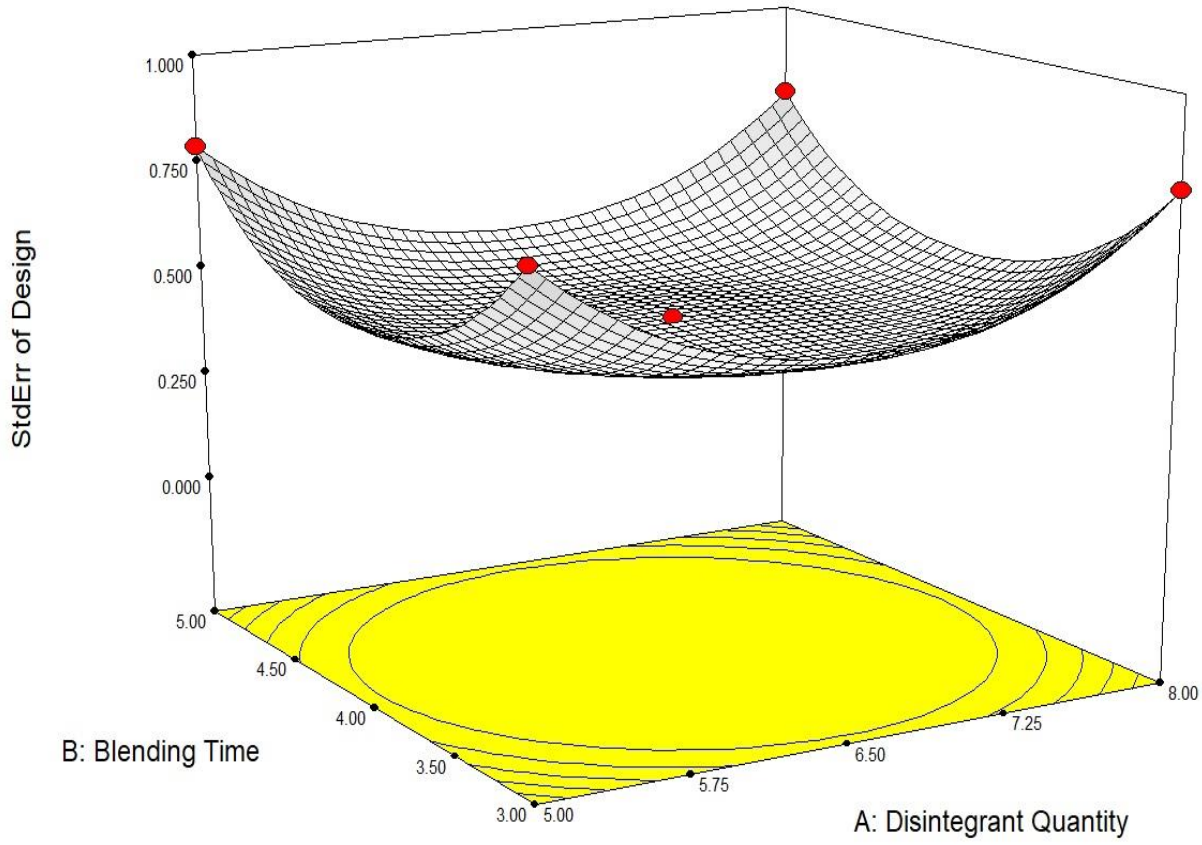


Figure 6.3: 3D contour plot indicating predicted standard error of the design space for RSM for the optimisation of dispersible tablets formulation

6.2.3.4 Effect on disintegration time response (cQA)

The effects of blending time and disintegrant quantity on disintegration time model were analysed using analysis of variance (ANOVA) partial sum of squares - type III, with the aid of statistical software package, Design Expert® version 7.0.0, where the level of significance was set at 5%. A quadratic full model procedure was used in fitting the data into the different possible prediction equations. The probability value (Prob > F) for each source of terms were examined to determine whether the value falls below 5% significance level to validate statistical significance of the model term. The ANOVA for the disintegration time data captured in the RSM is given in Table 6.5 below.

Table 6.5: Results of ANOVA for the RSM quadratic model for effects on the cQA

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Value	p-Value Prob > F	Remark
Model	4110.01	5	822.05	2284.50	< 0.0001	Significant
A	1991.61	1	1991.61	5534.72	< 0.0001	Significant
B	288.50	1	288.50	801.75	< 0.0001	Significant
AB	256.00	1	256.00	711.43	< 0.0001	Significant
A^2	1552.20	1	1552.200	4313.61	< 0.0001	Significant
B^2	0.24	1	0.24	0.68	0.4369	Insignificant
Lack of fit	052	3	0.17	0.35	0.7952	Insignificant

The Prob > F-value of < 0.0001 denotes that the model terms are significant, with 0.01% of the value occurring due to noise. In this instance, all model terms except B^2 are significant. According to the experimental runs represented in Table 6.3, all the formulation batches met the defined pharmacopoeial disintegration time limit of dispersible tablets (European Pharmacopoeia, 2013). The batch of tablets using a formula which included 5.50 % w/w of SSG and was mixed for 5.41 minutes disintegrated within 35 seconds while another batch containing the same amount of SSG but was mixed for 4 minutes disintegrated in 43 seconds. On the other hand, a batch that contained 4.38 % w/w SSG and was mixed for 4 minutes took the longest to disintegrate, 95 seconds (Table 6.3). The blending time and disintegrant quantity were therefore found to be, in fact, of significance in the outcome of disintegration time of the dispersible tablets.

In the full model, B^2 is not part of the fundamental terms that form the support structure of the model hierarchy, hence, it does not make any substantial statistical value to omit the term or adopt a reduced model. The adjusted R^2-value and the predicted value were 0.9885 and 0.9990 respectively for the full quadratic model. Reducing the model by backward elimination procedure by excluding B^2 resulted in the same adjusted R^2-value, the predicted R^2-value is 0.0002 less than that of a full model. This difference is insignificantly small and may be disregarded. Ultimately, the model predicted R^2-value of 0.9883 remains in reasonable harmony with the adjusted value, since the difference is less than 0.2.

The generated lack of fit model F-value of 0.35 confirms that the lack of fit is negligible relative to the pure error of the model, a validation that the model fits the supplied data. Moreover, 79.52% chance exists that the lack of fit F-value is a consequence of noise. The empirical second order polynomial equation was developed to adequately illustrate the relationship of the independent factors in influencing the response which is shown as Equation 6.4.

$$y = 557.55 - 118.16x_1 - 42.17x_2 + 5.33x_1x_2 + 6.64x_1 + 0.19x_2^2 \quad \textbf{Equation 6.4}$$

In judging the diagnostic details of the design, the normality of the distribution of the residuals of the data was investigated through a normal probability plot of the studentised residuals as shown in Figure 6.4.

