Development of a solid oral dosage form containing *Artemisia afra* extract

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ABSTRACT

Artemisia afra is a medicinal plant traditionally used in the form of a tea infusion. The process of preparing an infusion is time-consuming with potential variation in phytochemical compounds as well as poor stability after extraction. *Artemisia afra* is a popular medicinal plant, however, it is not yet available in a properly designed solid oral dosage form. A product in a solid oral dosage form containing *A. afra* will be beneficial, especially in view of the poor organoleptic properties of the water-based infusion or tea. By employing a scientific formulation approach such as the SeDeM Expert Diagram System (SeDeM EDS), the formulation time can be shortened to identify an optimised powder formulation for direct compression of tablets. In this study, a solid oral dosage form containing *A. afra* extract was formulated.

Artemisia afra was chemically characterised, and four phytochemical markers were identified to be quantified using a validated high-performance liquid chromatography (HPLC) analytical method. The method was applied to quantify the four selected *A. afra* phytochemical markers (selected based on peak heights) in preparations or powders containing *A. afra* with respect to morin hydrate equivalent values. The HPLC analytical method, using morin hydrate as an internal standard, was validated with regards to accuracy, precision, linearity, specificity, limit of detection and limit of quantification. Morin hydrate was added to all the *A. afra* samples to quantify each of the selected phytochemical marker molecules as milligram morin hydrate equivalents per gram of dry extract weight (mg MHE/g).

Aqueous *A. afra* extracts were prepared at four temperatures (25ºC, 50ºC, 70ºC, and 96ºC). The HPLC method was applied to determine the amount of mg MHE/g for the four selected phytochemical markers. Frozen *A. afra* extracts were freeze-dried to determine the dry extract powder yields. Results showed that extracts prepared at 96ºC yielded the highest dry powder extract and produced the highest amount of mg MHE/g for the four phytochemical marker molecules. Bulk aqueous *A. afra* extracts were subsequently prepared at 96ºC. Bulk *A. afra* frozen extracts were freeze-dried and the dry powder extract was used in combination with excipients to formulate a solid oral dosage form. Furthermore, extracts prepared from *A. afra* plant material, each derived from a different location, were compared with regards to phytochemical composition and dry extract powder yield. Variances in phytochemical composition between *A. afra* plants from different regions were observed, and the dry powder yields differed slightly.

The SeDeM EDS was employed to develop a directly compressible tablet containing *A. afra* extract. First, the powder flow properties of the dried *A. afra* extract powder were characterised using the SeDeM EDS. The values of 12 powder flow parameters were calculated and grouped

into five relevant SeDeM incidences, after which a polygon was drafted to obtain a graphical representation of the flow properties of the *A. afra* dry powder extract. Six excipients were also characterised using the SeDeM EDS, and corresponding polygons were constructed for each excipient. Based on excipient profiles, and the profile of the *A. afra* extract, tricalcium citrate was selected as the corrective excipient to compensate for the deficient properties of the *A. afra* extract. A small percentage of binder, lubricant and disintegrant was added to the tricalcium citrate (excipient to compensate for the deficient flow properties of the *A. afra* powder extract) and was again characterised with SeDeM EDS. Finally, the ratio of *A. afra* dry extract to excipient mixture required to formulate a final powder mixture for tabletting was calculated.

The formulated tablet mass was calculated based on 200 mg of dry *A. afra* powder extract inside each tablet. The *A. afra* dry powder extract was mixed with the corrective excipient mixture and compressed into 12 mm diameter flat faced tablets weighing 667 mg each. Tablets were packed into 13 containers of 60 tablets each, ready for 12 weeks stability testing and evaluation in terms of an assay, weight variation, hardness, friability, disintegration, and dissolution behaviour. All tablet samples complied with the BP specifications for uniformity of weight, friability, and disintegration. Assay results showed that the tabletting process immediately impacted the mg MHE/g phytochemical marker molecules 2 – 4, as they lost more than 35% in mg MHE/g after direct compression. Accelerated stability conditions of 25°C/60% relative humidity resulted in a slight reduction in mg MHE/g for phytochemical marker molecule 1 after 12 weeks, however a noticeable decrease in mg MHE/g was observed for phytochemical marker molecules 2 – 4 after 12 weeks. All four phytochemical marker molecules showed a more significant decrease in mg MHE/g at accelerated stability conditions of 40°C/75% relative humidity. Dissolution results showed that an increase in tablet hardness led to a reduced dissolution rate, and the reduction in MHE/g after the tabletting process shown by the assay results led to a maximum dissolution percentage of approximately 65% for phytochemical markers 2 – 3, and 48% for phytochemical marker 4. Tabletting had less of an impact on phytochemical marker 1.

Keywords: *Artemisia afra*, Medicinal plant, Solid Oral Dosage Forms, SeDeM Expert Diagram System (SeDeM EDS), High-Pressure Liquid Chromatography (HPLC).

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CHAPTER 1

INTRODUCTION, RESEARCH PROBLEM STATEMENT, AIMS AND OBJECTIVES

1.1 Introduction

1.1.1 The medicinal plant *Artemisia afra*

The *Artemisia* genus consists of approximately 500 species located in different regions worldwide (Bora & Sharma, 2011). The most well-known species is *Artemisia annua*, a medicinal plant with an established medicinal use in China as an antimalarial agent, which can be attributed to the presence of the phytochemical artemisinin. It is also grown in certain African countries where it is used to treat malaria. As opposed to *A. annua*, the species *A. afra* does not contain artemisinin. Despite the lack of artemisinin, many cultures on the African continent use *A. afra* in conjunction with other treatments against malaria (Amponsah, 2013). *Artemisia afra* is mainly consumed as medicine in the form of a tea infusion, which has been traditionally used in South Africa to treat illnesses such as colds, influenza, and bilharzia (Viljoen *et al.*, 2006).

1.1.2 Formulation of tablets as a solid oral dosage form

1.1.2.1 Manufacturing tablets using direct compression vs wet granulation

Direct compression is a simple tablet manufacturing method that includes only two steps, namely powder mixing and compression. Wet granulation is when a granulating fluid is added to a dry powder mixture and the wet mass is forced through a sieve to produce wet granules. Direct compression is a tablet manufacturing technique with certain advantages compared to that of the wet granulation tablet manufacturing technique. Direct compression allows for excellent stability of the active pharmaceutical ingredient (API) due to the absence of water, preventing hydrolytic degradation, which may occur with wet granulation. Therefore, direct compression is the best option for preparing tablets that contain hygroscopic and heat-labile APIs (Jivraj *et al*., 2000).

1.1.3 SeDeM Expert Diagram System

Traditionally, trial and error methods have been used to formulate solid oral dosage forms where the type and quantity of excipients were typically selected based on observations and prior experience. The SeDeM Expert Diagram System (SeDeM EDS) is a tool based on Quality by Design (QbD) principles and was initially developed specifically to be used in the formulation of directly compressed tablets. The SeDeM EDS identifies the powder properties that need to be corrected concerning poor flowability and compressibility of APIs by including specific excipients to optimise the tablet formulation for direct

compression. The SeDeM EDS comprises several parameters determined for the API and excipients to obtain a characteristic profile required for compression of all the powder components used in a tablet formulation. Based on the parameter profiles obtained for each powder in the tablet formulation, it is possible to identify excipients that can be used to compensate for deficient powder flow and compression properties (Nofrerias *et al.*, 2019; Scholtz *et al.*, 2017)

1.1.3.1 SeDeM EDS parameters and incidences

The SeDeM EDS uses a quantitative approach to characterise powder flow properties and powder compression characteristics. This system is based on 12 powder flow and powder compression-related parameters grouped into five incidences, as shown in Table 1.1. Three additional indices can be calculated to indicate whether a powder mixture can be compressed directly into a tablet as a solid oral dosage form. These indices include the parameter index (IP), parameter profile index (IPP), and good compressibility index (IGC) (Pérez *et al*., 2006; Suñé-Negre *et al*., 2008; Suñé Negre *et al*., 2005).

Table 1.1: SeDeM EDS powder flow and compression parameters grouped per incidence

SeDeM incidences	**SeDeM parameters**
Dimension	Tapped density (Dc), Bulk density (Da)
Compressibility	Inter-particle porosity (Ie), Carr's index (IC%), Cohesion index (Icd)
Flowability/powder flow	Hausner ratio (IH.), The angle of repose (α), Flowability (t")
Lubrication/stability	Loss on drying (% HR.), Hygroscopicity (%H),
Dosage/lubrication	Homogeneity index (Iθ), Particle size (%Pf)

1.1.3.2 SeDeM polygon

The values of the SeDeM EDS parameters are converted to corresponding radii and used to construct an irregular-shaped polygon graph. The constructed polygon gives a graphical overview of the parameters of the powder regarding suitability for direct compression. Radii of all SeDeM parameters range from 0 –10 and are grouped into five incidence factors, as shown in Figure 1.1. If a parameter has a radius value < 5, it means that the powder has unfavourable properties for that specific parameter, which predicts that poor powder performance could be expected in this specific area. If a specific incidence factor (consisting of 2 or 3 parameters) has a value < 5, it means that an excipient is required to be added to correct the properties of the powder to reach an incidence factor value of ≥ 5 (Scholtz *et al.*, 2017). The values of the five SeDeM incidence factors determine the quantity of the corrective excipient to be added to the API. Ultimately, the addition of the corrective excipient should ensure that all five incidence factors reach values of ≥ 5. The SeDeM EDS can also be used to identify suitable corrective excipients to be added to the API to compensate for the poor characteristics of the API intended for direct compression (Pérez *et al*., 2006).

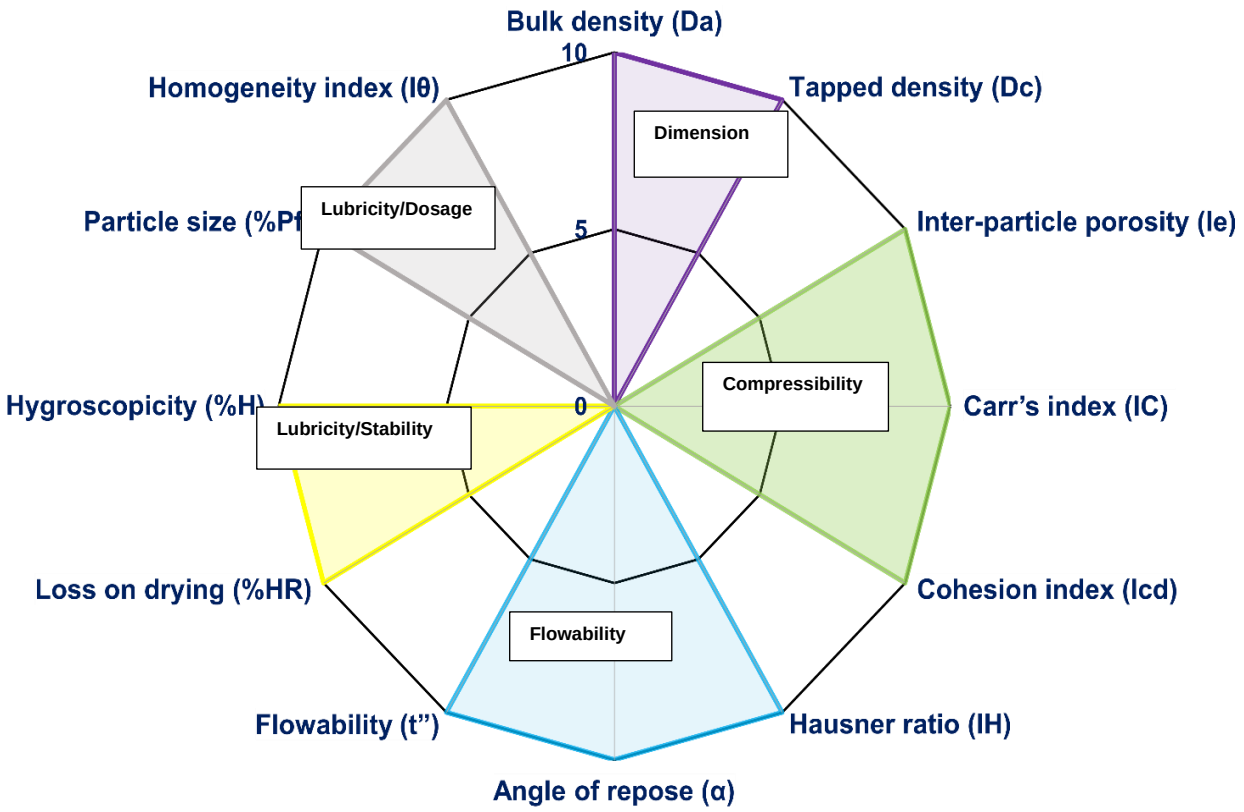

Figure 1.1: An example of a SeDeM EDS polygon showing the 12 parameters grouped into five incidences

1.2 Research problem

Artemisia afra is a medicinal plant traditionally used in the form of a tea infusion. Challenges associated with the preparation of an infusion tea are the variation in phytochemical contents due to factors such as the time of infusion, the temperature of the water and the weight of plant material used. *Artemisia afra* is a popular medicinal plant; however, it is not yet available in a properly designed solid oral dosage form that provides a consistent dose of a chemically verified extract of this plant (Thring & Weitz, 2006; Van Wyk, 2011).

A medicinal product in the form of a solid oral dosage form containing *A. afra* extract will be beneficial in providing a consistent dose and given the poor organoleptic properties of the water-based infusion or tea, namely the bitter taste, a solid oral dosage form will be beneficial. Furthermore, by employing a scientific formulation approach such as the SeDeM EDS, the formulation time for a tablet containing *A. afra* extract can be shortened by identifying the correct excipient powders for direct compression of the tablet.

1.3 Aim and objectives

This study aims to employ the SeDeM EDS to formulate a solid oral dosage form containing *A. afra*

extract. To reach this study aim, the following objectives are set:

- Validate a high-performance liquid chromatography (HPLC) analytical method to quantify the morin hydrate equivalents in four primary phytochemical marker molecules in the *A. afra* extract with regards to accuracy, precision, linearity, specificity, range, limit of detection (LOD), and limit of quantification (LOQ).
- Prepare an aqueous *A. afra* extract and chemically characterise this extract using HPLC by quantifying four main phytochemical marker molecules.
- Investigate the effect of water temperature during *A. afra* extract preparation on the yield and phytochemical composition.
- Compare *A. afra* plant material from different regions regarding dry powder yield and phytochemical composition.
- Characterise the powder flow properties of dried *A. afra* dry powder extract and selected excipients according to the SeDeM EDS parameters.
- Calculate the relevant SeDeM EDS incidences from the parameters obtained and optimise a directly compressible formulation.
- Prepare a directly compressible tablet containing *A. afra* dry powder extract based on the optimised SeDeM EDS powder formulation.
- Evaluate the tablets in terms of an assay, weight variation, hardness, friability, disintegration, and dissolution behaviour.
- Test the physical stability of the formulated tablets over 12 weeks. Tablets will be exposed to two different conditions, namely 25ºC/60% relative humidity (RH) and 40ºC/75% RH. Tablets will be evaluated in terms of an assay, weight variation, hardness, friability, disintegration, and dissolution behaviour after 1, 2, 3, 4, 8 and 12 weeks, respectively.

1.4 Chapter layout

The dissertation is divided into five chapters. Chapter 1 describes the research problem after a short introduction and provides the aim and objectives. Chapter 2 provides a literature study discussing the usage and regulation of complementary medicine, a description and usage of *A. afra* as a medicinal plant, tablet manufacturing methods, and affords background regarding the SeDeM EDS. Chapter 3 lists the materials and explains the methods. In Chapter 4 the results of the study are presented and discussed. Finally, chapter 5 offers a summary and recommendations.

CHAPTER 2

LITERATURE REVIEW ON HERBAL MEDICINES WITH THE FOCUS ON *A. AFRA*, SOLID ORAL DOSAGE FORMS AND THE SEDEM EDS

2.1 Introduction

A brief overview is given in this chapter on herbal medicines concerning their registration, usage, and the need for scientific methods to improve safety and efficacy. The literature overview specifically focuses on *A. afra*, a traditional herbal medicine commonly used as a herbal tea for the treatment of various illnesses. The health benefits and pharmacology of *A. afra* are provided, and the need for an alternative dosage form is discussed. Different solid oral dosage forms are highlighted and the state-of-the-art quality by design tablet formulation method, termed the SeDeM EDS, is discussed and explained.

2.2 Complementary and alternative medicine

2.2.1 Definitions

The World health organization (2021) describes complementary medicine, also named alternative medicine, as not considered part of the mainstream healthcare system. It is differentiated from traditional medicine because the treatment is not derived from any specific country's tradition. Complementary medicine can be defined as the diagnosis, treatment, and/or prevention of sickness, which complements mainstream medicine by satisfying a demand not met by orthodoxy or by diversifying the conceptual frameworks of medicine (Guantai & Addae-Mensah, 1999).

The United States National Institutes of Health classifies complementary and alternative medicine (CAM) into five groups, namely: (a) alternative medical systems, for example, acupuncture, homoeopathy and naturopathic medicine; (b) mind-body interventions such as meditation, cognitive behaviour therapy and prayer; (c) biologically based therapies for instance herbs and food supplements; (d) manipulative and body-based therapies such as massage and chiropractic techniques; and (e) energy flow therapies including Reiki and therapeutic touch that involve biofield and electromagnetic energy therapies (Koithan, 2009).

Subramani and Lakshmanaswamy (2017) compiled data on different complementary and alternative medicines used in patients with breast cancer. The different techniques and practices are summarised in Figure 2.1.

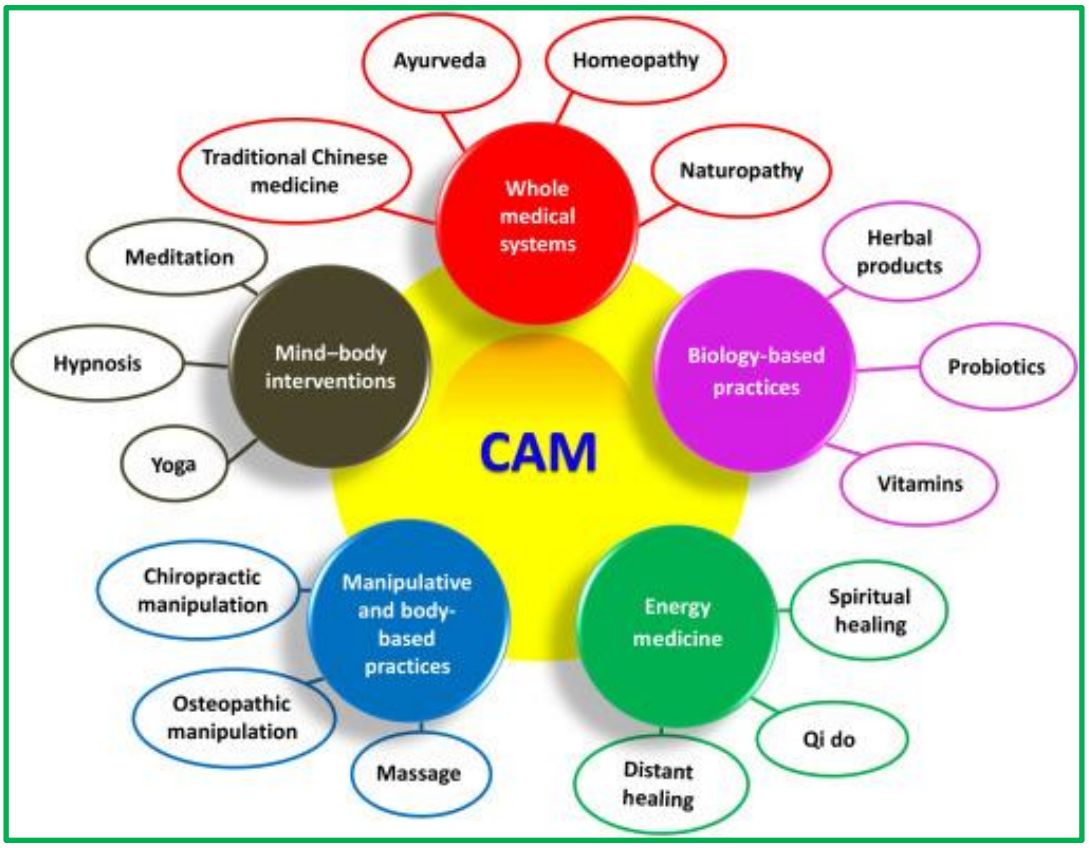

Figure 2.1: Illustration of the different techniques used as complementary and alternative medicines for the treatment of breast cancer (Subramani & Lakshmanaswamy, 2017)

2.2.2 Traditional medicine

Traditional medicine is based on the experience and beliefs of specific indigenous cultures of different countries worldwide. Traditional medicine includes practices handed down from generation to generation by cultures of different countries and they rely on the practices and knowledge gathered throughout decades, or even centuries, by their ancestors. These medications are used to treat, prevent and improve mental and physical health. Traditional medicine is used by cultures even with unproven effectiveness. In many instances, the health benefit of traditional medicines is based on theories and the mechanism of action cannot be explained. The World Health Organization (WHO) has a traditional medicine strategy ranging from 2014 to 2023. This strategy has objectives to integrate traditional medicine into health systems where appropriate, and implement policies, standards and regulations to ensure the quality and safety of traditional medicines. The WHO also aims to encourage sharing evidence-based information regarding traditional medicines (World health organization, 2021).

Traditional Chinese Medicine (TCM) is the most significant and best-known form of traditional medicine and is still widely used even in modern allopathic medicine. Traditional, complementary and alternative medications have received tremendous attention recently. During the Covid-19 pandemic, various traditional healers have claimed to treat Covid-19 with traditional medication, with some of the most

prevalent claims made by proponents of TCM. Treatment combinations of TCM with western medicine were also implemented (Ni *et al*., 2020). Officials issued TCM programs in 23 Chinese provinces in the hope of preventing Covid-19 infections and various traditional methods and formulas were used as an alternative technique for potential Covid-19 prevention (Luo *et al.*, 2020).

2.2.3 Herbal medicine

Herbal medicines are plants that contain active ingredients in the form of secondary metabolites (i.e., phytochemicals produced by the plant). Knowledge about herbal medicines is usually passed-on through generations through guidelines on the preparation of herbal remedies and combinations of herbal mixtures to treat different ailments and symptoms (Ahmad Khan & Ahmad, 2019; World health organization, 2021).

2.2.4 Custom CAM and herbal medicines

In 2015 the global sales of herbal medicine were approximately $100 billion, and the WHO reported that between 60% – 80% of the world's population used herbal medicine, mostly in developing countries (Ahmad Khan & Ahmad, 2019). The global demand for herbal medicines is growing and the trade of herbal drugs is expected to increase annually in developing countries such as India, where approximately 70% of the population benefited from CAM and herbal medicine (Vaidya & Devasagayam, 2007). In Germany, a nationwide online survey was conducted to determine the utilisation of herbal medicines, and it was found that 75.4% of Germans utilised herbal medicines sometime during the previous 12 months, and 86.7% have utilised herbal medicine sometime during their lifetime (Welz *et al*., 2019). A study in the United States reported that 60% of participants benefited CAM, with herbal medicine (31%) being the most prevalent (Velanovich *et al*., 2006). A survey on CAM and herbal medicine utilisation among university students in America showed that 58% of the students took advantage of at least one form of alternative medication (Johnson & Blanchard, 2006).

A study done in 2007 indicated that approximately 26.6 million (i.e., 52%) people living in South Africa at that stage utilised traditional medication, with the most prevalent users being black South Africans. It was also estimated that the trade of traditional medicines contributed roughly R 2.9 billion to the South African economy. However, the survey showed that traditional medicine was often more expensive than allopathic medicine available at local clinics and government-funded health facilities, which indicated that traditional medicine was not utilised per se as a cheaper alternative to standard medical treatment. Furthermore, the survey noted that some plant species might soon become endangered or extinct with the current utilisation of medicinal plants. The average consumer utilised about 157 g of herbal plant material per year and it was shown that harvesting plants or even parts (e.g., bark, roots, and bulbs) of the plant sometimes resulted in the destruction or death of the entire plant (Mander *et al.*, 2007).

2.2.5 Regulation

The regulation of CAM and herbal and traditional medicines differ vastly from country to country. A brief discussion on regulations according to country or region will be given in the following subsections.

2.2.5.1 The European Union

Within the European Union, the efficacy and safety of herbal medicinal products are controlled by the European Medicines Agency (EMA). The European Directive 2004/24/E.C. of 2004 states that herbal medicines need authorisation from the national regulatory authority (European parlement, 2004). In Europe, two categories were created for herbal products. The first category includes established herbal medicinal products, which are herbal products with recognised safety and efficacy profiles. The second category comprises herbal products for traditional use; for example, herbal products without recognised efficacy but accepted safety. The EMA must evaluate the safety and efficacy of the herbal product by using scientific literature. If insufficient data on safety is found, consumers are informed (Calapai, 2008).

2.2.5.2 The United States of America (USA)

In the USA, the Food and Drug Administration (FDA) regulates food supplements, botanical medicines, and herbs under the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (FDA, 2018). The USA law forbids the marketing of supplements for diagnosis, treatment, cure, or prevention of diseases. When products such as botanical herbs are intended to cure, prevent, mitigate, and diagnose disease, the FDA regulations require a pre-marketing approval process (Eisenberg, 2006). There are currently only a few FDA-approved botanical drugs, including Veregen® (sinecatechins ointment) used for genital warts and Mytesi® (crofelemer) to relieve diarrhoea in HIV patients on antiretroviral (ARV) medication (Patel *et al.*, 2013).

2.2.5.3 Canada

In Canada, Health Canada (HC) regulates supplements and botanical medicines under the Natural Health Products Regulations, which came into effect on January 1, 2004. Regulations for successful licensing include good manufacturing procedures, product quality and possible side effect reporting. Before health claims are made on product labels, evidence for safety and efficacy in published literature is required (Moss *et al*., 2006).

2.2.5.4 China

The Chinese National Department of Health is the governmental executive of complementary medicine regulated by the Chinese Food and Drug Administration (CFDA). Traditional Chinese Medicine (TCM) is not only sold as supplements but also as over-the-counter (OTC) medicine and prescription medicines (Dobos *et al*., 2005). Under the Chinese Food Law, TCM falls under a category classified as medicines.

TCM is popular and respected in China as an effective treatment of diseases. In recent years, TCM has also received worldwide attention after discovering the compound artemisinin utilised to treat malaria in the plant *Artemisia annua*. Other complementary supplements fall under the supplements category. Companies in China can make health claims on products if approved by the Chinese Food Safety Law. To receive approval substances must undergo toxicity testing. The State for Administration of Traditional Chinese Medicine (SATCM) provides guidelines and laws regarding TCM drug development and integration, and provides the qualifications and standards of education for TCM practitioners (Robinson, 2006).

2.2.5.5 Japan

In Japan, the Consumer Affairs Agency (CAA) regulates health foods. Health foods can fall into two categories namely: (1) foods in general and (2) foods with health claims. Under foods with health claims, vitamins and minerals can fall under a category that has food with nutrient function claims or under a group of products that claim to treat or cure diseases, which falls under food with specific health uses (Ohama *et al*., 2008). For a product to be registered for specific health uses, the health claims must be backed with scientific evidence to prove its safety and efficacy (Shimizu, 2003). The traditional medicine used in Japan is known as Kampo medicine. Kampo has its roots in TCM but has developed into a widely used system of medicines unique to Japan. Japanese Kampo medicines are regulated in a similar way as western prescription medications (Yu *et al*., 2006).

2.2.5.6 Regulation in South Africa

The South African Health Products Regulatory Authority (SAPHRA) regulates all health products in South Africa, including complementary medicine (SAPHRA, 2013). Products are divided into six categories of health disciplines, namely: (a) Aromatherapy; (b) Ayurveda; (c) Homeopathy; (d) Traditional Chinese Medicine; (e) Unani Tibb; and (f) Western Herbal Medicine Complementary Medicines. On November 15, 2013, SAHPRA, with the approval of the Minister of Health, decided that complementary medicines together with modern health supplements and traditional medicine that are not indigenous to South Africa should fall into class D medicine (Department of health, 2017). Complementary medicine must adhere to registration and licensing for manufacturing until distribution as outlined by section 22C (1)(b) of the Medicines Act. Companies must comply with all relevant regulations and provisions of the Medicines Act. Indications are based on low-risk outcomes, including maintenance of health, minor symptom relief or health enhancement with no reference to a disease. Registration by SAHPRA is subject to the substance's efficacy, quality and safety. Medicines used for a specific category are classified as either high-risk or low-risk based on indications, dosage form and composition. High-risk medicine requires clinical evidence to prove safety and efficacy, whereas low-risk medicines necessitate only traditional evidence. Only low-risk indications are allowed for health supplements (Fourie *et al*., 2017).

2.3 Artemisia afra

2.3.1 Botany

Artemisia afra (see Figure 2.2) is a well-known traditional medicinal plant in South Africa, and in folklore, the name "African wormwood" is mostly recognised, whereas other names such as "wildeals" (Afrikaans), “lengana” (Tswana), and “umhlonyane” (Zulu and Xhosa) are also used. The *A. afra* plant can grow up to two meters tall with a solid leafy and hairy stem. The soft green leaves are faced towards the stem, and the lighter green leaves are away from the stem. The *A. afra* plant produces yellow flower heads. *A. afra* is a traditional herbal remedy used as complementary medicine to treat symptoms of several diseases such as colds, influenza, bilharzia, and malaria (Liu *et al*., 2010; Van Wyk, 2011).

Figure 2.2: Photograph of the *A. afra* plant (photo taken at Bronkhorstspruit dam by PS Roets

2.3.2 Phytochemistry

Different phytochemical compounds have been identified in *A. afra,* including flavonoids (e.g., acacetin, apigenin, chrysoeriol, diosmetin, genkwanin, 7-methoxy acacetin, quercetin, kaempferol and luteolin), terpenoids (e.g., monoterpenoids, sesquiterpenes, glaucolides and guaianolides), chlorogenic acids (e.g., dicaffeoyl quinic acid derivatives, chlorogenic acid, etc.) and coumarins (e.g. scopoletin) (du Toit & van der Kooy, 2019). According to Avula *et al*. (2009), a mixture of different flavonoids such as acacetin, genkwanin, 7-methoxy acacetin, and sesquiterpene lactones seems to be responsible for the *in vitro* anti-plasmodial effect.

Liu *et al.* (2010) found the following compounds in *A. afra*, namely acetic acid, adenine, alanine, aspartic acid, caffeic acid, chlorogenic acid, choline, citric acid, 3,5-dicaffeoyl quinic acid, 1-O-ethyl-β-d-glucoside, formic acid, fumaric acid, α-glucose, β-glucose, glutamic acid, p-hydroxy benzoic acid, malic

acid, phosphatidylcholine, proline, quercetin, luteolin, rhamnose, succinic acid, sucrose, threonine, and valine. Other molecules identified include α-amyrin, 1α,4α-dihydroxybishopsolicepolide,12α,4α-dihydroxybishopsolicepolide, isoalantolactone, phytol, scopoletin and yomogiartemin (More *et al.*, 2012a).

It has been shown that *A. afra* plant material can retain its biological activity after being stored at room temperature under dark conditions for 12 –16 years. In addition, phenolic and flavonoid contents were higher in dried plant material than in fresh *A. afra* plant material (Amoo *et al*., 2012).

2.3.3 Pharmacology and health benefits

Different extracts from *A. afra* have shown pharmacological effects on several systems in the human body. Furthermore, pre-clinical and clinical studies have indicated efficacy in improving a wide range of symptoms including fever, coughing, and congestion. (du Toit & van der Kooy, 2019; Liu *et al.*, 2009; Ruppel, 2003).

Chloroform extracts of *A. afra* were effective against the malaria parasite *Plasmodium falciparum* at concentrations between 8.55 µg/mL and 12.35 µg/mL. However, they were inferior compared to that of *A. annua*, which showed activity against *P. falciparum* at concentrations between 0.050 µg/mL and 0.067 µg/mL, most likely due to the artemisinin content of *A. annua* (Amponsah, 2013; Kraft *et al*., 2003; Liu *et al*., 2010).

The pulmonary bronchodilation induced through the inhalation of *A. afra* aqueous extract steam is contributed to its high luteolin content. Steam inhalation with an aqueous extract made from dried *A. afra* leaves infused in 100 ml boiled saline solution (10 mg/mL and 50 mg/mL) did not lead to any significant change in tidal volume; however, nebulising with *A. afra* aqueous extract led to a statistically significant increase in tidal volume (i.e., an increase of 18%). Tidal volume can be described as the volume of gas inspired or expired during each respiratory cycle. Nebulising with *A. afra* extract containing a concentration of 250 ug/mL of luteolin increased lung compliance by 43.4% and reduced lung resistance by 8.5%. Compliance can be described as the volume change per unit of pressure change for lungs and is measured in millimetres per centimetre of water (ml/cm H_2O) (Joel Mjiqiza *et al*., 2013; Ruppel, 2003).

After administration in the form of an intravenously injected extract, the cardiovascular effects of *A. afra* were investigated in rabbits. The injected extract had a rapid onset of action (5 min) to reduce blood pressure, and the duration of action was 90 min. *A. afra* caused a gradual fall in both systolic and diastolic blood pressure. In addition, it showed a hypotensive effect *in vivo* and a biphasic dose-dependent effect of the heart *in vitro*. Higher doses showed cardiac depression, whereas lower doses caused cardiac stimulation followed by cardiac depression. In conclusion, this study indicated that

A. afra could potentially be effective in assisting with managing hypertensive conditions (Guantai & Addae-Mensah, 1999).

In a study investigating the hypoglycaemic effect of *A. afra*, male albino Wistar rats were injected with streptozotocin at 60 mg/kg body weight after an 18 hour fast to induce diabetes. After two weeks, the untreated rats had a significant increase in blood glucose and insulin concentrations, whereas the two groups of rats treated with *A. afra* extract at 50 mg/kg and 100 mg/kg body weight depicted normalised glucose and insulin levels. The researchers concluded that *A. afra* can be employed as a hypoglycaemic agent (Afolayan & Sunmonu, 2011). In another study, *A. afra* has been shown to significantly reduce blood glucose levels in diabetic Swiss albino mice. The mice were injected with alloxan (200 mg/kg) to cause drug-induced diabetes. Mice receiving an aqueous extract of *A. afra* at 500 mg/kg and 750 mg/kg, respectively, showed significantly decreased blood glucose levels compared to the untreated group (Issa & Hussen Bule, 2015b).

Chlorogenic acid, a compound found in *A. afra*, has an inhibitory effect on influenza A by acting as a neuraminidase blocker when tested on a cellular level and in animal tissue in pre-clinical studies (Ding *et al*., 2017). In chicken embryos, chlorogenic acid acted against infectious bursal disease virus (IBDV) by inhibiting virus replication and thereby reducing the histamine response, which led to a lower inflammatory response (Li *et al.*, 2021).

Five caffeoylquinic acid derivatives were isolated from the dried and powdered rhizomes of the *Elephantopus scaber* plant. Caffeoylquinic acid derivatives are also found in the *A. afra* plant. All five caffeoylquinic acid derivatives showed antiviral effects on the respiratory syncytial virus (RSV) and had equal or lower IC50 values than ribavirin, indicating sound antiviral effects. The phytochemical compounds 3,4-di-O-caffeoylquinic acid and 3,5-di-O-caffeoylquinic acid inhibited RSV by deterring virus-cell binding and cell-cell binding in the early and late stages of the viral replication cycle (Geng *et al.*, 2011).

van Vuuren and Muhlarhi (2017) tested extracts from five different plant species, including A*. afra*, for their effect against drug-resistant microbes. *A. afra* was the most promising plant extract and responded best against gram-positive drug-resistant bacteria, including *S. aureus*. Six compounds were identified by More *et al.* (2012b) in the fresh *A. afra* plant material: acacetin, scopoletin, phytol, betulinic acid, 12α,4α-dihydroxy-bishopsolicepolide, and α-amyrin. The above-mentioned compounds were tested for antimicrobial activity against gram-positive and harmful bacteria. The *A. afra* extract inhibited the growth of all the microbes included in the study at concentrations between 1.6 mg/mL to 25 mg/mL; and the six isolated compounds inhibited microbial growth at concentrations between 0.25 mg/mL and 1.0 mg/mL (More *et al.*, 2012b).

Finally, flavonoids in *A. afra* caused a dose-dependent sedative effect. These compounds can potentially bind to the GABA-benzodiazepine receptors leading to CNS-acting activity in the form of sedation (Stafford *et al*., 2005).

2.3.4 Toxicity

Artemisia afra exhibited a favourable safety profile when tested on mice and rats, respectively, and showed no acute toxicity or adverse effects when given to mice at dosages below 1 500 mg/kg of body weight. The maximum tolerated dose (MTD) from which all mice recovered was between 1 500 mg/kg and 2 500 mg/kg. The lowest dose that induced mortality was higher than 2 500 mg/kg. To test chronic toxicity, male Wistar rats were given *A. afra* extracts at doses of 0, 100, and 1 000 mg/kg daily for three months. The results showed a low potential for chronic toxicity even at these higher-than-normal doses. *A. afra* has a low potential to induce adverse effects and exhibits a hepato-protective effect in high doses (Mukinda & Syce, 2007).

A study was conducted to determine the mean lethal dose (LD50) where 60 healthy Swiss albino mice weighing 25 – 35 g were divided into ten groups of 6 (3 male and three female mice per group) and given a single dose of either 0, 1 000, 2 000, 3 000, 4 000, 5 000, 7 500, 10 000, or 12 000 mg/kg of an aqueous extract of *A. afra*. The LD50 dose obtained was 9 833.4 mg/kg, indicating that *A. afra* at therapeutic dosages of 500 – 1 000 mg/kg reduce blood glucose levels in rats and can be regarded as non-toxic (Issa & Hussen Bule, 2015a). Li *et al.* (2005) isolated three caffeoylquinic acid derivatives, also found in *A. afra* (du Toit & van der Kooy, 2019), and reported relatively low toxicity.

2.3.5 Administration of *A. afra*

The traditional way of consuming *A. afra* is to add a quarter cup of fresh leaves to boiling water for 10 min and take the resultant tea infusion by mouth (i.e., the oral route of administration). The *A. afra* infusion is typically sweetened with honey to mask the bad taste (Roberts, 1990; Van Wyk, 2011).

2.4 Solid oral dosage forms

2.4.1 The need for an alternative dosage form

A. afra is a popular medicinal plant; however it has poor organoleptic properties when prepared as an infusion (i.e., bitter taste) and is not yet commercially available as a solid oral dosage form such as a tablet or capsule (Thring & Weitz, 2006; Van Wyk, 2011). A possible solution to the poor organoleptic properties, namely the bitter taste, is to formulate a solid oral dosage form that contains an extract of this medicinal plant. Solid oral dosage forms offer numerous advantages compared to the traditional infusion preparations such as masked taste and improved stability, amongst others (Jivraj *et al*., 2000).

2.4.2 The advantages of solid oral dosage forms

The production of tablets and other solid oral dosage forms is generally energy-efficient, requires a relatively small labour force and produces relatively low amounts of waste products. Furthermore, dosage forms with relatively low variation between batches are highly repeatable. Quality control is relatively simple, and it is easy to up-scale production when the demand requires it (Jivraj *et al*., 2000; Teżyk *et al.*, 2016; Werani *et al*., 2004).

2.4.3 Tablet manufacturing

2.4.3.1 Tabletting by granulation

Granulation is a process where powder particle size is enlarged through agglomeration to transform fine, poor-flowing powders into coarse powders with improved flow properties. Granulation is also used to prevent the separation of constituents within a powder and improve the mixture's compaction and uniformity to provide better flow properties. Two granulation processes, namely dry and wet granulation, can be employed before compaction into tablets (Shanmugam, 2015).

