การศึกษาก่อนตั้งสูตรตำรับและการพัฒนาสูตรตำรับยาเม็ดแตกตัวในช่องปากของสารสกัดสมุนไพรพิกัดนวโกฐ

นางสาวปวีณา กำเหนิดนนท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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PREFORMULATION AND FORMULATION DEVELOPMENT OF ORODISPERSIBLE TABLETS CONTAINING PHIKUD NAVAKOT HERBAL EXTRACT

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmaceutics
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Thesis Title	PREFORMULATION AND FORMULATION DEVELOPMENT OF ORODISPERSIBLE TABLETS CONTAINING PHIKUD NAVAKOT HERBAL EXTRACT
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Field of Study	Pharmaceutics
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ปวีณา กำเหนิดนนท์ : การศึกษาก่อนตั้งสูตรตำรับและการพัฒนาสูตรตำรับยาเม็ดแตกตัวในช่องปากของสารสกัดสมุนไพรพิกัดนวโกฐ (PREFORMULATION AND FORMULATION DEVELOPMENT OF ORODISPERSIBLE TABLETS CONTAINING PHIKUD NAVAKOT HERBAL EXTRACT) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ภญ. ดร.นฤพร สุตัณฑวิบูลย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ภญ. ดร.จิตติมา ชัชวาลย์สายสินธ์, 125 หน้า.

งานวิจัยครั้งนี้มีวัตถุประสงค์ในการพัฒนาสูตรตำรับยาเม็ดแตกตัวในช่องปากของสารสกัดสมุนไพรพิกัดนวโกฐซึ่งเป็นยาสมุนไพรแผนโบราณที่มีการใช้ในประเทศไทยมายาวนานมากกว่า 100 ปี เพื่อเป็นการพัฒนายาสมุนไพรไทยแผนโบราณให้มีคุณภาพมาตรฐานและสะดวกต่อการใช้งาน โดยทำการศึกษาก่อนตั้งสูตรตำรับ และควบคุมคุณภาพของสารสกัดสมุนไพรพิกัดนวโกฐโดยการพัฒนาวิธีวิเคราะห์ด้วยเครื่องโครมาโทกราฟีของเหลวสมรรถนะสูงตามหลักเกณฑ์ของ AOAC Guidelines for Dietary Supplements and Botanicals ในการตรวจติดตามสารเคมีเชิงวิเคราะห์จำนวน 3 ชนิด คือ แกลลิค แอซิด วานิลิค แอซิด และ เฟรูลิค แอซิด ซึ่งเป็นสารเคมีจำพวกฟีนอลิค แอซิด ที่พบในสารสกัดพิกัดนวโกฐ ทำการแปรรูปสารสกัดพิกัดนวโกฐที่มีลักษณะข้นเหนียวให้เป็นผงแห้งด้วยวิธีการดูดซับเพื่อให้สะดวกต่อการนำไปใช้งาน การผลิตยาเม็ดแตกตัวในช่องปากของสารสกัดสมุนไพรพิกัดนวโกฐใช้กระบวนการผลิตด้วยวิธีตอกโดยตรง โดยใช้สารช่วยแตกตัวยิ่งยวดจำนวน 3 ชนิดในปริมาณที่ต่างกัน คือ Polyplasdone® XL-10, Explotab® และ Ac-Di-Sol® ปริมาณ 2%, 6% และ 10% และทำการประเมินคุณภาพของยาเม็ด จากนั้นนำสูตรตำรับที่ได้รับการคัดเลือกมาบรรจุในบรรจุภัณฑ์และศึกษาความคงสภาพที่สภาวะเร่งและสภาวะปกติตาม ASEAN Guideline on Stability Study of Drug Product ผลการทดลองที่เวลา 3 เดือนพบว่ามีการเปลี่ยนแปลงของปริมาณของสารเคมีเชิงวิเคราะห์มากกว่า 5% จากเวลาเริ่มต้น ในขณะที่ในสภาวะปกติพบว่าที่เวลา 3 เดือนและ 6 เดือน สารเคมีเชิงวิเคราะห์ทั้ง 3 ชนิดมีการเปลี่ยนแปลงไม่เกิน 5% จากเวลาเริ่มต้น ดังนั้นควรมีการศึกษาความคงสภาพในสภาวะปกติอย่างต่อเนื่องจนถึงเวลา 12 เดือนเพื่อเป็นข้อมูลในการกำหนดอายุของผลิตภัณฑ์ที่พัฒนาขึ้น