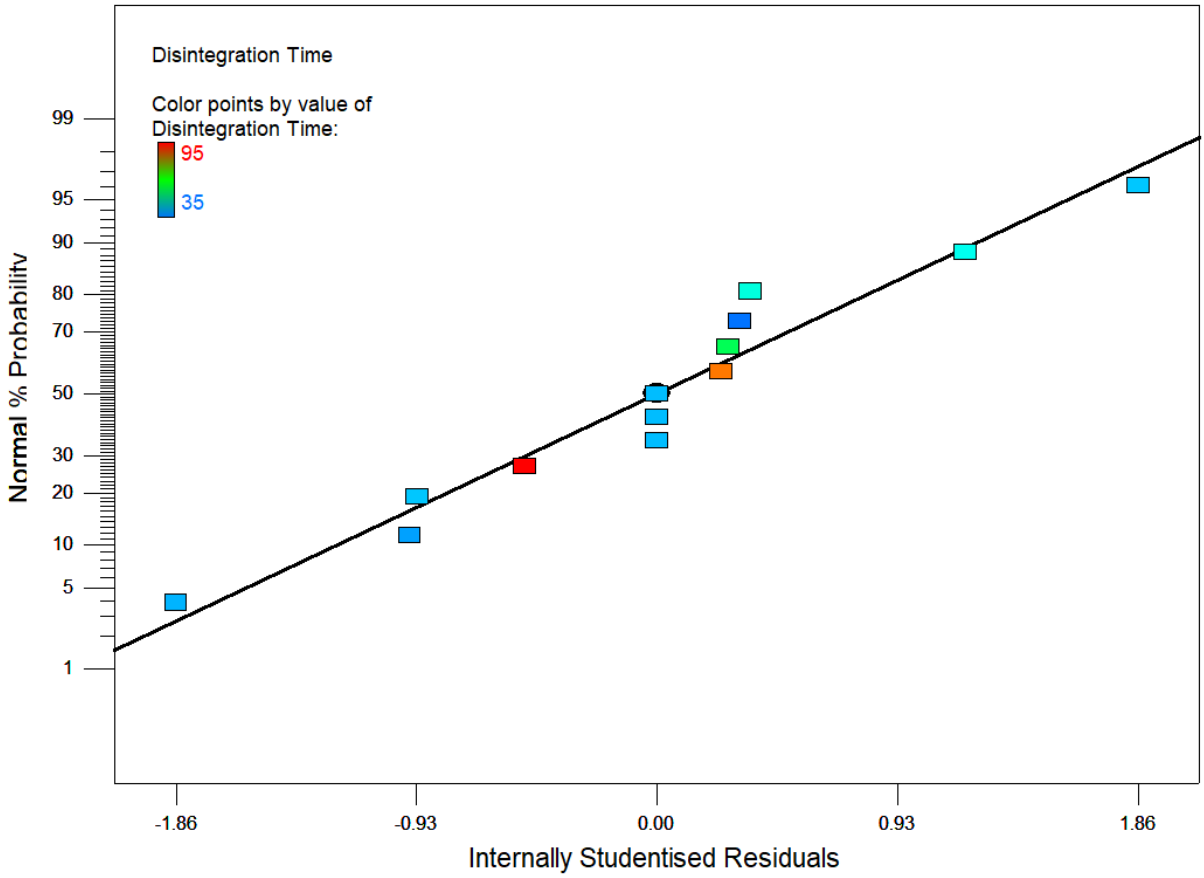


Figure 6.4: Normal probability plot of the residuals for disintegration time

The graph shows a fair distribution on either side of the plot line, a prediction that the residual points are approximately linear, demonstrating normality in the error term of the model. Although the residuals do not strictly fall within the straight line, they

however do not show trending towards one side of the graph. This implies that there might be other uncontrolled external factors that may be changing in the experiments and are influencing the response. Nevertheless, these factors appear to have negligible impact on the overall trend of the response.

Furthermore, predicted values of response weighed against actual experimental response values were used to determine suitability of the proposed model to make estimations within the design space. Figure 6.5 shows the predicted against actual data obtained from the experiments to check for constant error. A near-linear graph was produced as evidence that the disintegration time that was observed from experimental runs was in a reasonably descent range from that predicted by the model. This suggests that the model is an acceptable prediction tool in manufacturing dispersible tablets using the materials and conditions set in this study.