2.4.3.1.1 Dry granulation

Dry granulation is used for moisture-sensitive drugs or drugs that compress poorly after wet granulation. Firstly, a process called slugging, is used to produce a large tablet, which is then crushed to produce a powder consisting of coarse particles. Alternatively, a process termed roller compaction can be used to squeeze the powder between two rollers in order to produce flakes or a sheet of compact material, which is then crushed to produce a powder consisting of coarse particles. Figure 2.3 shows the steps involved in the process of dry granulation, from powder mixing to tablet compression. Setyawan *et al*. (2020) found that using dry granulation for ketoconazole resulted in an improved formulated product that circumvent the stability problems associated with wet granulation. Pneumatic dry granulation (PDG) is an advanced method that produces granules by mild compaction and passes a gas pneumatic system that separates the particle size fraction that are intended to be compressed into tablets (Sandler & Lammens, 2011; Shanmugam, 2015)

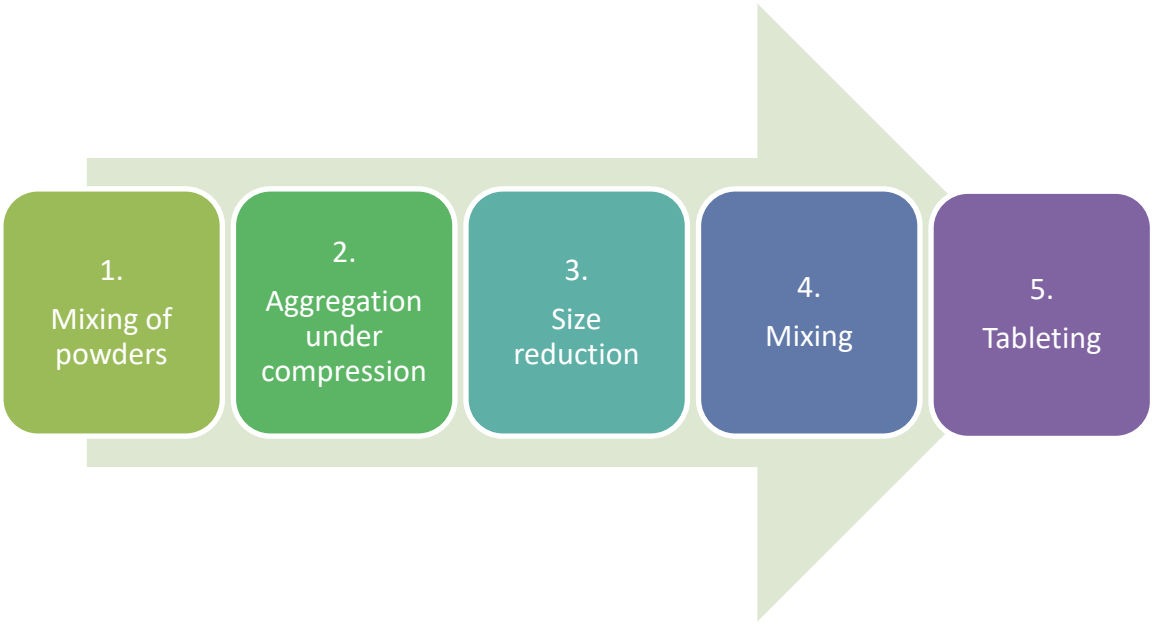

Figure 2.3: The steps involved in the tabletting process using dry granulation (Quinlan *et al*., 2015)

2.4.3.1.2 Wet granulation

Wet granulation is when a granulating fluid is added to a dry powder mixture and the wet mass is ten forced through a sieve to produce wet granules. Water is mainly used for ecological, toxic and economic reasons but can negatively impact drug stability. It furthermore requires a relatively long drying time. Alternatively, isopropanol or ethanol can be used (Rodriguez *et al*., 2002). The steps involved in tablet production using wet granulation are shown in Figure 2.4. An alternative form of wet granulation, titled melt granulation, can also be utilised. Melt granulation uses thermosetting polymers to form granules (Abberger, 2001). Steam granulation, on the other hand, is a method where steam is employed as a binder instead of water. Finally, freeze-granulation is a process where liquid nitrogen is used to instantly freeze particles into granules (Moritz & Nagy, 2002).

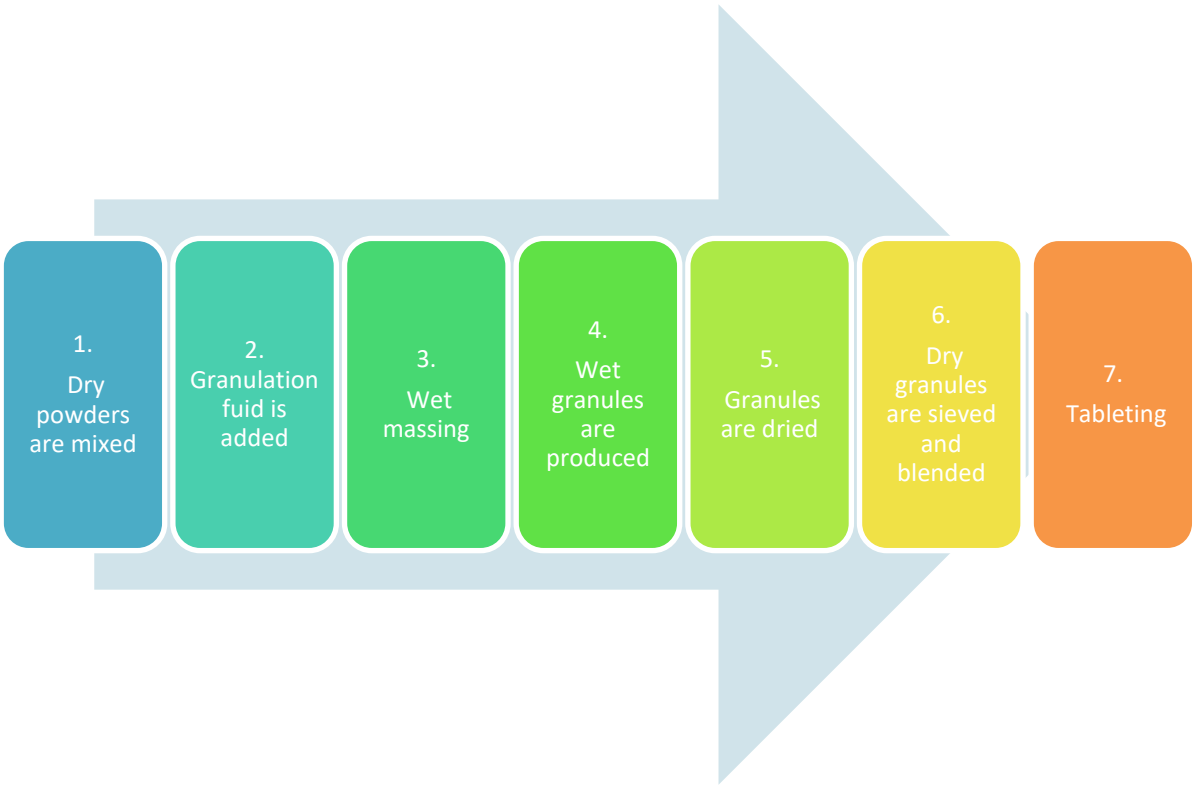

Figure 2.4: The steps involved in the tabletting process using wet granulation (Agrawal & Naveen, 2011)

2.4.4 Tabletting by direct compression

Direct compression is a simple tablet manufacturing method that includes only two steps, namely: powder mixing and direct compression. A powder mixture should have the ability to flow well to fill the die with a consistent mass in order to ensure tablet uniformity. Flowability can be tested via different methods, such as angle of repose, where a small angle of repose value indicates good flowability. The powder mixture should also possess good compressibility, which is affected by the powder mixture's bulk density and tapped density. (Martinello *et al*., 2006; Moritz & Nagy, 2002).

2.4.4.1 Advantages of tabletting by direct compression

Direct compression is the simplest method to produce tablets with the least number of steps involved. The process is time-efficient, and since no water or heat is needed, products remain more stable. In addition, the disintegration of tablets produced by direct compression is usually fast, which leads to faster drug release. Heat labile and hygroscopic powders can moreover be compressed into tablets using this method. Direct compression can also be easily applied to powder mixtures containing a high number of excipients (Jivraj *et al*., 2000; Pawar *et al*., 2014; Staniforth *et al*., 1981).

2.4.4.2 Disadvantages of tabletting by direct compression

Some fillers and dry binders used in tablet formulations for direct compression can be expensive. Technological difficulties can arise during the compression step and uniform colouring of tablets prepared from a mixture of dry powders can be complicated (Gaikwad & Kshirsagar, 2020). Excipients with larger particles are used to increase powder flowability that may cause segregation. Experimenting with compatible API combinations can be time-consuming and may consequently lead to higher costs during the formulation phase. Excipients are mostly needed to compensate for poor powder flow of APIs and ensure acceptable tablet compression, sometimes resulting in a limited amount of API that can be accommodated in the formulation. A powder formulation with a relatively low bulk density may lead to the production of tablets that do not display aesthetically correct dimensions, i.e., the tablets are too thin (Jivraj *et al.*, 2000).

2.4.5 Tablet excipients considered in this study

Tablet excipients usually are added to the APIs to enhance the tabletting process and to produce a high-quality tablet that complies with the requirements as determined by various pharmacopoeias including the British Pharmacopoeia (BP). Excipients are often classified according to their functions, e.g., filler, disintegrant, solution binder, dry binder, glidant, lubricant, or anti-adherent. In addition, certain excipients, for example cellulose, can be multifunctional because it acts as a filler, binder, and/or disintegrant simultaneously (Jivraj *et al*., 2000).

2.4.5.1 Emcompress® (Calcium Hydrogen Phosphate Dihydrate (DCP dihydrate)

Calcium Hydrogen Phosphate Dihydrate (DCP dihydrate) is considered a free-flowing powder that is commercially available as Emcompress®. It is insoluble in water and is also non-hygroscopic. Emcompress® is easy to compress with relatively low compaction pressure. Emcompress® exhibits a typical bulk density of 0.915 g/cm^3 and a tapped density of 1.17 g/cm^3 which indicated good compressibility (Jivraj *et al.*, 2000; Rowe, 2009).

2.4.5.2 Tricalcium citrate

Tricalcium citrate is commonly used in vitamin supplements as a source of calcium but has favourable properties when used as an excipient for the direct compression of tablets. Tricalcium citrate a is a brittle deforming filler material with excellent flow properties and has a high mechanical strength that produces high-quality tablets with acceptable properties. Hagelstein *et al*. (2018) determined that tricalcium citrate consists of particles with spherical shapes that form large agglomerates and have an average particle size of 135 µm. Loss of mass of tablets consisting of tricalcium citrate due to friability ranged from 0.1 – 0.6% w/w, which is considered acceptable. Furthermore, tablets prepared with tricalcium citrate disintegrated relatively quickly when combined with 0.5% w/w of the lubricant

magnesium stearate and showed good compressibility without any lubricant sensitivity (Hagelstein *et al*., 2018).

2.4.5.3 MicroceLac® 100

MicroceLac® 100 is a co-processed excipient consisting of approximately 75 % w/w alpha-lactose monohydrate and 25 % w/w microcrystalline cellulose (MCC). It was designed to formulate small, high-dose tablets from an API with poor powder flow properties. The two non-toxic fillers from which MicroceLac® 100 is prepared can also be used individually for the formulation of tablets by direct compression. The powder mixture is odourless, almost white, and is prepared through spray-drying the mixture of the two powders that it consists of. MicroceLac® 100 has particles with a spherical structure, a particle size distribution primarily between 32 – 250 µm, and is partially soluble in water. When exposed to relatively high humidity during storage, MicroceLac® 100 maintained its particle shape but showed molecular changes, including water-induced crystallisation when evaluated by scanning electron microscopy (Haware *et al*., 2015; Rowe, 2009).

2.4.5.4 Ludipress®

Ludipress® is a co-processed excipient consisting of a filler (93.4% w/w alpha-lactose monohydrate), a binder (3.2% w/w polyvinylpyrolidone), and a disintegrant (3.4% w/w crospovidone). Ludipress® show good flowability, primarily due to the spherical shape of the powder particles with smooth surfaces. It can be compressed with a relatively low force to produce tablets and is water-soluble. Ludipress® typically has a bulk density of 0.56 – 0.6 g/cm^{3,}(Rowe, 2009; Schmidt & Rubensdörfer, 1994).

2.4.5.5 Avicel® PH 200 (Microcrystalline cellulose)

Microcrystalline cellulose (MCC), branded as Avicel® PH 200, has a particle size of approximately 200 µm and is an effective binder-filler with good disintegration properties, high compressibility at a low compaction force, and has a high dilution value. Therefore, Avicel® PH 200 can be used as a filler, binder and disintegrant in tablet formulations when utilised in higher concentrations. In addition, Avicel® PH 200 has a significant effect on decreasing the friability of a tablet and can increase tablet compatibility and hardness (Damayanti *et al.*, 2018; Shangraw & Demarest, 1993).

MCC is a partially depolymerised cellulose primarily used as a diluent/binder providing good lubrication for solid oral dosage forms including tablets, and it can be ulilised in direct compression and wet-granulation formulations. It is purified with porous particles into a white, tasteless, odourless powder. MCC is hygroscopic with a large surface area and a low bulk density, giving it good binding properties. Commercially produced MCC can differ in moisture content and particle sizes, depending on the application (Rowe, 2009; Thoorens *et al*., 2014).

2.4.5.6 FlowLac® (Lactose monohydrate)

Lactose monohydrate (FlowLac®) is used as a filler-binder or a powder flow enhancer in tablet formulations for direct compression. FlowLac® can also be used as a filler and diluent in capsules. Lactose monohydrate has a white to off-white colour and a noticeable sweet taste (Rowe, 2009). Spray-dried lactose typically consists of 80 – 90% w/w alpha-lactose monohydrate and 10 – 20% w/w amorphous lactose. Spray-dried lactose monohydrate effectively enhances the flowability of granules (Huang *et al*., 2013). Stability tests were done on moisture-sensitive active ingredients (i.e. aspirin and niacinamide), which were tableted with either lactose monohydrate or anhydrous lactose, and both exhibited the same stability (Du & Hoag, 2001).

2.1.5.7 Kollidon® VA 64 (Vinylpyrrolidone-vinyl acetate copolymer or Copovidone)

Copovidone branded as Kollidon® VA 64 (vinylpyrrolidone-vinyl acetate copolymer) is a binder, granulation aid, and film-forming agent used as part of the formulation for controlled release tablets. The fine spherical powder is prepared by spray-drying and has a white to yellowish-white colour, a brief odour, and a faint taste. Copovidone can be added as a binder in wet granulation and for direct compression between 2 – 5% w/w of the formulation weight to improve cohesion, hardness, and elasticity. Copovidone typically has a bulk density of 0.24 – 0.28 g/cm^3, a tapped density of 0.35 – 0.45 g/cm^3, gains less than 10% mass when exposed to 50% relative humidity, and loses approximately 5% mass on drying (Rowe, 2009). In addition, copovidone particles contain plenty of damaged spheres that increase the surface area to enhance the binding of the dry powder but simultaneously lead to poor powder flow (Chaudhary *et al*., 2018). Kollidon® VA 64 implemented as a dry binder results in the production of tablets with good mechanical properties regarding friability and tablet hardness, and is effectively used for moisture-sensitive API's. It furthermore enhances plasticity when used at a concentration of 2 – 5% w/w. However, if used exclusively or at 85% w/w or higher, it causes high friability, lamination, and capping in tablets (Kolter & Flick, 2000).

2.1.5.8 Ac-Di-Sol® (Croscarmellose sodium)

Croscarmellose sodium branded as Ac-Di-Sol® is a carboxymethylcellulose sodium polymer used as a disintegrant in tablets. It is an odourless powder with white or greyish-white colour. Ac-Di-Sol® is used in wet-granulation and direct compression and can be added in concentrations between 0.5 – 5% w/w of the powder formulation for direct compression. It moreover has a bulk density of 0.529 g/cm^3 and a tapped density of 0.819 g/cm^3.

Ac-Di-Sol® is insoluble in water but can swell up to 8 times in volume after contact with water (Rowe, 2009). In a study, the disintegration times of tablets containing 12.5% w/w furosemide (hydrophobic API), Avicel® PH 200 and Ac-Di-Sol® (0, 0.0625% – 10 % w/w) were measured. The release of furosemide particles from the tablets was achieved and an overall faster mean disintegration time was

obtained with Ac-Di-Sol® incorporated in concentrations between 2.5% – 10% w/w compared to 0% – 1.25% w/w. It was, however, observed that tablets with 10% w/w Ac-Di-Sol® were affected by atmospheric moisture, leading to softening of the tablets (Marais *et al*., 2003).

2.4.5.9 Magnesium stearate

Magnesium stearate is a mixture of organic acids and magnesium. It is the most used lubricant in the pharmaceutical industry. Magnesium stearate provides lubrication by forming a thin layer on the particle surfaces of excipients and active ingredients, thereby preventing inter-particle bond formation. It is a white powder with a fine texture and a distinctive taste, with a brief odour caused by stearic acid. Magnesium stearate is used as a lubricant for tablets and capsules in the pharmaceutical industry and is also utilised in the food industry and in cosmetic products. Magnesium stearate has a bulk and tapped density of approximately 0.159 g/cm^3 and of 0.286 g/cm^3, respectively. It is a cohesive powder with poor flowability and is practically insoluble in water. Large quantities can cause mucosal irritation as well as a laxative effect; however, magnesium stearate is typically used in low percentages in powder formulations, thus limiting these side-effects. Incompatibilities arise when included with strong acids, alkalis, aspirin, and some vitamins (Lakio *et al*., 2013; Rowe, 2009).

2.4.6 The need for a scientific approach to formulate tablets

The traditional way of determining the composition of a powder mixture intended for tabletting is by using a trial-and-error method. Firstly, prior knowledge and experience are needed to select excipients from an available list to add to the formulation's APIs. Then, the selected powders are mixed and evaluated for direct compression, and if the process fails to produce acceptable tablets, other excipients will be selected for the powder mixture. Disadvantages of the trial-and-error method are the overspending of ingredients as well as that fact that this method is significantly time-consuming. Therefore, there is a great need for a reliable scientific method to formulate a powder mixture in a time-efficient and cost-effective manner. The SeDeM EDS was developed to address this need. The SeDeM EDS determines which powder flow properties of the API need adjustment to produce an optimal tablet powder formulation to be manufactured by direct compression (Han *et al*., 2018).

2.5 SeDeM Expert Diagram System (EDS)

The SeDeM EDS is a tool based on Quality by Design (QbD) principles that were initially developed to formulate directly compressible tablets. This system uses a quantitative approach to characterise powder flow and powder compression properties. Through these measurements, the system identifies which powder properties of the API need to be corrected by including excipients to optimise the formulation for direct compression. The SeDeM EDS is applied to both the API and each possible excipient in a tablet formulation to obtain a characteristic profile required for direct compression. Based

on the profiles attained for each powder and/or the powder mixture, it is possible to identify optimal excipient quantities to be used to compensate for the deficient properties of the API (Pérez *et al*., 2006; Suñé-Negre *et al*., 2008; Suñé Negre *et al*., 2005).

2.5.1 SeDeM parameters, incidences, and indices

The SeDeM EDS uses 12 powder flow parameters grouped into five incidences. The 12 powder flow parameters include: (a) bulk density (Da); (b) tapped density (Dc); (c) interparticle porosity (Ie) ; (d) Carr's index (Ic); (e) cohesion index (Icd); (f) Hausner ratio (IH); (g) angle of repose (α); (h) powder flow (%Pf); (i) loss on drying (% HR); (j) hygroscopicity (%H); (k) particle size below 50 µm (%Fm); and (l) homogeneity index (Iθ). The five incidences are the dimensional factor, compressibility factor, flowability/powder flow factor, lubricity/stability factor, and lubricity/dosage factor. Each incidence consists of two or three SeDeM EDS parameters, and the average radii of the SeDeM parameters for each incidence will provide an incidence value of between 0 and 10. For incidences below a value of 5, an amount of corrective excipient must be calculated to compensate for the incidence. The excipient that requires the lowest percentage to be added to the *A. afra* dry powder extract to compensate for weak properties is regarded as the best excipient. The *A. afra* dry powder extract and excipient will be mixed in a suitable ratio to provide an incidence value above 5 for all five incidences. Incidence values above five are considered suitable for direct compression. Three additional indices can be calculated to determine how well the powder mixture can be compressed into a tablet as a solid oral dosage form. These indices are the parameter index (IP), parameter profile index (IPP) and good compressibility index (IGC) (Pérez *et al*., 2006; Suñé-Negre *et al*., 2008; Suñé Negre *et al*., 2005).

2.5.2 SeDeM polygon

After obtaining the values of the 12 SeDeM EDS parameters, the converted to radii (ranging from 0 – 10) are used to construct an irregular-sided polygon. The polygon provides a graphical overview of the parameters of the powder regarding suitability for direct compression (Pérez *et al*., 2006).

2.5.3 SeDeM EDS applied in practice

The SeDeM EDS proved to be a reliable method to obtain a suitable formulation for direct compression of tablets. Application of the SeDeM EDS during the formulation of zidovudine tablets resulted in fewer resources and saved time, making it cost-effective while delivering high-quality tablets (Nofrerias *et al*., 2019). By combining and gathering parameters in a well-structured form to characterise powder properties, the SeDeM EDS is now considered one of the most successful methods used in pre-formulation studies (Dai *et al*., 2019).

Gülbağ *et al*. (2018) found that the SeDeM EDS provided an easy and effective method to formulate orally disintegrating tablets containing the API, memantine. Memantine has poor powder flow and

compressibility properties, but with proper calculation of the corrective excipients, the formulation was deemed suitable for direct compression to produce acceptable tablets. Gülbağ *et al.* (2018) also used the SeDeM EDS to formulate fast disintegrating tablets containing domperidone by calculating the optimal ratio of starch to glycine as excipients needed for direct compression. The optimal ratio of starch to glycine was calculated at 1:5, showing that the SeDeM EDS is an effective tool to predict the suitability of a powder mixture for direct compression in the pre-formulation phase (Singh *et al.*, 2019).

SeDeM EDS is primarily used for direct compression of tablets but has shown to be helpful in multiple-unit pellet systems (MUPS). in this study, three different APIs were used, namely ibuprofen, doxylamine, and paracetamol. SeDeM EDS was successfully applied on pellets containing different APIs, where after MUPS type tablets with good physical properties were produced (Hamman *et al*., 2019).

2.6 Summary

A. afra is a popular medicinal plant with pre-clinical and clinical studies proving its effectiveness; however, a traditional tea preparation is time-consuming and the organoleptic properties, such as the taste of the tea, are unfavourable, thus creating the need for an alternative dosage form. Developing a solid oral dosage form such as a tablet containing *A. afra* extract can address this need. The SeDeM EDS is an innovative, quality by design, scientific method that can be applied to produce high-quality tablets. SeDeM EDS can be applied in pre-formulation studies to reduce the financial cost of a trial-and-error approach when formulating a powder mixture for direct compression. Tabletting with direct compression is easy and cost-effective, and when combined with SeDeM EDS, the process will also be time efficient.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

To achieve the aim of this study, *A. afra* was chemically characterised and four phytochemical markers were identified to be quantified using a validated high-performance liquid chromatography (HPLC) analytical method. The method was applied to quantify the four selected *A. afra* phytochemical marker molecules in preparations or powders containing *A. afra* extract. Morin hydrate was used as an internal standard and was validated with regards to accuracy, precision, linearity, specificity, the limit of detection and the limit of quantification. Morin hydrate was added to all the *A. afra* samples to quantify each of the selected phytochemical marker molecules as milligram morin hydrate equivalents per gram of dry extract weight (mg MHE/g).

A. afra extracts were prepared at four temperatures (25ºC, 50ºC, 70ºC, and 96ºC). Liquid *A. afra* extracts were freeze-dried to determine the dry extract powder yields. The extract temperature that yielded the highest dry powder extract and had the highest amount of mg MHE/g for the four phytochemical marker molecules was consequently employed, and bulk aqueous *A. afra* extracts were prepared. Bulk *A. afra* extracts were freeze-dried and the dry powder extract used in combination with excipients to formulate a solid oral dosage form. Furthermore, extracts prepared from *A. afra* plant material, each attained from a different location, were compared with regards to phytochemical composition and dry powder extract yield.

The SeDeM EDS was employed to develop a directly compressible tablet containing *A. afra* extract. First, the powder flow properties of the dried *A. afra* extract powder was characterised using the SeDeM EDS. The values of 12 powder flow parameters were calculated and grouped into five relevant SeDeM incidences after which a polygon was drafted to obtain a graphical representation of the flow properties of the *A. afra* dry extract. Six excipients were also characterised using the SeDeM EDS, and corresponding polygons were constructed for each excipient. Based on excipient profiles, and the profile of the *A. afra* extract, the appropriate excipient was selected to compensate for the deficient properties of the *A. afra* extract. A small percentage of binder, lubricant and disintegrant was added to the corrective excipient (excipient to compensate for the deficient flow properties of the *A. afra* powder extract) and was again characterised with SeDeM EDS. Finally, the ratio of *A. afra* dry extract to excipient mixture needed to formulate a final powder mixture for tabletting was calculated and the powder mixture subjected to a final round of SeDeM EDS.

The formulated tablet mass was calculated based on 200 mg dry *A. afra* extract tablets. The *A. afra* extract was mixed with the excipients and compressed into 12 mm diameter tablets with a Korsch® XP1 tablet press with flat faced punches. Tablets were packed into 13 containers of 60 tablets each, ready for 12 weeks of stability testing and evaluation in terms of assay, weight variation, hardness, friability, disintegration, and dissolution behaviour.

Figure 3.1 is a graphical illustration to provide an overview of the experimental steps followed in this study. This figure shows two main chronological branches; branch 1 summarises experimental work that included temperature and region comparison tests for aqueous *A. afra* extracts, identification of four *A. afra* phytochemical marker molecules using HPLC, freeze-drying of *A. afra extracts*, yield calculations and validation of the internal standard. Branch 2 starts after the appropriate aqueous extract temperature for the *A. afra* plant material was identified, and experimental work included bulk extract preparation, freeze-drying, and powder flow enhancement of bulk extracts. In addition, the *A. afra* dry powder extract and six excipients were subjected to powder flow studies to determine the 12 SeDeM EDS parameters of each, where after SeDeM polygons were created for each powder. Finally, the appropriate excipients were mixed with the dry *A. afra* powder extract in an optimal ratio. Tablets were compressed and stability experiments were performed on all tablet formulations.

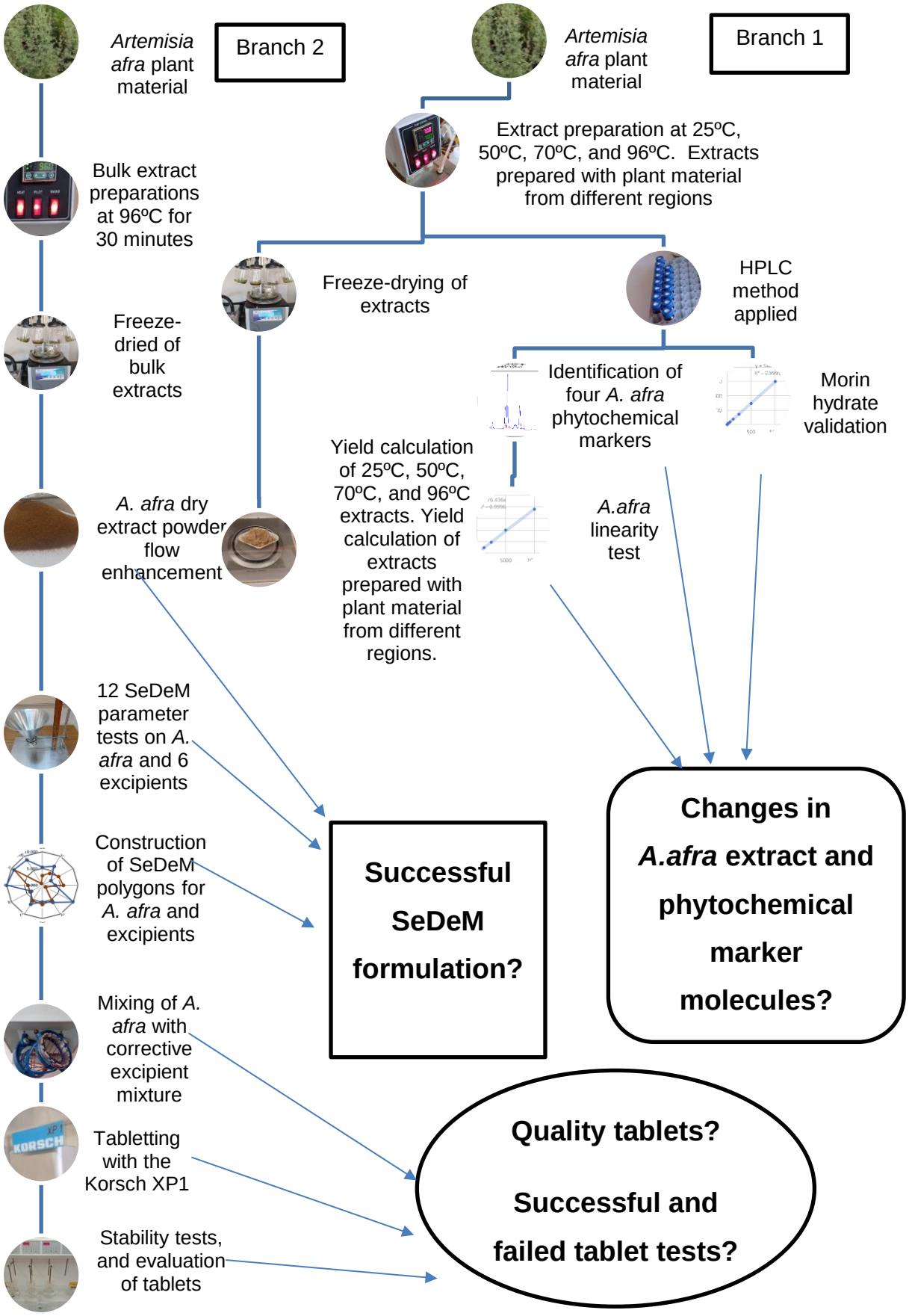

Figure 3.1: A graphical illustration and overview of the experimental layout of this study

3.2 Materials

The materials, suppliers and batch numbers of materials used in the study are given in Table 3.1.

Table 3.1: Materials and information on the materials used in the study

Type of material	Material name	Supplier	Batch number
Internal standard	90% Morin hydrate	Sigma-Aldrich	MKCK2209
Solvent	Methanol	Merck (LiChrosolve gradient grade)	STBH4057
Solvent	Formic acid	Supelco (LCMS grade)	1026770
Plant material	*Artemisia afra* leaves and twigs	Bronkhorstspruit Bay area, and Potchefstroom Airfield area, South Africa	Herbarium specimen numbers PUC0015455 PUC0015456
Disintegrant	Croscarmellose sodium (Ac-di-sol)	FMC International, little island, Co. Cork, Ireland.	T017C
Lubricant	Magnesium stearate	Warren Chem Specialties, Cape Town, South Africa	624489
Wetting agent and solvent	Deionised water	Prepared in house using a Rephile water purification system	
Binder/Filler	Ludipress®	BASF The Chemical Company, Ludwigshafen, Germany	16355416K0
	Avicel® PH 200	FMC International Wallingstown. Little Island. Corc	M939C
	Emcompress®	BASF The Chemical Company, Ludwigshafen, Germany	84512
	Tricalcium citrate	Jungbunzlauer, Ladenburg, Germany	8051454
	Kollidon® VA 64	BASF The Chemical Company, Ludwigshafen, Germany	93520356 v0
	MicroceLac® 100	Meggle Group, Wasserburg, BG Excipients & Technology	30020

3.3 Analytical method preparation and validation using morin hydrate

3.3.1 High-Pressure Liquid Chromatography (HPLC) method

An HPLC method was developed and validated with regards to range, linearity, accuracy, precision, LOD and LOQ. The method was developed on a Shimadzu i-Nexera LC-2040 (Japan) HPLC instrument. Various solvent systems and gradient profiles were tested using different columns to separate the phytochemical constituents. An Agela XBD C18 (2) 150 mm X 2.1 mm (Agela Technologies, China) column provided the best separation at a 0.4 mL/min flow rate and an oven temperature of 30°C. The injection volume was 10 µL. A stepwise gradient system was employed consisting of water containing 0.1% v/v formic acid (A) and methanol (MeOH) containing 0.1% v/v formic acid (B). A gradient was established that comprised for the first 6 min 30% v/v (B), which was increased to 40% v/v (B) at 6 – 17 min, 100% v/v (B) at time 17 – 19 min, and 30% v/v (B) at time 19 – 21 min for a total run time of 21 min.

3.3.2 Preparation of morin hydrate stock solution

Morin hydrate (plant flavanol) was used as an internal standard after meeting different criteria, including the following: it is not naturally present in *A. afra* and does not interfere or overlap with other compounds during HPLC analysis. A stock solution of morin hydrate was prepared by weighing 50.2 mg of morin hydrate in a volumetric flask and making it up with methanol to a volume of 50 mL to provide a solution with a concentration of 1.004 mg/mL. The stock solution was covered with foil and stored in a fridge at a temperature between 2 – 8ºC. Serial dilutions from the stock solution were prepared to provide dilutions with a concentration range between 0.98 – 1 004 µg/mL of morin hydrate.

3.3.3 Validation of analytical method with morin hydrate

The analytical method was validated to ensure that the results obtained from the HPLC chromatograms were accurate, reliable, and reproducible. Method validation provided reliability during the analysis and quantification of the four *A. afra* phytochemical marker molecules. Quantification was expressed as morin hydrate equivalents per gram of dry extract weight (MHE/g). Morin hydrate was validated with regards to linearity, range, accuracy, precision (LOD), and (LOQ).

3.3.3.1 Linearity and range

Linearity indicates the ability of a method to provide reliable results where the analyte concentration is directly proportional within the tested range. The range is the interval from the highest to the lowest analyte concentration (Shabir, 2003). Linearity was determined by injecting ten serial dilutions in duplicate over a predetermined concentration range (0.98 µg/mL – 1004 µg/mL). The dilutions were prepared using a pipet to transfer 5 mL of the stock solution into a 10 mL volumetric flask and making

it up to 10 mL by adding methanol, providing a 50% dilution. Five mL was withdrawn from the diluted solution and made up to 10 mL for the next serial dilution. This process was repeated 10 times to obtain morin hydrate concentrations of 1 004, 502, 251, 125.5, 62.75, 31.38, 15.69, 7.84, 3.92, 1.96, and 0.98 µg/mL. The dilutions were shaken and sonicated for 5 min. To determine the linearity of the calibration curve, Equation 3.1 was used. Generally, a correlation coefficient (r^2) higher than 0.998 is acceptable (Singh, 2013).

$$y = mx + c \qquad \text{Eq. 3.1}$$

Where y is the peak area value derived from the chromatogram, m is the slope of the standard curve, x is the concentration of the analyte injected, and c is the intercept.

3.3.3.2 Precision

Precision indicates how repeatable the method is by showing the degree of scattering between a series of measurements from multiple samples. The percentage relative standard deviation (%RSD) between the samples should not exceed 2% (Shabir, 2003). Intra-day precision was done with triplicate injections of three different morin hydrate concentrations (0.98, 15.68 and 251 µg/mL), three times during the same day, whereas inter-day precision was done by injecting three different concentrations (0.98, 15.68 and 251 µg/mL) in triplicate, daily, for three consecutive days to determine the %RSD.

3.3.3.3 Accuracy

Accuracy shows how close the result of the analytical method is to the actual value. Accuracy was determined after comparing triplicate injections of a low, intermediate and a high concentration with the actual value. Accuracy was measured by calculating the percentage recovery. An average recovery of 100 ± 2% was required (Shabir, 2003). Higher accuracy is generally associated with fewer errors (Singh, 2013).

3.3.3.4 Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection (LOD) is the lowest detectable concentration of an analyte. Limit of quantification (LOQ) is the lowest analyte concentration where reliable results can be obtained with acceptable accuracy and precision. LOD can be calculated with Equation 3.2 and LOQ with Equation 3.3. In both calculations, S is the slope of the morin hydrate calibration curve, and S.D. is the standard deviation based on the blank, also known as background noise (Shabir, 2003).

$$\text{LOD} = 3.3 \times \frac{\text{SD}}{\text{S}} \qquad \text{Eq. 3.2}$$

$$\text{LOQ} = 10 \times \frac{\text{SD}}{\text{S}} \qquad \text{Eq. 3.3}$$

3.4 Artemisia afra

3.4.1 Collection and handling and storage of *A. afra* plant material

The *A. afra* plant material used for formulation studies was collected in February of 2021 at the Bronkhorstspruit dam area in the province of Gauteng, South Africa. The plant material was transported to the Potchefstroom Campus of the North-West University (NWU), South Africa. A plant sample was taken to the NWU Botany department, where the herbarium specimen number (PUC0015456) was deposited. All twigs and leaves were stripped from the fresh plant material after removing foreign materials such as grass and ground. The twigs and leaves were spread out in a dark room and air dried for 4 weeks at 25ºC. Approximately 500 g of fresh twigs and leaves were weighed and kept separately to determine the water loss due to drying. All the plant material used to prepare bulk *A. afra* extracts came from one batch of plant material. Approximately 3 kg of dried twigs and leaves were sieved and packed into brown boxes to be stored in a cool, dry room. The plant material from Bronkhorstspruit was readily available in large amounts, and was subsequently selected for bulk extract preparation

To determine the variation in the phytochemical composition of *A. afra* plants as a function of location, three batches of air-dried *A. afra* twigs and leaves were collected from three different locations. Batch 1 was a commercial sample of *A. afra* plant material obtained from SUNfarming South Africa Pty (location 1). Batch 2 was collected from the wild in the Potchefstroom Airfield area, South Africa (location 2, herbarium specimen number PUC0015455), whereas batch 3 was collected from the wild in the Bronkhorstspruit Bay area, South Africa (location 3, herbarium specimen number PUC0015456).

3.4.2 Identification of four phytochemicals in the traditional *A. afra* tea infusion

An aqueous extract was prepared by adding *A. afra* twigs and leaves to water a temperature of 96°C for 10 min (traditional method). The 4 phytochemical markers with the highest peaks were identified with HPLC analysis.

3.4.3 Linearity analysis for *A. afra*

An *A. afra* extract was prepared, and a range of diluted solutions analysed with HPLC to prove that the selected phytochemical marker molecules within the extract exhibited linearity regarding instrument response and concentration within a particular concentration range. Nine serial dilutions of the extract were prepared and analysed. A standard curve was constructed from the data obtained and linear regression was used to confirm linearity.

3.4.4 Plant material and sample preparation

To identify the most appropriate temperature for bulk extract preparation, volumes of 100 mL distilled water was added to 250 mL glass beakers and placed inside a water bath with the temperature set on either 25ºC, 50ºC, 70ºC, or 96ºC. An external thermometer was used to measure the temperature inside the glass flask. When the water reached the required temperature, approximately 5 g of *A. afra* plant material was added to the 250 mL beaker, stirred for 10 s and infused for 30 min to prepare an infusion or tea. Using a syringe, 5 mL of aqueous extract was withdrawn from each infusion and filtered through a 0.45 µm syringe filter into an HPLC vial. The same method of preparation was used to determine phytochemical variation between three batches obtained from different locations, the only difference being that extracts were all prepared at 96ºC.

3.4.5 Yield calculation of dry powder extract

To determine the dry powder yield for each *A. afra* aqueous extract prepared, the remaining liquid infusions (95 mL) were filtered, frozen at -80°C, and freeze-dried for 72 h using a VirTis advantage benchtop freeze-dryer (S.P. Industries, Inc., PA, USA). The dried powders were weighed individually. Dried extracts were placed into sealed vessels and placed in a desiccator with external silica to protect them from humidity and contamination. Extracts were stored in a cool and dark environment. The percentage yield of each dry powder extract prepared was calculated using Equation 3.4.

$$\% \text{ yield} = \frac{\text{mass of dry extract}}{\text{mass of dry plant material}} \times 100 \qquad \text{Eq. 3.4}$$

3.5 Quantification of phytochemical marker molecules

3.5.1 Morin hydrate added to *A. afra* infusions (teas)

Using a pipette, a 500 µL sample was withdrawn from each *A. afra* aqueous extract prepared at different temperatures (25ºC, 50ºC, 70ºC, or 96ºC), or extracts prepared with *A. afra* plant material from different regions (as described in section 3.3.2.4) and inserted into an HPLC vial. Each vial was spiked with 200 µL of the morin hydrate stock solution (as described in section 3.3.2.1), which resulted in a total volume of 700 µL per vial to be analysed by HPLC.

3.5.2 Calculation of four *A. afra* phytochemical marker concentrations

The linear regression equation was used to calculate the concentration for each one of the four *A. afra* phytochemical marker molecules. A 1,004 mg/mL morin hydrate solution (200 µL) was added to the *samples of the A. afra* extract (500 µL). To compensate for dilution, the concentration was multiplied

by a factor of 1.4 resulting from adding the morin hydrate stock solution to the vials. Equation 3.5 was used to calculate all marker molecule concentrations.

$$x = \frac{y - c}{m} \times 1.4 \qquad \text{Eq. 3.5}$$

Where x was the concentration of the extract to be calculated, y was the peak area value derived from the chromatogram, c the intercept, and m the slope of the morin hydrate standard curve.