ภาควิชา	วิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม	ลายมือชื่อนิสิต .....
		ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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KEYWORDS: PHIKUD NAVAKOT / ORODISPERSIBLE TABLETS / QUALITY CONTROL / STABILITY / HERBAL MEDICINE

PAVENA KUMNERDNON: PREFORMULATION AND FORMULATION DEVELOPMENT OF ORODISPERSIBLE TABLETS CONTAINING PHIKUD NAVAKOT HERBAL EXTRACT. ADVISOR: NARUEPORN SUTANTHAVIBUL, Ph.D., CO-ADVISOR: JITTIMA CHATCHAWALSAISIN, Ph.D., 125 pp.

The main purpose of this research was to develop orodispersible tablets containing Phikud Navakot Extract (NVK-E) with consistent quality and convenience. Phikud Navakot is a traditional Thai herbal medicine that has been used in Thailand for more than 100 years. Preformulation studies of NVK-E and excipients were carried out. High performance liquid chromatography (HPLC) method was validated according to AOAC Guidelines for Dietary Supplements and Botanicals to determine existing three analytical markers, i.e. gallic acid, vanillic acid and ferulic acid. The viscous NVK-E was transformed to more processable powder by adsorption on the selected excipient. Orodispersible tablets containing NVK-E were developed by direct compression method using three superdisintegrants, i.e. Polyplasdone® XL-10, Explotab® and Ac-Di-Sol® at different percentages of 2%, 6% and 10% in formulations. Quality control of tablets were performed. The stability study of the chosen formulation was evaluated in the intended package for commercialization according to ASEAN Guideline on Stability Study of Drug Product under accelerated and long term storage conditions. At 3 months under accelerated storage condition, more than 5% deviated from initial values which failed to meet the requirement. However, at 3 and 6 months under long term storage condition, analytical markers still retained values of not more than 5% from initial values. Thus, their values must be monitored real-time up to 12 months under long term storage condition for the conclusion on shelf-life of the finished product.

Department:	Pharmaceutics and Industrial Pharmacy	Student's Signature .....
		Advisor's Signature .....
Field of Study:	Pharmaceutics	Co-Advisor's Signature .....
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LIST OF ABBREVIATIONS

%	=	percentage
%LA	=	Percentage of label amount
RSD	=	Related Standard Deviation
SD	=	Standard Deviation
R	=	Correlation coefficient
conc.	=	Concentration
ρ	=	density
°C	=	degree Celsius
kg	=	kilogram
mg	=	milligram
ml	=	milliliter
mm	=	millimeter
µl	=	microliter
µm	=	micrometer
µM	=	micromolar
nm	=	nanometer
min	=	minute
sec	=	second
tab	=	tablet
v/v	=	volume by volume
DMSO	=	Dimethylsulfoxide
DPPH	=	2,2-diphenyl-1-Picrylhydrazyl
NVK-E	=	Phikud Navakot Extract
HorRat	=	Horwitz Ratio
RP	=	Reverse Phase
DAD	=	Diode-Array Detector
HPLC	=	High Performance Liquid Chromatography
DSC	=	Differential Scanning Calorimeter

pH	=	the negative logarithm of the hydrogen ion concentration
GAE	=	Gallic Acid Equivalence
HER	=	Herb to Extract Ratio
ODTs	=	Orodispersible Tablets
TLC	=	Thin layer chromatography
CE	=	Capillary Electrophoresis
GC	=	Gas Chromatography
Av	=	Average
AV	=	Acceptance Value