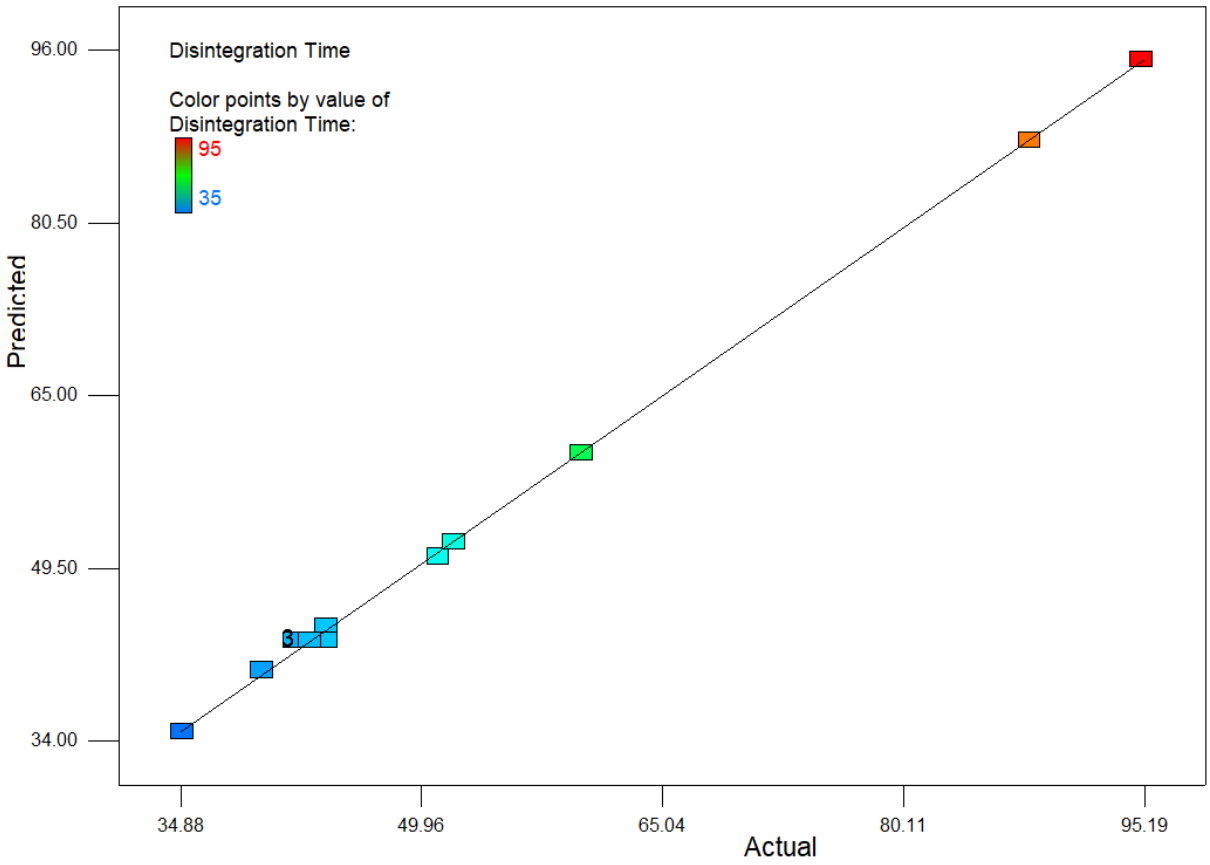


Figure 6.5: Predicted versus actual value plot for disintegration time

A plot of externally studentised residuals was generated to check the consistence of each run, in relation to other runs - a way to detect outliers. Outliers are observations that appear to distinctly deviate from the rest of the sample members due to different causes factors (Grubbs, 1969). Shalabh (2009) reports that using externally studentised residuals instead of other types of residuals increases the sensitivity in detecting outliers. As observed in Figure 6.6, all the response values are found within the 95% confidence interval and satisfy the three-sigma rule, with no outliers. The 13 runs all show externally studentised residual values within the calculated limits of -4.56 and +4.56.

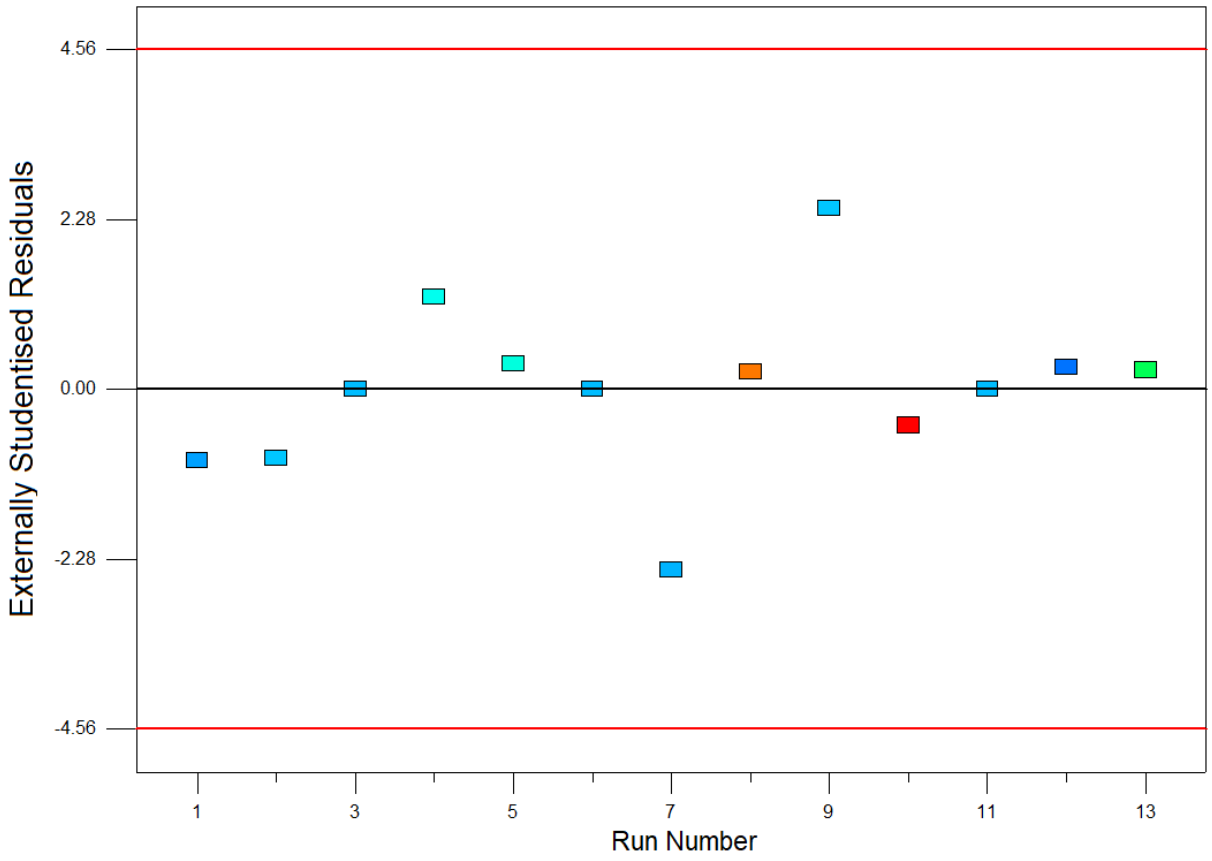


Figure 6.6: Externally studentised residual plot for disintegration time response

As mentioned earlier, both disintegrant quantity and blending time factors have significant influence in determining the disintegration time. The response values will vary depending on the different levels of these two variables. An interaction exists as shown by the interaction graph in Figure 6.7. The two non-parallel lines intersect, indicating that the effect of one factor is dependent on the level of the other.