3.5.3 Calculation of mg morin hydrate equivalents (MHE) for each selected phytochemical marker molecule of *A. afra*

The milligram morin hydrate equivalents per gram of dry extract weight (mg MHE/g) was calculated for each of the four phytochemical marker molecules for teas made at 25ºC, 50ºC, 70ºC, and 96ºC, and for teas prepared with plant material from different regions at 96ºC. The weight of each freeze-dried yield sample that was calculated with Equation 3.4, was used in the formula. The concentration of each marker molecule calculated with Equation 3.5 was also used. The volume of 100 mL was the same for all extracts and was thus a constant in the equation to calculate the mg MHE/g shown in Equation 3.6 (Aryal *et al.*, 2019; Uddin *et al.*, 2012; Zhishen *et al.*, 1999).

$$C = \frac{cV}{m} \qquad \text{Eq. 3.6}$$

Where C is the content mg morin hydrate equivalent per gram of dry extract weight (mg MHE/g); m is the mass of the dried powder extract yield (g) calculated with Equation 3.4; c is the concentration of the phytochemical marker obtained from the morin hydrate calibration curve in milligram per millilitre (mg/mL) calculated with Equation 3.5; and V the volume of the extract (100 mL) (Bhandari & Rajbhandari, 2015; KUMAR[1] *et al.*, 2010).

3.6 Preparation of bulk *A. afra* extracts

After the appropriate extraction temperature was identified, multiple bulk extracts were prepared by adding 25 g *A. afra* twigs and leaves (Bronkhorstspruit batch) to 500 mL purified water that was infused for 30 min at the identified temperature. The aqueous infusions were filtered, frozen overnight at -80 ºC, and freeze-dried. Based on the number of experiments to be conducted during SeDeM characterisation, formulation and tablet production, it was calculated that a minimum amount of 250 g dry *A. afra* extract was needed for SeDeM EDS characterisation and tabletting.

3.6.1 Enhancement of *A. afra* extract powder flow

A two-step freeze-drying and sieving technique was used to enhance the flow of the *A. afra* extract powder. Approximately 5 g of the fluffy, low density, highly hygroscopic *A. afra* bulk extract powder

obtained from freeze-drying the infusion or liquid extract was wetted with a small amount of distilled water, frozen at -80 °C overnight, and freeze-dried for 72 h for a second time. An HPLC phytochemical analysis was done to compare the four phytochemical markers of the original dry extract to the freeze-dried extract obtained by the two-step freeze-dried process. Approximately 300 g of dry extract was wetted with 500 mL of purified water, frozen overnight at -80°C, and freeze-dried, providing one batch of approximately 300 g enhanced *A. afra* extract powder. The dry powder extract was sieved for 10 min with the Fritsch Analysette vibratory sieve shaker (Laborette model, Germany) in sieve sizes ranging between 710 µm and 45 µm. Powder particles within the size range of 45 – 710 µm were used for formulation studies. The sieved bulk extract of approximately 260 g was placed into a glass container and stored inside a sealed plastic container surrounded with silica until formulation.

3.7 Characterisation of *A. afra* powder extract and six excipients with the SeDeM EDS

3.7.1 Measurement of 12 SeDeM parameters

The 12 parameters used by the SeDeM EDS are all indicators of powder flow characteristics or compressibility, and the measurements were conducted as required by the SeDeM EDS. The SeDeM parameters that were determined include bulk density (Da), tapped density (Dc), interparticle porosity (Ie), Carr's index (Ic), cohesion index (Icd), Hausner ratio (I.H.), angle of repose (α), powder flow (%Pf), loss on drying (% H.R.), hygroscopicity (%H), particle size below 45 µm (%Fm), and the homogeneity index (Iθ) (Suñé-Negre *et al.*, 2008).

3.7.1.1 Bulk density (Da)

For most powders, a quantity of 100 g was added to a 250 mL graduated cylinder. For powders having density values lower than 0.4 g/cm^3, where the unsettled volume of 100 g was more than what could be contained in a 250 mL graduated cylinder, 50 g of powder was used. The unsettled volume was measured, and bulk density was calculated using Equation 3.7 (Suñé-Negre *et al.*, 2008).

$$Da = m/V_a \qquad \text{Eq. 3.7}$$

Where: Da is the bulk density, m is the mass of powder weighed; and V_a is the unsettled powder volume.

3.7.1.2 Tapped density (Dc)

The tapped volume of the powders was determined using an Erweka® tapped density tester (SVM 121/221, Erweka, Germany). The same 250 mL graduated cylinder used for bulk volume was used. The powder sample was tapped for 3 min (300 taps/min), and the settled volume was measured; after

that, the sample was tapped for periods with 1 min intervals until the tapped volume changed with less than 2% (Scholtz *et al.*, 2017). The tapped density was determined using Equation 3.8.

$$Dc = m/V_c \quad \text{Eq. 3.8}$$

Where: Dc is the tapped density; m is the mass of the powder weighed; and V_c is the tapped volume of the powder.

3.7.1.3 Cohesion index (Icd)

The cohesion index (Icd) was determined by compressing each powder at a maximum compression force using a Korsch® XP1 single station tablet press (Korsch®, Germany) with a 10 mm flat faced punch and die set. The hardness of 10 tablets was determined using an Erweka® TBH425 tablet hardness tester (Erweka, Germany). For powders unable to be compressed due to excessive force being required or issues in powder flow, a 3.5% w/w mixture of colloidal silicon dioxide (0.14%), talc (2.36%), and magnesium stearate (1.00%) was added (Perez *et al*. 2006). The value of the Icd is equal to the average crushing strength of the ten tablets measured in Newton (N). The SeDeM upper limit is 200 N (Nofrerias *et al.*, 2019).

3.7.1.4 Carr's index (I.C.)

Carr's index is also known as Carr's compressibility index and measures the bridge strength and stability of a powder (Aulton & Taylor, 2013). Equation 3.9 was used to calculate Carr's index.

$$IC = \frac{Dc-Da}{Dc} \times 100 \quad \text{Eq. 3.9}$$

Where: I.C. is Carr's Index; Da is the bulk density; and Dc is the tapped density.

3.7.1.5 Inter-particle porosity (Ie)

The inter-particle porosity (Ie) was calculated using the bulk density and tapped density values. Equation 3.10 was used to determine the Ie.

$$Ie = \frac{Dc - Da}{Dc \times Da} \quad \text{Eq. 3.10}$$

Where: Ie is Inter-particle porosity; D_c is the tapped density; and D_a is the bulk density (Suñé-Negre *et al.*, 2008).

3.7.1.6 Hausner ratio (IH)

The Hausner ratio was determined using bulk and tapped density values in Equation 3.11).

$$IH = \frac{Dc}{Da} \quad \text{Eq. 3.11}$$

Where I.H. is the Hausner ratio, Dc is the bulk density, and Da is the tapped density (Suñé-Negre *et al.*, 2008)

3.7.1.7 Angle of repose (α)

A mass of 100 g powder was allowed to flow through a funnel attached 20 cm above a level table. First, the diameter as well as the height of the powder cone were determined. Next, the radius was calculated by halving the measured diameter (Scholtz *et al.*, 2017). Finally, the angle of repose was determined using Equation 3.12.

$$\tan(\alpha) = \frac{h}{r} \quad \text{Eq. 3.12}$$

Where h is the height of the powder cone; and r is the radius of the powder. (B.P., 2021).

3.7.1.8 Flowability (t")

To test flowability, an Erweka® GLA powder and granulate flow tester was used where 100 g of powder was allowed to flow through a funnel with a 15 mm opening. The time taken for the powder to completely flow through the opening was noted in seconds. Results were calculated using Equation 3.13 to present results in grams per second (g/s). The SeDeM system allows up to 20 s for the cylinder to empty (B.P., 2021; Pérez *et al*., 2006).

$$t" = \frac{\text{grams}}{\text{Second}} \quad \text{Eq. 3.13}$$

Flowability (t") will be quantified in grams per second (g/s).

3.7.1.9 Loss on drying (% H.R.)

Three accurately weighed powder samples of 1 – 2 g for all the excipients and *A. afra* were dried in an oven at 105 ± 2ºC for 180 min (Pérez *et al.*, 2006). The loss on drying for each powder was measured as a percentage mass lost after drying, calculated with Equation 3.14 (BP, 2021).

$$\%HP = 100 - \left(\frac{\text{Powder mass after drying}}{\text{powder mass before drying}}\ 100\right) \quad \text{Eq. 3.14}$$

3.7.1.10 Hygroscopicity (%H)

Hygroscopicity (%H) was determined by measuring the average increase in weight of three 1 – 2 g powder samples after being placed in a climatic chamber (Binder KMF240) for 24 h at a temperature of 22 ± 1°C and a relative humidity of 76% ± 2% (Pérez *et al.*, 2006). The difference between the initial

weight of the powder and the weight after 24 h was expressed as a percentage using Equation 3.15 (Scholtz *et al.*, 2017).

$$\%H = 100 - \left(\frac{\text{Powder mass after climate rooms}}{\text{Powder mass before climate rooms}} \times 100\right) \quad \text{Eq. 3.15}$$

3.7.1.11 Percentage of particles measuring <45 µm l (%Pf)

In the original published article on the SeDeM EDS, a sieve test described by Pérez *et al.* (2006) was used to calculate the percentage of particles smaller than 50 µm; however, a quicker and easier method with the use of laser diffraction was used by Scholtz *et al.* (2017). A Malvern® Mastersizer 2000 instrument that was fitted with a Hydro 2000SM dispersion unit was employed together with a dispersant (either water, ethanol or cyclohexane) in which the sample is insoluble. All results were grouped in different particle size fractions, namely, the percentage particles between 0 µm – 45 µm, 46 µm – 106 µm, 106 µm – 212 µm, 212 µm – 355, 355 – 500 µm, 500 – 710 µm and larger than 710 µm. This data was used to determine the parameter of particle size smaller than 45 µm as well as the homogeneity index (Scholtz *et al.*, 2017).

3.7.1.12 Homogeneity index (Iθ)

The size and shape of the particles play an important role in mixing a powder with the excipients. Particle size also affects compressibility. It can similarly affect the dosage form's active ingredient and dissolution behaviour (BP, 2021). Equation 3.16 was used to determine the homogeneity index.

$$I\Theta = \frac{F_m}{100 + (d_m - d_{m-1})F_{m-1} + (d_{m+1} - d_m)F_{m+1} + (d_m - d_{m-2})F_{m-2} + \ldots + (d_m - d_{m-n})F_{m-n} + (d_{m+n} - d_m)F_{m+n}} \quad \text{Eq. 3.16}$$

Where: Iθ is the relative homogeneity index; F_m is the % of particles in the majority range; F_{m-1} the % of particles in the range immediately below the majority range; F_{m+1} the % of particles in the range immediately above the majority range; n is the order number of the fraction understudy within a series, with respect to the majority fraction; d_m is the mean diameter of the particles in the majority fraction; d_{m-1} is the mean diameter of the particles in the fraction of the range immediately below the majority range; and d_{m+1} is the mean diameter of the particles in the fraction of the range immediately above the majority range (Pérez *et al.*, 2006; Suñé-Negre *et al.*, 2008). (Aguilar-Díaz, García-Montoya, Pérez-Lozano, Suñé-Negre, Minarro*, et al.*, 2014)

3.7.2 Calculation of SeDeM EDS radius values and indices and polygon construction

After the 12 SeDeM EDS parameters were calculated, the results were converted into radius values between 0 – 10 to create a SeDeM EDS polygon. Polygons were constructed for the *A. afra* powder (API) and for all six excipients that were subject to SeDeM EDS characterisation. The polygon provides a visual illustration of the strengths and weaknesses of each powder. Each excipient polygon was

overlaid with an *A. afra* polygon to visually compare the 12 parameters. A radius value of 10 is the upper limit for each SeDeM parameter (Nofrerias *et al.*, 2019; Scholtz *et al.*, 2017).

The 12 SeDeM parameters were grouped into five incidences: dimensional factor, compressibility factor, flowability/powder flow factor, lubricity/stability factor, and lubricity/dosage factor. Table 3.2 shows which parameters are grouped into each of the five incidences and the factor applied to calculate the radius values for each parameter (Nofrerias *et al.*, 2018; Suñé-Negre *et al.*, 2008).

Table 3.2: Incidences, conversion factors and limits for the 12 parameters to obtain radius values (r) (Pérez *et al*., 2006)

Incidence	Parameter	Limit value (v)	Radius (r)	Factor applied to v
Dimensions	Bulk density	0 – 1	0 – 10	10v
	Tapped density	0 – 1	0 – 10	10v
Compressibility	Inter-particle porosity	0 – 1.2	0 – 10	10v/1.2
	Carr's index	0 – 50	0 – 10	v/5
	Cohesion index	0 – 200	0 – 10	v/20
Flowability/powder flow	Hausner ratio	3 – 1	0 – 10	(10 - (10v/3)
	Angle of repose	50 – 0	0 – 10	10 - (v/5)
	Powder flow	20 – 0	0 – 10	10 - (v/2)
Lubricity/stability	Loss on drying	10 – 0	0 – 10	10 – v
	Hygroscopicity	20 – 0	0 – 10	10 - (v/2)
Lubricity/dosage	Particles < 50 µ	50 – 0	0 – 10	10 - (v/5)
	Homogeneity index	0 – 2 x 10-2	0 – 10	500v

3.7.3 Additional SeDeM EDS indices

Based on the SeDeM EDS, additional indices were calculated to evaluate the suitability of the powder mixtures with respect to direct compression. The indices are the parameter index (IP), parameter profile index (IPP), and good compressibility index (IGC).

Parameter index (IP)

The parameter index (IP) was calculated with Equation 3.17.

$$(IP) = \frac{\text{No. } p \geq 5}{\text{No. Pt}} \qquad \text{Eq. 3.17}$$

Where: No. p is the number of parameters ≥ 5 and No. Pt is the total number of parameters. A value for IP ≥ 0.5 is considered suitable for direct compression (Pérez *et al.*, 2006).

Parameter profile index (IPP)

The parameter profile index (IPP) was calculated with Equation 3.18.

(IPP) = Mean value of all the parameters Eq. 3.18

A value of IPP ≥ 5 is considered suitable for direct compression.

Good compression index (IGC)

The good compression index was determined with Equation 3.19.

$$(IGC) = IPP \times f \qquad \text{Eq. 3.19}$$

IPP is the Parameter profile index, and f is the reliability factor determined by Equation 20.

$$f = \frac{\text{polygon area}}{\text{circle area}} \qquad \text{Eq. 3.20}$$

A value of IGC ≥ 5 is considered acceptable for direct compression (Pérez *et al.*, 2006)

3.8 Corrective excipients for *A. afra* tablet mixture

3.8.1 Calculation of corrective excipient amount

The profile of the *A. afra* dry powder extract in terms of direct compression was evaluated, and the deficient properties of the powder were identified. Then, a corrective quantity for the potential corrective excipients was calculated based on the deficient properties. The five incidences (dimensional factor, compressibility, flowability/ powder flow, lubricity/stability, and lubricity/dosage) of the *A. afra* powder extract were used to calculate the corrective excipient quantities to correct all the problem areas (i.e. incidences below a value of 5). The percentage corrective excipient to be included in the formulation was calculated using Equation 3.21 (Pérez *et al.*, 2006).

$$(CP = 100 - (\frac{RE-R}{RE-RP}X100) \qquad \text{Eq. 3.21}$$

Where: CP is the % of corrective excipient needed; RE is the mean-incidence radius value of the corrective excipient; R is the mean incidence value to be obtained in the blend (5); and RP is the mean-incidence radius value of the *A. afra* powder to be corrected (Suñé-Negre *et al.*, 2008).

3.8.2 Selection of the most appropriate corrective excipient

Excipients that were unable to compensate for the poor characteristics of the *A. afra* extract were excluded from consideration as a primary corrective excipient. The excipients deliberated suitable as a corrective excipient for *A. afra* extract powder were compared. The excipient that allowed the highest percentage of *A. afra* in the powder formulation was furthermore identified.

3.8.3 Selection of a lubricant, disintegrant, and binder

The 3.5% w/w lubricant mixture of colloidal silicon dioxide (0.14%), talc (2.36%), and magnesium stearate (1.00%) recommended by the SeDeM EDS was used as a lubricant for the corrective mixture

(Perez *et al.* 2006). Croscarmellose sodium (5% w/w) was used as the disintegrant, and Kollidon® VA 64 (3% w/w) was added as a binder to the corrective mixture.

3.8.4 SeDeM EDS applied to the corrective mixture

The corrective powder mixture was prepared by mixing the primary corrective excipient with the lubricant, binder and disintegrant in the Turbula® mixer (type T2B, Willy A. Bachofen Machinenfabrik, Switzerland) for 5 min at a speed of 69 rpm. The mixture was again subjected to SeDeM EDS characterisation. Incidences and additional indices were calculated to determine what percentage *A. afra* can be added to obtain the final powder mixture intended for tablet manufacturing.

3.9 Preparation of tabletting powder mixture and tabletting

3.9.1 Mixing of the final powder mixture and tabletting

After SeDeM EDS characterisation, the final corrective mixture (70% w/w) was added to the *A. afra* dry powder extract (30% w/w) and was mixed in a glass container for 5 min at a speed of 69 rpm with the Turbula® mixer (type T2B, Willy A. Bachofen Machinenfabrik, Switzerland). The final tablet formula contained 30% w/w *A. afra* dry powder extract.

3.9.2 Calculation of *A. afra* dosage and tablet weight

The method used to determine the quantity of *A. afra* extract per tablet is described here. The dried *A. afra* twigs and leaves had a loss in weight of approximately 75% after four weeks of air-drying. A quarter cup of fresh leaves and twigs (traditional measuring method) weighed approximately 8 g (2 g when dried). A traditional tea was prepared, and the extract was frozen overnight at -80ºC where after it was freeze-dried. The yield was approximately 400 mg. With the API (*A. afra*) making up 30% of the final tablet mixture, a single tablet containing 400 mg of *A. afra* dry extract will have a mass of approximately 1 333 mg, representing a relatively large tablet. The majority of patients may experience difficulty in swallowing a large tablet. Therefore, based on this consideration, the decision was taken to formulate tablets containing 200 mg *A. afra* extract, implying that two tablets will represent the corresponding quantity of *A. afra* extract on average consumed if a traditional *A. afra* tea infusion was to be prepared.

3.9.3 Compression of *A. afra* extract containing tablets

Tabletting was done using a Korsch® XP1 single punch tablet press (Korsch, Germany), connected to a PharmaResearch® unit. Tablets were formulated to contain 200 mg *A. afra* extract using 12 mm diameter flat faced punch and die tooling. The formulated tablet weight was 667 mg. A tablet batch of approximately 800 tablets was compressed at 10 strokes/min. Tablets were packed into 13 amber

containers comprising 60 tablets each. Silica bags were inserted inside each container, ready for stability testing and evaluation in terms of assay, weight variation, hardness, friability, disintegration, and dissolution behaviour.

3.9.4 Evaluation of tablets and stability testing

The tablets were evaluated with respect to physical characteristics and dissolution behaviour according to the specifications of the BP (2021). Approximately 24 h after tabletting, the first container with 60 tablets (week 0) was evaluated with regards to weight variation, hardness, friability, disintegration, and dissolution behaviour. In addition, an assay was done to compare the tablets with the original dry extract.

Climatic chambers (Binder KMF240) were used to test stability under the following conditions: 25°C/60% relative humidity and 40°C/75% relative humidity. Six labelled containers containing 60 tablets each were placed in the respective climatic chambers. Stability testing was conducted for 3 months (12 weeks). Sampling was conducted at the following time points: 1, 2, 3, 4, 8, and 12 weeks. One of the six containers was removed from each of the two climatic chambers at each time point for stability evaluation. Stability evaluation included the same tests as for the tablets at week 0.

3.9.4.1Assay

Currently, there are no official assay specifications for tablets containing *A. afra*. Ten tablets were crushed with a pestle in a mortar to produce a powder for assay purposes. A quantity of approximately 667 mg crushed powder (weight of one tablet) was transferred to a 50 mL volumetric flask, made up to 50 mL with distilled water, and stirred for 20 min in an ultra-sonic bath to disperse and dissolve the extract. A quantity of 667 mg of tablet powder theoretically contained 200 mg of *A. afra* dry extract. A volume of 5 mL from each dispersion was filtered and the selected marker molecules were quantified using HPLC. Results were expressed as a percentage loss of MHE/g as a function of time. Graphical illustrations were obtained with Microsoft® Excel to illustrate phytochemical marker breakdown throughout the 12 weeks of stability testing.

3.9.4.2 Friability

The friability of the tablets was determined using an Erweka® friabilator (Type TAR 220, Erweka GmbH, Heusenstamm, Germany). The friability test was conducted according to the specifications given in the BP (2021). Ten tablets were dusted and weighed with a Mettler Toledo® balance (Mettler, Switzerland, Model PB303-S) and rotated 100 times at 25 rpm for a total of 4 min. The tablets were removed, dusted, and weighed again. If tablets were broken or cracked, the sample failed the test. If the tablets did not lose more than 1% of their initial weight, they complied with specifications (BP, 2021).

Equation 3.18 was used to determine the percentage friability.

$$\text{Friability (\%)} = \frac{\text{- Weight after friability}}{\text{Initial tablet weight}} \times 100 \qquad \text{Eq. 3.18}$$

3.9.4.3 Disintegration

The specified apparatus and method described in the BP (2021) was used. The disintegration test was conducted using an Erweka® disintegration apparatus (Type ZT 323, Erweka GmbH, Heusenstamm, Germany). As specified for conventional tablets, an upper time limit of 15 min was allowed for complete tablet disintegration.

3.9.4.4 Crushing strength

The crushing strength was determined for 10 randomly selected tablets from each container. The tablet hardness of these tablets was measured in Newton (N), using an Erweka® TBH425 tablet hardness tester (Erweka GmbH, Heusenstamm, Germany).

3.9.4.5 Uniformity of tablet weight

The test for uniformity of weight was conducted according to the specifications from Appendix XII C of the BP (2021). For tablets with a mass of 250 mg or higher, it was required that no more than two tablets may deviate with more than 5% from the average tablet weight. Twenty tablets were randomly selected and gently brushed to remove any powder. Each tablet was weighed individually using a Mettler Toledo® balance (Mettler, Switzerland, Model PB303-S). The average tablet mass and percentage standard deviation (%RSD) were subsequently calculated.

3.9.4.6 Dissolution

Dissolution studies were conducted using Apparatus 2 of the BP (2021) fitted with paddles. Dissolution studies were done three-fold at 37ºC with a Distek® water bath dissolution system (model 2500, Distek® Inc., North Brunswick, the USA). As a dissolution medium, 900 mL of deionised water was used with a paddle speed of 50 rpm. Samples of 5 mL were withdrawn with a syringe at time intervals of 2, 5, 10, 15, 30, 60, 90, 120, 180, and 240 min and filtered through a 0.45 µm filter. Upon withdrawal of the sample at 240 min, the paddle speed was increased to 150 rpm for 15 min, and a sample was withdrawn to represent the infinity sample. HPLC analyses was conducted to quantify the marker molecules in the dissolution samples. Results were expressed as a percentage marker molecule released at each time interval. Dissolution profiles were constructed using Microsoft® Excel.

3.10 Summary

Experimental methods that were applied to conduct this study were discussed in this chapter. These methods included the technique applied to prepare an aqueous extract of *A. afra* plant material. Also, the SeDeM EDS methods applicable to the characterisation of the *A. afra* extract powder and the

preparation for an optimised powder mixture intended for direct compression is discussed. The approaches used to manufacture *A. afra* containing tablets as well as methods applicable to the evaluation of the tablets after manufacturing and stability testing were moreover discussed. As part of the evaluation of the tablets, an HPLC assay method based on the determination of morin hydrate equivalents per gram of dry weight (MHE/g) was also discussed.

The SeDeM EDS methods that were used to characterise the *A. afra* dry extract powder as well as various excipients were discussed. Methodology related to the assay of the prepared *A. afra* containing tablets were also presented.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter provides the results obtained from the research project, which have been processed, interpreted and discussed. The HPLC validation results for morin hydrate as an internal standard and the linearity results for the four phytochemical marker molecules identified in the *A. afra* plant are included. The A. *afra* yield percentages and the variation in quantity (i.e. mg morin hydrate equivalents per gram of dry extract) for each phytochemical marker molecule due to different aqueous extract temperatures are compared and discussed. The appropriate temperature for bulk extract preparation was identified. The phytochemical composition of *A. afra* plant material from three different regions were compared with regards to the dry powder extract yield and the amount of each of the four phytochemical marker molecules present in the extract.

The bulk *A. afra* dry powder extracts were prepared by an infusion technique followed by double freeze-drying. Sieving was used to obtain a fraction of the *A. afra* dry powder with improved powder flow properties. The *A. afra* dry powder extract and six powder excipients were characterised with the SeDeM Expert Diagram System (EDS) to provide a profile of powder flow properties. The suitability for direct compression was evaluated for each powder. Weaknesses in the *A. afra* powder profile was identified, and calculations were done. Six selected excipients were compared to identify the best excipient to mix with the *A. afra* powder in order to correct its weak powder flow properties. The identified excipient was again characterised with SeDeM EDS after a small percentage of a mixture of binder, disintegrant and a lubricant was added to prepare the final corrective mixture. Calculations were done to determine the percentage *A. afra* dry powder extract in the final tablet mixture. The *A. afra* dry extract was mixed with the corrective powder mixture and was finally characterised with SeDeM EDS to verify suitability for direct compression.

Tablets containing 200 mg *A. afra* extract were prepared and evaluated according to the BP criteria. Results for mass variation, friability, disintegration and dissolution behaviour are provided and discussed. Assay results from 200 mg dry *A. afra* extract were compared to that of the equivalent powder content of one tablet. The tests mentioned above were conducted on tablets 24 h after manufacturing as well as on tablets subjected to 12 weeks stability testing at two different accelerated stability conditions. Tablets subjected to accelerated stability conditions were evaluated after 1, 2, 3, 4, 8 and 12 weeks of exposure to these conditions. Subsequently, the results from the tablet evaluation tests over 12 weeks were compared and discussed.

4.2 Validation of the morin hydrate HPLC analytical method

To validate the analytical method using morin hydrate as an internal standard, the linearity, range, accuracy, precision, the limit of detection (LOD), and the limit of quantification were determined.

4.2.1 Linearity and range

Figure 4.1 depicts the regression data obtained from the average HPLC peak area values (y-axis) plotted as a function of concentration after 11 serial dilutions of morin hydrate (x-axis) were injected to obtain a standard curve. There was a linear relationship between the instrument response and analyte concentration with a correlation coefficient (R^2) of 0.999 over a range of 0.98 – 1 004 µg/mL. This R^2 value achieved complied with the requirement of ≥ 0.998 (Singh, 2013).

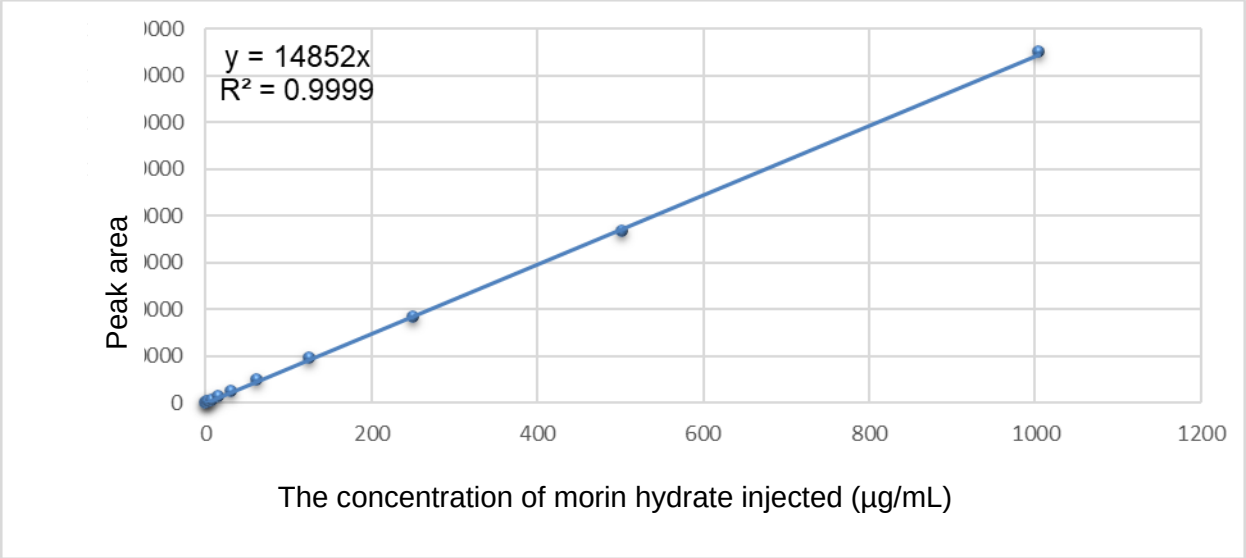

Figure 4.2: standard curve for morin hydrate between the range of 0.98 – 1 004 µg/mL

4.2.2 Precision

4.2.2.1 Intra-day precision

The retention times, peak area values, standard deviation (SD) and %RSD values were calculated for 3 morin hydrate concentrations (0.98, 15.68, and 251 µg/mL) obtained at three different time points within the same day. The results for intra-day precision are provided in Table 4.1. The % RSD values complied with the intra-day requirement of a % RSD < 2% (Shabir, 2003).

Table 4.1: Intra-day precision results for morin hydrate HPLC analytical method

(µg/mL)	Retention Time	Peak area	Average peak area	Standard deviation (SD)	Percentage relative standard deviation (% RSD)
0.98	13.6	16282	16301	168	1.03
	13.6	16516			
	13.7	16105			
15.68	13.6	250729	250306	359	0.14
	13.6	249850			
	13.7	250339			
251	13.6	3688477	3703175	11772	0.32
	13.6	3703751			
	13.7	3717297			

4.2.2.2 Inter-day precision

The retention times, peak area values, SD and %RSD values were calculated for 3 morin hydrate concentrations (0.98, 15.68, and 251 µg/mL) obtained over three days. The results for inter-day precision are provided in Table 4.2. The % RSD values complied with the inter-day requirement of a % RSD < 2% (Shabir, 2003).

Table 4.2: Inter-day precision results for morin hydrate HPLC analytical method

(µg/mL)	Retention Time	Peak area	Average peak area	Standard deviation	Percentage relative standard deviation (% RSD)
0.98	13.9	16083	16217	96	0.59
	13.6	16301			
	13.8	16267			
15.68	13.8	250761	250676	274	0.11
	13.6	250306			
	13.7	250961			
251	13.8	3648808	3691371	31075	0.84
	13.6	3703175			
	13.7	3722129			

4.2.3 Accuracy

Accuracy as expressed by recovery percentages derived and calculated for three morin hydrate concentrations (0.98, 15.68, and 251 µg/mL) are shown in Table 4.3. The average recovery complied with the requirement of 100 ± 2% (Shabir, 2003; Singh, 2013).

Table 4.3: Accuracy results for morin hydrate HPLC analytical method

Concentration (µg/mL)	Peak area (AUC)	Peak area (average)	% Recovery
0.98	16083	16199	100.11
	16301		
	16267		
15.68	250761	252175	99.86
	250306		
	250961		
251	3648807	3650043	99.68
	3703175		
	3722128		

4.2.4 Limit of detection (LOD) and limit of quantification (LOQ)

The SD determined was used to calculate the limit of detection (LOD) and limit of quantification (LOQ). The LOD was calculated as 0.066 µg/mL and LOQ as 0.201 µg/mL.

4.3 Artemisia afra

4.3.1 Identification of four phytochemicals in the *A. afra* tea infusion

An HPLC fingerprint was developed for *A. afra* extract to identify the 4 largest peaks that represent 4 phytochemical marker molecules that could be quantified throughout the study to represent the concentration of the extract. The chromatogram obtained for *A. afra* extract indicating the largest peaks of 4 phytochemical markers is shown in Figure 4.2. These 4 phytochemical marker molecules were quantified to compare changes in phytochemical composition of the *A. afra* extract after extraction at different temperatures and during stability studies. The 4 phytochemical marker molecules were quantified by determining the amount of mg MHE/g.

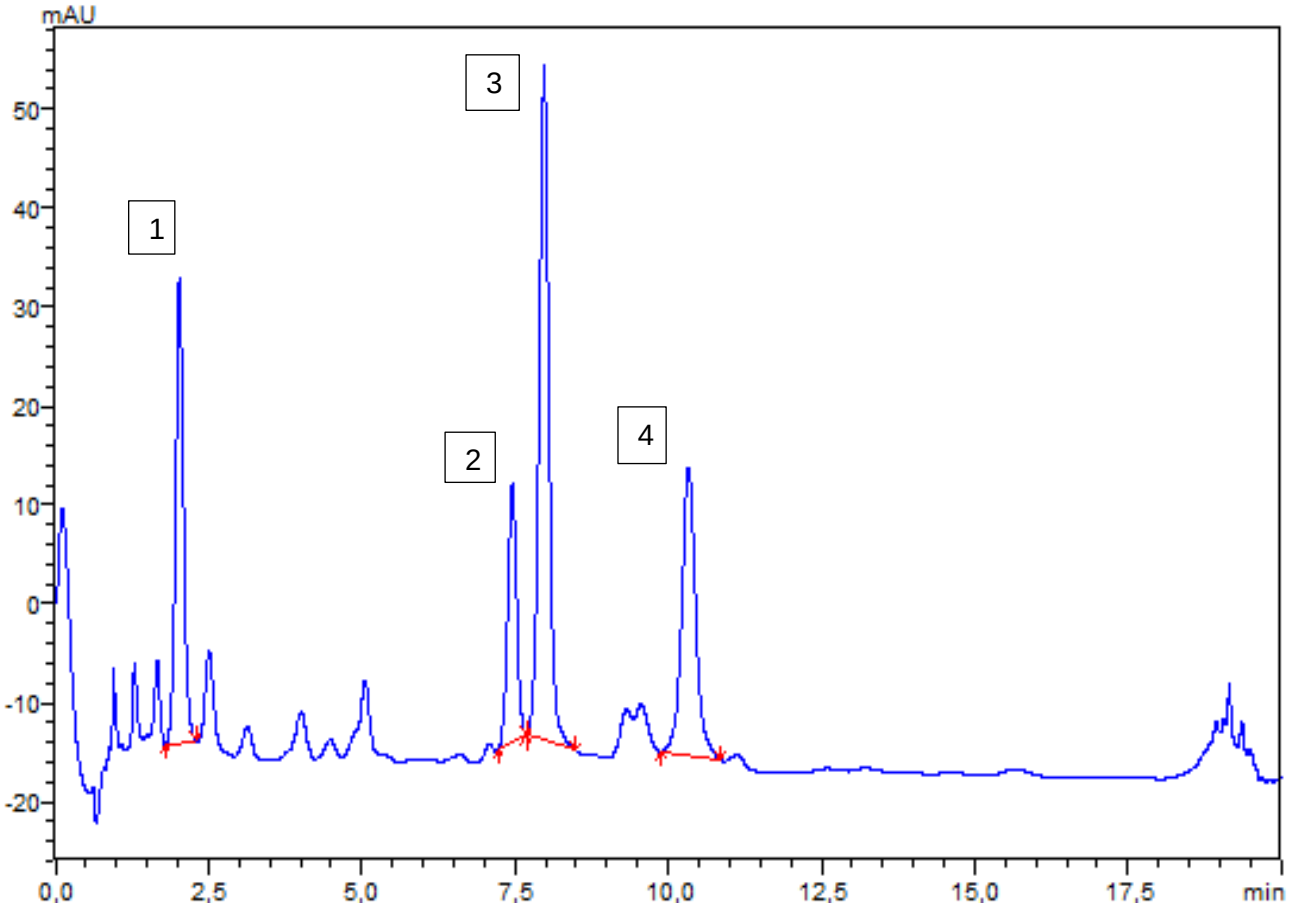

Figure 4.2: Chromatogram of *A. afra* with the peaks of four selected phytochemical marker molecules

4.3.2 Linearity for phytochemical marker molecules in *A. afra* extract

Linearity for the standard curves of each of the four phytochemical markers was determined. The regression data obtained from the average peak area values (y-axis) plotted as a function of concentration after 9 serial dilutions (39.063 – 10000 µg/mL) of *A. afra* (x-axis) were analysed and are shown in Figures 4.3 – 4.6. The standard curve slope was 764.36 for phytochemical marker 1; 541.97 for phytochemical marker 2; 1 573 for phytochemical marker 3; and 967.65 for phytochemical marker 4. There was a linear relationship between the instrument response and analyte concentration with a R^2 value of 0.999 over a range of 39.063 – 10 000 µg/mL for all 4 phytochemical markers. This obtained R^2 value complied with the requirement of $R^2 \geq 0.998$ (Singh, 2013).

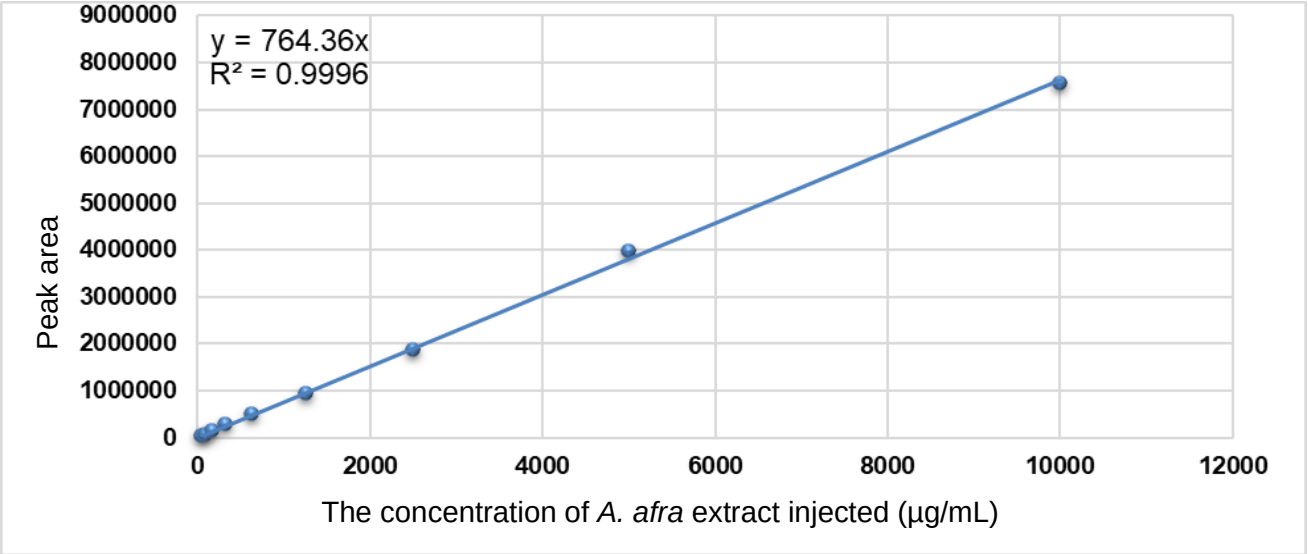

Figure 4.3*:* Regression data of *A. afra* phytochemical marker 1

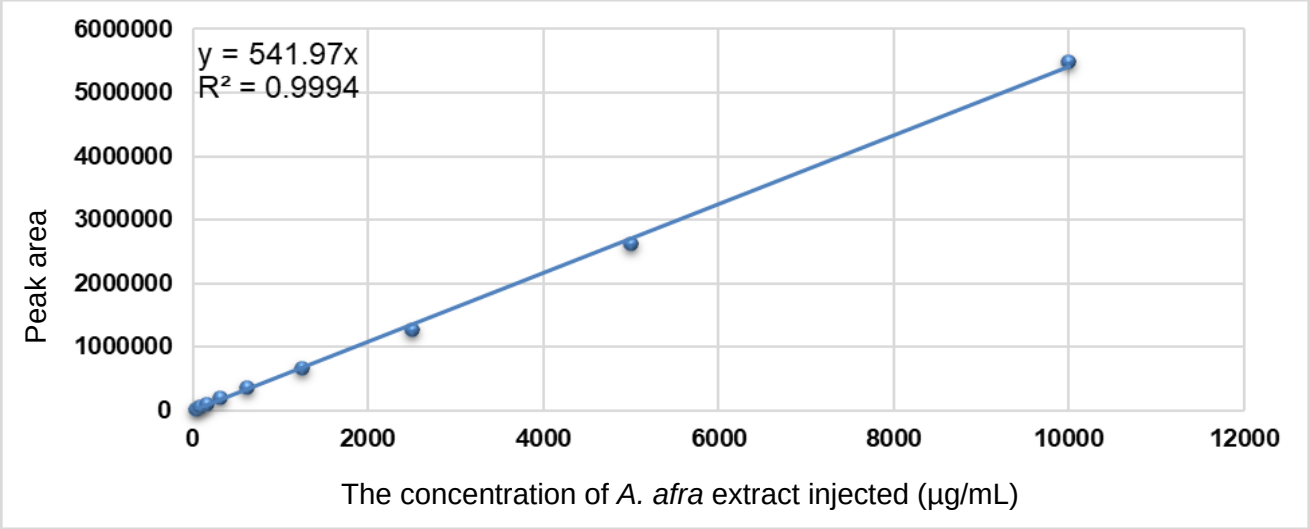

Figure 4.4*:* Regression data of *A. afra* phytochemical marker 2

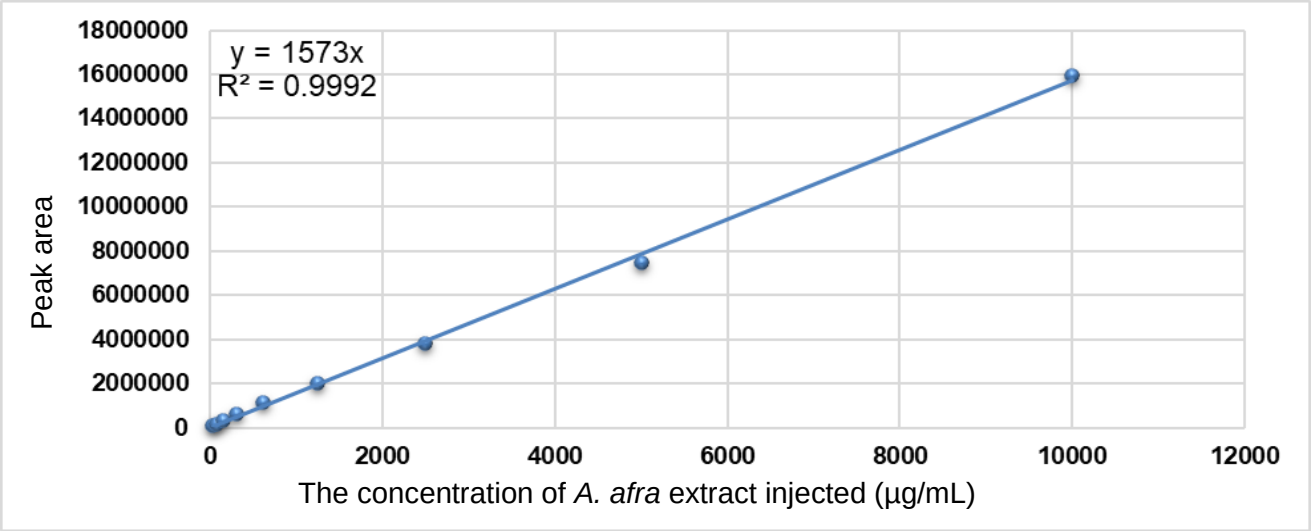

Figure 4.5*:* Regression data of *A. afra* phytochemical marker 3

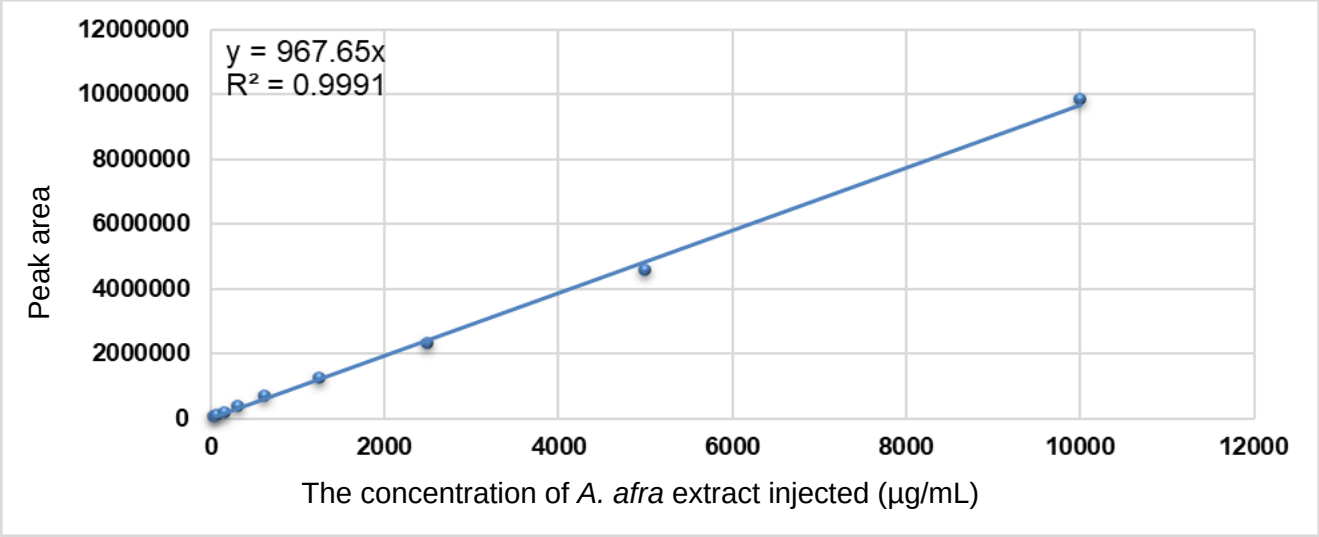

Figure 4.6*:* Regression data of *A. afra* phytochemical marker 4

4.3.3 Yield results of *A. afra* extracts prepared at different temperatures

The *A. afra* extract prepared at 96ºC exhibited the highest yield (1.354 g), followed by 70ºC (1.225 g), 50ºC (0.678 g), and 25ºC (0.233 g) as shown in Table 4.4. The results indicated that a higher yield of *A. afra* dry powder extract can be produced at the highest water temperature of 96ºC investigated as compared to the lower temperatures studied, namely 70ºC, 50ºC and 25ºC.

Table 4.4: Yield results for *A. afra* dry extracts at four different temperatures

Extract temperature (ºC)	Weight of *A. afra* twigs and leaves (g)	Water (mL)	Time (min)	Extract yield (g)	Yield percentage (%)
25	4.759	95	30	0.233	4.69
50	4.757	95	30	0.678	14.25
70	4.753	95	30	1.225	24.50
96	4.756	95	30	1.354	28.47

4.3.4 Quantification of four phytochemical marker molecules using morin hydrate equivalents

The average concentrations (µg/mL) were calculated by using the average peak area values of each phytochemical marker molecule on the HPLC chromatograms and the slope of the morin hydrate standard curve.

4.3.4.1 Calculating the quantity morin hydrate equivalents per gram of dry extract weight for *A. afra* extracts prepared at four different temperatures

The results in Table 4.6 show that *A. afra* extracts prepared at 96 ºC produced a higher amount of mg MHE/g compared to the *A. afra* extracts prepared at the lower temperatures.

Table 4.5: Average concentrations and mg of morin hydrate equivalents per gram of dry extract weight for *A. afra* extracts prepared at four different temperatures

***A. afra* phytochemical marker and temperature**	**Average concentration (mg/mL)**	**Mg MHE/g of dry extract weight**
phytochemical marker 1 25ºC	0.029	11.927
phytochemical marker 2 25ºC	0.002	0.907
phytochemical marker 3 25ºC	0.048	19.597
phytochemical marker 4 25ºC	0.002	0.967
Morin hydrate added to *A. afra* extract at 25ºC	0.074	69.131
phytochemical marker 1 50ºC	0.063	8.889
phytochemical marker 2 50ºC	0.007	1.029
phytochemical marker 3 50ºC	0.139	19.431
phytochemical marker 4 50ºC	0.014	1.968
Morin hydrate added to *A. afra* extract at 50ºC	0.073	70.369
phytochemical marker 1 70ºC	0.109	8.471
phytochemical marker 2 70ºC	0.021	1.629
phytochemical marker 3 70ºC	0.307	23.769
phytochemical marker 4 70ºC	0.05	3.913
Morin hydrate added to *A. afra* extract at 70ºC	0.073	69.571
phytochemical marker 1 96ºC	0.146	10.210
phytochemical marker 2 96ºC	0.067	4.732
phytochemical marker 3 96ºC	0.498	34.933
phytochemical marker 4 96ºC	0.148	10.415
Morin hydrate added to *A. afra* extract at 96ºC	0.073	70.658

4.3.4.2 Superimposed HPLC chromatograms for *A. afra* aqueous extracts prepared at different temperatures

HPLC chromatograms for different *A. afra* aqueous extracts prepared at different temperatures are superimposed in Figure 4.7. The difference in peak heights for each of the four phytochemical markers is visually presented. The morin hydrate peak (number 5) remained relatively constant as an equal amount of morin hydrate (200 µL) was added to each *A. afra* HPLC vial to serve as an internal standard. Extracts prepared at 96ºC exhibited the highest peaks for all four phytochemical markers, followed by preparation at 70ºC, 50ºC, and 25ºC, respectively. The *A. afra* extract prepared at 96 ºC gave the highest percentage yield (27.08%), highest peak heights, and higher mg MHE/g, which confirmed that the traditional method of preparing *A. afra* with boiling water (approximately 96ºC) is superior to lower temperatures in terms of phytochemical extraction. Consequently, bulk extracts of *A. afra* plant material for further use in this study were prepared at 96ºC.

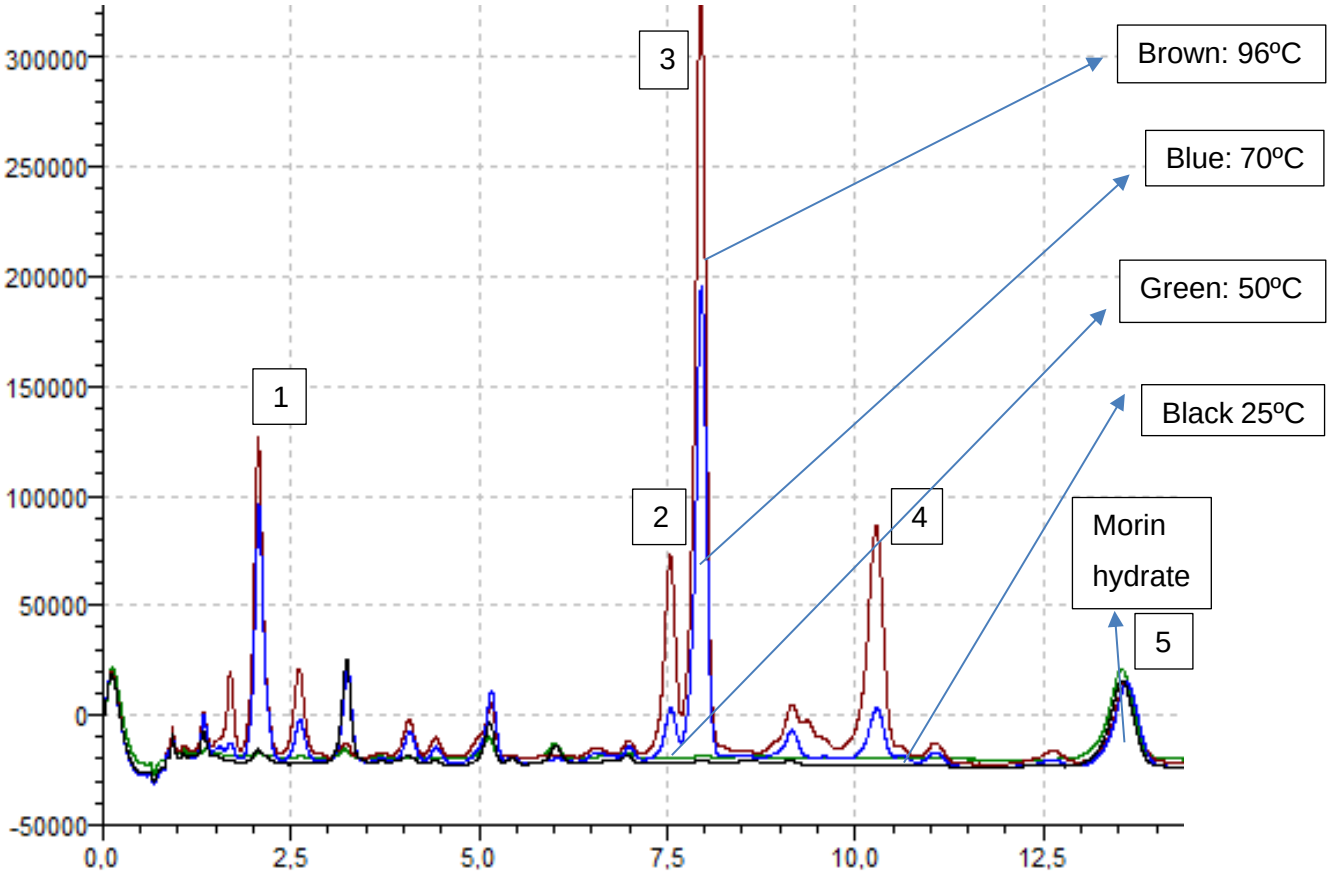

Figure 4.7: Superimposed HPLC chromatograms of the four *A. afra* phytochemical markers prepared at 4 different temperatures as well as morin hydrate

4.3.5 Visual differences and yield results of *A. afra* extracts from different regions

Figure 4.8 shows the visual differences between the 3 *A. afra* plant materials obtained from different locations, their aqueous extracts, and filtered extracts. The commercially grown plant material from SUNfarm SA Pty (A) had a distinctly lighter colour compared to the sample collected from the

Potchefstroom airfield area (B) and the plant material from the Bronkhorstspruit Bay area (C) was the darkest in colour.

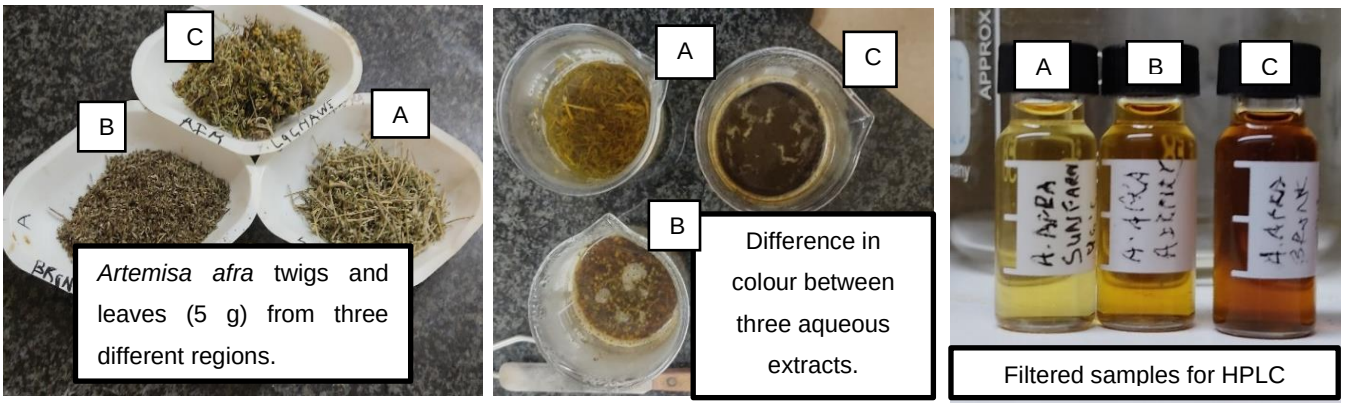

Figure 4.8: Visual differences between the three batches (from different regions) of *A. afra* plant materials, aqueous extracts, and filtered extracts

4.3.5.1 Yield results of *A. afra* extracts from different regions

Table 4.6 shows the dry *A. afra* powder extract results prepared with plant material from three different locations. The SUNfarming SA Pty extract yielded 1.312 g, which was the highest, followed by the extract made from the Bronkhorstspruit area plant material (1.164 g), and the plant material from the Potchefstroom airfield displayed the lowest yield (1.140).

Table 4.6: Yield results for *A. afra* extracts prepared with plant material from three different regions

***Artemisia afra* plant material source**	***A. afra* twigs and leaves (g)**	**Water (mL)**	**Time (min)**	**Extract yield (g)**	**Yield percentage (%)**
SUNfarming S.A Pty	4.756	95	30	1.312	27.59
Potchefstroom Airfield area	4.753	95	30	1.140	23.97
Bronkhorstspruit area	4.752	95	30	1.164	24.5

4.3.5.2 Calculation of mg of morin hydrate equivalents per gram of dry extract weight for *A. afra* extracts prepared with plant material from three different regions

There was variation in the amount of mg MHE/g for all 4 phytochemical marker molecules of *A. afra* extracts prepared from plant materials from 3 different regions. The *A. afra* extract prepared from the plant material obtained from SUNfarming SA Pty rendered the highest concentration of all 4 chemical marker molecules; with chemical marker molecule 1 being the most abundant. Notably, for the Airfield and Bronkhorstspruit samples, the highest concentration was found in chemical marker 3 and not chemical marker 1 when compared to the SUNfarming SA Pty sample. Average concentrations and

mg MHE/g of dry extract weight for all 4 chemical marker molecules from all the extracts can be seen in Table 4.7.

Table 4.7: Average concentrations and mg MHE/g of dry extract weight for *A. afra* extracts prepared with plant material from three different regions

***Artemisia afra* phytochemical marker and temperature**	**Average concentration (mg/mL)**	**Mg MHE/g of dry extract weight**
SUNfarming South Africa Pty phytochemical marker 1	0.219	24.335
SUNfarming South Africa Pty phytochemical marker 2	0.050	5.540
SUNfarming South Africa Pty phytochemical marker 3	0.219	23.848
SUNfarming South Africa Pty phytochemical marker 4	0.097	10.801
Morin hydrate added to SUNfarming South Africa Pty	0.078	75.011
Potchefstroom Airfield area phytochemical marker 1	0.058	6.431
Potchefstroom Airfield area phytochemical marker 2	0.036	3.960
Potchefstroom Airfield area phytochemical marker 3	0.127	14.049
Potchefstroom Airfield area phytochemical marker 4	0.051	5.671
Morin hydrate added to Potchefstroom Airfield area	0.077	74.988
Bronkhorstspruit area phytochemical marker 1	0.532	5.911
Bronkhorstspruit area phytochemical marker 2	0.026	2.898
Bronkhorstspruit area phytochemical marker 3	0.119	13.235
Bronkhorstspruit area phytochemical marker 4	0.056	6.261
Morin hydrate added to Bronkhorstspruit area	0.078	74.988

4.3.5.3 HPLC chromatograms of four *A. afra* phytochemical marker molecules prepared with plant material from three different regions, spiked with morin hydrate.

Figure 4.9 shows the chromatograms for each *A. afra* extract prepared with plant material obtained from a different region. The peaks of the 4 phytochemical marker molecules are numbered from 1 – 4, and morin hydrate is peak number 5. The chromatograms demonstrate that all 3 plant batches contained all 4 phytochemical marker molecules, but the peak heights were different.

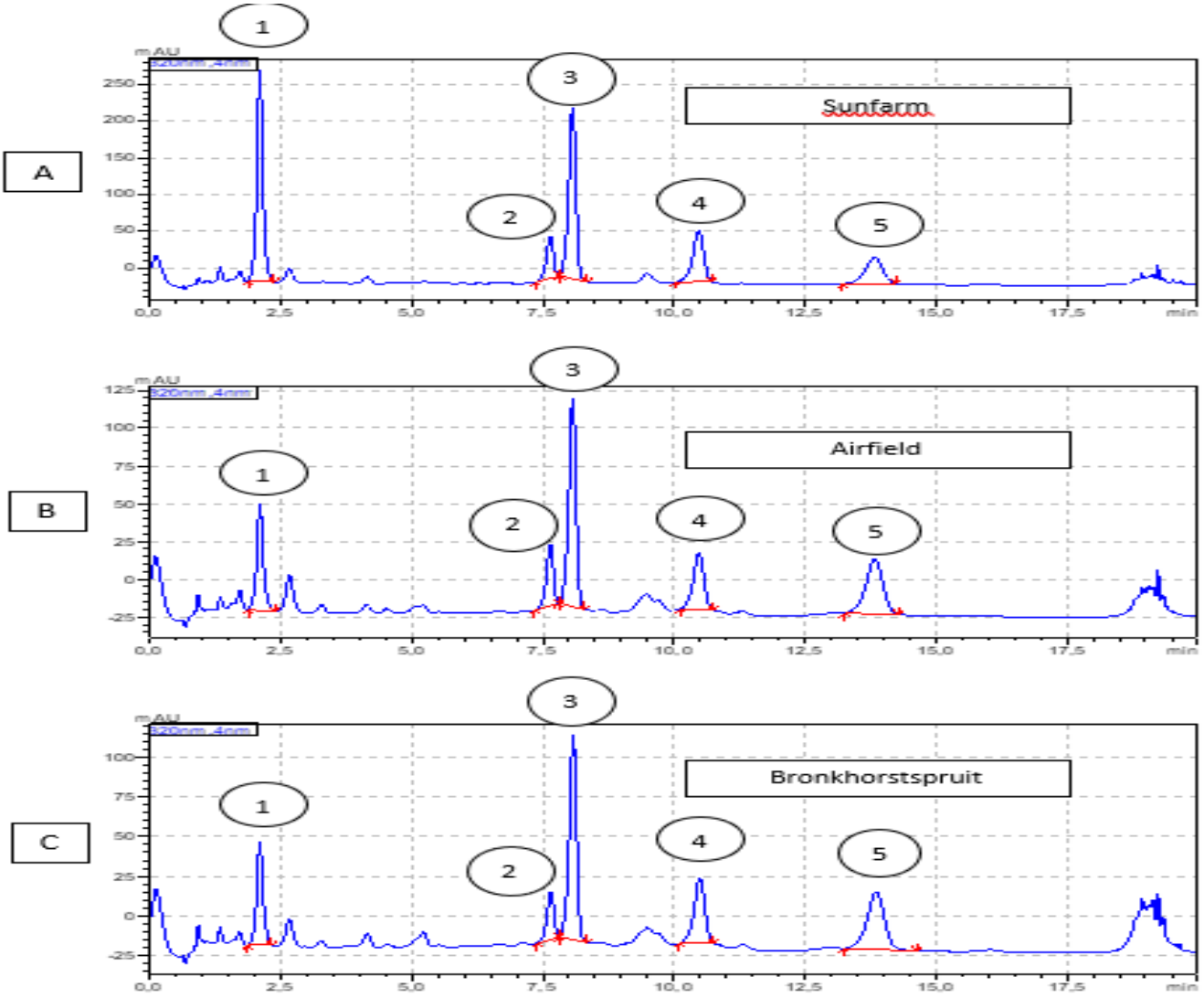

Figure 4.9*:* Chromatogram of *A. afra* samples, SUNfarming SA Pty (A), Potchefstroom airfield and (B), and Bronkhorstspruit (C), including the four phytochemical marker peaks (1 – 4) and the internal standard morin hydrate (5)

4.3.6 Preparation of bulk *A. afra* extracts

Bulk extracts were all prepared at 96ºC for 30 min. Twenty-five grams of *A. afra* twigs and leaves were infused in 500 mL water for 30 min. Extracts were frozen overnight at -80ºC, and the frozen extracts were consequently freeze-dried. The original *A. afra* dry powder extracts presented low density and

poor powder flow properties. The powder could not pass regular powder flow tests, for example the angle of repose or flow speed due to the powder not being able to flow through a 15 mm opening. Considering the overall flow behaviour, the *A. afra* powder presented with very poor flow properties as shown in Table 4.8 (Sunil *et al.*, 2012). The original *A. afra* extract depicted a poor Hausner ratio of 1.51, and a Carr's index of 33.7, indicating very poor powder flow. The angle of repose test indicated very, very poor powder flow as the angle of repose test could not be completed successfully.

Table 4.8: *Artemisia afra* powder flow properties before double freeze-drying and sieving, the ranges in bold represent the ranges applicable to the *A. afra* extract bulk extract (Sunil *et al.*, 2012).

Flow	Carr Index (Ic)	Hausner ratio (IH)	The angle of Repose (θº)
Excellent	≤ 10	1.00-1.11	25-30
Good	11-16	1.12-1.18	31-35
Fair	16-20	1.19-1.25	36-40
Passable	21-25	1.26-1.34	41-45
Poor	26-31	1.35-1.45	46-55
Very poor	**32-37**	**1.46-1.59**	56-65
Very, very poor	>38	>1.6	**>66**

4.3.7 Enhancement of *A. afra* extract powder flow properties

Double freeze-drying was implemented and resulted in a powder with a higher bulk density and improved flowability. The extract was sieved in sieve fractions ranging between 45 µm and 710 µm, and the sieved *A. afra* dry powder extract (45 µm – 710 µm) exhibited better flowability compared to the original extract. It was also possible to determine an angle of repose value for this powder, indicating improved flow properties. It is evident from the data in Table 4.9 that the powder flow properties of the *A. afra* bulk extract improved after double freeze-drying and sieving. It was possible to conduct all powder flow tests required for SeDeM EDS with this *A. afra* extract. The Carr's Index (29.344) and the Hausner ratio (1.415) improved from 'very poor' to 'poor', and more importantly, the powder extract was able to flow through a 15 mm opening to complete the angle of repose test with a value of 21.350, representing excellent powder flow.

Table 4.9: Improvement in *A. afra* extract powder flow after double freeze-drying and sieving, with the bold values representing the ranges applicable to *A. afra* powder exhibiting improved powder flow

Flow	Carr Index (Ic)	Hausner ratio (IH)	The angle of Repose (θº)
Excellent	≤ 10	1.00-1.11	**25-30**
Good	11-16	1.12-1.18	31-35
Fair	16-20	1.19-1.25	36-40
Passable	21-25	1.26-1.34	41-45
Poor	**26-31**	**1.35-1.45**	46-55
Very poor	32-37	1.46-1.59	56-65
Very very poor	>38	>1.6	>66

4.4 SeDeM Expert Diagram System (EDS)

4.4.1 Measurement of 12 SeDeM parameters, incidences, and polygon construction of *A. afra*

4.4.1.1 Evaluation of *A. afra* powder flow properties

SeDeM EDS was implemented by subjecting the *A. afra powder* extract to various powder flow tests to determine 12 SeDeM EDS parameter values. Each parameter value was converted into a radius value between 0 – 10 and six of the 12 parameters had radius values higher than five as seen in Table 4.10. The 12 parameters were grouped into 5 SeDeM incidences. The double freeze-drying and sieving led to a flowability incidence value above 5. The other 4 incidences, namely dimension, compressibility, lubricity/stability, lubricity/dosage, portrayed values below 5 and needed to be corrected with excipients to be suitable for direct compression.

It is evident from the results in Table 4.10 that the dimensional factor exhibited the lowest value (3.5), with both parameter radii (r) values smaller than 5. The low bulk density value of 0.29 (r = 2.896) indicated that the *A. afra* powder extract occupied a large volume in the 250 mL graduated cylinder, whereas the low tapped density value of 0.41 (r = 4.099) specified that the powder extract had fairly little room for powder consolidation when tapped (Abdullah & Geldart, 1999).

The compressibility incidence was 4.05 and included two SeDeM parameter radius values above 5 namely inter-particle porosity (r = 5.222), and Carr's index (r = 5.869). The cohesion index (Icd) value of 21.27 N was the SeDeM parameter in the compressibility incidence with the lowest radius value (1.064), showing that the *A. afra* extract could not be compressed into tablets with sufficient mechanical strength without the addition of an excipient to compensate for this deficiency.

The flowability incidence was the only incidence above 5, with all 3 SeDeM parameter radius values higher than 5. The good flowability can be ascribed to the double freeze-drying and sieving. The low Hausner ratio (IH) value (1.415) acquired for the *A. afra* extract contributed to a radius value of 5.285. A low IH value indicates less powder cohesion (Dutta & Dullea, 1990). On average, the *A. afra* powder extract presented with a flow rate of 9.167 g/s when allowed to flow through a 15 mm diameter opening and, when converted, corresponded with a SeDeM radius value of 5.417. The angle of repose (21.350°) had a radius of 5.730. An angle of repose value below 30° indicates a free-flowing powder (Al-Hashemi & Al-Amoudi, 2018).

The lubricity/stability incidence failed at 4.01, with both parameter radii values below 5. The (%HR) parameter showed a 6.068% loss in weight (r = 3.932), and the (%H) parameter was at 11.81% increase in weight (r = 4.095).

The lubricity/dosage incidence failed by a small margin at 4.88. The (Iθ) was poor at 0.0012 (r = 0.594), but the percentage particles smaller than 45 µm (%Pf) was low, resulting in a high radius of 9.174. The low %Pf was mainly due to the sieving method, where most particles smaller than 45 µm were removed and discarded.

Table 4.10: SeDeM values, polygon radii, and incidence factor values of *A. afra* bulk extract

Parameter	**SeDeM Value**	**SeDeM limit**	**Polygon radius**	**Incidence**
Bulk density (Da)	0.290	0-1	2.896	Dimension 3.5 (FAIL)
Tapped density (Dc)	0.410	0-1	4.099	
Inter-particle porosity (Ie)	0.627	0-1.2	5.222	Compressibility 4.05 (FAIL)
Carr's index (Ic)	29.344	0-50	5.869	
Cohesion index (Icd)	21.27	0-200	1.064	
Hausner ratio (IH)	1.415	1-3	5.282	Flowability 5.48
The angle of repose (α)	21.350	50-0	5.730	
Powder flow (t'')	9.167	20-0	5.417	
Loss on drying (%HR)	6.068	10-0	3.932	Lubricity/Stability 4.01 (FAIL)
Hygroscopicity (%H)	11.81	20-0	4.095	
Particles < 45µm (%Pf)	4.130	50-0	9.174	Lubricity/Dosage 4.88 (FAIL)
Homogeneity index (Iθ)	0.0012	0 – 2 x 10-2	0.594	

Subaequently, a polygon was constructed for the *A. afra* dry powder extract to illustrate the radius values of all 12 parameters. The three SeDeM EDS parameters with the lowest radius values are indicated with blue arrows in the *A. afra* polygon (Figure 4.10). The 3 lowest parameters were bulk density (2.896), homogeneity index (0.594), and cohesion index (1.065). The SeDeM EDS parameters

representing the best values can also be identified when studying the polygon, with percentage particles under 45 µm scoring the highest, most likely due to the sieving applied to the *A. afra* dry powder extract to remove the small particles. Other SeDeM parameters with radii slightly above 5 included powder flow speed, Hausner ratio, Carr's index, angle of repose, and Inter-particle porosity.

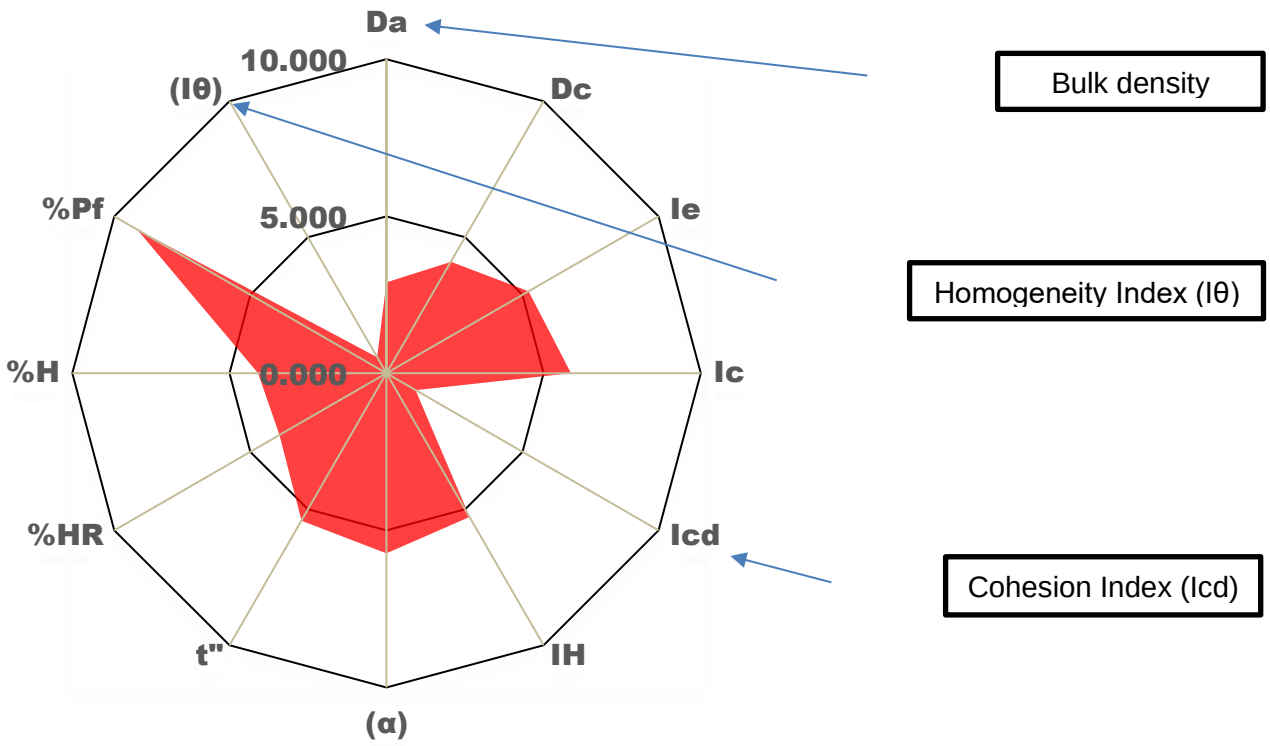

Figure 4.10: Polygon of 12 SeDeM parameters of *A. afra* dry powder extract with the three weakest parameters indicated with blue arrows

4.4.2 Additional SeDeM EDS indices for *A. afra* dry powder extract

Six of the 12 SeDeM parameter radii were above a value of 5, leading to a successful parameter index value of 0.5. The parameter profile index (IPP = 4.448) and good compressibility Index (IGC = 4.234) failed to meet the minimum requirements of having a value equal to or higher than 5. The reliability factor (f) used in the equation to calculate IGC was 0.952. Four of the five incidence factors presented with values smaller than 5. From these incidence factors it was clear that the *A. afra* dry powder extract was unsuitable for direct compression.

4.4.3 Measurement of 12 SeDeM parameters and incidences of excipients

Six excipients were subjected to the SeDeM EDS powder flow tests to identify the appropriate excipient to correct the poor powder flow properties of the *A. afra* powder extract. The selected excipients were tricalcium citrate, Ludipress®, MicroceLac® 100, Kollidon® VA 64, Emcompress®, and Avicel® PH 200. The 12 SeDeM parameters and five incidences were calculated for all six excipients, and polygons were constructed for each excipient. SeDeM radius values higher than 10 were converted to 10.

4.4.2.1 Kollidon® VA 64

The dimensional factor for Kollidon® VA 64 was 3.31, as shown in Table 4.11, indicating that Kollidon® VA 64 was not suitable as a filler, as it was impossible to correct the poor dimensional factor of *the A. afra* dry powder extract (3.50). The low density of Kollidon® VA 64 resulted in a poor flowability incidence value of 1.99. Furthermore, the powder was unable to flow through a 15 mm opening to determine flow speed (t") and angle of repose (α); therefore, a minimum radius value of zero was assigned to these parameters. The incidences for compressibility (6.07), lubricity/stability (7.41), and lubricity/dosage (5.51) were all above the minimum value of 5.

Table 4.11: SeDeM values, polygon radii, and incidence factor values of Kollidon® VA 64

Parameter	SeDeM Value	SeDeM limit	Polygon radius	Incidence
Bulk density (Da)	0.300	0-1	0.3	Dimension 3.31 (FAIL)
Tapped density (Dc)	0.362	0-1	3.623	
Inter-particle porosity (Ie)	0.573	0-1.2	4.778	Compressibility 6.07
Carr's index (Ic)	17.197	0-50	3.439	
Cohesion index (Icd)	200	0-200	10	
Hausner ratio (IH)	1.208	1-3	5.974	Flowability 1.99 (FAIL)
The angle of repose (α)	0	50-0	0	
Powder flow (t")	0	20-0	0	
Loss on drying (%HR)	4.189	10-0	5.811	Lubricity/Stability 7.41
Hygroscopicity (%H)	4.153	20-0	9.010	
Particles < 45µm (%Pf)	10.87	50-0	7.826	Lubricity/Dosage 5.51
Homogeneity index (Iθ)	0.0064	0 – 2 x 10-2	3.189	

A polygon of Kollidon® VA 64 (green) and a polygon of the *A. afra* powder extract (red) are superimposed in Figure 4.11. Kollidon® VA 64 was able to compensate for only three weak *A. afra* SeDeM parameters, namely cohesion index (Icd), hygroscopicity (%H), and loss on drying (%HR).

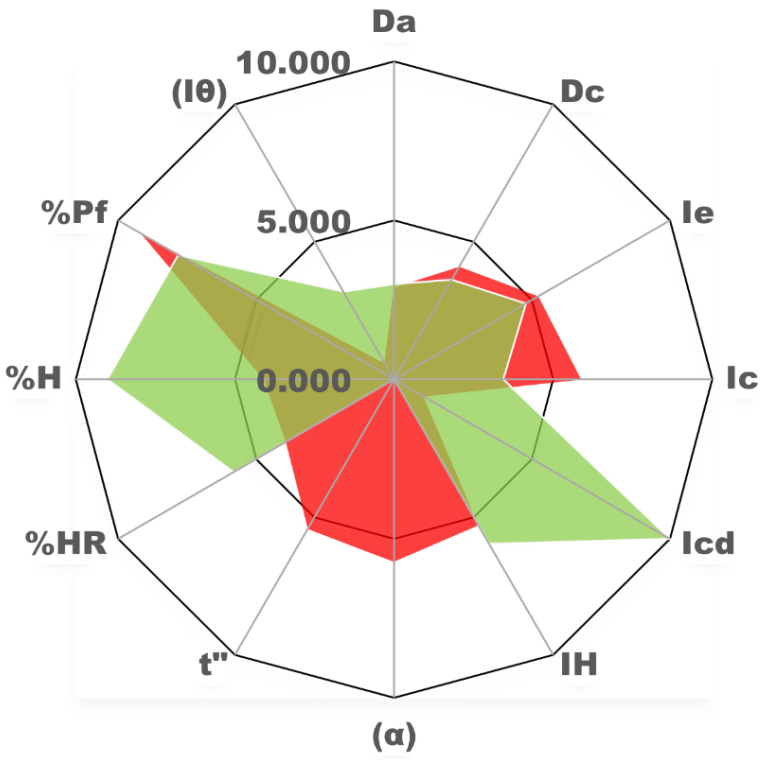

Figure 4.11: Superimposed polygon of *A. afra* powder extract (red) and Kollidon® VA 64 (green)

4.4.2.2 Emcompress®

The dimensional incidence for Emcompress® was high at 9.71, as shown in Table 4.12. A high dimensional incidence is mainly due to the high density of the powder (Rowe, 2009). Lubricity/stability presented a value above 9, which may be attributed to a maximum radius value of 10 for the hygroscopicity (%H) parameter. Flowability was 6.96, with all three contributing parameter radius values above 5. The primary deficiency for Emcompress® is the high percentage of powder particles smaller than 45 µm (51.28% < 45µm) and low Homogeneity index (2.508) that resulted in a poor lubricity/dosage incidence value of 1.25. Compressibility (4.14) was also below 5. The poor compressibility and lubricity/dosage incidences rendered Emcompress® unsuitable for correcting all poor indices of the *A. afra* dry power extract.

Table 4.12: SeDeM values, polygon radii, and incidence factor values of Emcompress®

Parameter	SeDeM Value	SeDeM limit	Polygon radius	Incidence
Bulk density (Da)	0.942	0-1	9.417	Dimension 9.71
Tapped density (Dc)	1.027	0-1	10	
Inter-particle porosity (Ie)	0.090	0-1.2	0.750	Compressibility 4.14 (FAIL)
Carr's index (Ic)	8.330	0-50	1.666	
Cohesion index (Icd)	200	0-200	10	
Hausner ratio (IH)	1.093	1-3	6.358	Flowability 6.96
The angle of repose (α)	18.256	50-0	6.349	
Powder flow (t")	3.667	20-0	8.167	
Loss on drying (%HR)	1.837	10-0	8.163	Lubricity/Stability 9.08
Hygroscopicity (%H)	0	20-0	10	
Particles < 45µm (%Pf)	51.280	50-0	0	Lubricity/Dosage 1.25 (FAIL)
Homogeneity index (Iθ)	0.005	0 – 2 x 10-2	2.508	

Figure 4.12 is a superimposed polygon of the *A. afra* powder extract (red), and Emcompress® (yellow), and illustrates the shortcomings of the Emcompress® parameters radii values with regards to particles < 45µm (%Pf), Homogeneity Index (Iθ), Inter-particle porosity (Ie), and Carr's index (Ic).