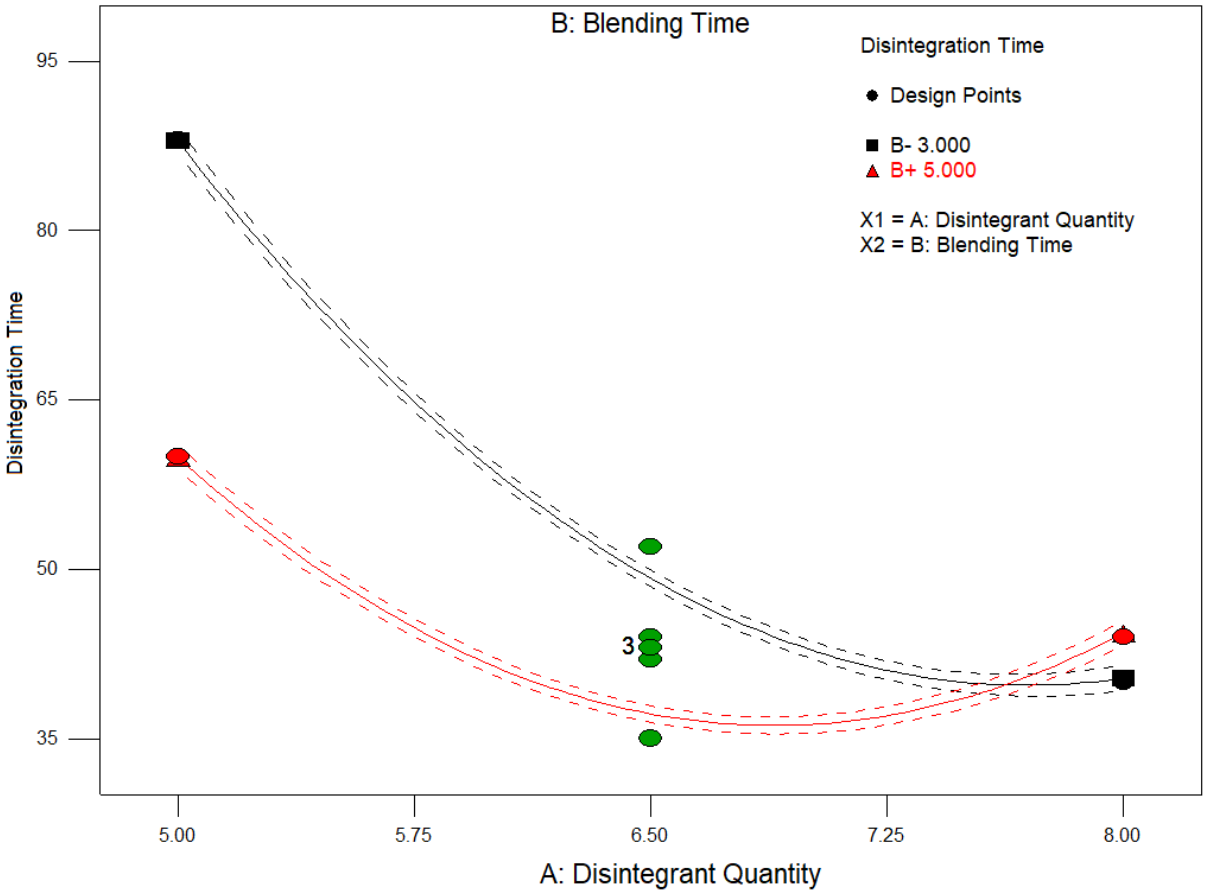


Figure 6.7: Interaction graph of effect of independent variables on response

A rotatable 3D representation of the design space displaying how the disintegration time varies as a function of SSG quantity and powder mixing time is depicted in Figure 6.8. The response surface plot exhibits the evidence that both the input factors influence the product cQAs. The lowest point of the response curve represents the most favourable response where the least disintegration time lies. Although not as proficient in determining response values and coordinates as the contour plot, the rotating and tilting ability of the 3D graph allows for the estimation of lowest point on the response surface. In this instance, it appears to lie within a design space between 6.50 - 7.25 and 4.50 - 5.00 of A and B axes values respectively. The 3D surface offers a full perspective of the response in relation to the factors involved.

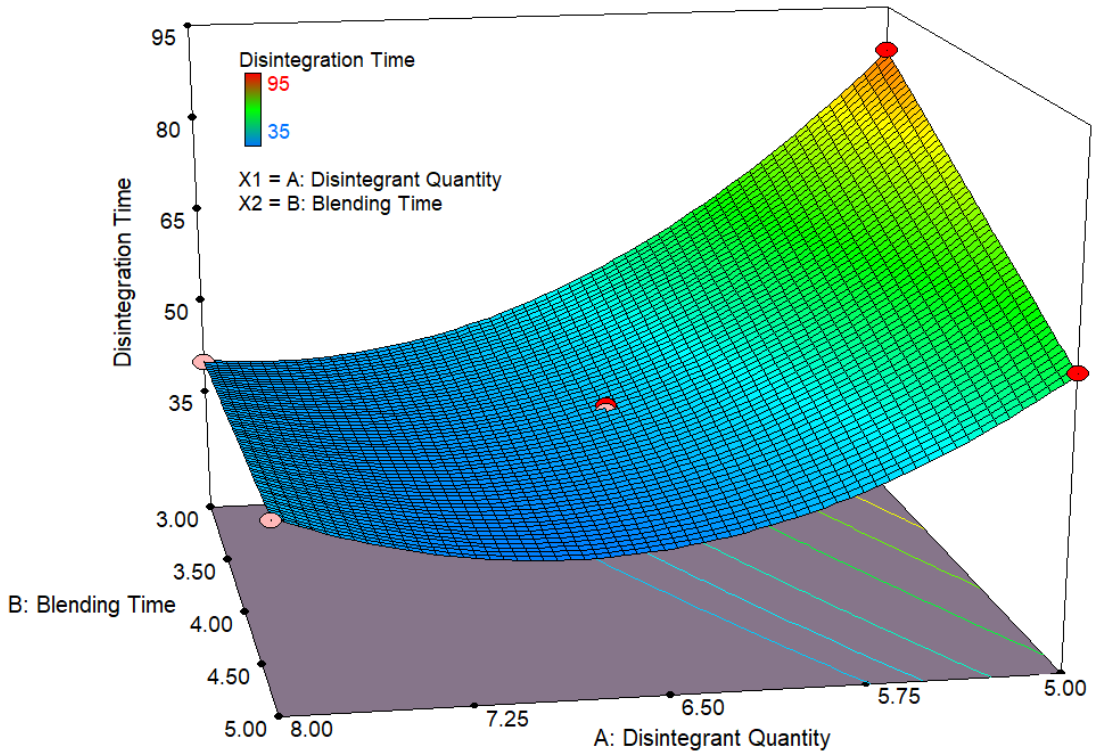


Figure 6.8: A 3D display of the response surface of disintegration as a function of disintegrant quantity and blending time

6.2.4 Process optimisation

6.2.4.1 Statistical Optimisation

Optimisation of the manufacturing process was achieved following a numerical optimisation technique using desirability function approach, a method developed by Derringer and Suich (1980). Design-Expert® version 7.0.0 software (Stat-Ease Inc., Minneapolis, USA) was applied in the process as described by Myers and colleagues (2009).