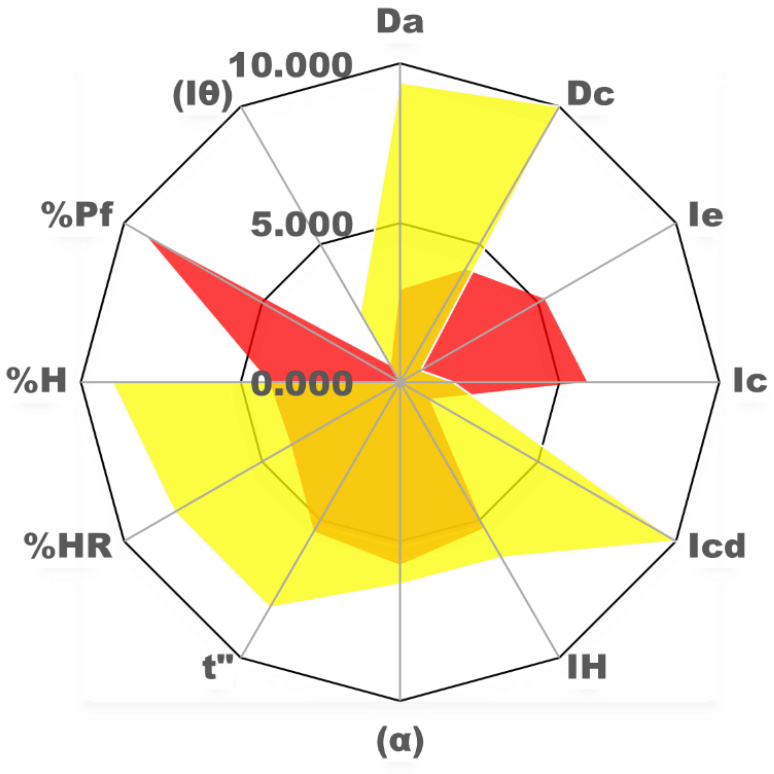

Figure 4.12: Superimposed polygon of *A. afra* powder extract (red) and Emcompress® (yellow)

4.4.2.3 Avicel® PH 200

Avicel® PH 200 rendered radius values higher than 5 for 8 of the 12 SeDeM parameters as shown in Table 4.13. Four of the incidences were higher than 5, namely compressibility (5.47), flowability (7.17), lubricity/stability (7.66), and lubricity/dosage (7.81). Unfortunately, the dimension incidence (4.19) was below 5, thus indicating Avicel® PH 200 unsuitable from being used successfully as a remedial excipient to correct the low *A. afra* dimension incidence of 3.50.

Table 4.13: SeDeM values, polygon radii, and incidence factor values of Avicel® PH 200

Parameter	SeDeM Value	SeDeM limit	Polygon radius	Incidence
Bulk density (Da)	0.373	0-1	3.731	Dimension 4.19
Tapped density (Dc)	0.440	0-1	4.399	
Inter-particle porosity (Ie)	0.407	0-1.2	3.389	Compressibility 5.47
Carr's index (Ic)	15.173	0-50	3.035	
Cohesion index (Icd)	200	0-200	10	
Hausner ratio (IH)	1.179	1-3	6.070	Flowability 7.17
The angle of repose (α)	15.396	50-0	6.932	
Powder flow (t")	2.967	20-0	8.517	
Loss on drying (%HR)	4.189	10-0	8.811	Lubricity/Stability 7.66
Hygroscopicity (%H)	0.977	20-0	9.512	
Particles < 45µm (%Pf)	4.98	50-0	9.004	Lubricity/Dosage 7.81
Homogeneity index (Iθ)	0.013	0 – 2 x 10-2	6.611	

A superimposed polygon was constructed for Avicel® PH 200 and *A. afra dry* powder extract, as shown in Figure 4.13. Avicel® PH 200 fell short on bulk density (Da) and tapped density (Dc) parameters.

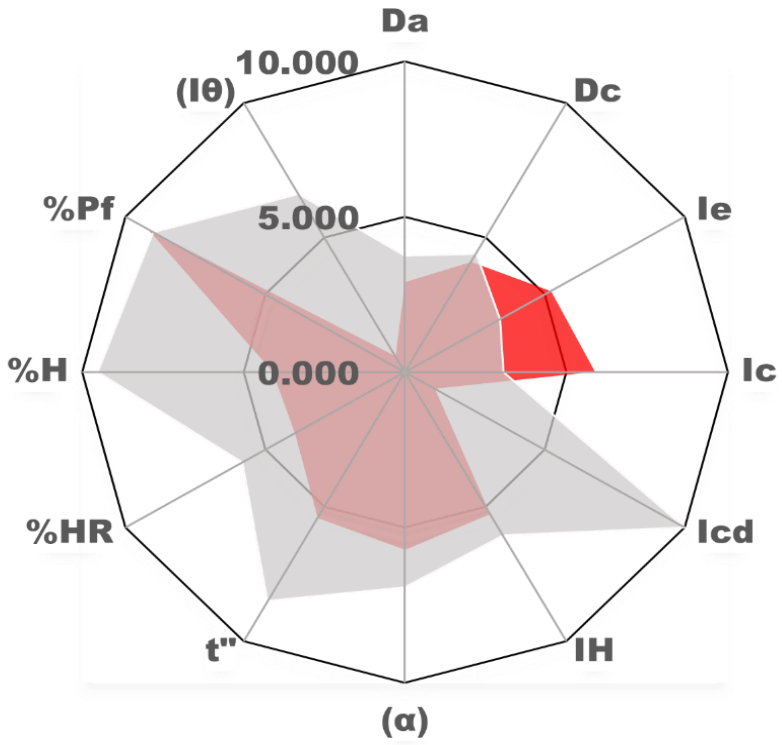

Figure 4.13*:* Superimposed polygon of *A. afra* powder extract (red) and Avicel® PH 200 (grey)

4.2.2.4 MicroceLac® 100

MicroceLac® 100 presented 8 of 12 SeDeM parameters exhibiting radius values above 5. All 5 incidences were above a value of 5 as shown in Table 4.14. The highest incidence was lubricity/stability (8.20), followed by flowability (6.04), lubricity/dosage (5.71), dimension (5.38), and compressibility (5.12). Based on these results, MicroceLac® 100 was considered suitable as a corrective excipient. The powder was able to theoretically correct all the incidences of the *A. afra* dry powder extract to above 5, whereas Avicel® PH 200 had a dimension incidence below 5 and could not correct the weak dimension factor od *A. afra* (3.50).

Table 4.14: SeDeM values, polygon radii, and incidence factor values of MicroceLac® 100

Parameter	SeDeM Value	SeDeM limit	Polygon radius	Incidence
Bulk density (Da)	0.496	0-1	4.959	Dimension 5.38
Tapped density (Dc)	0.580	0-1	5.803	
Inter-particle porosity (Ie)	0.293	0-1.2	2.444	Compressibility 5.12
Carr's index (Ic)	14.546	0-50	2.909	
Cohesion index (Icd)	200	0-200	10	
Hausner ratio (IH)	1.170	1-3	6.099	Flowability 6.04
The angle of repose (α)	19.521	50-0	6.096	
Powder flow (t")	8.133	20-0	5.933	
Loss on drying (%HR)	2.746	10-0	7.254	Lubricity/Stability 8.20
Hygroscopicity (%H)	1.707	20-0	9.147	
Particles < 45µm (%Pf)	8.770	50-0	8.246	Lubricity/Dosage 5.71
Homogeneity index (Iθ)	0.006	0 – 2 x 10-2	3.176	

Figure 4.14 is a superimposed polygon for MicroceLac® 100 and *A. afra* dry powder extract. It is evident that MicroceLac® 100 compensates well for the poor SeDeM parameters of *A. afra* extract, namely loss on drying (%HR), hygroscopicity (%H), cohesion index (Icd), and to a lesser degree bulk density (Da) and tapped density (Dc).

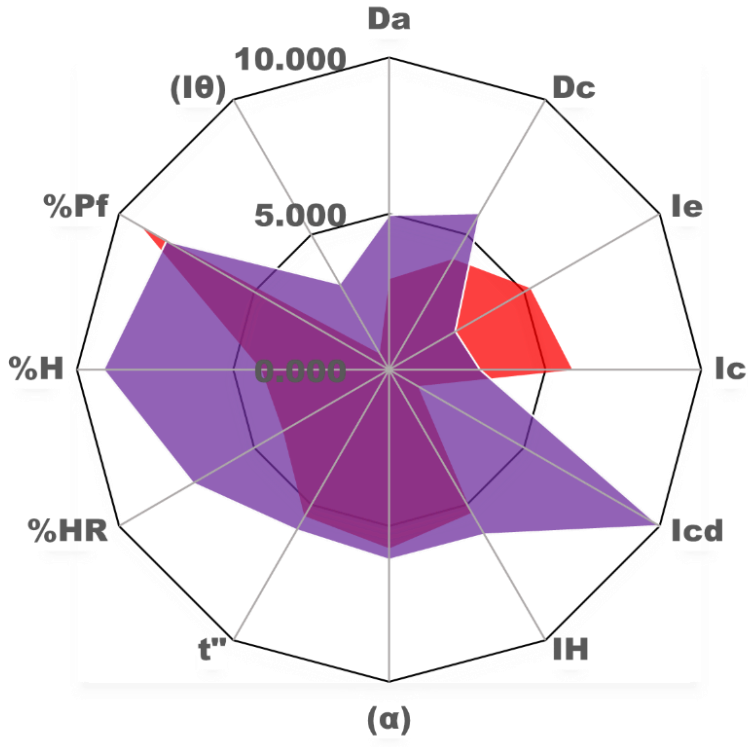

Figure 4.14*:* Superimposed polygon of *A. afra* powder extract (red) and MicroceLac® 100 (purple)

4.2.2.5 Ludipress®

Ludipress® presented with 8 SeDeM EDS parameters with radius values above 5, as seen in Table 4.15. All 5 incidences were above a value of 5, namely lubricity/dosage (5.23), compressibility (5.29), dimension (5.60), flowability (6.27), and lubricity/stability (6.23). Ludipress® was considered suitable to be utilised as a corrective excipient for the *A. afra* dry powder extract. Similar to MicroceLac® 100, the powder is able to theoretically correct all the *A. afra* dry powder extract incidences below 5 when utilised as a corrective excipient.

Table 4.15: SeDeM values, polygon radii, and incidence factor values of Ludipress®

Parameter	SeDeM Value	SeDeM limit	Polygon radius	Incidence
Bulk density (Da)	0.511	0-1	5.111	Dimension 5.60
Tapped density (Dc)	0.610	0-1	6.098	
Inter-particle porosity (Ie)	0.317	0-1.2	2.639	Compressibility 5.29
Carr's index (Ic)	16.183	0-50	3.237	
Cohesion index (Icd)	200	0-200	10	
Hausner ratio (IH)	1.193	1-3	6.023	Flowability 6.27
The angle of repose (α)	22.766	50-0	5.447	
Powder flow (t'')	5.30	20-0	7.350	
Loss on drying (%HR)	5.457	10-0	4.543	Lubricity/Stability 6.23
Hygroscopicity (%H)	4.153	20-0	7.923	
Particles < 45µm (%Pf)	4.310	50-0	9.138	Lubricity/Dosage 5.23
Homogeneity index (Iθ)	0.002	0 – 2 x 10-2	1.312	

Ludipress® compensated well for some poor parameters of the *A. afra* powder extract as illustrated by the superimposed polygon in Figure 4.15. The cohesion index (Icd), hygroscopicity (%H), and powder flow (t'') can be substantially improved, whereas bulk density (Da) and tapped density (Dc) can be compensated for slightly.

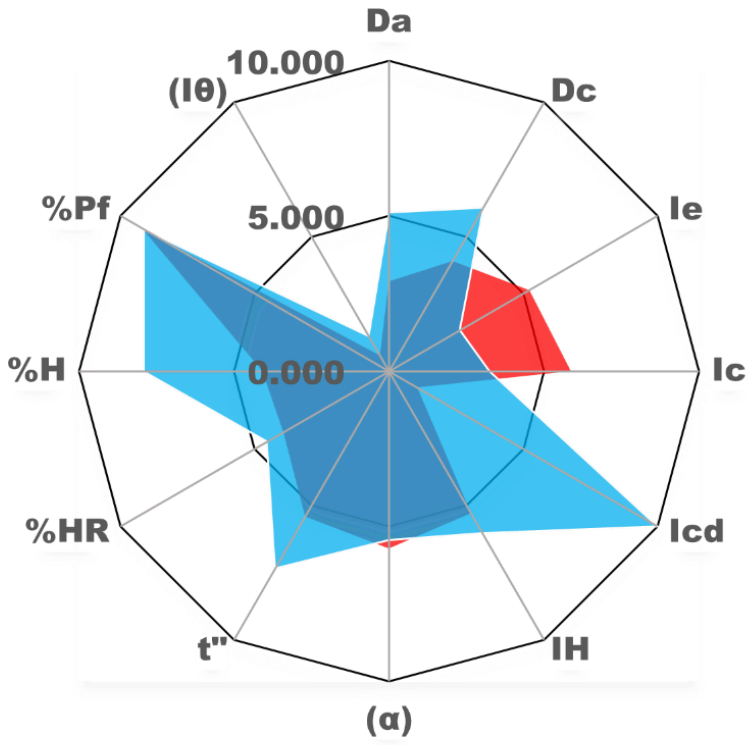

Figure 4.15: Superimposed polygon of *A. afra* powder extract (red) and Ludipress® (light blue)

4.2.2.6 Tricalcium citrate

Eight of the 12 SeDeM parameters were above 5 for tricalcium citrate. All incidences were also above 5, as shown in Table 4.16. The highest incidence factor was lubricity/dosage (8.85), mainly due to tricalcium citrate having a low water solubility (Hagelstein *et al.*, 2018), followed by the lubricity/stability (6.77), dimension (5.96), flowability (5.83), and compressibility (5.43) incidences. Tricalcium citrate was theoretically the most appropriate excipient to be used as a corrective excipient to compensate for the weak powder flow characteristics of the *A. afra* dry powder extract. Tricalcium citrate providing high incidence values for the incidences where the *A. afra* scored the lowest and can be used in the lowest concentration to correct all four *A. afra* incidences below 5. As is the case with MicroceLac® 100 and Ludipress®, Tricalcium citrate was able to correct all four *A. afra* dry powder extract incidences, whereas the other three excipients was unable to correct all the incidences.

Table 4.16: SeDeM values, polygon radii, and incidence factor values of tricalcium citrate

Parameter	SeDeM Value	SeDeM limit	Polygon radius	Incidence
Bulk density (Da)	0.578	0-1	5.377	Dimension 5.96
Tapped density (Dc)	0.654	0-1	6.536	
Inter-particle porosity (Ie)	0.330	0-1.2	2.750	Compressibility 5.43
Carr's index (Ic)	17.739	0-50	3.548	
Cohesion index (Icd)	200	0-200	10	
Hausner ratio (IH)	6.833	1-3	5.948	Flowability 5.83
The angle of repose (α)	25.257	50-0	4.949	
Powder flow (t")	6.833	20-0	6.583	
Loss on drying (%HR)	6.459	10-0	3.541	Lubricity/Stability 6.77
Hygroscopicity (%H)	0	20-0	10	
Particles < 45µm (%Pf)	4.98	50-0	9.004	Lubricity/Dosage 8.85
Homogeneity index (Iθ)	0.017	0 – 2 x 10-2	8.70	

Tricalcium citrate compensated well for most of the deficient SeDeM parameters of the *A. afra* powder extract. Figure 4.16 is a superimposed polygon that illustrates how the tricalcium citrate parameters with high radius values could improve the low radius values of *A. afra*. The low parameter values of the *A. afra* dry extract, namely the cohesion index (Icd) and homogeneity index (Iθ), can easily be compensated for by the cohesion index and homogeneity index values of tricalcium citrate. The parameters of hygroscopicity (%H), bulk density (Da), and tapped density (Dc) could also be improved considerably.

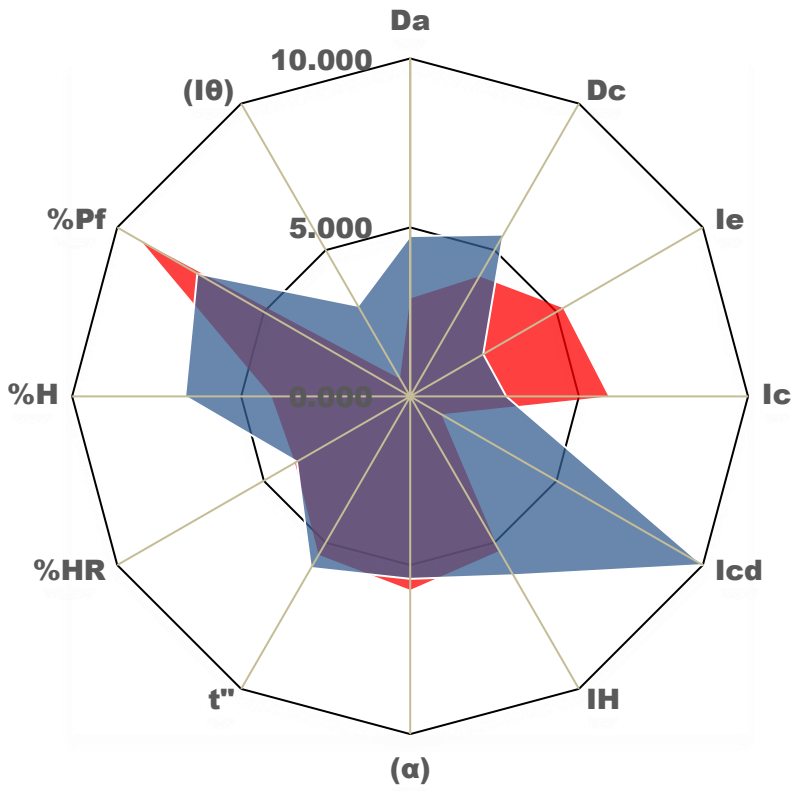

Figure 4.16*:* Superimposed polygon of *A. afra* powder extract (red) and tricalcium citrate (dark blue)

4.3 Comparison and calculation of corrective excipients

SeDeM EDS is typically implemented to correct the poor flow and compressibility properties of a powder as indicated by its poorest incidence factor (Suñé-Negre *et al.*, 2008). In the case of the *A. afra* dry powder extract, the dimensional factor was the poorest; however, solving the poorest incidence factor does not necessarily mean the other three incidences below 5 will automatically be corrected; thus, corrective excipient percentages for all incidences under 5 were calculated (Aguilar-Díaz, García-Montoya, Pérez-Lozano, Suñé-Negre, Miñarro*, et al.*, 2014). A theoretical comparison was made in Table 4.17, which included the six excipients and compared all incidences. The aim was to calculate the minimum amount of each excipient needed to correct all the poor incidences to ≥ 5. Table 4.15 shows the minimum amount of each excipient to correct each specific incidence factor. Avicel® PH 200 was unable to correct the dimensional factor, no matter how high the percentage of the excipient in the mixture, thus if the excipient possessed an incidence value below 5 for a specific incidence, it will not be able to correct that poor incidence value (also below 5) of the *A. afra* dry powder extract. Emcompress® was unable to correct the compressibility factor, lubricity/dosage factor, and lubricity/stability factor. The only three excipients appropriate for corrective excipients were MicroceLac® 100, tricalcium citrate, and Ludipress®. The minimum corrective excipient percentage was calculated. When the compressibility incidence was corrected, all the other deficient incidence values were also improved to become above 5. The minimum required corrective excipient percentage for MicroceLac® 100 was 88.79% and would thus allow the powder mixture to contain only 11.21% of *A.*

afra dry powder extract. The minimum corrective excipient percentage required from Ludipress® was 76.61%, allowing 23.39% *A. afra* dry powder extract in the mixture. Tricalcium citrate proved to have the best powder properties to compensate for the deficient properties of *A. afra* and allowed 31.16% of *A. afra* dry powder extract to be included in the mixture as it only requires 68.84% of tricalcium citrate as a corrective excipient to compensate for all the poor indices of the API.

Table 4.17: Calculation of minimum excipient percentage required from each excipient to correct all *A. afra* powder extract indices, the results in bold show the minimum percentage excipient required to correct all the *A. afra* incidences

Dimension						
Excipient	**Kollidon® VA 64**	**Avicel PH® 200**	**Microce-lac® 100**	**Tricalcim citrate**	**Emcom-press®**	**Ludi-press®**
The radius of corrective excipient	6.81	4.19	5.38	5.96	9.71	5.60
The radius of *A. afra* indices to be corrected	3.50	3.50	3.50	3.50	3.50	3.50
Minimum excipient required %	45.317	> 100	79.787	60.976	24.155	71.429
Compressibility						
Excipient	**Kollidon® VA 64**	**Avicel® PH 200**	**Microce-lac® 100**	**Tricalcium citrate**	**Emcom-press®**	**Ludi-press®**
The radius of corrective excipient	6.81	5.47	5.12	5.43	4.14	5.29
The radius of *A. afra* indices to be corrected	4.05	4.05	4.05	4.05	4.05	4.05
Minimum excipient required %	34.420	66.901	**88.785**	**68.841**	> 100	**76.613**

Table 4.17 (cont): Calculation of minimum excipient percentage required from each excipient to correct all *A. afra* powder extract indices, the results in bold show the minimum percentage excipient required to correct all the *A. afra* incidences

Lubricity/Stability						
Excipient	**Kollidon® VA 64**	**Avicel® PH 200**	**Microce-lac® 100**	**Tricalcium citrate**	**Emcom-press®**	**Ludi-press®**
The radius of corrective excipient	3.31	7.66	8.20	6.77	9.08	6.23
The radius of *A. afra* indices to be corrected	4.01	4.01	4.01	4.01	4.01	4.01
Minimum excipient required %	> 100	27.123	23.628	35.870	19.527	44.595
Lubricity/Dosage						
Excipient	**Kollidon® VA 64**	**Avicel® PH 200**	**Microce-lac® 100**	**Tricalcium citrate**	**Emcom-press®**	**Ludi-press®**
The radius of corrective excipient	7.41	8.31	5.71	8.95	1.25	6.23
The radius of *A. afra* indices to be corrected	4.88	4.88	4.88	4.88	4.88	4.88
Minimum excipient required %	4.743	3.499	14.458	2.948	> 100	8.889

4.4 Calculation of total tablet mass to include a dose of 200 mg of *A. afra* powder extract per tablet

The total tablet mass needed to contain 200 mg *A. afra* powder extract for each appropriate excipient was calculated and is shown in Table 4.18. To produce a tablet with 200 mg *A. afra* extract using MicroceLac® 100, the tablet would theoretically weigh 1784 mg. If Ludipress® were used, one tablet would weigh 855 mg, which is considerably lighter than a tablet produced with MicroceLac® 100. Tricalcium citrate was the excipient that allowed the tablet production with the lowest mass, with one tablet containing 200 mg of *A. afra* extract weighing 642 mg. Smaller tablets are associated with

improved patient compliance and are easier to produce and pack than larger tablets. The aimed dosage regimen was two tablets containing 200 mg each, three times per day.

Table 4.18: Comparison of theoretical tablet weight, using three different excipients, to produce a tablet containing 200 mg *A. afra* dry powder extract

Excipient	Minimum % excipient in tablet	Maximum % *A. afra* in tablet	Tablet mass to contain 200 mg *A. afra*
Ludipress®	76.61	23.39	855
Tricalcium citrate	68.84	31.16	642
MicroceLac® 100	88.875	11.125	1784

4.5 Corrective mixture

4.5.1 Selection of a lubricant, disintegrant, and binder

A 3.5% w/w of a lubricant mixture comprised of colloidal silicon dioxide (0.14% w/w), talc (2.36% w/w), and magnesium stearate (1.00% w/w) was used to enhance compressibility, similar to what was used when the SeDeM powder flow properties were evaluated (Nofrerias *et al.*, 2019; Pérez *et al.*, 2006). Besides this suggested lubricant mixture, Kollidon® VA 64 3% w/w as a binder and 5% w/w Ac-di-sol® as the disintegrant were also included in the corrective mixture to increase the chance of proper tablet binding and fast disintegration. The final corrective powder mixture consisted primarily of the corrective excipient tricalcium citrate (88.5% w/w), which had to be mixed with the *A. afra* extract powder to prepare direct compression tablets.

4.5.2 Mixing of powders for tablet formulation including the filler, lubricant, disintegrant, and binder

The minimum amount of tricalcium citrate required for a successful theoretical SeDeM EDS direct compression tablet formulation for *A. afra* extract was predicted to be 67.9% w/w of the total tablet composition (which correlates to 88.5% w/w of the excipient mixture). This percentage was rounded up to 70% w/w to compensate for small changes when the percentages of lubricant, disintegrant, and binder were added to the corrective excipient. Thus 30% of *A. afra dry powder* extract (API) was included in the final powder formulation for the tablet. The corrective mixture was mixed for 5 min in the Turbula® mixer at 47 rpm; after that, SeDeM studies were conducted on the final formulation to verify its suitability for direct compression. The corrective mixture comprised tricalcium citrate (88.5% w/w), Ac-di-sol® (5% w/w), Kollidon® VA 64 (3% w/w), colloidal silicon dioxide (0.14% w/w), talc (2.36% w/w), and magnesium stearate (1.00% w/w).

The corrective powder mixture without *A. afra* extract powder was subjected to SeDeM EDS parameter tests. The results are shown in Table 4.19. Seven of the 12 SeDeM EDS parameters were above a value of 5 for the corrective mixture. Most notably, the homogeneity index (Iθ) was considerably lower for the final corrective mixture (88.5% w/w tricalcium citrate) when compared to that of tricalcium citrate alone. The lower homogeneity index was expected because six different powders with different properties were mixed together, causing a broader spread in powder particle size. All 5 SeDeM EDS indices, however, possessed values above a value of 5, which indicated that the powder mixture could be directly compressed into tablets.

Table 4.19: SeDeM values, polygon radii, and incidence factor values of the corrective mixture of tricalcium citrate (88.5% w/w), Ac-di-sol® (5% w/w), Kollidon® VA 64 (3% w/w), colloidal silicon dioxide (0.14% w/w), talc (2.36% w/w), and magnesium stearate (1.00% w/w)

Parameter of excipient powder mixture	SeDeM Value	SeDeM limit	Polygon radius	Incidence
Bulk density (Da)	0.517	0-1	5.199	Dimension 5.72
Tapped Density (Dc)	0.628	0-1	6.276	
Inter-particle Porosity (Ie)	0.340	0-1.2	2.75	Compressibility 5.45
Carr's Index (Ic)	17.584	0-50	3.431	
Cohesion Index (Icd)	200	0-200	10	
Hausner Ratio (I.H.I.H.)	1.213	1 -3	5.976	Flowability 5.84
Angle Of Repose (α)	22.978	50-0	5.404	
Powder Flow (t")	7.700	20-0	3.150	
Loss on drying (% HR HR.)	6.620	10-0	3.380	Lubricity/Stability 6.35
Hygroscopicity (%H)	1.360	20-0	9.320	
Particles < 45µm (%Pf)	15.040	50-0	6.992	Lubricity/Dosage 5.24
Homogeneity Index (Iθ)	0.007	0 – 2 x 10-2	3.490	

Figure 4.17 is an illustration of 3 superimposed polygons where the *A. afra* powder extract is represented by the red polygon, the blue polygon represents tricalcium citrate, and the yellow polygon represents the final corrective mixture consisting of tricalcium citrate (88.5% w/w), Ac-di-sol® (5% w/w), Kollidon® VA 64 (3% w/w), colloidal silicon dioxide (0.14% w/w), talc (2.36% w/w), and magnesium stearate (1.00% w/w). Comparison of the polygon of the final corrective mixture with the polygon of tricalcium citrate shows that two parameter radius values were visibly lower, namely, particles < 45µm (%Pf) and homogeneity index (Iθ). However, the lower values were not considered to cause problems, since these two parameters make up the lubricity/dosage incidence, which only needed a slight correction in the *A. afra* extract powder, as the lubricity/dosage incidence of the API is close to 5 (4.88).

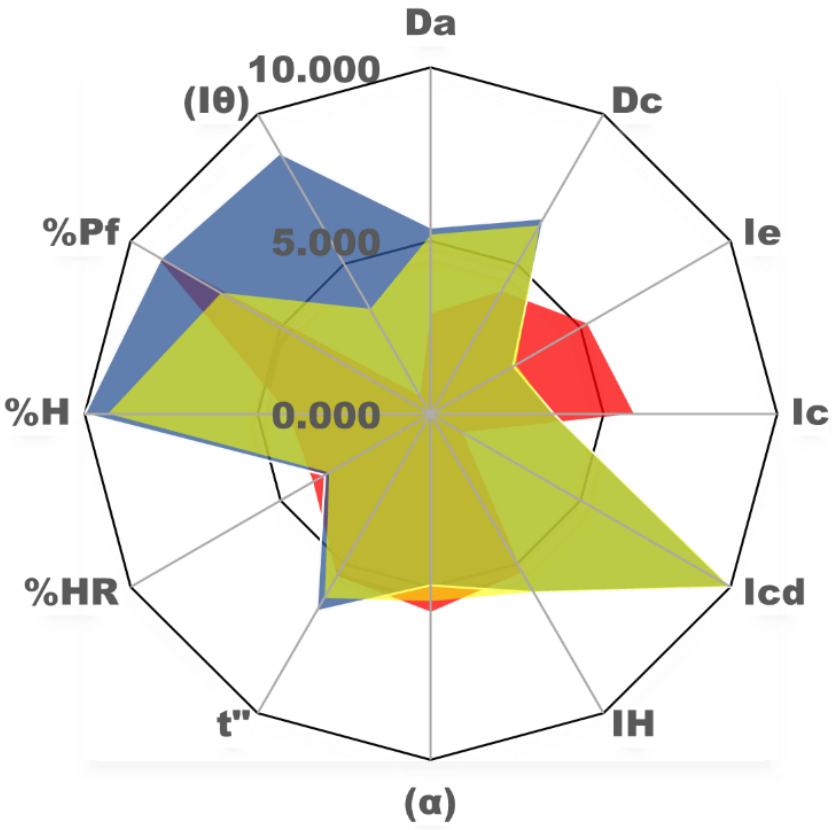

Figure 4.17: Superimposed polygons for *A. afra* powder extract (red), tricalcium citrate (blue), and final corrective mixture (yellow)

The minimum excipient percentage required for the final corrective excipient mixture was calculated, and the results are shown in Table 4.20. The incidences for dimension and compressibility were most likely be the determining factors for the minimum excipient required and showed to be correct as the compressibility incidence required a minimum of 67.86% of excipient to be included. Based on these results and the likelihood of success in direct compression if this corrective mixture is to be used, a final powder mixture containing 30% of *A. afra* dry powder extract could be prepared using the suggested corrective powder mixture.

Table 4.20: Calculation of minimum excipient percentage required from the final corrective mixture to correct all *A. afra* powder extract indices.

SeDeM incidence				
Processed parameter	**Dimension**	**Compressibility**	**Lubricity/ Stability**	**Lubricity/ Dosage**
The radius of corrective excipient	5.72	5.45	6.67	5.24
The radius of *A. afra* indices to be corrected	3.50	4.05	4.01	4.88
Calculated excipient %	**67.57%**	**67.86%**	**37.22%**	**33.33%**

The final tablet mixture was mixed for 5 min in the Turbula® mixer and was again subjected to SeDeM EDS analysis to characterise the suitability with regards to direct compression of the final tablet mixture containing 30% w/w *A. afra* powder extract. The results in Table 4.21 show that all five incidence values were above a value of 5, indicating that the corrective excipient mixture could compensate for the low incidence values of the *A. afra* dry powder extract. Seven of the 12 SeDeM EDS parameters possessed values above 5. All three additional indices were acceptable with a parameter index (IP) of 0.58, a parameter profile index (IPP) of 5.32, and a good compressibility index (IGC) of 5.06.

Table 4.21*:* SeDeM values, polygon radii, and incidence values of the final tablet mixture containing 30% w/w of *A. afra* powder extract and 70% w/w corrective excipient

Parameter of final powder mixture	SeDeM Value	SeDeM Limit	Polygon radius	Incidence
Bulk density (Da)	0.474	0-1	4.739	Dimension 5.13
Tapped Density (Dc)	0.552	0-1	5.525	
Inter-particle Porosity (Ie)	0.300	0-1.2	2.5	Compressibility 5.11
Carr's Index (Ic)	14.218	0-50	2.844	
Cohesion Index (Icd)	200	0-200	10	
Hausner Ratio (I.H.I.H.)	1.166	1- 3	6.114	Flowability 5.80
Angle Of Repose (α)	22.977	50-0	5.405	
Powder Flow (t'')	8.267	20-0	5.867	
Loss on drying (% HR HR.)	6.129	10-0	3.871	Lubricity/Stability 5.26
Hygroscopicity (%H)	6.690	20-0	6.655	
Particles < 45µm (%Pf)	13.660	50-0	7.268	Lubricity/Dosage 5.18
Homogeneity Index (Iθ)	0.0062	0 – 2 x 10-2	3.088	

Figure 4.18 shows three superimposed polygons where the red polygon represents the *A. afra* dry powder extract, the yellow polygon represents the corrective excipient mixture, and the green polygon represents the final tablet mixture intended for direct compression. The final tablet mixture consisted of *A. afra* dry powder extract (30% w/w), tricalcium citrate (61.95% w/w), Ac-di-sol® (3.5% w/w), Kollidon® VA 64 (2.1% w/w), colloidal silicon dioxide (0.098% w/w), talc (1.652% w/w), and magnesium stearate (0.7% w/w). The superimposed polygons show how the corrective excipient mixture (yellow) compensated well for the weak SeDeM EDS parameters of the *A. afra* dry powder extract (red), to provide an improved powder in the form of a final tablet mixture (green). The final tablet mixture had good powder flow properties suitable for direct compression.

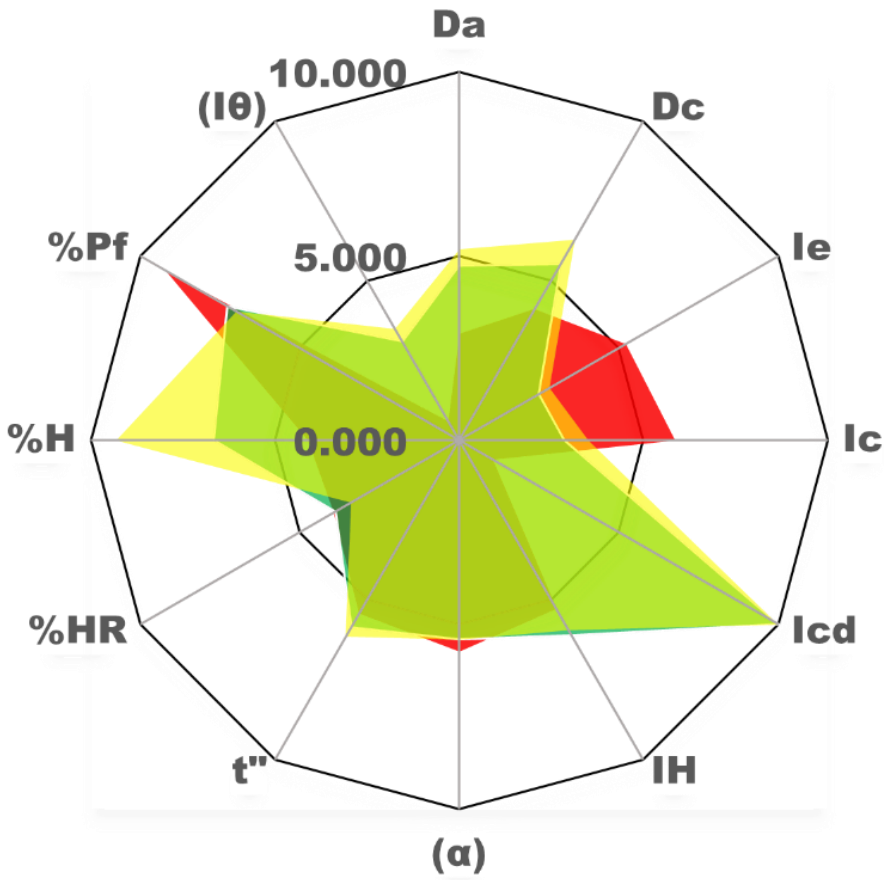

Figure 4.18: Superimposed polygons for *A. afra* powder extract (red), the corrective excipient (yellow), and final tablet mixture (green)

4.6 Tabletting

To compress tablets containing 200 mg *A. afra* (30% w/w) dry powder extract, a quantity of 466.67 mg of corrective excipient was required. The final tablet mass was approximately 667 mg. Firstly, tablets were compressed with a 12 mm diameter punch using the manual hand wheel, tablet weight and hardness were measured, and adjustments were made after every compressed tablet until a satisfactory tablet was produced. After tablet weight and hardness were set, a tablet batch of 797 tablets was compressed on an automatic setting at ten strokes per minute. Tablets were packed into 13 amber glass containers, each containing 60 tablets. Silica bags were inserted inside each container as shown in Figure 4.19. The containers were kept in a cool and dark place for 24 h before being subjected to stability testing and evaluation, including an assay, weight variation, hardness, friability, disintegration, and dissolution behaviour.

Figure 4.19: Tablets containing 200 mg *A. afra* dry powder extract, silica bag, and an example of an amber container that were used during stability testing

4.7 Tablet evaluation

4.7.1 Uniformity of tablet weight

Table 4.22 shows the results for the uniformity of tablet weight over 12 weeks. None of the tablets deviated by more than 5% from the average tablet weight, and thus all tablets complied with the required BP (2021) specifications in Appendix XII C . The tablets exposed to 40ºC/75% relative humidity exhibited a slight decrease in average tablet weight over 12 weeks, which can be explained by the higher temperature (40ºC) that caused a loss of tablet moisture which was absorbed by the silica bag inside each container.

Table 4.22: Uniformity of tablet weight results for *A. afra* tablets

Time in climatic chamber	Average mass (mg) ± %RSD after exposure to 25ºC/60% relative humidity	Average mass (mg) ± %RSD exposure to 40ºC/75% relative humidity
Week 0	666.9 ± 0.22%	666.9 ± 0.22%
Week 1	666.3 ± 0.40%	666.5 ± 0.26%
Week 2	667.1 ± 0.32%	666.1 ± 0.42%
Week 3	666.9 ± 0.31%	666.3 ± 0.40%
Week 4	665.9 ± 0.36%	665.8 ± 0.39%
Week 8	665.7 ± 0.22%	663.7 ± 0.35%
Week 12	666.4 ± 0.29%	663.1 ± 0.63%

4.7.2 Crushing strength

Table 4.23 shows the crushing strength results for *A. afra* tablets at two different climatic chamber conditions over 12 weeks; the average tablet hardness (N) and standard deviation for ten tablets are given. The average tablet hardness of tablets not exposed to accelerated stability conditions (week 0) was 109.7 N ± 2.71 %RSD. Tablets exposed to 25ºC/60% relative humidity showed a slight increase in average tablet hardness from 109.7 N ± 2.71% RSD to 122.9 N ± 6.35% RSD over 12 weeks, whereas the tablets exposed to 40ºC/75% relative humidity showed a more pronounced increase in tablet hardness, as the average tablet hardness increased from 109.7 N N ± 2.71% RSD to 160 N ± 5.93% RSD after 12 weeks. A possible reason for an increase in tablet hardness after exposure to 40ºC can be due to tablets losing moisture when drying out, and the silica bags absorbing the moisture. After three weeks, the tablet hardness seemed to have reached a plateau at this storage condition. The results imply that the average hardness of the *A. afra* tablets increased with an increase in temperature and relative humidity.

Table 4.23: Crushing strength results for *A. afra* tablets from two different climatic chamber conditions over 12 weeks

Time in climatic chamber	Crushing strength (N) ± %RSD after exposure to 25ºC/60% relative humidity	Crushing strength (N) ± %RSD after exposure to 40ºC/75% relative humidity
Week 0	109.7 ± 2.71%	109.7 ± 2.71%
Week 1	114.6 ± 2.98%	156.8 ± 5.22%
Week 2	113.8 ± 3.66%	150.3 ± 5.54%
Week 3	116.6 ± 3.52%	160.5± 2.60%
Week 4	113.7 ± 2.81%	161.8 ± 5.55%
Week 8	116.0 ± 5.76%	164.7 ± 5.79%
Week 12	122.9 ± 6.35%	160.0 ± 5.93%

4.7.3 Disintegration time

The disintegration time results in Table 4.24 show that all tablets complied with the BP requirements for disintegration, as on every occasion, all six tablets were entirely disintegrated before the upper time limit of 900 seconds was reached (BP, 2021). Tablets exposed to 40ºC/75% relative humidity had a more significant increase in disintegration time compared to tablets exposed to 25ºC/60% relative humidity. After just one week, disintegration time increased from 426 seconds (week 0) to 489 seconds (week 1), which correlates with the significant increase in tablet hardness over one week. An increase in disintegration time is expected as the tablet hardness increased over time and tablets that harden usually exhibit an increase in disintegration time (Dor & Fix, 2000).

Table 4.24: Disintegration time results for *A. afra* tablets over 12 weeks in seconds

Time in climatic chamber	Disintegration time for 6 tablets after exposure to 25ºC/60% relative humidity	Disintegration time for 6 tablets after exposure to 40ºC/75% relative humidity
Week 0	426 s	426 s
Week 1	429 s	489 s
Week 2	430 s	491 s
Week 3	432 s	518 s
Week 4	434 s	516 s
Week 8	431 s	521 s
Week 12	436 s	515 s

4.7.4 Friability

The friability test did not cause any tablets to break or crack. There was, however, a slight loss in percentage tablet mass after tablets were dusted and weighed, as shown in Table 4.25. The loss in tablet mass percentage over 12 weeks was within the limit (≤1% loss) specified by the BP (BP, 2021).

Table 4.25: Friability results for *A. afra* tablets over 12 weeks

Time in climatic chamber	Percentage loss in mass after exposure to 25ºC/60% relative humidity	Percentage loss in mass after exposure to 40ºC/75% relative humidity
Week 0	0.20%	0.20%
Week 1	0.29%	0.26%
Week 2	0.12%	0.14%
Week 3	0.21%	0.12%
Week 4	0.18%	0.22%
Week 8	0.21%	0.14%
Week 12	0.29%	0.44%

4.7.5 Assay

4.7.5.1 Tablets exposed to 25ºC/60% relative humidity for 12 weeks

Table 4.26 shows the assay results for tablets exposed to 25ºC/60% relative humidity over 12 weeks. The average morin hydrate equivalents per gram of dry extract weight were determined for all four phytochemical marker molecules in the tablets. They were compared to the amount of mg morin hydrate equivalents per gram of dry extract weight (mg MHE/ g) in the original dry *A. afra* powder extract. The mg MHE/g for all 4 phytochemical marker molecules can be compared with the assay where only 200 mg dry *A. afra* powder extract was used to evaluate

Table 4.26: Assay data for *A. afra* tablets exposed to 25ºC/60% relative humidity

***Artemisia afra* phytochemical marker molecule in extract or tablet**	**Average concentration (mg/mL)**	**Mg MHE/g of dry extract weight**
Phytochemical marker 1 in *A. afra* 200 mg extract	0.033	8.283
Phytochemical marker 2 in *A. afra* 200 mg extract	0.022	5.480
Phytochemical marker 3 in *A. afra* 200 mg extract	0.083	20.804
Phytochemical marker 4 in *A. afra* 200 mg extract	0.049	12.265
Morin hydrate added for assay	0.080	80.125
Phytochemical marker 1 in week 0 tablets	0.033	8.261
Phytochemical marker 2 in week 0 tablets	0.014	3.499
Phytochemical marker 3 in week 0 tablets	0.049	12.345
Phytochemical marker 4 in week 0 tablets	0.023	5.801
Morin hydrate added for assay	0.079	78.817
Phytochemical marker 1 in week 1 tablets	0.034	8.406
Phytochemical marker 2 in week 1 tablets	0.014	3.495
Phytochemical marker 3 in week 1 tablets	0.053	13.168
Phytochemical marker 4 in week 1 tablets	0.022	5.580
Morin hydrate added for assay	0.078	78.474
Phytochemical marker 1 in week 2 tablets	0.033	8.243
Phytochemical marker 2 in week 2 tablets	0.014	3.443

Table 4.26 (cont.): Assay data for *A. afra* tablets exposed to 25ºC/60% relative humidity

***Artemisia afra* phytochemical marker molecule in extract or tablet**	**Average concentration (mg/mL)**	**Mg MHE/g of dry extract weight**
Phytochemical marker 3 in week 2 tablets	0.048	11.997
Phytochemical marker 4 in week 2 tablets	0.022	5.571
Morin hydrate added for assay	0.079	78.753
Phytochemical marker 1 in week 3 tablets	0.033	8.212
Phytochemical marker 2 in week 3 tablets	0.014	3.445
Phytochemical marker 3 in week 3 tablets	0.054	13.405
Phytochemical marker 4 in week 3 tablets	0.024	5.893
Morin hydrate added for assay	0.079	79.074
Phytochemical marker 1 in week 4 tablets	0.033	8.223
Phytochemical marker 2 in week 4 tablets	0.014	3.419
Phytochemical marker 3 in week 4 tablets	0.054	13.418
Phytochemical marker 4 in week 4 tablets	0.023	5.757
Morin hydrate added for assay	0.080	79.552
Phytochemical marker 1 in week 8 tablets	0.031	7.869
Phytochemical marker 2 in week 8 tablets	0.013	3.154
Phytochemical marker 3 in week 8 tablets	0.051	12.758
Phytochemical marker 4 in week 8 tablets	0.020	4.884
Morin hydrate added for assay	0.082	81.906
Phytochemical marker 1 in week 12 tablets	0.031	7.715
Phytochemical marker 2 in week 12 tablets	0.009	2.337
Phytochemical marker 3 in week 12tablets	0.036	9.012
Phytochemical marker 4 in week 12 tablets	0.013	3.272
Morin hydrate added for assay	0.080	80.400

The loss in the mg MHE/g was expressed as percentages in Figure 4.20. The tabletting process immediately affected the mg MHE/g dry extract of phytochemical marker molecules 2 – 4. Twenty-four hours after tabletting, phytochemical markers 2 – 3 decreased in mg MHE/g dry extract by approximately 35%, and phytochemical marker 4 exhibited a more considerable decrease of 52.2%. *A. afra* contains plant phenols, metal-containing compounds such as magnesium stearate has been shown to cause complexation when combined with phenols (Bharate *et al.*, 2016). Magnesium stearate can form several other degradants (Hotha *et al.*, 2016). It has also been shown that compression on a tablet press can accelerate incompatibilities between an API and excipient as seen with theophylline and starch 1500 (Mazurek-Wadołkowska *et al.*, 2016). Whether magnesium stearate and the compression process contributed to the decrease in the content of certain phytochemical marker molecules in this study is an important consideration and should therefore be investigated in future studies.

The mg MHE/g of phytochemical marker 1 remained practically unchanged after the dry *A. afra* powder extract was mixed with excipients and compressed into tablets, indicating that not all the phytochemical marker molecules were negatively affected by the tabletting process.

It can be observed from Figure 4.20 that phytochemical marker 1 exhibited a 5% reduction in mg MHE/g of dry extract after 8 weeks and a 7% decrease after 12 weeks of exposure to 25°C/60% relative humidity. The quantity of phytochemical marker 2 (expressed as mg MHE/g of dry extract) decreased by 6% from week 0 to week 8 and an additional 12% from week 8 to week 12. The loss in the content of phytochemical marker 3 followed a similar trend as phytochemical marker 2, with a 20% decrease in mg MHE/g of dry extract over 12 weeks. Phytochemical marker 4 exhibited the highest percentage loss (21%) between week 0 and week 12.

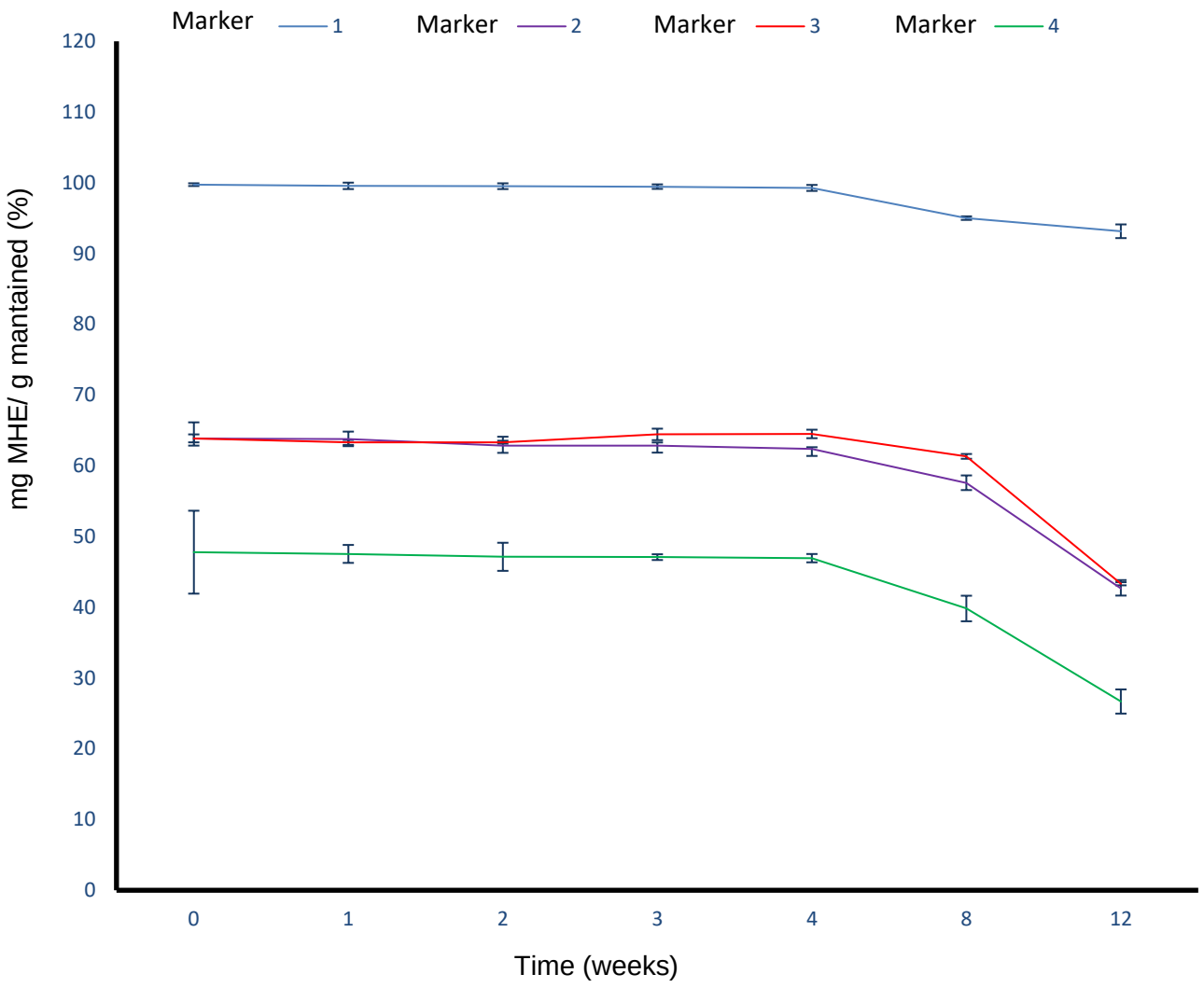

Figure 4.20: Percentage loss of mg MHE/g for 4 *A. afra* phytochemical marker molecules exposed to 25ºC/60% relative humidity over 12 weeks plotted as a function of time

4.7.5.2 Tablets exposed to 40ºC/75% relative humidity for 12 weeks

Table 4.27 shows the assay results for tablets exposed to 40ºC/75% relative humidity. At this accelerated stability storage condition, all four phytochemical marker molecules showed a substantial loss in mg MHE/g of dry extract as a function of time.

Table 4.27: Assay data for *A. afra* tablets exposed to 40ºC/75% relative humidity

***Artemisia afra* phytochemical marker molecule in extract or tablet**	**Average concentration (mg/L)**	**Mg MHE/ g of dry extract weight**
Phytochemical marker 1 in *A. afra* 200 mg extract	0.033	8.283
Phytochemical marker 2 in *A. afra* 200 mg extract	0.022	5.480
Phytochemical marker 3 in *A. afra* 200 mg extract	0.083	20.804
Phytochemical marker 4 in *A. afra* 200 mg extract	0.049	12.265
Morin hydrate added for assay	0.080	80.125
Phytochemical marker 1 in week 0 tablets	0.033	8.261
Phytochemical marker 2 in week 0 tablets	0.014	3.499
Phytochemical marker 3 in week 0 tablets	0.049	12.345
Phytochemical marker 4 in week 0 tablets	0.023	5.801
Morin hydrate added for assay	0.079	78.817
Phytochemical marker 1 in week 1 tablets	0.033	8.253
Phytochemical marker 2 in week 1 tablets	0.013	3.282
Phytochemical marker 3 in week 1 tablets	0.046	11.390
Phytochemical marker 4 in week 1 tablets	0.021	5.320
Morin hydrate added for assay	0.078	78.256
Phytochemical marker 1 in week 2 tablets	0.033	8.172
Phytochemical marker 2 in week 2 tablets	0.012	2.926
Phytochemical marker 3 in week 2 tablets	0.040	10.040
Phytochemical marker 4 in week 2 tablets	0.019	4.749
Morin hydrate added for assay	0.083	82.605
Phytochemical marker 1 in week 3 tablets	0.031	7.743
Phytochemical marker 2 in week 3 tablets	0.009	2.359
Phytochemical marker 3 in week 3 tablets	0.034	8.376

Table 4.27 (cont.): Assay data for *A. afra* tablets exposed to 40ºC/75% relative humidity

***Artemisia afra* phytochemical marker molecule in extract or tablet**	**Average concentration (mg/L)**	**Mg MHE/ g of dry extract weight**
Phytochemical marker 4 in week 3 tablets	0.016	3.911
Morin hydrate added for assay	0.087	87.170
Phytochemical marker 1 in week 4 tablets	0.031	7.722
Phytochemical marker 2 in week 4 tablets	0.008	2.117
Phytochemical marker 3 in week 4 tablets	0.029	7.130
Phytochemical marker 4 in week 4 tablets	0.012	2.894
Morin hydrate added for assay	0.078	77.633
Phytochemical marker 1 in week 8 tablets	0.030	7.523
Phytochemical marker 2 in week 8 tablets	0.005	1.225
Phytochemical marker 3 in week 8 tablets	0.020	4.904
Phytochemical marker 4 in week 8 tablets	0.005	1.283
Morin hydrate added for assay	0.083	83.224
Phytochemical marker 1 in week 12 tablets	0.029	7.299
Phytochemical marker 2 in week 12 tablets	0.001	0.304
Phytochemical marker 3 in week 12tablets	0.005	1.316
Phytochemical marker 4 in week 12 tablets	0.003	0.710
Morin hydrate added for assay	0.082	82.467

Figure 4.21 shows the percentage loss of mg MHE/g of phytochemical marker molecules in tablets exposed to 40ºC/75% relative humidity. It is evident that phytochemical marker 1 showed a much smaller loss in percentage mg MHE/g compared to phytochemical markers 2 – 4. Phytochemical marker 1 decreased by 12% over 12 weeks, whereas phytochemical markers 2 – 4 decreased by ≥ 43% at this accelerated stability condition.

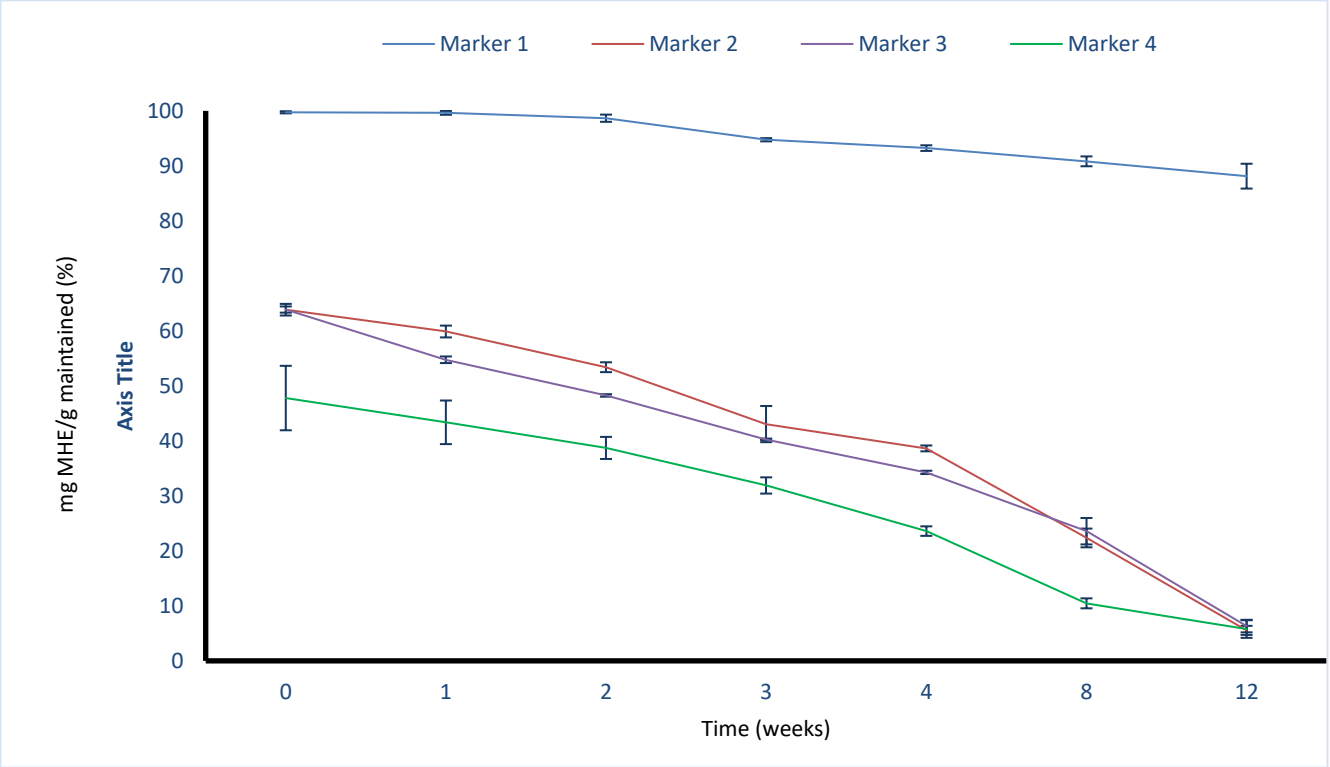

Figure 4.21: Percentage loss of mg MHE/g for 4 *A. afra* phytochemical marker molecules exposed to 40ºC/ 75% relative humidity over 12 weeks plotted as a function of time

4.7.6 Dissolution

4.7.6.1 *Artemisia afra* phytochemical marker molecules exposed to 25ºC/60% relative humidity

The release profile of all four phytochemical marker molecules as a function of time can be observed in Figure 4.22. At week zero, 98.72% of phytochemical marker molecule 1 was released after 240 min, whereas only 89.28% op phytochemical marker molecule 1 was released at the same time point after 12 weeks of exposure to 25ºC/ 60% relative humidity. The decrease in percentage dissolution of all four phytochemical marker molecules after 240 min was likely caused by an increase in tablet hardness from week 0 (109.7 N ± 2.71%) to week 12 (122.9 N ± 6.35%). The release of phytochemical marker 2 followed a different release trend as phytochemical marker molecule 1, as the release rate was slower, and a lower percentage dissolution was reached. At week zero, 63.22% of phytochemical marker 2 was dissolved after 240 min, whereas after 12 weeks, the release was slightly lower at 59.59%. The relatively lower percentage dissolution of marker molecule 2 (59.59%) at the end of the dissolution test (i.e., 240 min) as compared to that of marker molecule 1 (89.28%) can be explained by the decrease in its concentration and mg MHE/g in the tablets as observed with the assay. A marked decrease in mg MHE/g in phytochemical marker molecules 2 – 4 was observed after compression of the tablets. When tablets (24 h after compression) containing 200 mg *A. afra* dry powder extract were compared to 200 mg of the *A. afra* dry powder extract by means of an assay, the tablets contained a lower amount of mg MHE/g compared to the original *A. afra* extract.. The loss of this phytochemical marker molecules

is unknown at this stage, but potential degradation during compression could have occurred. The release profile of phytochemical marker 3 followed a release trend similar to that of phytochemical marker molecule 2. At week zero, 58.96% dissolution was reached at the 240 min time point, compared to 54.20% at week 12. The relatively lower percentage dissolution of marker molecule 3 at 240 min as compared to that of marker molecule 1 can also be explained by the decrease in its mg MHE/g in the tablets, as observed with the assay. Phytochemical marker molecule 4 experienced the greatest decrease in mg MHE/g after the tabletting process and showed the lowest percentage dissolution after 240 min, as a mere 46.46% was dissolved at week 0, and only 43.97% dissolved at week 12.

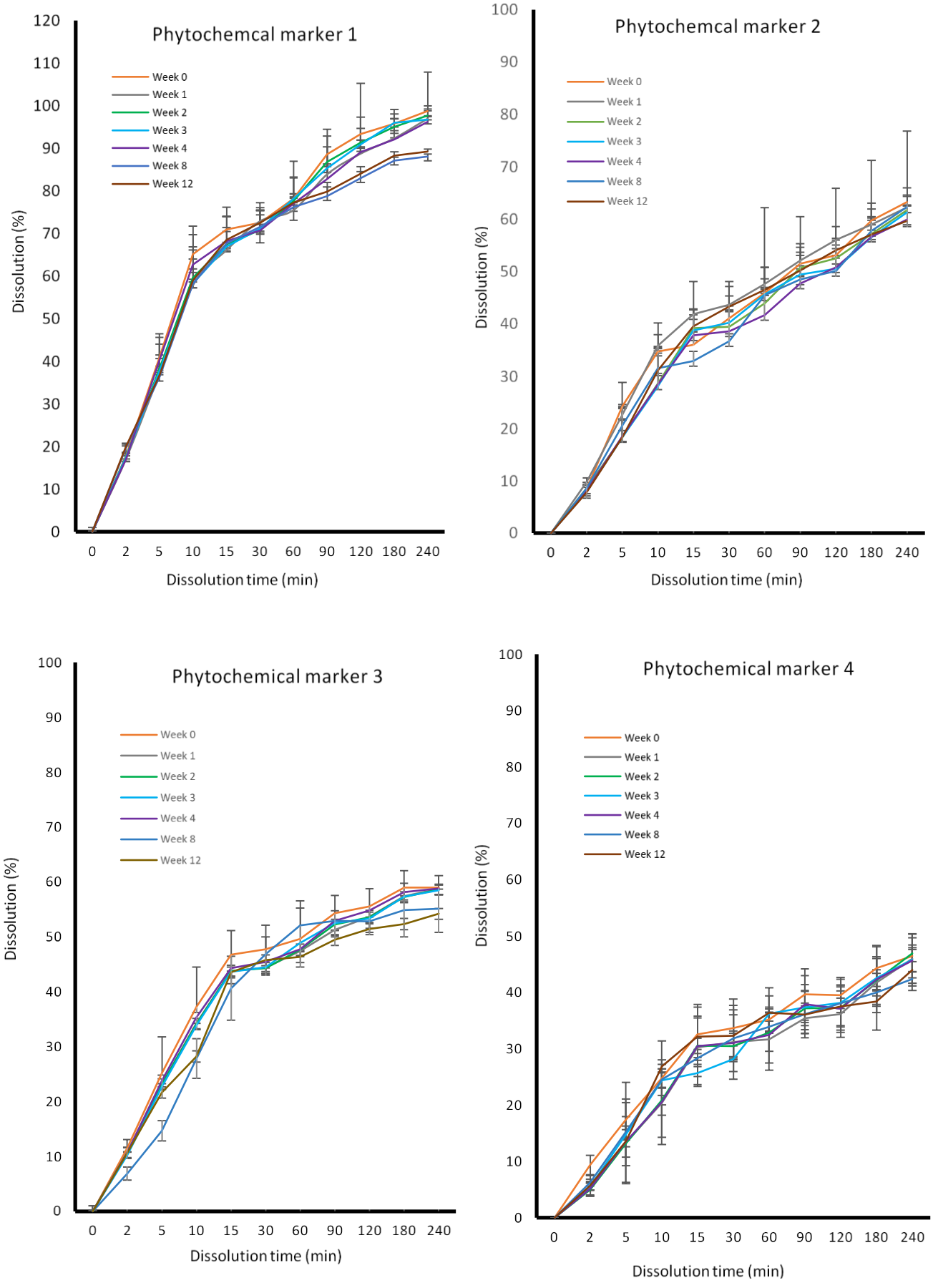

Figure 4.22*:* Percentage dissolution of *A. afra* phytochemical marker molecules 1 – 4 at 25ºC/60% relative humidity plotted as a function of time

Table 4.28 shows the 240 min area under the curve (AUC) values and relative standard deviation (± RSD) for the dissolution curves of every marker molecule over 12 weeks of dissolution studies after exposure to accelerated stability testing conditions at 25ºC/60% relative humidity. The results showed a slight decrease in AUC (µg/mL per min) for all four phytochemical marker molecules from week 0 to week 12. This reduction correlates with the slower release of all phytochemical marker molecules due to the increase in tablet hardness over 12 weeks (Table 4.23). It can be that a harder tablet led to a slower disintegration speed of the tablets, resulting in a longer dissolution time. Phytochemical marker 4 showed the highest reduction in AUC, from 134397 µg/mL per min ± 5.23% RSD at week 0 to 89719 µg/mL per min ± 4.93% RSD at week 12, which correlates with the reduction seen in the assay results.

Table 4.28: Area under the curve (AUC) in µg/mL per min for dissolution curves and relative standard deviation (± %RSD) values for tablets exposed to 25ºC/60% relative humidity

	Phytochemical marker 1	**Phytochemical marker 2**	**Phytochemical marker 3**	**Phytochemical marker 4**
Dissolution week	**AUC (µg/mL per min) ± %RSD**	**AUC (µg/mL per min) ± %RSD**	**AUC (µg/mL per min) ± %RSD**	**AUC (µg/mL per min) ± %RSD**
Week 0	61 676 ± 2.93	112 937 ± 4.13	113 622 ± 4.10	134 397 ± 5.23
Week 1	78179 ± 9.45	93599 ± 12.82	128106 ± 4	96918 ± 5.83
Week 2	65287 ± 3.31	118905 ± 4.43	130546 ± 4.04	103798 ± 6.12
Week 3	62658 ± 3.42	113332 ± 3.41	124280 ± 3.13	107077 ±0.34
Week 4	64001 ± 3.00	112372 ± 4.78	119346 ± 4.06	99531 ± 6.40
Week 8	63730 ± 2.11	113128 ± 1.04	112295 ± 1.99	115455 ± 2.75
Week 12	61353 ± 3.46	90600 ± 7.82	92335 ± 4.68	89719 ± 4.93

4.7.6.2 *Artemisia afra* phytochemical marker molecules exposed to 40ºC/75% relative humidity

The release of phytochemical marker molecules 1 – 4 from the tablets exposed to an accelerated stability condition of 40ºC/75% relative humidity is depicted in Figure 4.23. The dissolution results showed a notable slower release of all phytochemical marker molecules at week 12 compared to its release observed at week 0. Dissolution for phytochemical marker molecule 1 at week 12 was 86.20% after 240 min, compared to 98.72% dissolution at week 0, indicating that release was notably slower after tablets were exposed to an accelerated stability storage condition of 40°C/75% relative humidity. The slower release of all four phytochemical marker molecules can be explained by the increase in hardness of the tablets when exposed to this accelerated stability condition. The increase in tablet hardness from week 0 to week 12 was substantial as the hardness increased from 109.7 N ± 2.71% RSD to 160.0 N ± 5.93% RSD. Prior to exposure to the accelerated stability storage condition (week 0), phytochemical marker 2 was 63.22% dissolved at 240 min, but after 12 weeks of exposure to the accelerated stability condition, dissolution could reach only 53.01% after 240 min. The relatively lower

percentage dissolution of phytochemical marker molecules 2 – 4 at 240 min as compared to that of marker molecule 1 can also be explained by the decrease in its concentration and mg MHE/g in the tablets as observed with the assay. After the assay, it was noted that a decrease of mg MHE/g in phytochemical markers 2 – 4 was observed after the tabletting process was finished. Phytochemical marker molecule 3 also showed a slower dissolution release rate after 12 weeks of drug release studies in comparison to the dissolution profile at week 0. Approximately 59% of phytochemical marker molecule 3 was dissolved after 240 min at week 0, which can also be explained by the loss during tablet compression as seen in the assay test. The accelerated storage condition of 40°C/75% relative humidity led to a substantial decrease in weekly dissolution percentages at the 240 minute time interval, namely 56.97% dissolution after 4 weeks, 52.44% dissolution after 8 weeks and only 49.08% dissolution after 12 weeks. Phytochemical marker molecule 4 reached only 38.3% dissolution at 240 min after 12 weeks of exposure, compared to 46.46% dissolution at week 0. The low dissolution percentages can also be explained by the reduction in mg MHE/g in phytochemical marker 4 observed after the assay. Phytochemical marker 1 was not influenced by the process of powder formulation and tabletting; however, phytochemical marker molecules 2 – 4 saw a steep decrease in concentration (35% – 52.2%) after an assay was conducted at week zero, 24 h after tabletting. When observing the results n Figure 4.24, it is evident that exposure to an accelerated storage condition of 40°C/75% relative humidity had a far more significant impact on the release of phytochemical marker molecules compared to 25°C/60% relative humidity, as the dissolution was slower over 12 weeks, and a lower total dissolution was achieved after 240 min. The decrease in dissolution rate can possibly be attributed to a more than 50 N increase in tablet hardness over 12 weeks (Table 4.23).

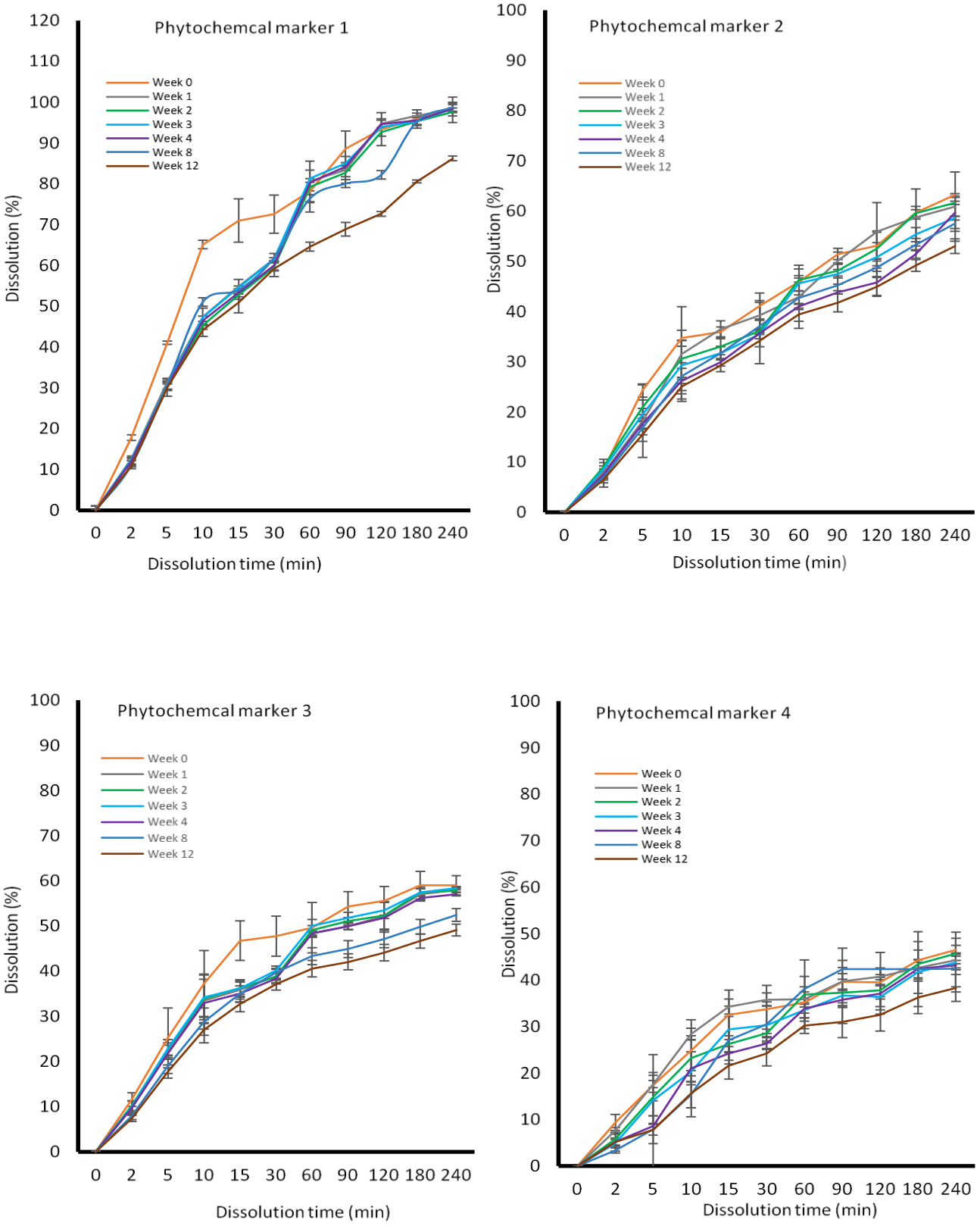

Figure 4.23: Percentage dissolution of A. afra phytochemical marker molecules 1 – 4 at 25ºC/60% relative humidity plotted as a function of time

Table 4.29 shows the 240 min area under the curve (AUC) values in µg/mL per min for dissolution curves and the relative standard deviation (± %RSD) for tablets exposed to 40ºC/75% relative humidity for all four marker molecules over a 12-week period of dissolution studies after exposure to an accelerated stability testing condition of 40ºC/75% relative humidity. The AUC of all phytochemical marker molecules showed a reduction after 12 weeks. The decrease correlates with the dissolution results that showed a lower percentage dissolution after 12 weeks, likely due to an increased tablet hardness of approximately 50 N from week 0 to week 12. Phytochemical marker molecules 2 – 4 showed a more significant decrease in content compared to phytochemical marker molecule 1, which correlated with the assay results, where phytochemical marker molecules 2 – 4 showed a faster reduction in phytochemical marker content over 12 weeks.

Table 4.29: Area under the curve (AUC) in µg/mL per min for dissolution curves and relative standard deviation (± %RSD) values for tablets exposed to 40ºC/75% relative humidity

	Phytochemical marker 1	**Phytochemical marker 2**	**Phytochemical marker 3**	**Phytochemical marker 4**
Dissolution week	**AUC (µg/mL per min) ± %RSD**	**AUC (µg/mL per min) ± %RSD**	**AUC (µg/mL per min) ± %RSD**	**AUC (µg/mL per min) ± %RSD**
0	78 179 ± 2.93	93 599 ± 4.13	113 622 ± 4.10	134 397 ± 5.23
Week 1	65287 ± 2.53	118905 ± 3.21	128106 ± 0.54	96918 ± 1.13
Week 2	62658 ± 1.76	113332 ± 0.69	130546 ± 0.84	103798 ± 0.37
Week 3	64001 ± 1.56	112372 ± 0.57	124280 ± 0.67	107077 ± 5.33
Week 4	63730 ± 2.49	113128 ± 2.43	119346 ± 0.93	99531 ± 0.33
Week 8	61353 ± 1.21	90600 ± 3.31	112295 ± 3.48	115455 ± 1.83
Week 12	54235 ± 1.92	59662 ± 4.72	92335 ± 4.97	89719 ± 8.36

4.8 Summary

Artemisia afra aqueous extracts prepared at 96ºC produced a higher yield of dry powder extract and rendered a similar amount or more mg morin hydrate equivalents per gram of dry extract weight (mg MHE/g) when compared to extracts prepared at 25ºC, 50ºC, and 70ºC. Bulk *A. afra* dry powder extracts were produced by double freeze-drying and sieving the bulk powder. The bulk *A. afra* dry powder extract and 6 powder excipients were subjected to SeDeM EDS. Tricalcium citrate was identified as the most appropriate corrective excipient to correct the poor powder flow properties (i.e., low incidences of the *A. afra* dry powder extract). Tricalcium citrate was mixed with a small amount of disintegrant, lubricant and binder to make a corrective powder mixture characterised using the SeDeM EDS. The corrective mixture (70% w/w) was mixed with the *A. afra* dry powder extract (30% w/w) and was subjected to a last round of SeDeM EDS. All 5 SeDeM incidences and 3 additional indices were acceptable for tabletting using direct compression. Tablets weighing 667 mg were compressed and

was subjected to stability testing and evaluation in terms of an assay, weight variation, hardness, friability, disintegration, and dissolution behaviour at week 0 and for 12 weeks after the tablets were exposed to accelerated stability conditions of 25°C/60% relative humidity and 40°C/75% relative humidity. All tablet samples complied with the BP specifications for uniformity of weight, friability, and disintegration. Assay results at 25°C/60% relative humidity showed a slight reduction in mg MHE/g for phytochemical marker molecule 1; however, a more noticeable decrease in mg MHE/g was observed for phytochemical marker molecules 2 – 4. All four phytochemical markers showed an accelerated decrease in content at 40°C/75% relative humidity, but phytochemical marker molecule 2 – 4 exhibited a faster decline in concentration over the 12-week period. The formulation, mixing with excipients, and tabletting might have impacted the content of phytochemical marker molecules 2 – 4, as observed in the assay results. Dissolution results showed that increased tablet hardness reduced the overall dissolution rate and that complete dissolution for phytochemical markers 2 – 4 was not possible, as the reduction in concentration after the tabletting process shown by the assay results, led to the highest possible dissolution of approximately 65% for phytochemical markers 2 and 3; and a highest possible dissolution of approximately 48% for phytochemical marker 4.

CHAPTER 5

FINAL SUMMARY AND RECOMMENDATIONS

5.1 Final summary

The study employed the SeDeM Expert Diagram System (EDS) to formulate a solid oral dosage form containing *A. afra* extract. An HPLC analytical method was validated regarding linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ), with morin hydrate used as the internal standard. The method successfully quantified four prominent phytochemical marker molecules inside the *A. afra* extract. The quantity of each phytochemical marker was expressed as mg morin hydrate equivalents per gram of dry extract weight (mg MHE/ g). Four aqueous *A. afra* extracts were prepared at different temperatures (25ºC, 50ºC, 70ºC, or 96ºC) for 30 min. The mg MHE/g were determined for all four-phytochemical markers at each selected temperature, and the dry powder mass yield of each extract was measured. Extracts prepared at 96ºC produced the highest dry powder yield and had the highest overall mg MHE/g, supporting the traditional preparation method where boiling water (± 96ºC) is used to prepare *A. afra* tea.

Three aqueous *A. afra* extracts were prepared at 96ºC for 30 min with plant material derived from different regions (SUNfarm S.A pty, Potchefstroom airfield, Bronkhorstspruit). The same four phytochemical marker molecules were present, but the quantity as expressed by mg MHE/g was different for each extract, indicating variances in phytochemical composition between *A. afra* plants from different regions. The dry powder extract yields were measured, and small differences in yield mass and powder colours were observed. A batch of bulk *A. afra* dry powder extract with good powder flow properties was produced after double freeze-drying, and powder sieving was implemented. Bulk *A. afra* aqueous extracts were made using one large batch of *A. afra* plant material gathered from the Bronkhorstspruit Bay area as the plant material at Bronkhorstspruit was available in large quantities to produce a sufficient amount of *A. afra* dry powder extract for formulation studies and tableting.

The *A. afra* dry extract powder was characterised with the SeDeM EDS, and a polygon was constructed using the12 SeDeM EDS parameter values namely bulk density (Da), tapped density (Dc), interparticle porosity (Ie), Carr's index (Ic), cohesion index (Icd), Hausner ratio (IH), angle of repose (α), powder flow (%Pf), loss on drying (% HR), hygroscopicity (%H), particle size below 45 µm (%Fm), and the homogeneity index (Iθ). The 12 SeDeM EDS parameters were divided into 5 incidences namely dimension, compressibility, flowability, lubricity/stability, lubricity/dosage. Results indicated that the dry *A. afra* powder extract was not suited for direct compression as four of the five *A. afra* SeDeM EDS incidences needed correction. Subsequently, six powder excipients were subjected to the SeDeM EDS to identify the appropriate excipient to correct the poor powder flow properties of the *A. afra* powder

extract. Ludipress®, MicroceLac® 100, Kollidon® VA 64, tricalcium citrate, Emcompress®, and Avicel® PH 200 were characterised using SeDeM EDS, and polygons for each excipient were constructed and superimposed with the *A. afra* polygon. Three powder excipients were suitable as corrective excipients (tricalcium citrate, Ludipress®, and MicroceLac® 100). Tricalcium citrate had the most appropriate SeDeM EDS powder profile and was subsequently chosen as the corrective excipient that allowed the highest percentage *A. afra* dry powder extract inclusion into the tabletting mixture.