The software interface was commanded to restrain variable factors within specific ranges and simultaneously minimise disintegration time. The aim of optimisation was to predict ideal conditions that will facilitate manufacture of a batch of dispersible tablets with lowest possible disintegration time within the given factor boundaries. Table 6.6 expresses the constraints of the optimisation variables. From the 100 computed optimisation factor combinations and predicted responses, the one with the highest desirability ranking is revealed in Table 6.7. The contour plots and 3D surface

images in Figure 6.9 and Figure 6.10 respectively provide a graphical target of desirability and response as a function of variable factors. The optimum values of response parameters are both given in Table 6.7, together with the input variables, and are conspicuously labeled in the subsequent figure.

Table 6.6: Constraints of the optimisation variables

Name	Goal	Lower Limit	Upper Limit	Importance
Disintegrant Quantity	is in range	5 %w/w	8 %w/w	3
Blending Time	is in range	3 mins	5 mins	3
Disintegration Time	minimize	35 secs	95 secs	5

Table 6.7: Predicted input variable values and corresponding response for the preparation of confirmatory batch of dispersible tablets

Disintegrant Quantity (%w/w)	Blending Time (mins)	Disintegration Time (secs)	Desirability
6.89	5.00	36.17	0.98

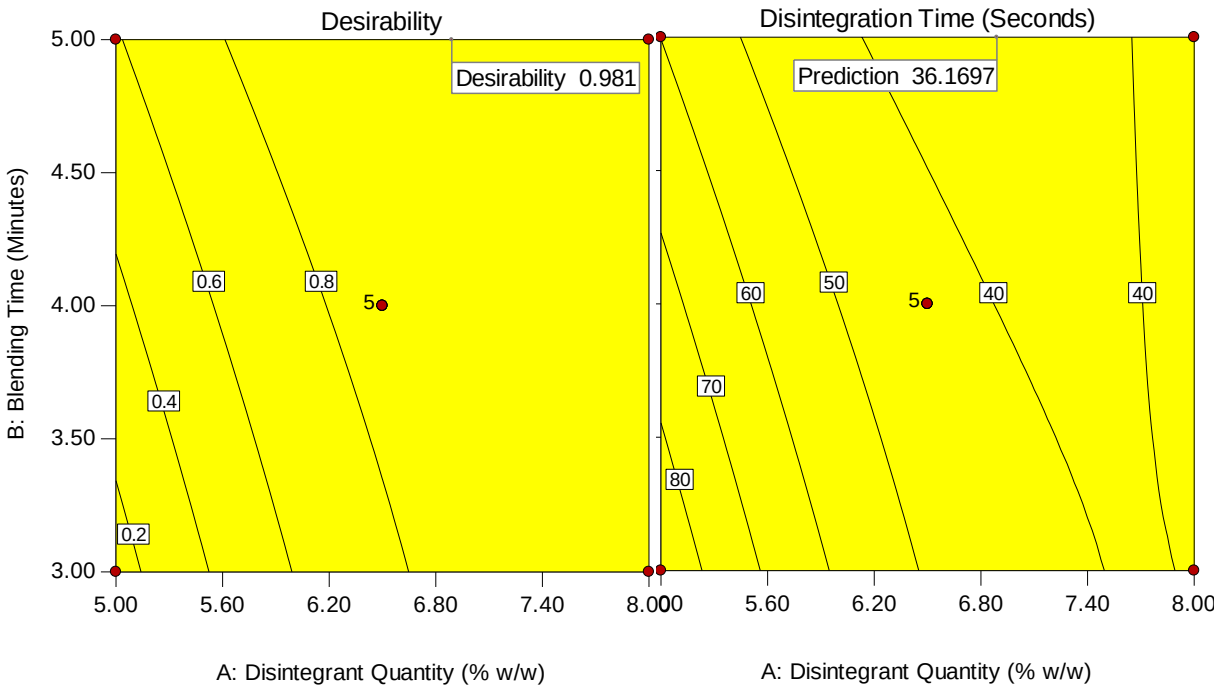


Figure 6.9: Contour plot depicting process optimisation for desirability and response as functions of variables