The corrective excipient (tricalcium citrate, 88.5% w/w) was supplemented with a 3.5% w/w of a SeDeM EDS lubricant mixture comprised of colloidal silicon dioxide (0.14% w/w), talc (2.36% w/w), and magnesium stearate (1.00% w/w), together with Kollidon® VA 64 (3% w/w) as a binder, and Ac-di-sol® (5% w/w) as a disintegrant. The corrective mixture was characterised with SeDeM EDS and the results indicated that a minimum of 67.86% w/w corrective excipient was needed to correct all five *A. afra* SeDeM incidences to above 5 to compensate for the poor powder flow properties of the *A. afra* dry powder extract. The minimum required corrective excipient (67.86% w/w) was not used, but a slightly higher percentage (70% w/w) to ensure that all five incidence values of the final tablet mixture exceeded a value of 5. The corrective powder mixture (70% w/w) was mixed with the *A. afra* dry powder extract (30% w/w) to prepare a tabletting powder mixture. The powder was subject to one last SeDeM EDS characterisation cycle. All 5 SeDeM incidences were above a value of 5 (dimension (5.13), compressibility (5.11), flowability (5.80), lubricity/stability (5.26), lubricity/dosage (5.18)), and the three additional indices (parameter index (IP) of 0.58, a parameter profile index (IPP) of 5.32, and a good compressibility index (GCP) of 5.06) were all acceptable. The results showed that the tabletting mixture exhibited good powder flow properties suitable for direct compression.

Tablets weighing 667 mg and containing 200 mg *A. afra* dry powder extract were compressed. These tablets were packed in amber containers, and silica bags were added. Containers were kept in a cool and dark room for 24 h, where after they were subjected to 12 weeks stability testing (25ºC/60% relative humidity or 40ºC/75% relative humidity) and tablet evaluation tests. Tablets complied with the BP requirements for uniformity of tablet weight, disintegration time, and friability. Tablet hardness increased after exposure to the accelerated stability conditions at 25ºC/60% relative humidity and increased even more after exposure to 40ºC/75% relative humidity. Dissolution studies showed that exposure to 25ºC/60% relative humidity had a small impact on releasing the four phytochemical marker molecules into the dissolution medium. However, exposure to 40ºC/75% relative humidity had a more pronounced impact in reducing dissolution rate, leading to a lower dissolution percentage for all four phytochemical marker molecules over 240 min of dissolution.

The assay results indicate that phytochemical marker molecules 2 – 4 are less stable than phytochemical marker 1, and experienced possible compatibility problems with powder excipients. Assay results showed that the powder mixing and tabletting process immediately impacted the mg MHE/g of phytochemical markers molecules 2 – 4, as they lost more than 35% in mg MHE/g at week 0,

even before exposure to accelerated stability conditions. However, phytochemical marker 1 did not decrease in mg MHE/g after mixing and tabletting. Phytochemical marker 1 was the most stable marker throughout the assay studies and had the lowest weekly reduction in mg MHE/g after exposure to accelerated stability conditions. Exposure to 40ºC/75% relative humidity caused a notably faster weekly reduction in mg MHE/g in all phytochemical markers, compared to 25ºC/60% relative humidity exposure.

5.2 Recommendations for future studies

Based on the results and findings of the study, the following recommendations are made for future studies concerning formulation experiments with *A. afra*:

- The commercially farmed *A. afra* plant material from SUNfarm S.A pty showed a larger extract powder yield and had more mg MHE/g than the other *A. afra* plant material. It is worth investigating how nutrients in soil impact the phytochemical composition of *A. afra* plants.
- The freeze-drying method applied to frozen extract was highly time-consuming, and it is advised to investigate spray-drying as a possible alternative.
- Investigate using mass spectrometry (MS) instead of HPLC to obtain more accurate results and possibly identify the four phytochemical markers used in this study.
- Possible compatibility issues arose between *A. afra* dry powder extract and powder excipients. Conducting compatibility studies between *A. afra* dry powder extract and all excipients should be considered for future studies.
- It appears that the complete dose of the phytochemical marker molecules were dissolved after 240 min throughout the 12 weeks of dissolution studies. Consider increasing dissolution time to 360 min or more.

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ADDENDUMS

ADDENDUM A: MORIN HYDRATE VALIDATION RESULTS

Table A1: inter-day precision results for morin hydrate

Inter-day Precision Day One					
Mass (w/v)	**Ret Time**	**Peak Area**	**Mean**	**STDEVP**	**RSD**
(µg/mL)	**(min)**	**(AUC)**	**(AUC)**		**(%)**
0.98	13.86	16000	16083	119.51	0.74
	13.84	16252			
	13.84	15997			
15.68	13.83	251290	250761	377.69	0.15
	13.80	250431			
	13.80	250563			
251	13.78	3648497	3648808	916.69	0.03
	13.78	3650053			
	13.78	3647873			
Mean	13.83				
STDEVP	0.03				
% RSD	0.21				
Inter-day Precision Day Two					
Mass (w/v)	**Ret Time**	**Peak Area**	**Mean**	**STDEVP**	**RSD**
(µg/mL)	**(min)**	**(AUC)**	**(AUC)**		**(%)**
0.98	13.59	16037	16282	252.54	1.55
	13.6	16630			
	13.59	16181			
15.68	13.58	250742	250729	403.04	0.16
	13.58	251217			
	13.57	250230			
251	13.55	3697191	3688478	7449.76	0.20
	13.57	3678992			
	13.52	3689250			
Mean	13.57411				
STDEVP	0.021398				
% RSD	0.157639				
Inter-day Precision Day Three					
Mass (w/v)	**Ret Time**	**Peak Area**	**Mean**	**STDEVP**	**RSD**
((µg/mL)	**(min)**	**(AUC)**	**(AUC)**		**(%)**
0.98	13.824	16661	16267	292.52	1.79
	13.795	15960			
	13.78	16182			
15.68	13.772	250857	250961	178.08	0.07
	13.758	250815			
	13.761	251212			
251	13.734	3745372	3722129	16449.75	0.44
	13.75	3711345			
	13.74	3709669			
Mean	13.76822				
STDEVP	0.026745				
% RSD	0.19425				

Table A2: Intra-day precision results for morin hydrate

Intra-day precision run one					
Mass (w/v)	**Ret Time**	**Peak Area**	**Mean**	**STDEVP**	**RSD**
(µg/mL)	**(min)**	**(AUC)**	**(AUC)**		**(%)**
0.98	13.598	16037	16282	252.53	1.55
	13.6	16630			
	13.587	16181			
15.68	13.58	250742	250729	403.03	0.16
	13.577	251217			
	13.572	250230			
251	13.553	3697191	3688478	7449.76	0.20
	13.573	3678992			
	13.527	3689250			
Mean	13.57				
STDEVP	0.02				
% RSD	0.16				

Intra-day precision run two					
Mass (w/v)	**Ret Time**	**Peak Area**	**Mean**	**STDEVP**	**RSD**
(µg/mL)	**(min)**	**(AUC)**	**(AUC)**		**(%)**
0.98	13.60	16776	16516.33	204.22	1.24
	13.57	16496			
	13.57	16277			
15.68	13.58	249695	249850.3	1223.15	0.49
	13.61	248436			
	13.62	251420			
251	13.63	3720635	3703751	13814.4	0.37
	13.63	3703822			
	13.65	3686797			
Mean	13.62				
STDEVP	0.02				
% RSD	0.17				

Intra-day precision run three					
Mass (w/v)	**Ret Time**	**Peak Area**	**Mean**	**STDEVP**	**RSD**
(µg/mL)	**(min)**	**(AUC)**	**(AUC)**		**(%)**
0.98	13.67	16106	16105.67	26.54	0.16
	13.68	16138			
	13.69	16073			
15.68	13.69	249066	250339	1161.75	0.46
	13.70	250076			
	13.69	251875			
251	13.71	3780045	3717297	44386.26	1.19
	13.71	3684421			
	13.70	3687426			
Mean	13.69389				
STDEVP	0.011666				
% RSD	0.085192				

Table A3: Linearity results for morin hydrate

Sample	(µg/ml)	Ret Time	AUC	Mean
Std 1	0.98	13.863	16331	16199
		13.845	16067	
Std 2	1.96	13.841	33398	33702
		13.831	34006	
Std 3	3.92	13.812	65479	66125
		13.822	66771	
Std 4	7.84	13.81	127163	128150
		13.816	129137	
Std 5	15.69	13.817	254049	252175
		13.819	250301	
Std 6	31.38	13.826	496495	493546
		13.831	490597	
Std 7	62.75	13.822	954083	964089
		13.833	974095	
Std 8	125.5	13.84	1892481	1893808
		13.841	1895135	
Std 9	251	13.824	3651816	3650033
		13.83	3648250	
Std 10	502	13.812	7296412	7307130
		13.816	7317848	
Std 11	1004	13.799	15045682	14998579.5
		13.801	14951477	

ADDENDUM B: LINEARITY RESULTS FOR *A. AFRA*

Table B1: Linearity results for *A. afra*

Artemisia afra Linearity marker 1

Sample	(µg/ml)	Ret Time	AUC	Mean
Std 1	39.063	1.995	3414	3445
		2.01	3476	
Std 2	78.125	2.011	7082	7005
		2.02	6928	
Std 3	156.25	2.014	13733	13991.5
		2.034	14250	
Std 4	312.5	2.015	28009	27831.5
		1.993	27654	
Std 5	625	2.024	56328	51724.5
		2	47121	
Std 6	1250	2.015	94693	94442.5
		1.997	94192	
Std 7	2500	2.009	188091	188884
		2.024	189677	
Std 8	5000	20.26	404129	397356.5
		20.23	390584	
Std 9	10000	2.03	795898	757054.5
		2.004	718211	

R^2	
Range	0.999724
Linearity	0.999565

Artemisia afra Linearity marker 2

Sample	(µg/ml)	Ret Time	AUC	Mean
Std 1	39.063	7.451	2210	2287.5
		7.44	2365	
Std 2	78.125	7.459	4631	4750
		7.445	4869	
Std 3	156.25	7.485	9962	9925
		7.467	9888	
Std 4	312.5	7.437	19584	19555.5
		7.447	19527	
Std 5	625	7.44	33348	35936.5
		7.455	38525	
Std 6	1250	7.449	65035	65462
		7.46	65889	
Std 7	2500	7.464	126066	126335.5
		7.45	126605	
Std 8	5000	7.457	266077	262341.5
		7.459	258606	
Std 9	10000	7.441	516977	548634
		7.453	580291	

R^2	
Range	0.999593
Linearity	0.999593

Artemisia afra Linearity marker 3

Sample	(µg/ml)	Ret Time	AUC	Mean
Std 1	39.063	7.989	8045	8092
		7.98	8139	
Std 2	78.125	7.995	15522	15640.5
		7.983	15759	
Std 3	156.25	8.019	32397	32189
		8.003	31981	
Std 4	312.5	7.956	61084	61091.5
		7.963	61099	
Std 5	625	7.959	103509	111575
		7.972	119641	
Std 6	1250	7.969	198795	198331
		7.978	197867	
Std 7	2500	7.982	383390	379179
		7.968	374968	
Std 8	5000	7.974	753730	745547
		7.976	737364	
Std 9	10000	7.962	1532597	1595421
		7.97	1658244	

R^2	
Range	0.999372
Linearity	0.999752

Artemisia afra Linearity marker 4

Sample	(µg/ml)	Ret Time	AUC	Mean
Std 1	39.063	10.393	4140	4101
		10.38	4062	
Std 2	78.125	10.386	9449	9099.5
		10.369	8750	
Std 3	156.25	10.419	17465	17877
		10.4	18289	
Std 4	312.5	10.308	36485	35504.5
		10.32	34524	
Std 5	625	10.32	63753	67512
		10.328	71271	
Std 6	1250	10.33	121763	121795.5
		10.34	121828	
Std 7	2500	10.345	233689	231981
		10.332	230273	
Std 8	5000	10.333	464968	457992
		10.338	451016	
Std 9	10000	10.328	949141	982277.5
		10.33	1015414	

R^2	
Range	0.999363
Linearity	0.999363

ADDENDUM C: YIELDS RESULTS FROM *A. AFRA* BULK EXTRACTS

Table C1*:* Yields results from *A. afra* bulk extracts

Extract No	*A. afra* twigs and leaves (g)	H20 mL	Yield (g)	Extract No	*A. afra* twigs and leaves (g)	H2O mL	Yield (g)
1	25	500	6.7	28	25	500	6.6
2	25	500	6.7	29	25	500	6.7
3	25	500	6.8	30	25	500	6.7
4	25	500	6.6	31	25	500	6.8
5	25	500	6.8	32	25	500	6.8
6	25	500	6.7	33	25	500	6.6
7	25	500	6.6	34	25	500	6.7
8	25	500	6.8	35	25	500	6.7
9	25	500	6.7	36	25	500	6.8
10	25	500	6.6	37	25	500	6.7
11	25	500	6.8	38	25	500	6.8
12	25	500	6.8	39	25	500	6.6
13	25	500	6.7	40	25	500	6.7
14	25	500	6.8	41	25	500	6.8
15	25	500	6.6	42	25	500	6.8
16	25	500	6.7	43	25	500	6.7
17	25	500	6.6	44	25	500	6.8
18	25	500	6.8	45	25	500	6.8
19	25	500	6.9	46	25	500	6.7
20	25	500	6.7	47	25	500	6.8
21	25	500	6.7	48	25	500	6.6
22	25	500	6.8	49	25	500	6.7
23	25	500	6.6	50	25	500	6.8
24	25	500	6.7	51	25	500	6.7
25	25	500	6.8	52	25	500	6.8
26	25	500	6.8	53	25	500	6.8
27	25	500	6.6	54	25	500	6.6

ADDENDUM D: RESULTS FOR DENSITY STUDIES, ANGLE OF REPOSE, FLOWABILITY, LOSS ON DRYING AND HYGROSCOPICITY

Table D1: Results for density studies, angle of repose, flowability, loss on drying and hygroscopicity of *A. afra*

Artemisia afra				
	Value 1	**Value 2**	**Value 3**	**Average**
P (Nett weight)	50.05	49.96	50.04	50.02
Va (Initial volume powder)	174.00	172.00	172.00	172.67
Vc (Final volume powder)	123.00	121.00	122.00	122.00
h (hight of the cone)	32.00	32.00	32.00	32.00
r (radius of the cone)	82.30	81.50	81.80	81.87
h/r	0.389	0.393	0.391	0.391
t" (flowability) (sek) (15 mm)	9.20	9.10	9.20	9.17
LOD (before)	1.040	1.010	1.090	1.047
LOD (after)	0.980	0.940	1.030	0.983
LOD	5.77	6.93	5.50	6.07
% H (Hygroscopicity)	11.82	11.11	12.50	11.81
flowability (sek)	9.20	9.10	9.20	9.167
flowability (g/s)	10.90	11.00	10.90	10.933

Table D2: Results for density studies, angle of repose, flowability, loss on drying and hygroscopicity of tricalcium citrate

Tricalcium citrate				
	Value 1	**Value 2**	**Value 3**	**Average**
P (Nett weight)	100.26	99.78	100.03	100.02
Va (Initial volume powder)	188.00	186.00	184.00	186.00
Vc (Final volume powder)	154.00	153.00	152.00	153.00
h (hight of the cone)	32.00	32.00	32.00	32.00
r (radius of the cone)	67.00	68.00	68.50	67.83
h/r	0.478	0.471	0.467	0.472
t" (flowability) (sek) (15 mm)	6.80	6.70	7.00	6.83
LOD (before)	1.540	1.450	1.670	1.55
LOD (after)	1.440	1.350	1.570	1.45
LOD	6.49	6.90	5.99	6.46
% H (Hygroscopicity)	0.00	0.00	0.00	0.00
flowability (sek)	6.80	6.70	7.00	6.83
flowability (g/s)	14.70	14.90	14.30	14.63

Table D3: Results for density studies, angle of repose, flowability, loss on drying and hygroscopicity of Ludipress®

Ludipress®				
	Value 1	**Value 2**	**Value 3**	**Average**
P (Nett weight)	100.13	96.96	100.04	100.04
Va (Initial volume powder)	198.00	193.00	196.00	195.67
Vc (Final volume powder)	166.00	162.00	164.00	164.00
h (hight of the cone)	30.00	30.00	30.00	30.00
r (radius of the cone)	72.00	70.00	72.50	71.50
h/r	0.417	0.429	0.414	0.420
t" (flowability) (sek) (15 mm)	5.30	5.20	5.40	5.30
LOD (before)	1.350	1.190	1.320	1.287
LOD (after)	1.280	1.120	1.250	1.217
LOD	5.19	5.88	5.30	5.46
% H (Hygroscopicity)	4.23	4.05	4.18	4.15
flowability (sek)	5.30	5.20	5.40	5.300
flowability (g/s)	18.90	19.20	18.50	18.867

Table D4: Results for density studies, angle of repose, flowability, loss on drying and hygroscopicity of MicroceLac 100

MicroceLac® 100				
	value 1	**value 2**	**value 3**	**Average**
P (Nett weight)	100.14	99.89	100.02	100.02
Va (Initial volume powder)	202.00	200.00	203.00	201.67
Vc (Final volume powder)	172.00	171.00	174.00	172.33
h (hight of the cone)	28.00	28.00	28.00	28.00
r (radius of the cone)	81.00	78.00	78.00	79.00
h/r	0.346	0.359	0.359	0.355
t" (flowability) (sek) (15 mm)	8.20	8.00	8.20	8.13
LOD (before)	1.390	1.520	1.160	1.357
LOD (after)	1.360	1.480	1.120	1.320
LOD	2.16	2.63	3.45	2.746
% H (Hygroscopicity)	1.44	1.85	1.83	1.71
flowability (sek)	8.20	8.00	8.20	8.133
flowability (g/s)	12.20	12.50	12.20	12.300

Table D5: Results for density studies, angle of repose, flowability, loss on drying and hygroscopicity of Avicel PH 200

Avicel® PH200				
	value 1	**value 2**	**value 3**	**Average**
P (Nett weight)	50.02	49.96	50.05	50.01
Va (Initial volume powder)	134.00	133.00	135.00	134.00
Vc (Final volume powder)	114.00	113.00	114.00	113.67
h (hight of the cone)	19.00	19.00	19.00	19.00
r (radius of the cone)	69.50	69.00	68.50	69.00
h/r	0.273	0.275	0.277	0.275
t" (flowability) (sek) (15 mm)	2.90	3.00	3.00	2.97
LOD (before)	2.036	2.037	2.038	2.037
LOD (after)	1.951	1.950	1.954	1.952
LOD	4.17	4.27	4.12	4.189
% H (Hygroscopicity)	0.98	0.97	0.98	0.98
flowability (sek)	2.90	3.00	3.00	2.967
flowability (g/s)	34.50	33.30	33.30	33.700

Table D6: Results for density studies, angle of repose, flowability, loss on drying and hygroscopicity of Emcompress®

Emcompress®				
	value 1	**value 2**	**value 3**	**Average**
P (Nett weight)	100.37	100.13	99.61	100.04
Va (Initial volume powder)	110.00	108.00	101.00	106.33
Vc (Final volume powder)	98.00	98.00	96.00	97.33
h (hight of the cone)	21.00	21.00	21.00	21.00
r (radius of the cone)	64.00	63.00	64.00	63.67
h/r	0.328	0.333	0.328	0.330
t" (flowability) (sek) (15 mm)	3.80	3.60	3.60	3.67
LOD (before)	1.410	1.860	1.300	1.523
LOD (after)	1.380	1.840	1.270	1.497
LOD	2.13	1.08	2.31	1.84
% H (Hygroscopicity)	0.00	0.00	0.00	0.00
flowability (sek)	3.80	3.60	3.60	3.667
flowability (g/s)	26.30	27.80	27.80	27.300

Table D7: Results for density studies, angle of repose, flowability, loss on drying and hygroscopicity of Kollidon VA 64

Kollidon® VA 64				
	value 1	**value 2**	**value 3**	**Average**
P (Nett weight)	50.06	50.02	49.98	50.02
Va (Initial volume powder)	168.00	166.00	166.00	166.67
Vc (Final volume powder)	138.00	138.00	138.00	138.00
h (hight of the cone)	28.00	28.00	28.00	28.00
r (radius of the cone)	185.00	185.00	192.00	187.33
h/r	0.151	0.151	0.146	0.150
t" (flowability) (sek) (15 mm)	no flow	no flow	no flow	no flow
LOD (before)	2.036	2.037	2.038	2.037
LOD (after)	1.951	1.950	1.954	1.952
LOD	4.17	4.27	4.12	4.189
% H (Hygroscopicity)	4.23	4.05	4.18	4.15
flowability (sek)	2.90	3.00	3.00	2.967
flowability (g/s)	34.50	33.30	33.30	33.700

Table D8: SeDeM EDS cohesion index results

Artemisia afra	**Cohesion Index**		**Tricalcium citrate**	**Cohesion Index**		**Kollidon® VA 64**	**Cohesion Index**
1	23		1	454		1	242
2	21		2	456		2	250
3	24		3	476		3	246
4	21		4	422		4	256
5	18		5	461		5	242
Average	**21.40**		**Average**	**453.80**		**Average**	**247.20**
Ludipress®	**Cohesion Index**		**Avicel® PH 200**	**Cohesion Index**		**Emcompress®**	**Cohesion Index**
1	319		1	274		1	272
2	310		2	255		2	249
3	334		3	260		3	280
4	330		4	270		4	287
5	338		5	275		5	287
Average	**326.20**		**Average**	**266.80**		**Average**	**275.00**
MicroceLac® 100	**Cohesion Index**		**Excipient corrective mix**	**Cohesion Index**		***Artemisia afra* final tablet mix**	**Cohesion Index**
1	341		1	445		1	245
2	353		2	437		2	238
3	355		3	449		3	242
4	347		4	435		4	249
5	340		5	442		5	241
Average	**347.20**		**Average**	**441.60**		**Average**	**243.00**

ADDENDUM E: MALVERN MASTERSIZER DATA SHEETS AND HOMOGENEITY CALCULATIONS

MASTERSIZER 2000

Result Analysis Report

Sample Name: Artemisia afra Bronk Extract - Average
SOP Name: A. afra ext Bronk
Measured: 03 June 2021 10:14:21 AM
Sample Source & type: PR
Measured by: Pieter Roets
Analysed: 03 June 2021 10:14:22 AM
Sample bulk lot ref: 001 (Sample 3)
Result Source: Averaged

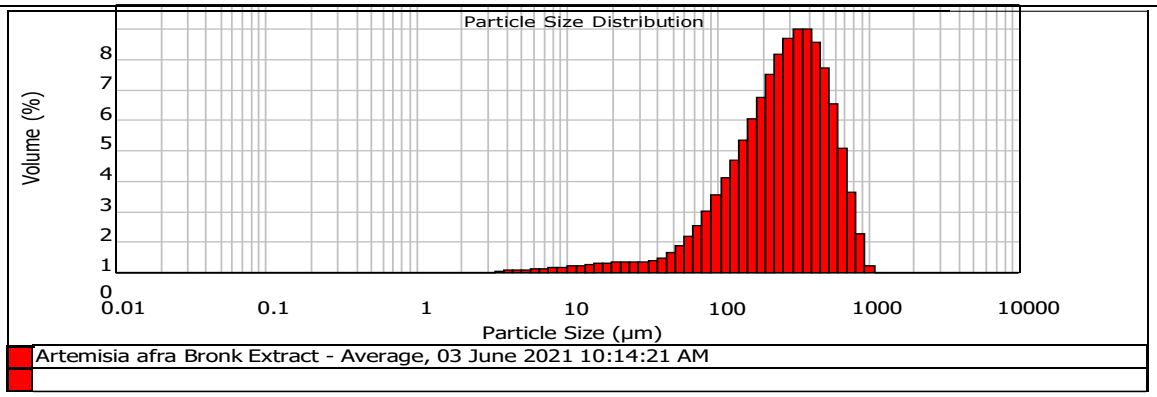
Particle Name: Quercetin
Accessory Name: Hydro 2000SM (A)
Analysis model: General purpose
Sensitivity: Enhanced
Particle RI: 1.767
Absorption: 0
Size range: 0.020 to 2000.000 um
Obscuration: 13.38 %

Dispersant Name: Cyclohexane
Dispersant RI: 1.468
Weighted Residual: 0.832 %
Result Emulation: Off

Concentration: 0.2757 %Vol
Span : 1.801
Uniformity: 0.56
Result units: Volume

Specific Surface Area: 0.0432 m²/g
Surface Weighted Mean D[3,2]: 138.915 um
Vol. Weighted Mean D[4,3]: 317.700 um

d(0.1): 89.327 um **d(0.5):** 283.569 um **d(0.9):** 600.157 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.22	120.226	3.69	1258.925	00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.25	138.038	4.35	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.28	158.489	5.03	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.30	181.970	5.76	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.32	208.930	6.48	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.33	239.883	7.16	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.33	275.423	7.70	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.35	316.228	8.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.04	34.674	0.39	363.078	7.97	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.07	39.811	0.47	416.869	7.54	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.08	45.709	0.62	478.630	6.70	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.09	52.481	0.84	549.541	5.51	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.10	60.256	1.15	630.957	4.08	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.11	69.183	1.54	724.436	2.62	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.13	79.433	2.00	831.764	1.25	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.16	91.201	2.52	954.993	0.21	10000.000	
0.091	0.00	0.955	0.00	10.000	0.19	104.713	3.09	1096.478	0.00		
0.105		1.096		11.482		120.226		1258.925			

Figure E1: Malvern Mastersizer data sheet for *A. afra* dry powder extract

MASTERSIZER 2000

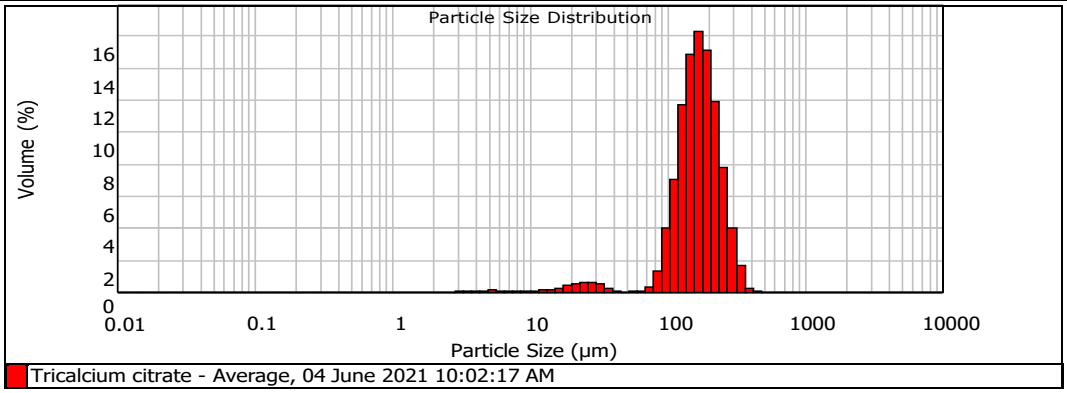
Result Analysis Report

Sample Name: Tricalcium citrate - Average
SOP Name: A. afra ext Bronk
Measured: 04 June 2021 10:02:17 AM
Sample Source & type: PR
Measured by: Pieter Roets
Analysed: 04 June 2021 10:02:18 AM
Sample bulk lot ref: 001 (sample1)
Result Source: Averaged

Particle Name:	Accessory Name:	Analysis model:	Sensitivity:
Titanium Dioxide	Hydro 2000SM (A)	General purpose	Enhanced
Particle RI:	**Absorption:**	**Size range:**	**Obscuration:**
2.741	0.1	0.020 to 2000.000 um	15.11 %
Dispersant Name:	**Dispersant RI:**	**Weighted Residual:**	**Result Emulation:**
Cyclohexane	1.468	1.771 %	Off

Concentration:	Span :	Uniformity:	Result units:
0.2627 %Vol	0.880	0.287	Volume
Specific Surface Area:	**Surface Weighted Mean D[3,2]:**	**Vol. Weighted Mean D[4,3]:**	
0.0521 m²/g	115.135 um	172.887 um	

d(0.1): 105.590 um **d(0.5):** 168.059 um **d(0.9):** 253.519 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.10	120.226	11.65	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.16	138.038	14.82	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.26	158.489	16.27	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.38	181.970	15.07	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.50	208.930	11.85	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.58	239.883	7.79	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.58	275.423	3.94	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.04	30.200	0.48	316.228	1.68	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.07	34.674	0.26	363.078	0.25	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.08	39.811	0.01	416.869	0.03	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.09	45.709	0.00	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.09	52.481	0.00	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.09	60.256	0.00	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.08	69.183	0.30	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.07	79.433	1.32	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.06	91.201	3.99	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.07	104.713	6.99	1096.478	0.00		
0.105	0.00	1.096		11.482		120.226		1258.925			

Operator notes:

Figure E2: Malvern Mastersizer data sheet for tricalcium citrate

MASTERSIZER 2000

Result Analysis Report

Sample Name: Microcelac 100 - Average
SOP Name: A. afra ext Bronk
Measured: 04 June 2021 10:13:00 AM
Sample Source & type: PR
Measured by: Pieter Roets
Analysed: 04 June 2021 10:13:02 AM
Sample bulk lot ref: 001 (sample1)
Result Source: Averaged

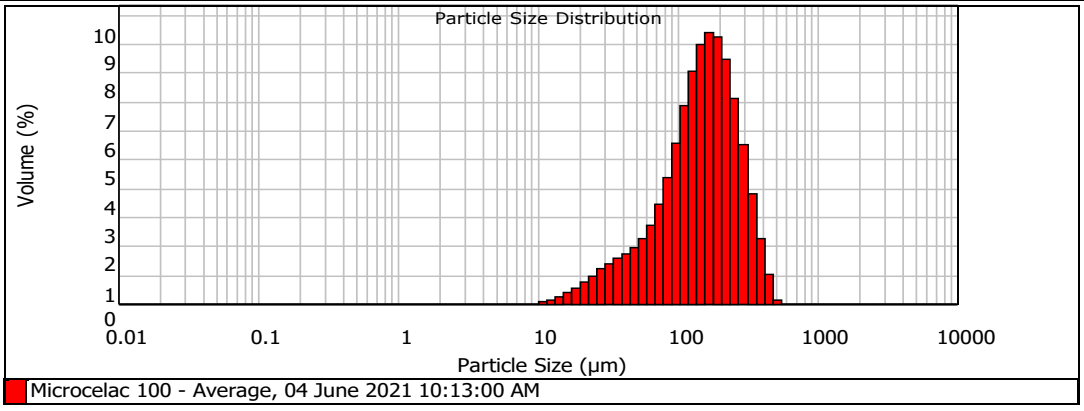
Particle Name: Titanium Dioxide
Accessory Name: Hydro 2000SM (A)
Analysis model: General purpose
Sensitivity: Enhanced
Particle RI: 2.741
Absorption: 0.1
Size range: 0.020 to 2000.000 um
Obscuration: 13.99 %

Dispersant Name: Cyclohexane
Dispersant RI: 1.468
Weighted Residual: 0.648 %
Result Emulation: Off

Concentration: 0.2119 %Vol
Span : 1.613
Uniformity: 0.491
Result units: Volume

Specific Surface Area: 0.0598 m²/g
Surface Weighted Mean D[3,2]: 100.418 um
Vol. Weighted Mean D[4,3]: 164.168 um

d(0.1): 49.302 um **d(0.5):** 151.420 um **d(0.9):** 293.540 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.13	120.226	8.04	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.23	138.038	8.96	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.37	158.489	9.40	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.55	181.970	9.23	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.75	208.930	8.45	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.98	239.883	7.13	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	1.19	275.423	5.52	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	1.39	316.228	3.81	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	1.56	363.078	2.26	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	1.73	416.869	1.03	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	1.93	478.630	0.14	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	2.24	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	2.71	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	3.42	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	4.38	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	5.55	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.08	104.713	6.84	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.08	120.226	6.84	1258.925	0.00		

Operator notes:

Figure E3: Malvern Mastersizer data sheet for MicroceLac® 100

MASTERSIZER 2000

Result Analysis Report

Sample Name: Ludipress - Average
SOP Name: A. afra ext Bronk
Measured: 04 June 2021 10:22:54 AM
Sample Source & type: PR
Measured by: Pieter Roets
Analysed: 04 June 2021 10:22:55 AM
Sample bulk lot ref: 001 (sample1)
Result Source: Averaged

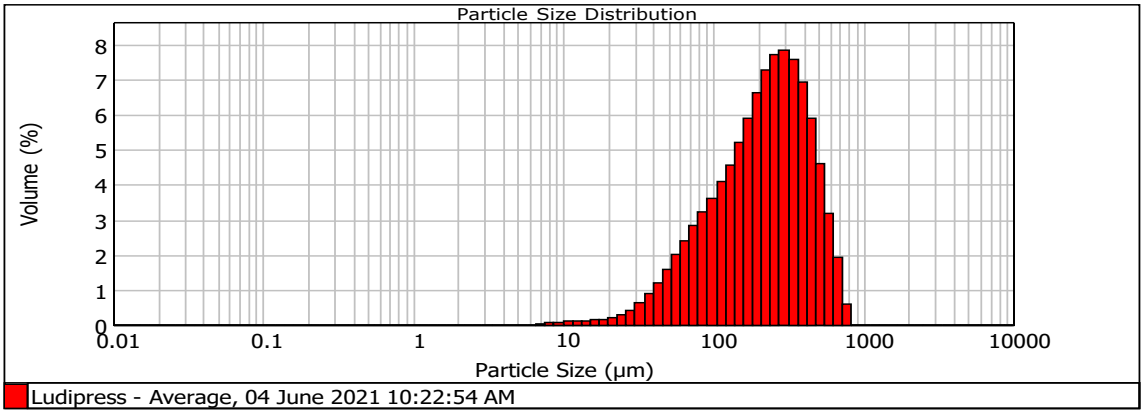
Particle Name: Titanium Dioxide
Accessory Name: Hydro 2000SM (A)
Analysis model: General purpose
Sensitivity: Enhanced
Particle RI: 2.741
Absorption: 0.1
Size range: 0.020 to 2000.000 um
Obscuration: 12.87 %

Dispersant Name: Cyclohexane
Dispersant RI: 1.468
Weighted Residual: 0.791 %
Result Emulation: Off

Concentration: 0.2654 %Vol
Span : 1.853
Uniformity: 0.572
Result units: Volume

Specific Surface Area: 0.0436 m²/g
Surface Weighted Mean D[3,2]: 137.478 um
Vol. Weighted Mean D[4,3]: 252.343 um

d(0.1): 67.515 um **d(0.5):** 223.718 um **d(0.9):** 482.168 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.11	120.226	4.58	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.13	138.038	5.20	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.14	158.489	5.89	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.17	181.970	6.61	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.21	208.930	7.26	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.30	239.883	7.72	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.43	275.423	7.86	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.62	316.228	7.60	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.88	363.078	6.93	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	1.21	416.869	5.88	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	1.59	478.630	4.60	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	2.00	549.541	3.18	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	2.41	630.957	1.91	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.01	69.183	2.82	724.436	0.60	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.07	79.433	3.22	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.08	91.201	3.63	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.10	104.713	4.07	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925			

Operator notes:

Figure E4: Malvern Mastersizer data sheet for Ludipress®

MASTERSIZER 2000

Result Analysis Report

Sample Name: Avicel PH200 - Average
Sample Source & type: PR
Sample bulk lot ref: 001 (sample2)

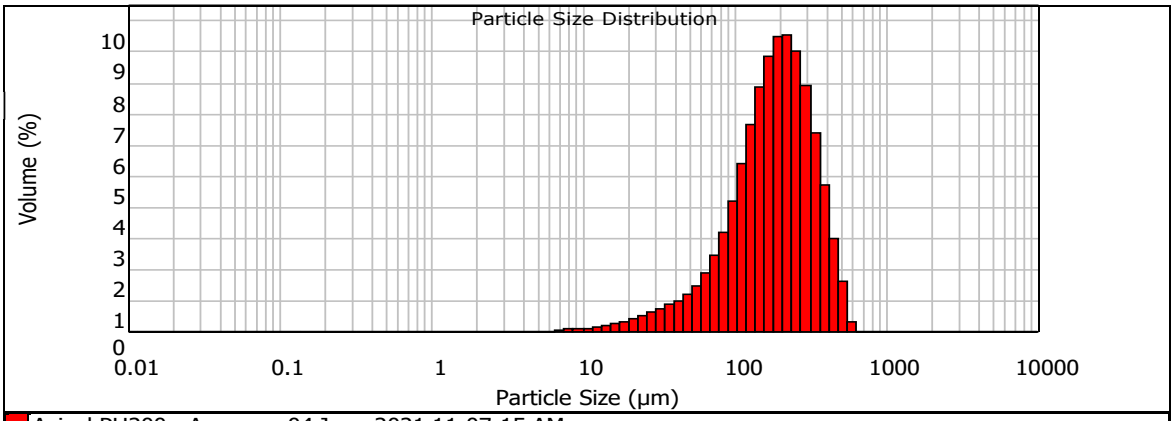
SOP Name: A. afra ext Bronk
Measured by: Pieter Roets
Result Source: Averaged

Measured: 04 June 2021 11:07:15 AM
Analysed: 04 June 2021 11:07:16 AM

Particle Name:	Accessory Name:	Analysis model:	Sensitivity:
Titanium Dioxide	Hydro 2000SM (A)	General purpose	Enhanced
Particle RI:	**Absorption:**	**Size range:**	**Obscuration:**
2.741	0.1	0.020 to 2000.000 um	14.21 %
Dispersant Name:	**Dispersant RI:**	**Weighted Residual:**	**Result Emulation:**
Cyclohexane	1.468	0.815 %	Off

Concentration:	Span :	Uniformity:	Result units:
0.2699 %Vol	1.540	0.472	Volume
Specific Surface Area:	**Surface Weighted Mean D[3,2]:**	**Vol. Weighted Mean D[4,3]:**	
0.0478 m²/g	125.479 um	203.508 um	

d(0.1): 71.019 um **d(0.5):** 187.180 um **d(0.9):** 359.338 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.12	120.226	6.62	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.16	138.038	7.86	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.22	158.489	8.86	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.29	181.970	9.47	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.39	208.930	9.54	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.49	239.883	9.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.61	275.423	7.91	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.72	316.228	6.39	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.85	363.078	4.69	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	1.00	416.869	2.98	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	1.19	478.630	1.59	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	1.46	549.541	0.31	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	1.85	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.03	69.183	2.42	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.06	79.433	3.20	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.07	91.201	4.19	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.09	104.713	5.36	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925	0.00		

Operator notes:

Figure E5: Malvern Mastersizer data sheet for Avicel® PH 200

MASTERSIZER 2000

Result Analysis Report

Sample Name:
Emcompress - Average

SOP Name:
A. afra ext Bronk

Measured:
04 June 2021 09:43:27 AM

Sample Source & type:
PR

Measured by:
Pieter Roets

Analysed:
04 June 2021 09:43:28 AM

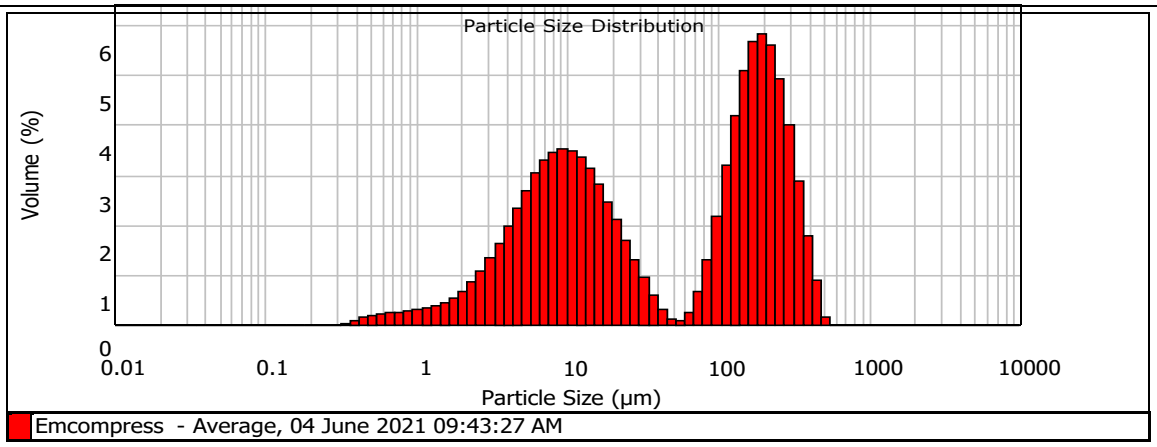
Sample bulk lot ref:
001 (sample1)

Result Source:
Averaged

Particle Name:	Accessory Name:	Analysis model:	Sensitivity:
Titanium Dioxide	Hydro 2000SM (A)	General purpose	Enhanced
Particle RI:	**Absorption:**	**Size range:**	**Obscuration:**
2.741	0.1	0.020 to 2000.000 um	12.35 %
Dispersant Name:	**Dispersant RI:**	**Weighted Residual:**	**Result Emulation:**
Water	1.330	0.535 %	Off

Concentration:	Span :	Uniformity:	Result units:
0.0153 %Vol	8.287	2.94	Volume
Specific Surface Area:	**Surface Weighted Mean D[3,2]:**	**Vol. Weighted Mean D[4,3]:**	
0.631 m²/g	9.511 um	105.366 um	

d(0.1): 4.131 um **d(0.5): 32.416 um** **d(0.9): 272.779 um**



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.33	11.482	3.35	120.226	4.20	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.37	13.183	3.12	138.038	5.07	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.44	15.136	2.82	158.489	5.65	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.54	17.378	2.46	181.970	5.83	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.67	19.953	2.09	208.930	5.58	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.84	22.909	1.70	239.883	4.93	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	1.06	26.303	1.31	275.423	3.98	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	1.32	30.200	0.94	316.228	2.88	3311.311	0.00
0.030	0.00	0.316	0.01	3.311	1.63	34.674	0.59	363.078	1.79	3801.894	0.00
0.035	0.00	0.363	0.09	3.802	1.97	39.811	0.30	416.869	0.89	4365.158	0.00
0.040	0.00	0.417	0.13	4.365	2.34	45.709	0.11	478.630	0.13	5011.872	0.00
0.046	0.00	0.479	0.17	5.012	2.69	52.481	0.08	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.21	5.754	3.02	60.256	0.24	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.23	6.607	3.28	69.183	0.64	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.25	7.586	3.46	79.433	1.30	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.27	8.710	3.53	91.201	2.18	954.993	0.00	10000.000	
0.091	0.00	0.955	0.30	10.000	3.49	104.713	3.19	1096.478	0.00		
0.105	0.00	1.096		11.482		120.226		1258.925			

Figure E6: Malvern Mastersizer data sheet for Emcompress®

MASTERSIZER 2000

Result Analysis Report

Sample Name:
Kolidon VA64 - Average

SOP Name:
A. afra ext Bronk

Measured:
04 June 2021 10:31:12 AM

Sample Source & type:
PR

Measured by:
Pieter Roets

Analysed:
04 June 2021 10:31:13 AM

Sample bulk lot ref:
001 (sample1)

Result Source:
Averaged

Particle Name:
Titanium Dioxide

Accessory Name:
Hydro 2000SM (A)

Analysis model:
General purpose

Sensitivity:
Enhanced

Particle RI:

Absorption:

Size range:

Obscuration:

2.741

0.1

0.020 to 2000.000 um

15.24 %

Dispersant Name:
Cyclohexane

Dispersant RI:
1.468

Weighted Residual:
0.433 %

Result Emulation:
Off

Concentration:

Span :

Uniformity:

Result units:

0.1374 %Vol

1.668

0.52

Volume

Specific Surface Area:

Surface Weighted Mean D[3,2]:

Vol. Weighted Mean D[4,3]:

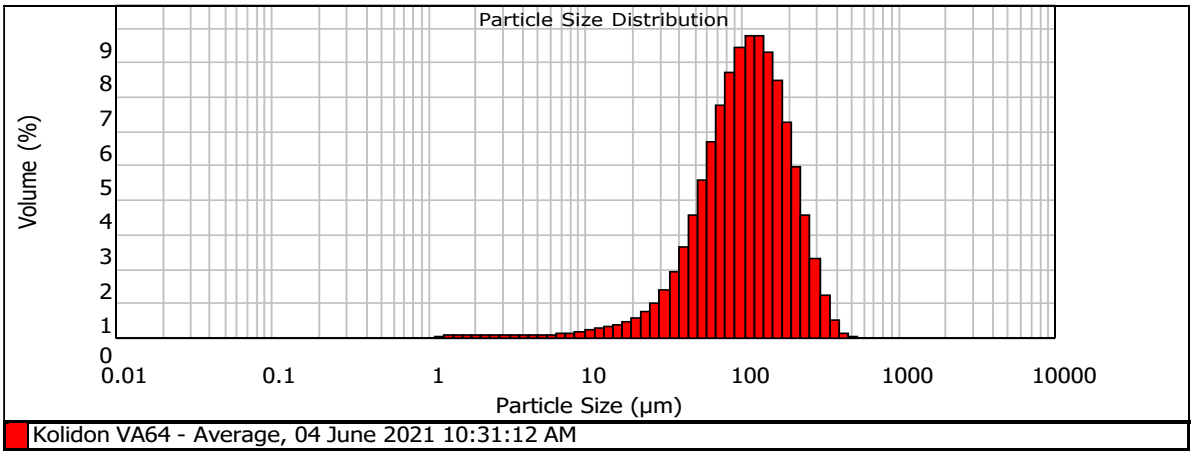
0.0977 m²/g

61.439 um

122.825 um

d(0.1): 43.128 um **d(0.5):** 108.395 um **d(0.9):** 223.976 um

---



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.02	11.482	0.26	120.226	8.74	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.07	13.183	0.31	138.038	8.28	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.08	15.136	0.37	158.489	7.44	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.08	17.378	0.44	181.970	6.27	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.09	19.953	0.55	208.930	4.94	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.09	22.909	0.72	239.883	3.55	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.08	26.303	0.99	275.423	2.29	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.08	30.200	1.38	316.228	1.25	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.08	34.674	1.92	363.078	0.51	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.07	39.811	2.64	416.869	0.10	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.07	45.709	3.54	478.630	0.01	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.08	52.481	4.56	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.09	60.256	5.66	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.11	69.183	6.74	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.14	79.433	7.70	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.17	91.201	8.41	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.22	104.713	8.78	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925	0.00		

Operator notes:

Figure E7: Malvern Mastersizer data sheet for Kollidon® VA 64

MASTERSIZER 2000

Result Analysis Report

Sample Name:
TCC 88.5% Ac-di-Sol 5% Kollidon VA64

SOP Name:
A. afra ext Bronk

Measured:
08 September 2021 10:58:13 AM

Sample Source & type:
RP

Measured by:
Neil Barnard

Analysed:
08 September 2021 10:58:14 AM

Sample bulk lot ref:
001

Result Source:
Averaged

Particle Name:	Accessory Name:	Analysis model:	Sensitivity:
Titanium Dioxide	Hydro 2000SM (A)	General purpose	Enhanced
Particle RI:	**Absorption:**	**Size range:**	**Obscuration:**
2.741	0.1	0.020 to 2000.000 um	13.97 %
Dispersant Name:	**Dispersant RI:**	**Weighted Residual:**	**Result Emulation:**
Cyclohexane	1.468	0.868 %	Off

Concentration:	Span :	Uniformity:	Result units:
0.1294 %Vol	1.636	0.484	Volume
Specific Surface Area:	**Surface Weighted Mean D[3,2]:**	**Vol. Weighted Mean D[4,3]:**	
0.0954 m²/g	62.885 um	150.575 um	

d(0.1): 29.916 um **d(0.5):** 146.052 um **d(0.9):** 268.815 um

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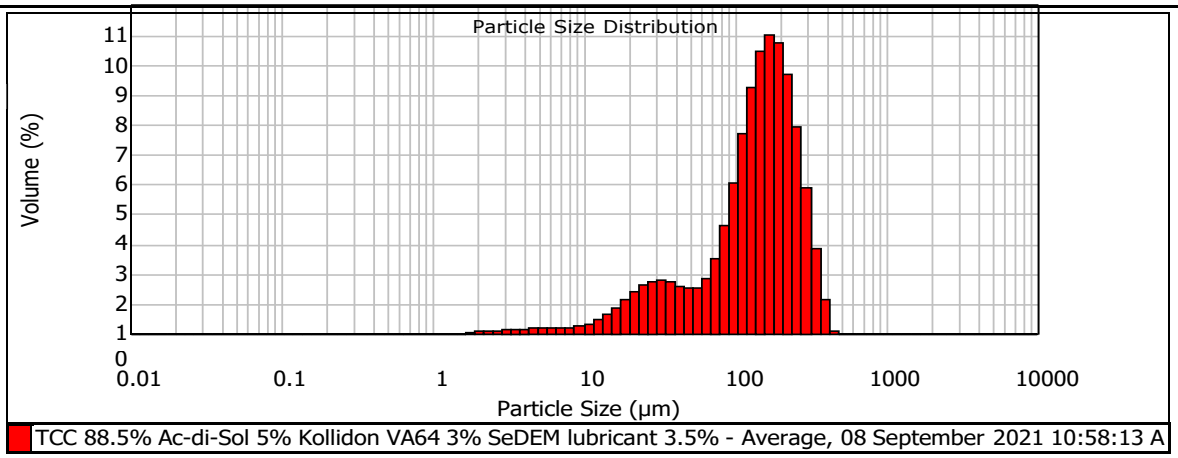

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.45	120.226	8.26	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.64	138.038	9.47	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.87	158.489	10.02	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.03	17.378	1.14	181.970	9.75	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.07	19.953	1.40	208.930	8.67	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.09	22.909	1.62	239.883	6.93	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.10	26.303	1.76	275.423	4.88	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.12	30.200	1.79	316.228	2.84	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.14	34.674	1.71	363.078	1.12	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.16	39.811	1.59	416.869	0.09	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.17	45.709	1.49	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.18	52.481	1.54	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.19	60.256	1.85	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.19	69.183	2.53	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.21	79.433	3.62	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.25	91.201	5.05	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.33	104.713	6.69	1096.478	0.00		
0.105		1.096		11.482		120.226		1258.925			

Figure E8: Malvern Mastersizer data sheet for corrective excipient powder mixture

MASTERSIZER 2000

Result Analysis Report

Sample Name:
Afra tablet mix - Average

SOP Name:
A. afra ext Bronk

Measured:
16 August 2021 11:59:25 AM

Sample Source & type:
PR

Measured by:
Neil Barnard

Analysed:
16 August 2021 11:59:26 AM

Sample bulk lot ref:
Sample 2

Result Source:
Averaged

Particle Name:	Accessory Name:	Analysis model:	Sensitivity:
Yellow pigment	Hydro 2000SM (A)	General purpose	Enhanced
Particle RI:	**Absorption:**	**Size range:**	**Obscuration:**
2.187	0.1	0.020 to 2000.000 um	13.75 %
Dispersant Name:	**Dispersant RI:**	**Weighted Residual:**	**Result Emulation:**
Cyclohexane	1.468	1.379 %	Off

Concentration:	Span :	Uniformity:	Result units:
0.1067 %Vol	1.831	0.557	Volume
Specific Surface Area:	**Surface Weighted Mean D[3,2]:**	**Vol. Weighted Mean D[4,3]:**	
0.112 m²/g	53.708 um	140.577 um	

d(0.1): 29.650 um **d(0.5):** 129.015 um **d(0.9):** 265.830 um

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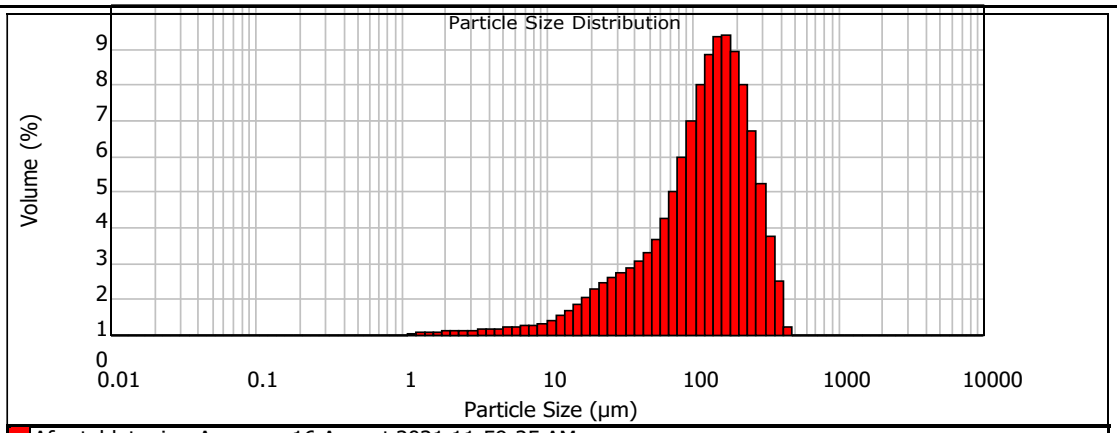

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.01	11.482	0.52	120.226	7.83	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.07	13.183	0.67	138.038	8.35	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.07	15.136	0.85	158.489	8.40	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.08	17.378	1.05	181.970	7.94	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.09	19.953	1.25	208.930	7.01	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.11	22.909	1.44	239.883	5.70	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.12	26.303	1.61	275.423	4.24	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.14	30.200	1.75	316.228	2.73	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.15	34.674	1.88	363.078	1.52	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.17	39.811	2.05	416.869	0.19	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.18	45.709	2.29	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.20	52.481	2.68	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.21	60.256	3.25	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.24	69.183	4.01	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.27	79.433	4.96	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.32	91.201	5.99	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.40	104.713	7.00	1096.478	0.00		
0.105		1.096		11.482		120.226		1258.925			

Figure E9: Malvern Mastersizer data sheet for final tabletting powder mixture

Table E1: Homogeneity index results for *A. afra*, excipients, corrective excipient mixture, and final tabletting powder mixture

Artemisia afra					
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	4.58	Fm+5	(dm+5)-dm	621
500µm - 710µm	605	13.56	Fm+4	(dm+4)-dm	446
355µm - 500µm	427.5	19.06	Fm+3	(dm+3)-dm	268.5
300µm - 355µm	327.5	9.68	Fm+2	(dm+2)-dm	168.5
212µm - 300µm	256	17.7	Fm+1	(dm+1)-dm	97
106µm - 212µm	159	22.31	Fm	dm	0
45µm - 106µm	75.5	8.98	Fm-1	dm-(dm-1)	83.5
0µm - 45µm	22.5	4.13	Fm-2	dm-(dm-2)	136.5
Iθ =	**0.001189**		**%< 45=**	**4.13%**	
Tricalcium citrate					
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	0	Fm+5	(dm+5)-dm	621
500µm - 710µm	605	0	Fm+4	(dm+4)-dm	446
355µm - 500µm	427.5	0.4	Fm+3	(dm+3)-dm	268.5
300µm - 355µm	327.5	2.85	Fm+2	(dm+2)-dm	168.5
212µm - 300µm	256	20.85	Fm+1	(dm+1)-dm	97
106µm - 212µm	159	65.40	Fm	dm	0
45µm - 106µm	75.5	6.11	Fm-1	dm-(dm-1)	83.5
0µm - 45µm	22.5	4.05	Fm-1	dm-(dm-1)	136.5
Iθ =	**0.017333**		**%< 45=**	**4.05%**	
		Ludipress®			
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	0.81	Fm+5	(dm+5)-dm	621
500µm - 710µm	605	7.87	Fm+4	(dm+4)-dm	446
355µm - 500µm	427.5	16.61	Fm+3	(dm+3)-dm	268.5
300µm - 355µm	327.5	9.39	Fm+2	(dm+2)-dm	168.5
212µm - 300µm	256	19.11	Fm+1	(dm+1)-dm	97
106µm - 212µm	159	26.74	Fm	dm	0
45µm - 106µm	75.5	13.17	Fm-1	dm-(dm-1)	83.5
0µm - 45µm	22.5	4.31	Fm-2	dm-(dm-2)	136.5

Iθ =	**0.002625**		**%< 45**	**4.31%**	
MicroceLac® 100					
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	0	Fm+5	(dm+5)-dm	621
500µm - 710µm	605	0.02	Fm+4	(dm+4)-dm	446
355µm - 500µm	427.5	3.91	Fm+3	(dm+3)-dm	268.5
300µm - 355µm	327.5	5.2	Fm+2	(dm+2)-dm	168.5
212µm - 300µm	256	18.25	Fm+1	(dm+1)-dm	97
106µm - 212µm	159	42.85	Fm	dm	0
45µm - 106µm	75.5	20.99	Fm-1	dm-(dm-1)	83.5
0µm - 45µm	22.5	8.77	Fm-2	dm-(dm-2)	136.5
Iθ =	**0.006352**		**%< 45=**	**8.77%**	
Avicel® PH 200					
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	0	Fm+5	(dm+5)-dm	175
500µm - 710µm	605	1.27	Fm+4	(dm+4)-dm	0
355µm - 500µm	427.5	9.25	Fm+3	(dm+3)-dm	177.5
300µm - 355µm	327.5	8.32	Fm+2	(dm+2)-dm	277.5
212µm - 300µm	256	22.58	Fm+1	(dm+1)-dm	349
106µm - 212µm	159	38.76	Fm	dm	446
45µm - 106µm	75.5	14.85	Fm-1	dm-(dm-1)	529.5
0µm - 45µm	22.5	4.98	Fm-1	dm-(dm-1)	582.5
Iθ =	**0.000969**		**%< 45=**	**4.98%**	
Kollidon® VA 64					
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	0	Fm+5	(dm+5)-dm	175
500µm - 710µm	605	0	Fm+4	(dm+4)-dm	0
355µm - 500µm	427.5	0.77	Fm+3	(dm+3)-dm	177.5
300µm - 355µm	327.5	1.84	Fm+2	(dm+2)-dm	277.5
212µm - 300µm	256	9.46	Fm+1	(dm+1)-dm	349
106µm - 212µm	159	39.34	Fm	dm	446
45µm - 106µm	75.5	37.72	Fm-1	dm-(dm-1)	529.5
0µm - 45µm	22.5	10.87	Fm-2	dm-(dm-2)	582.5
Iθ =	**0.000821**		**%< 45**	**10.87%**	

Emcompress®					
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	0	Fm+7	(dm+7)-dm	621
500µm - 710µm	605	0.02	Fm+6	(dm+6)-dm	446
355µm - 500µm	427.5	3.19	Fm+5	(dm+5)-dm	268.5
300µm - 355µm	327.5	3.88	Fm+4	(dm+4)-dm	168.5
212µm - 300µm	256	12.49	Fm+3	(dm+3)-dm	97
106µm - 212µm	159	24.31	Fm+2	(dm+2)-dm	0
45µm - 106µm	75.5	4.82	Fm+1	(dm+1)-dm	83.5
0µm - 45µm	22.5	51.28	Fm	dm	136.5
Iθ =	**0.005016**		**%< 45=**	**51.28%**	
		Corrective excipient mixture			
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	0	Fm+5	(dm+5)-dm	621
500µm - 710µm	605	0	Fm+4	(dm+4)-dm	446
355µm - 500µm	427.5	1.54	Fm+3	(dm+3)-dm	268.5
300µm - 355µm	327.5	4.12	Fm+2	(dm+2)-dm	168.5
212µm - 300µm	256	17.88	Fm+1	(dm+1)-dm	97
106µm - 212µm	159	44.64	Fm	dm	0
45µm - 106µm	75.5	16.78	Fm-1	dm-(dm-1)	83.5
0µm - 45µm	22.5	15.04	Fm-2	dm-(dm-2)	136.5
Iθ =	**0.006979**		**%< 45=**	**15.04%**	
Final tablet mixture (30% w/w *A. afra*/70% w/w corrective excipient mixture)					
Homogeneity index	Iθ				
Sieve sizes		AVG Diameter	% in Frac		Difference in Diameter
710µm - 850µm	780	0	Fm+5	(dm+5)-dm	621
500µm - 710µm	605	0	Fm+4	(dm+4)-dm	446
355µm - 500µm	427.5	2.06	Fm+3	(dm+3)-dm	268.5
300µm - 355µm	327.5	3.81	Fm+2	(dm+2)-dm	168.5
212µm - 300µm	256	14.73	Fm+1	(dm+1)-dm	97
106µm - 212µm	159	41.74	Fm	dm	0
45µm - 106µm	75.5	26.42	Fm-1	dm-(dm-1)	83.5
0µm - 45µm	22.5	13.2	Fm-2	dm-(dm-2)	136.5
Iθ =	**0.0062**		**%< 45=**	**13.2%**	

ADDENDUM F: ASSAY RESULTS FOR *A. AFRA* TABLETS

Table F1: Assay results for *A. afra* tablets exposed to 25ºC/60% humidity and %RSD

	Phytochemical marker 1		**Phytochemical marker 2**		**Phytochemical marker 3**		***Phytochemical 4***	
	Peak area	**%Left + %RSD**	**Peak area**	**%Left + %RSD**	**Peak area**	**%Left + %%SD**	**Peak area**	**%Left + %RSD**
200 MG extract	3514694	100 ± 1.36%	2325311	100 ± 2.28%	8827911	*100 ± 0.85%*	*5204507*	*100 ± 2.55%*
Week 0 tablets	3505403	99.74 ± 0.19%	1484693	63.85 ± 1.05%	5238663	*59.34 ± 0.56%*	*2461749*	*47.3 ± 5.86%*
Week 1 tablets	3499718	99.57 ± 0.46%	1482916	63.77 ± 1.28%	5368880	*63.29 ± 0.35%*	*2474306*	*47.54 ± 1.27%*
Week 2 tablets	3497749	99.52 ± 0.41%	1460925	62.83 ± 0.43%	5090739	*63.33 ± 0.21%*	*2453104*	*47.13 ± 1.99%*
Week 3 tablets	3489513	99.46 ± 0.31%	1461677	62.86 ± 0.24%	5686464	*64.43 ± 0.81%*	*2450467*	*47.08 ± 0.41%*
Week 4 tablets	3489171	99.27 ± 0.43%	1450757	62.39 ± 1.07%	5693892	*64.49 ± 0.60%*	*2442972*	*46.94 ± 0.69%*
Week 8 tablets	3339073	95.00 ± 0.25%	1338524	57.56 ± 1.19%	5413891	*61.32 ± 0.34%*	*2072677*	*39.82 ± 1.81%*
Week 12 tablets	3273868	93.15 ± 0.97%	991792	42.65 ± 0.35%	3824250	*43.31± 0.22%*	*1388635*	*26.68 ± 1.71%*

Table F2: Assay results for *A. afra* tablets exposed to 40ºC/75% humidity and %RSD

	Phytochemical marker 1		**Phytochemical marker 2**		**Phytochemical marker 3**		***Phytochemical 4***	
	Peak area	**%Left + %RSD**	**Peak area**	**%Left + %RSD**	**Peak area**	**%Left + %%SD**	**Peak area**	**%Left + %RSD**
200 MG *A. afra* extract	3514694	100 ± 1.36%	2325311	100± 2.28%	8827911	*100 ± 0.85%*	*5204507*	*100 ± 2.55%*
Week 0 tablets	3505403	99.74 ± 0.19%	1484693	63.85 ± 1.05%	5238663	*59.34 ± 0.56%*	*2461749*	*47.3 ± 5.86%*
Week 1 tablets	3501933	99.63 ± 0.35%	1392517	59.89 ± 1.07%	48332260	*54.75 ± 0.3%*	*2257388*	*43.37 ± 3.96%*
Week 2 tablets	3467834	98.67 ± 0.65%	1241516	53.39 ± 0.89%	4260596	*48.26 ± 0.22%*	*2015074*	*38.72 ± 2.01%*
Week 3 tablets	3489513	94.75 ± 0.29%	1001111	43.05 ± 3.28%	3554464	*40.26 ± 0.15%*	*1659749*	*31.89 ± 1.48%*
Week 4 tablets	3276744	93.23 ± 0.51%	898221	38.63 ± 0.53%	3025548	*34.27 ± 0.30%*	*1227970*	*23.59 ± 0.87%*
Week 8 tablets	3192270	90.83 ± 0.89%	507188	22.35 ± 1.70%	2081052	*23.57 ± 2.39%*	*544506*	*10.46 ± 0.91%*
Week 12 tablets	3172524	88.13 ± 2.26%	128988	5.55 ± 0.82%	558630	*6.34 ± 1.14%*	*1388635*	*5.79 ± 1.71%*

ADDENDUM G DISSOLUTION RESULTS FOR *A. AFRA* TABLETS

Table G1: Dissolution results for *A. afra* tablets exposed to 25ºC/60% humidity

Week 0 Marker 1		
Time	Dissolution %	±SD %
0	0.00	0.00
2	17.76	0.70
5	41.05	0.38
10	65.08	1.04
15	70.97	5.26
30	72.55	4.68
60	78.19	5.11
90	88.61	4.30
120	93.36	4.01
180	95.83	2.25
240	98.72	1.24

Week 0 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	8.45	0.73
5	24.24	4.53
10	34.66	5.52
15	35.93	2.47
30	41.07	4.21
60	45.86	4.90
90	51.43	3.13
120	53.10	3.18
180	59.68	3.39
240	63.22	2.76

Week 0 Marker 3		
Time	Dissolution %	±SD %
0	0	0
2	11.54	1.53
5	25.19	6.62
10	37.24	7.28
15	46.75	4.41
30	47.71	4.44
60	49.62	5.60
90	54.27	3.30
120	55.54	3.20
180	59.00	3.03
240	58.96	2.15

Week 0 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	9.37	1.71
5	17.41	6.61
10	24.83	6.59
15	32.54	5.29
30	33.71	5.14
60	35.18	5.65
90	39.63	4.56
120	39.53	3.10
180	44.27	4.05
240	46.46	3.90

Week 1 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	17.02	4.55
5	37.06	0.01
10	57.83	1.76
15	66.13	0.00
30	72.92	3.98
60	75.55	7.39
90	83.92	5.99
120	88.78	6.24
180	92.38	4.14
240	97.02	3.64

Week 1 Marker 2		
Time	Dissolution %	±SD %
0	0	0
2	0.00	0.85
5	9.74	1.18
10	22.62	2.05
15	35.84	6.24
30	41.85	4.38
60	43.65	3.25
90	47.55	8.32
120	52.10	9.84
180	56.01	12.28
240	58.90	14.56

Week 1 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	10.76	1.31
5	23.21	5.88
10	34.26	6.79
15	43.76	3.78
30	44.34	3.37
60	47.41	4.50
90	51.25	2.97
120	53.61	3.29
180	57.42	3.06
240	58.72	2.93

Week 1 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	5.12	1.10
5	13.38	7.10
10	20.39	6.08
15	30.26	5.22
30	31.15	6.59
60	31.64	5.44
90	35.39	2.72
120	36.14	4.13
180	41.78	4.25
240	46.00	4.38

Week 2 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	17.51	0.78
5	38.32	8.14
10	59.63	10.07
15	67.49	6.60
30	70.82	5.28
60	77.67	5.38
90	86.83	3.54
120	91.34	3.33
180	95.12	1.91
240	97.75	1.03

Week 2 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	8.52	0.77
5	18.31	5.67
10	28.56	5.83
15	39.20	3.63
30	39.45	3.96
60	43.86	4.73
90	50.67	2.99
120	52.53	3.41
180	57.15	3.00
240	61.73	2.56

Week 2 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	10.65	1.48
5	23.14	6.24
10	34.11	6.85
15	43.83	3.80
30	44.28	3.82
60	47.65	5.19
90	52.23	3.03
120	53.53	3.14
180	57.23	2.53
240	58.63	2.52

Week 2 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	4.95	0.59
5	13.23	7.16
10	20.89	6.52
15	30.55	5.31
30	30.46	5.81
60	32.87	8.51
90	37.20	5.50
120	37.29	3.67
180	42.11	3.45
240	46.96	4.27

Week 3 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	17.40	0.82
5	37.87	8.33
10	58.27	10.44
15	66.87	5.38
30	71.11	5.89
60	78.25	5.91
90	85.38	3.84
120	90.99	3.85
180	95.97	1.18
240	96.71	2.82

Week 3 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	7.90	1.27
5	18.30	5.93
10	28.10	5.74
15	38.71	3.93
30	40.22	3.16
60	45.58	1.31
90	49.43	3.62
120	50.52	2.03
180	56.49	3.26
240	61.25	3.35

Week 3 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	10.23	1.32
5	22.69	5.98
10	33.90	7.16
15	43.66	4.32
30	44.46	3.80
60	48.87	1.70
90	52.46	3.10
120	53.30	2.92
180	57.36	2.76
240	58.49	2.68

Week 3 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	5.50	0.61
5	14.81	0.86
10	24.37	1.36
15	25.65	2.30
30	28.18	0.15
60	36.24	3.10
90	37.38	4.00
120	38.13	4.22
180	42.55	1.46
240	45.79	2.12

Week 4 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	17.00	0.75
5	40.33	3.72
10	62.74	4.11
15	68.21	5.72
30	70.71	4.60
60	77.11	1.51
90	82.84	2.85
120	89.16	2.75
180	92.07	0.35
240	96.21	3.00

Week 4 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	8.09	1.15
5	18.52	6.03
10	28.44	6.96
15	37.79	3.94
30	38.59	3.89
60	41.67	5.27
90	47.71	2.76
120	50.78	3.60
180	56.65	3.82
240	59.87	2.66

Week 4 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	10.74	1.49
5	23.79	5.97
10	35.34	7.35
15	44.33	3.70
30	45.44	3.88
60	47.77	5.19
90	52.91	2.82
120	54.81	3.35
180	58.18	2.91
240	58.78	2.49

Week 4 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	5.16	1.31
5	13.56	7.54
10	20.53	7.53
15	30.49	6.86
30	31.00	5.06
60	32.45	4.96
90	37.90	5.11
120	37.12	4.17
180	42.29	5.83
240	45.69	4.03

Week 8 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	19.64	0.84
5	36.29	1.62
10	58.19	2.65
15	67.63	2.83
30	71.54	2.84
60	76.26	2.01
90	78.78	2.12
120	82.96	1.60
180	87.09	0.88
240	88.07	0.57

Week 8 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	8.56	1.16
5	20.60	1.21
10	31.50	3.71
15	32.92	1.84
30	36.70	1.41
60	45.46	0.40
90	48.42	0.62
120	50.07	1.36
180	57.77	1.11
240	62.26	0.43

Week 8 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	6.83	1.19
5	14.64	1.86
10	27.81	3.61
15	40.62	5.81
30	46.83	3.16
60	52.06	4.50
90	52.93	1.82
120	52.80	2.00
180	54.91	4.89
240	55.10	4.31

Week 8 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	6.24	0.57
5	15.27	0.99
10	24.54	1.36
15	28.33	1.51
30	31.80	1.32
60	33.91	1.02
90	36.15	1.46
120	38.08	0.91
180	39.95	0.57
240	42.38	1.26

Week 12 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	19.91	0.85
5	36.79	1.65
10	58.99	2.68
15	68.55	2.87
30	72.52	2.88
60	77.30	2.04
90	79.85	2.15
120	84.10	1.63
180	88.28	0.90
240	89.28	0.58

Week 12 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	7.72	1.99
5	18.34	3.26
10	31.11	4.56
15	39.50	3.30
30	43.28	3.85
60	46.43	15.72
90	50.19	5.09
120	54.03	4.54
180	57.08	4.79
240	59.59	2.89

Week 12 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	10.62	2.19
5	21.63	4.11
10	28.21	9.16
15	43.53	3.27
30	45.74	4.22
60	46.32	2.08
90	49.43	5.25
120	51.41	3.98
180	52.34	4.01
240	54.20	3.38

Week 12 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	5.70	1.80
5	13.63	4.35
10	26.86	0.40
15	32.11	3.64
30	32.31	4.57
60	36.40	0.08
90	36.07	4.12
120	37.51	3.43
180	38.36	5.05
240	43.97	3.62

Table G2: Dissolution results for *A. afra* tablets exposed to 40ºC/75% humidity

Week 0 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	17.76	0.70
5	41.05	0.38
10	65.08	1.04
15	70.97	5.26
30	72.55	4.68
60	78.19	5.11
90	88.61	4.30
120	93.36	4.01
180	95.83	2.25
240	98.72	1.24

Week 0 Marker 2		
Time	Dissolution %	±SD %
0	8.45	0
2	24.24	0.21
5	34.66	1.28
10	35.93	1.57
15	41.07	1.02
30	45.86	2.57
60	51.43	2.55
90	53.10	1.14
120	59.68	2.65
180	63.22	1.27
240	8.45	0.24

Week 0 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	11.54	1.53
5	25.19	6.62
10	37.24	7.28
15	46.75	4.41
30	47.71	4.44
60	49.62	5.60
90	54.27	3.30
120	55.54	3.20
180	59.00	3.03
240	58.96	2.15

Week 0 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	9.37	1.71
5	17.41	6.61
10	24.83	6.59
15	32.54	5.29
30	33.71	5.14
60	35.18	5.65
90	39.63	4.56
120	39.53	3.10
180	44.27	4.05
240	46.46	3.90

Week 1 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	12.49	0.24
5	31.50	0.62
10	47.41	2.14
15	54.78	1.80
30	61.53	0.35
60	80.56	4.98
90	83.37	3.27
120	94.74	1.20
180	96.70	0.62
240	98.11	3.12

Week 1 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	7.77	2.73
5	18.13	7.23
10	31.50	9.45
15	36.50	1.66
30	39.25	2.87
60	42.90	6.26
90	50.09	1.69
120	55.89	5.81
180	58.72	5.69
240	60.88	6.90

Week 1 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	9.81	1.45
5	22.49	1.10
10	33.42	4.79
15	35.83	1.97
30	38.91	1.45
60	48.32	0.64
90	49.97	0.78
120	52.24	2.96
180	57.05	1.55
240	58.00	0.49

Week 1 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	7.59	0.70
5	17.66	0.78
10	28.41	1.20
15	34.29	1.65
30	35.79	1.41
60	35.85	1.63
90	39.72	1.69
120	40.76	1.74
180	42.67	1.16
240	44.31	0.80

Week 2 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	12.26	0.23
5	30.51	0.57
10	45.27	2.40
15	52.65	1.87
30	59.62	0.32
60	79.27	5.45
90	82.65	2.76
120	92.65	1.24
180	95.29	0.90
240	97.57	1.73

Week 2 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	8.95	0.93
5	20.89	1.45
10	30.55	3.67
15	32.97	1.63
30	36.10	0.42
60	46.21	0.42
90	48.16	1.53
120	52.58	3.48
180	59.53	0.75
240	61.64	0.32

Week 2 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	10.19	1.13
5	22.69	1.39
10	33.83	5.47
15	36.22	1.40
30	38.74	0.79
60	49.05	1.05
90	51.04	0.82
120	52.36	3.08
180	57.48	0.88
240	57.86	0.50

Week 2 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	5.82	1.27
5	14.94	0.92
10	23.26	3.81
15	26.23	1.77
30	28.60	0.82
60	36.83	1.69
90	37.33	2.80
120	37.76	3.42
180	43.46	0.88
240	45.70	0.20

Week 3 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	12.26	0.27
5	30.69	0.31
10	47.30	2.23
15	54.51	1.68
30	61.42	0.74
60	81.27	5.56
90	85.00	1.59
120	93.87	1.32
180	95.29	0.33
240	98.39	0.37

Week 3 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	8.48	0.78
5	19.49	1.19
10	29.28	5.00
15	31.68	1.36
30	35.43	0.84
60	45.63	1.53
90	47.44	1.95
120	50.88	2.87
180	55.32	1.39
240	58.61	0.38

Week 3 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	9.88	1.32
5	22.84	1.99
10	34.22	4.95
15	36.19	1.92
30	40.18	0.45
60	50.02	1.44
90	51.80	1.24
120	53.45	1.81
180	57.48	0.74
240	58.41	0.35

Week 3 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	4.86	0.90
5	14.22	5.26
10	20.26	7.77
15	29.35	5.00
30	30.30	5.01
60	33.55	2.40
90	36.72	6.03
120	36.37	3.73
180	41.78	4.68
240	43.77	5.21

Week 4 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	11.92	0.26
5	30.30	0.33
10	46.50	2.24
15	53.24	1.86
30	59.90	0.35
60	80.20	5.04
90	84.21	2.47
120	94.55	1.08
180	95.54	1.44
240	98.36	1.85

Week 4 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	7.50	0.81
5	17.65	1.09
10	26.20	3.62
15	29.88	0.78
30	35.66	6.09
60	40.99	0.60
90	43.80	0.32
120	45.78	2.81
180	51.44	0.82
240	59.62	3.09

Week 4 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	9.64	0.90
5	21.82	1.39
10	32.91	5.76
15	35.06	1.98
30	38.31	1.12
60	48.33	0.87
90	49.90	0.63
120	51.78	3.16
180	56.20	0.48
240	56.97	0.36

Week 4 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	5.16	0.89
5	8.58	11.52
10	21.03	3.52
15	24.23	1.47
30	26.32	1.24
60	33.84	0.95
90	35.75	1.64
120	37.09	3.46
180	42.46	1.97
240	43.20	0.98

Week 8 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	11.12	0.51
5	30.68	0.34
10	51.01	3.29
15	53.95	3.31
30	61.91	0.80
60	76.35	1.52
90	80.00	0.61
120	82.17	2.67
180	95.28	1.20
240	98.70	0.13

Week 8 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	6.95	0.56
5	16.73	1.30
10	27.13	1.48
15	31.71	1.27
30	37.05	1.20
60	42.69	1.31
90	45.23	1.78
120	48.75	1.80
180	53.31	1.21
240	57.48	1.50

Week 8 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	7.90	0.73
5	18.96	1.58
10	28.81	3.02
15	34.97	1.79
30	39.67	1.42
60	43.31	1.92
90	44.91	1.89
120	47.14	1.95
180	49.84	1.64
240	52.44	1.39

Week 8 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	3.36	0.52
5	7.91	1.31
10	15.33	2.77
15	27.07	5.14
30	30.51	4.01
60	38.11	6.20
90	42.30	4.53
120	42.37	3.61
180	42.36	8.07
240	42.41	5.02

Week 12 Marker 1		
Time	Dissolution %	±SD %
0	0	0
2	10.82	0.22
5	30.12	2.17
10	44.14	1.51
15	50.87	2.51
30	59.12	1.85
60	64.59	1.08
90	68.85	1.71
120	72.59	0.55
180	80.55	0.33
240	86.20	0.59

Week 12 Marker 2		
Time	Dissolution %	±SD %
0	0.00	0
2	6.41	0.51
5	15.43	1.20
10	25.02	1.36
15	29.24	1.17
30	34.17	1.11
60	39.37	1.21
90	41.70	1.65
120	44.95	1.66
180	49.16	1.11
240	53.01	1.38

Week 12 Marker 3		
Time	Dissolution %	±SD %
0	0.00	0
2	7.39	0.68
5	17.74	1.48
10	26.96	2.82
15	32.73	1.68
30	37.12	1.33
60	40.54	1.79
90	42.03	1.77
120	44.12	1.83
180	46.65	1.54
240	49.08	1.30

Week 12 Marker 4		
Time	Dissolution %	±SD %
0	0.00	0
2	5.22	1.99
5	7.82	3.03
10	15.57	4.97
15	21.62	2.97
30	24.20	2.69
60	30.16	1.66
90	30.97	3.41
120	32.48	3.46
180	36.28	3.54
240	38.30	2.85

ADDENDUM H: ETHICS APPROVAL

Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 086 016 9698
Web: http://www.nwu.ac.za

North-West University Health Research Ethics Committee (NWU-HREC)

Tel: 018 299-1206
Email: Ethics-HRECApply@nwu.ac.za (for humanstudies)

30 March 2021

RESEARCH ETHICS COMMITTEE LETTER OF DECISION: NO RISK

Based on the review by the North-West University Health Research Ethics Committee (NWU-HREC) on 30/03/2021, the NWU-HREC hereby clears your study as a no risk study. This implies that the NWU-HREC grants its permission that, provided the general conditions specified below are met, the study may be initiated,using the ethics number below.

Study title: Development of a solid oral dosage form containing *Artemisia afra* extract
Principal Investigator/Study Supervisor/Researcher: Prof JH Steenekamp

Student: PS Roets - 24240850

Ethics number

Application Type: Single study

Risk: **No Risk**

N	W	U	-	0	0	1	7	3	-	2	1	-	A	1

General conditions:

The following general terms and conditions will apply:

- *The commencement date indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-HREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research;*
  - *withdraw or postpone clearance if:*
    - *any unethical principles or practices of the study are revealed or suspected;*
    - *it becomes apparent that any relevant information was withheld from the NWU-HREC or that information has been false or misrepresented;*
    - *submission of the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or*
    - *new institutional rules, national legislation or international conventions deem it necessary.*

Please note: Due to the nature of the study i.e. (laboratory work involving the development of a solid dosageform of a specific plant extract), this study will be able to proceed during the current alert level, following receiptof this approval letter. No additional COVID-19 restrictions have been placed on the study except that the researcher must ensure that before proceeding with the study that all research team members have reviewedthe North-West University COVID-19 Occupational Health and Safety Standard Operating Procedure.

The NWU-HREC would like to remain at your service and wishes you well with your study. Please do not hesitate to contact the NWU-HREC for any further enquiries or requests for assistance.

Yours
sincerely,

Digitally
signed by Prof
Petra Bester

Date: 2021.03.30
11:51:43 +02'00'

NWU-HREC Chairperson

Digitally signed by Gordon Wayne Towers Date: 2021.03.30 09:35:30 +02'00'

Head of the Faculty of Health Sciences Ethics Office for Research, Training and Support