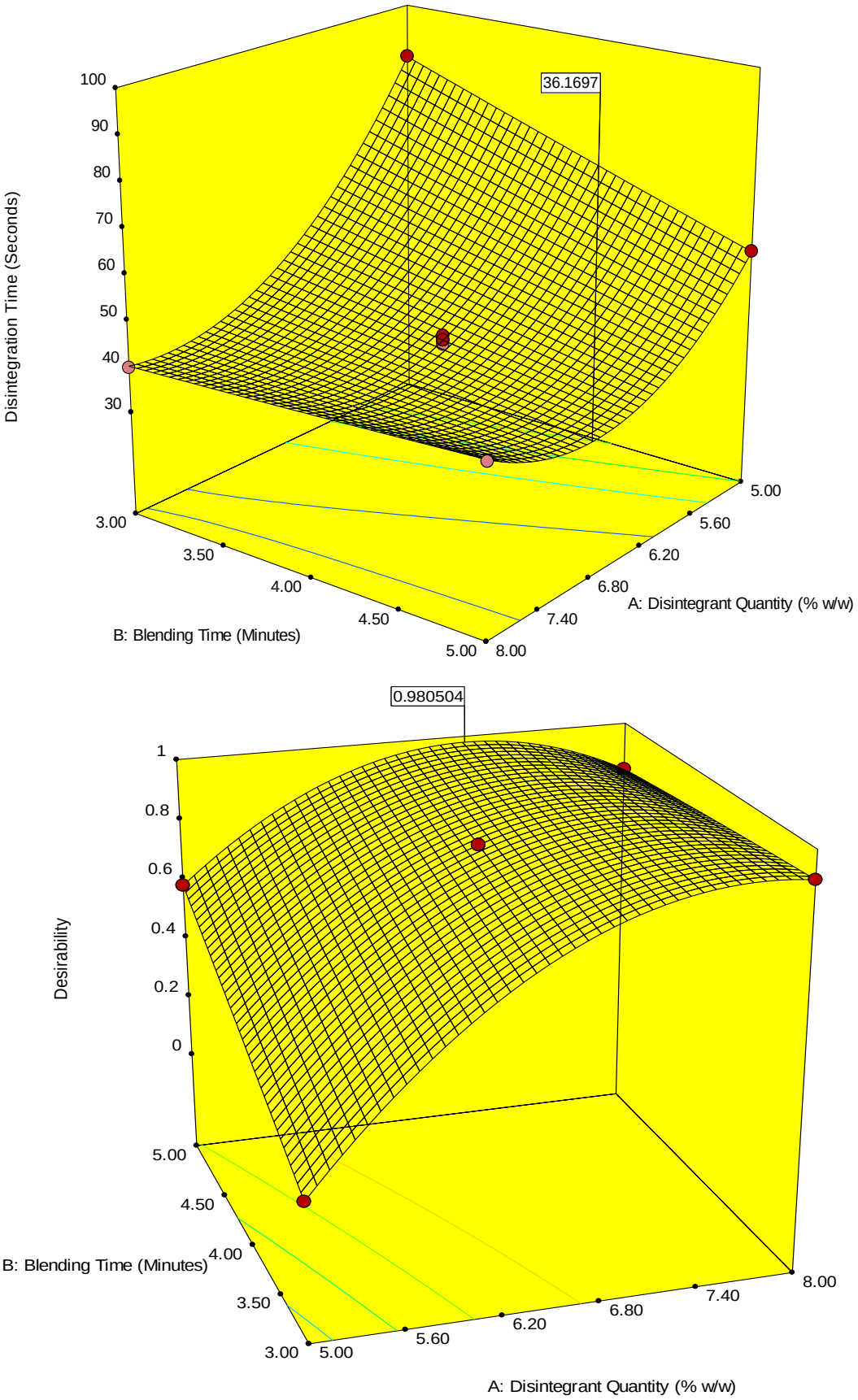


Figure 6.10: 3D surface for process optimisation for desirability and response

6.2.3.2 Analysis of confirmatory batch

Predicted input variable values from solution number 1 (Table 6.7) were used for the actual preparation of the optimised batch. The aim of the exercise was to manufacture a confirmatory batch of dispersible tablets using the optimised parameters. The actual measured response of the batch should agree with the hypothesised product cQAs values to affirm if the developed model is accurate and can be used for prediction of similar manufacturing settings.

The batch was manufactured using materials and equipment described in Sections 5.2.1 and 5.2.2 respectively, where SSG composed 6.89% w/w of the total mass in the formula, and the powder blend was mixed for five minutes after addition of magnesium stearate (Table 6.7). The dispersible tablets were manufactured and their cQA was assessed using the method and equipment described in Section 5.2.4.5. A confidence interval of 95% was used for the response. The mean (n=100) of the actual batches was compared to the predicted mean value as summarised in Table 6.8, and the prediction error was found to be 2.2% more that the predicted value. The actual mean value of 36.95 was also within the 95% predicted interval (PI) and closer to the higher PI limit. The percentage prediction error was calculated using Formula 6.5.

$$\% \text{ prediction error} = \frac{\text{Experimental Value - Predicted value}}{\text{Predicted Value}} \times 100 \quad \textbf{Equation 6.5}$$

Table 6.8: Summary of predicted and actual response of the hypothesised model at the optimised conditions

Response	Predicted Mean	95% PI low	n	Actual Mean	95% PI high	Std Dev
Disintegration Time (Secs)	36.17	35.40	100	36.95	37.00	0.599865

Physical characteristics of the resultant dosage form manufactured using optimised process conditions were assessed. The confirmatory batch of tablets comply with the set limits and the required compendial specifications. Although within the required boundaries, friability parameter, however, appears to have increased towards the upper USP recommended margin. Table 6.9 summarises the observed physical

parameters of the tablets prepared using the optimised formulation composition and verifies that the proposed RSM model holds an accurate estimation ability for the manufacturing settings stipulated above.

Table 6.9: Physical characteristics of dispersible tablets manufactured under optimised conditions

Parameters	Results
Weight variation (mg)	915.34 ± 4.28
Breaking Force (N)	38.9 ± 1.09
Friability (% w/w)	0.92 ± 0.59
Disintegration Time (secs)	36.95 ± 0.60
Levofloxacin content (%)	101 ± 1.44
Pyrazinamide content (%)	102 ± 1.91

6.3 CONTROL STRATEGY AND QUALITY RISK MITIGATION

Control strategy has been described in several ICH guidelines, and is summarised as system that involves a set of controls that spring from understanding product and its manufacturing process. The control strategy normally involves monitoring of material attributes, the design of the manufacturing process as well as the product (International Conference on Harmonisation, 2012). The International Conference on Harmonisation (2005) classifies control strategy and risk reduction as part of quality risk management, which is a methodical procedure for determining and evaluating risk of quality during the drug product lifecycle. Risk mitigation concentrates on avoidance or reduction of quality risk factors that may cause the product to fall outside acceptable limits. Control strategy involves improving detectability of quality risks.

As previously revealed by the FMEA analysis and the QbD, the sources of cQAs variability are the product quality risks. These risks were identified as input attributes such as disintegration quantity in the formulation as well as the length of time taken to mix the powder blends after the addition of magnesium stearate. These variables were found to have distinct effects on the disintegration time of the dispersible tablets and are therefore from the critical points of the design space. The FMEA analysis concept was exploited in drawing up the risk mitigation and control strategy. Table 6.10 is a

summarised extract from Table 5.7 in the previous chapter. It has been brought forward as a reference to show how the identified attributes contributed to the product quality risk before implementation of control strategy.

Table 6.10: Extract of FMEA analysis showing high quality risk factors

Attributes	Severity	Occurrence	Detection	RPN
% Disintegrant	8	7	3	168
Blending time	7	7	3	174

Disintegrant concentration has a significant impact on the disintegration time of tablets in both conventional and fast disintegrating tablets. This effect is widely exploited in dispersible tablets, hence the advent of superdisintegrants (Augsburger*, et al.*, 2007). The type and concentration of various types of disintegrant alter the manner of tablet swelling. One of the disintegrants whose disintegration ability is substantially influenced by its concentration is SSG (Rudnic*, et al.*, 1982).

A study that was conducted to determine the impact of disintegrants in tablet formulation discovered that disintegration time improved as disintegrant quantity increased, albeit to a certain concentration level (Marais*, et al.*, 2003). These findings are consistent with results of this study as evidenced by Figure 6.10. SSG generally has spherically shaped particles which accounts for its good flowability. On another hand, its disintegration ability is less negatively impacted by mixing with hydrophobic lubricant such as magnesium stearate (Lerk*, et al.*, 1982).

The preliminary formulation design outlined in Table 5.2 was used to determine the disintegrant that offered the most favourable performance and SSG was selected through the assessment of the outcomes. The significance of concentration was then demonstrated through the application of DoE. The range of concentration in combination with blending time has been determined through the establishment of design space. This knowledge lowers the potential effects and cause of product failure. The updated FMEA analysis of disintegrant type and concentration after implementing the control strategy is given in Table 6.11, and the contrast with the previous analysis shown in Figure 6.11.

The DoE helped to detect possible failure and mitigate the impact of product failure that could have occurred due to incorrect extent of lubrication. In running the preformulation batches, lubrication was timed to occur for three minutes. Although the batches passed the disintegration test, scrutinizing the effect of lubrication mixing time improved the rate of tablet disintegration. The degree of lubrication can be altered mainly in two ways by either changing the concentration or lubricant mixing time. The latter method was exploited in this study.

Prolonged lubricant blending time using magnesium stearate decreases tablet hardness and disintegration time due to a phenomenon known as surface coverage that results in decreased tablet inter-particular forces. It is therefore necessary to strike a balance between the detrimental and beneficial effects of magnesium stearate (Moreton, 2006). This fact is revealed by the apparent decrease in tablet hardness as exhibited by lower breaking force of 38.9 ± 1.09 N for tablets manufactured using the powder blend that was lubricated for five minutes, compared to the lowest breaking force of 42.0±1.42 N for tablets produced using three minutes lubricated powder blend (Batch 4).

It is noted that the two contrasted batches were manufactured also contained different concentrations of disintegrant. However, Adjei and colleagues (2017) report that disintegration concentration has little or no effect on tablet hardness. If this study can be verified, it can also be concluded that the increase in mixing time to five minutes from the initial three minutes caused the increase in friability seen in the batch of tablets manufactured under optimised conditions. Shah and Mlodozeniec (1977) express that such findings may supply some rationale for the challenges frequently encountered in the manufacturing process and drug product characteristics of scale-up of solid dosage forms.

The potential cause of product failure is reduced by identifying and monitoring the effects involved in change of lubricant mixing time. The DoE was used to establish the trend of impact of lubricant blending time. The FMEA analysis after implementing control strategy was recalculated and displayed in Table 6.11. The new value was contrasted with the previous analysis shown as in Figure 6.11.

Table 6.11: Updated FMEA analysis showing high-risk modes

Attributes	Severity	Occurrence	Detection	RPN
% Disintegrant	4	3	1	12
Blending time	5	2	2	20

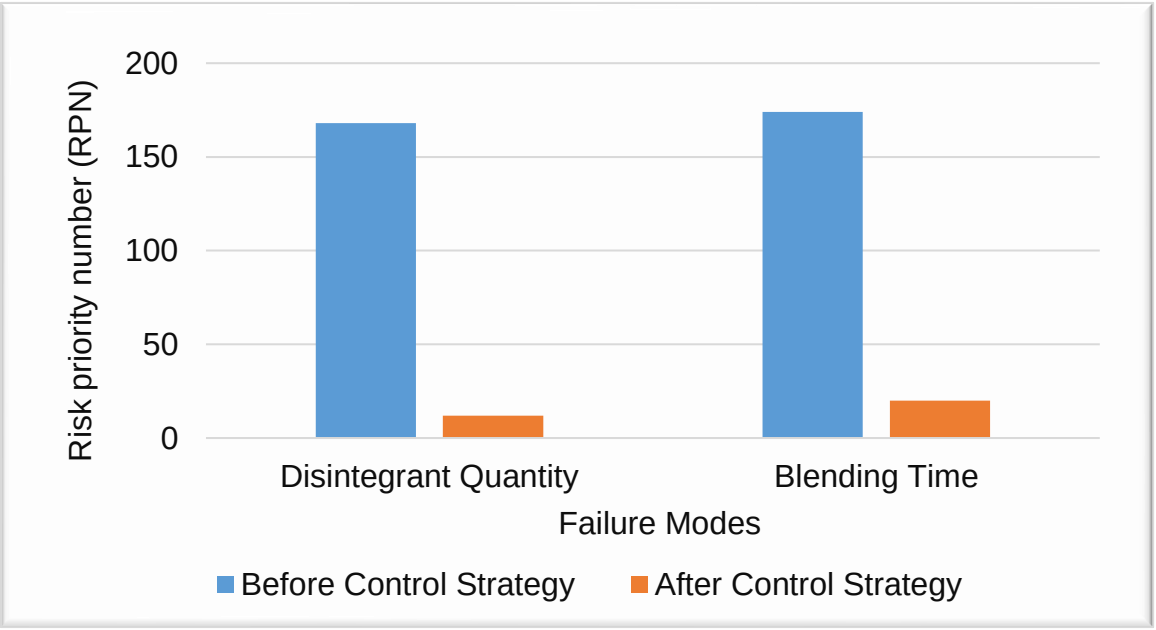


Figure 6.11: Comparison of RPN of failure modes pre- and post-implementation of control strategy

The FMEA analysis post control strategy implementation for the previously high-risk failure modes show relatively low RPN values, evidence that product risk management has been successfully applied. If scaling-up is required, the design space may need to be modified to suit the changes, and quality risk management should be performed using the technique described in ICH Q11 guidelines (2012) to promote continual quality refinement of product lifecycle. In-process quality control system should include ensuring that consistency in performance of the tableting machine instrumentation is maintained by routinely inspecting the instruments to confirm if the desired settings are in place. Tablets should be randomly assessed during processing to determine if they meet the described quality criteria.

During the process of identifying the product cQAs for the drug quality attributes in the previous chapter, tablet thickness and breaking force values were set to be measured using the batch that is manufactured within the optimised model and after implementing control strategy. Table 6.12 presents the update to the cQAs for the dispersible tablets recorded in Table 5.5.

Table 6.12: Updated cQAs of the dispersible tablets after implementing the control strategy

Quality Attributes	Target	cQA?	Justification
Thickness	3.91±0.23 mm	No	The dispersible tablet will be dissolved in water before swallowing, size is not a crucial factor
Breaking force	38.9 ± 1.09 N	No	This range allowed for acceptable disintegration time and friability

All parameters, including material attributes, process factors and product quality should be monitored for consistency as a risk mitigation strategy. These parameters should be maintained within design space limits and any deviations should be investigated promptly. Above all, all processes should be governed by the general rules of quality control outlined in the current good manufacturing practice guide (cGMP).

6.4 CONCLUSION

The formulation parameters were then optimised using RSM approach to offer a combination of input variable levels that simultaneously satisfy the response criteria within a defined range. The application and reliability of the data generated by this type of optimisation model lies in accuracy, precision and reproducibility of the experimental conditions. The technique is therefore highly sensitive to variability, and as such, has limited potential wide application.

The aims of the chapter were fully met. The relationship between cMAs (type and concentration of disintegrant), cPPs (disintegrant blending time) as well as cQA (disintegration time) and the extent to which they interact and influence each other for

the product to meet the QTPP was established using DoE within a RSM model. The use of SSG at 6.89 %w/w and blending the lubricant for five minutes yielded a batch of dispersible tablets that achieved complete disintegration in an average of 36.95 seconds, with physical characteristics summarised in Table 6.9. The value was within the range predicted by the model, hence it was acceptable.

The control strategies were implemented as described in ICH guidelines Q11 (2009) to ensure that the cQAs remains within specifications in subsequent model application. Control strategy involves planning the experimental process while applying the understanding of the material attributes, the process parameters as well as the factors that affect the finished product. Possible failure was detected though the outcomes of DoE.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

Over the past decade, there has been a significant rise in interest and research in paediatric TB, which has led to the development of novel drugs and drug combinations to treat TB and MDR-TB. Bedaquiline and delamanid are some of the novel anti-tuberculosis drugs that have been recently been approved for use in South Africa. Delamanid is especially meant for treatment of MDR-TB in children. There is, however, still a shortfall of adequate paediatric MDR-TB options, other than using these drugs at reduced doses (by breaking the dosage for, or otherwise) to deliver the drugs to children. The major challenge that both health care professionals and caregivers are faced with lies on the unavailability of paediatric-friendly dosage forms.

Levofloxacin and pyrazinamide are some of the long-standing drugs that have been effective in treatment of MDR-TB. This study sought to develop a FDC containing levofloxacin and pyrazinamide in the form of a dispersible tablet form for the treatment of MDR-TB in children below the age of eight years. The study followed a systematic approach where the physicochemical characteristics of the drug substances were examined through analytical techniques such as HPLC, IR spectroscopy and TGA. A framework of QbD was set as a guideline regulating the methodology of the research and the optimisation segment was accomplished with the aid of a RSM model. The physicochemical and pharmacokinetic properties of the APIs were explored as a foundation to define the QTPP of the product, which fulfills the concept of beginning with the end in mind, the pillar of QbD.

The HPLC method to simultaneously quantify levofloxacin and pyrazinamide was developed and validated in compliance with the guidelines outlined by Food and Drug Administration (1994) in agreement with the International Conference on Harmonisation (1995). The linearity, accuracy and precision of the method were found to be valid within a concentration range of 0.05 µg/ml and 0.10µg/ml for both drug compounds. The statistical % RSD, as well as % bias for the experimental analyses were maintained below 5% to facilitate confirmation of accuracy and precision of the HPLC method. All the forced degradation studies which included oxidative, hydrolytic

and photolytic degradation showed that the method had the ability to resolve between the analyte and decomposition products. The technique therefore proved to be stability-indicating under constant chromatographic conditions.

Preformulation studies were performed as a crucial step of building quality into a product. The characteristics of the APIs were investigated as individuals to identify their general behaviour and in the presence of each other, to determine the extent of potential interactions if they existed. This was achieved through the establishment of powder densities, IR spectroscopy and TGA. The micromeritic evaluation results showed that although the API powders did not show the best flowability, direct compression as a tableting method was feasible with the incorporation of MCC as a diluent. MCC possesses good flow properties and is often incorporated in dosage forms to promote mechanical strength. Thermal analyses of levofloxacin and pyrazinamide showed that both substances exhibited thermal stability at normal formulation temperatures. The DSC thermograms suggested that the substances may show potential interactions at higher temperatures, approximately above 160ºC.

Characteristics of excipients were studied further before the actual manufacturing formulation design was developed. As a test run, three batches containing different superdisintegrants consisting of 5% w/w of the total tablet weight were prepared (F1-F3), after which three more formulations containing 8% w/w of the disintegrants were prepared (F4-F6). Scaling up the concentration of the disintegrants was a method used to check whether higher disintegrant quantity would boost the disintegration time response as suggested in literature. The batch containing 8% w/w of SSG appeared to perform better than all the other batches in terms of disintegration rate and was therefore selected as a target for optimisation.

This preliminary batch offered a basis for the set-up of the QTPP for the formulation. The cQA was identified from the target QTPP as being the tablet disintegration time. This was achieved by determining the attributes of raw materials (cMAs) and processing factors (cPPs) that alter the cQAs to levels that compromise safety and efficacy of the drug product. A quality risk assessment method called Failure Mode and Effects Analysis (FMEA) effectively aided in ranking the potential failure mode according to priority. Quantity of disintegrant as well as lubricant blending time were

implicated as the possible failure modes that needed immediate attention compared to others (Davis*, et al.*, 2008).

Optimisation of the manufacturing process employed the RSM mathematical model where the central composite statistical design model with two factors and a single response was used to set up DoE for the formulation process. The technique first determined the relationship of cMAs, cPPs and the cQA in the formula and manipulated this relationship in creating a design space. The effects of SSG and lubricant blending time factors were found to overlap, which implies that they could not be manipulated as individuals to make a change on the response. A statistical analysis software generated contour and 3D rotatable surface response plots which displayed the effects of input variables on the response at varying levels.

The outcomes of the optimisation studies demonstrate that the disintegration time of the FDC dispersible tablet do heavily rely on the SSG concentration and the period taken to mix the lubricant with the powder blend. The best disintegration time achieved after optimising the processing conditions was 36.95 seconds, obtained by using 6.89% w/w of SSG and blending magnesium stearate for five minutes. The response values predicted by the model and the actual confirmatory experimental values were within proximity of the 5% acceptable error, an indication that the model was accurate and reliable. A control strategy to mitigate future product quality risks were put in place and involve a rich background knowledge of the product. The plan also involves a continuous assessment of risk modes from material attributes, processing and product properties.

Future experimental analysis that would be vital to conduct would comprise accelerated stability-indicating tests following the guidelines stipulated by Food and Drug Administration (2003). The results of such a study would reveal possible long-term compatibilities or interactions between the APIs as well as API and excipients, where existence of interactions will lead to development of alternative or advanced methods of formulation that would enable compatibility. DoE would investigate more parameters such as the impact of concentration of lubricant, mixing time of lubricant beyond five minutes in addition to effect of the rotation rate of cube mixer during powder blending phase and compression force. Moreover, it would be of interest to

explore the disintegration behaviour of the FDC formulated using the novel commercial premixed and ready-for-use excipient matrix.

Recommendations for this study would be to further investigate and formulate paediatric-friendly dosage forms using various drugs that are already approved for the treatment of MDR-TB. It is encouraged that various combinations of two or more of these drugs be formulated into FDCs. This would further ameliorate the pill burden that is currently faced nationwide. Is also recommended that the dosage forms be furnished in a range of strengths to provide for all the age groups of children.

In conclusion, FDC dispersible tablets containing 150 mg of levofloxacin and 300 mg of pyrazinamide for treatment of MDR-TB in children were formulated, manufactured and evaluated. The aim of the study was therefore fulfilled by meeting all the objectives that were set in the beginning of the study. The tablets passed all the relevant quality criteria that governed the scope of this study. However, potential limitations of the study include the fact that laboratory scale equipment such as the mixer and tablet press were used, and they may not be representation of the conditions in industry.

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