CHAPTER I
INTRODUCTION

Phikud Navakot is a traditional Thai herbal medicine that is widely used in Thailand for more than 100 years. It consists of equal quantities of 9 herbs including *Angelica sinensis* (Kot-Chieng), *Angelica dahurica* (Kot-Sor), *Ligusticum sinense* (Kot-Hua-Bua), *Atractylodes lancea* (Kot-Khe-Ma), *Artemisia annua* (Kot-Chu-La-Lum-Pha), *Picrorhiza kurrooa* (Kot-Garn-Prow), *Saussurea lappa* (Kot-Gra-Duk), *Nardostachys grandiflora* (Kot-Cha-Da-Mung-Sri) and *Terminalia chebula* (Kot-Poong-Pla). Phikud Navakot is used for the treatment of circulation disorder and as an anthelmintic and analgesic agent (1). Phikud Navokot is a major ingredient in "Yahom Navakot" which is listed in the National List of Essential Medicine for the treatment of circulation disorder (2). The recent study showed that Phikud Navakot extract has a lipid lowering effect (3) and antioxidant activity (4). From these results and the increasing popularity of herbal and traditional medicines suggest that the pharmacological activities, standardization and quality control of Phikud Navakot Extract require evaluation. In recent years, a number of pharmaceutical research has focused on new dosage forms with convenience of use which may improve the quality of life. The orodispersible tablet is one of the pharmaceutical dosage forms that are widely used and preferred commercial products (5). Orodpersible tablets are tablets that rapidly dissolve in the oral cavity. It can be easily administered with or without water. They are preferable for patients who are suffering from swallowing disorders such as disable or mentally ill patients as well as pediatric and elderly patients (6). Efficient quality control of NVK-E raw material and NVK-E in the pharmaceutical dosage forms are an important part of pharmaceutical product development to assure the safety, quality and efficacy of the herbal product. Many analytical techniques have been utilized to control the quality of herbal products, including HPLC, TLC, GC and capillary electrophoresis, depending on the nature of the active constituents to be analyzed (7). These methods are often unsuccessful due to the complex nature of

herbal products. Therefore, the quality control of NVK-E and NVK-E in the pharmaceutical dosage form is quite challenging.

In this study, the quality control of NVK-E was evaluated and the orodispersible tablets containing NVK-E was developed. The quality control of the tablets were determined during the manufacturing process and the chosen formulation was tested for the further stability study according to the ASEAN Guideline on Stability Study of Drug Product.

The purposes of this study are as follows:

1. To control the quality of NVK-E.
2. To formulate orodispersible tablets containing NVK-E and perform the quality control of the product.
3. To evaluate the stability of orodispersible tablets containing NVK-E.

CHAPTER II
LITERATURE REVIEWS

1. Phikud Navakot

Phikud Navakot is a traditional Thai herbal medicine that has been used in Thailand for more than 100 years. It consists of equal quantities of 9 herbs: *Angelica sinensis* (Kot-Chieng), *Angelica dahurica* (Kot-Sor), *Ligusticum sinense* (Kot-Hua-Bua), *Atractylodes lancea* (Kot-Khe-Ma), *Artemisia annua* (Kot-Chu-La-Lum-Pha), *Picrorhiza kurrooa* (Kot-Garn-Prow), *Saussurea lappa* (Kot-Gra-Duk), *Nardostachys grandiflora* (Kot-Cha-Da-Mung-Sri) and *Terminalia chebula* (Kot-Poong-Pla). Phikud Navakot is used for treatment of hiccups and circulation disorder, and as an antihelminthic and analgesic agent (1). Phikud Navokot is a major ingredient in "Yahom Navakot" which is listed in the National List of Essential Medicine for the treatment of circulation disorder (2). The previous study shows that 80% ethanolic extract of Phikud Navakot exhibits cardioprotective activity, anti-oxidant activity and anti-platelet aggregration activity (8). Phikud Navakot extract decreases vasorelaxation due to carbachol in the rat aorta, which supports the use of Phikud Navakot against dizziness and fainting (9). Previous study suggests that ethanolic Phikud Navakot extract has a lipid lowering effect that occurs through an enhancement of LDL-R expression and the inhibition of HMG-CoA reductase expression (3). The recent study suggests that Phikud Navakot 1:1 ethanol to water extract and Phikud Navakot water extract exhibit antioxidant activities against DPPH, $O_2^{\bullet-}$, $OH^{\bullet}$, $NO^{\bullet}$ and H_2O_2 (4).

The study suggested that the extraction of Phikud Navakot with 80% ethanol at 100°C for 3 hours exhibited the preferable %yield, total phenolic content, cardioprotective activity, anti-oxidant activity and anti-platelet aggregration activity. Phikud Navakot 80% ethanolic extracted from this study obtained Herb to Extract Ratio (HER) of 3.3:1 (8).

According to the National List of Essential Medicine, 212 g of Yahom Navakot contains 36 g of Phikud Navakot. The administration of Yahom Navakot is 3-6 g divided 3 times daily which is equivalent to Phikud Navakot 0.5-1 g daily. From the

previous study, the extraction of 80% ethanolic extract Phikud Navakot obtained Herb to Extract Ratio (HER) of 3.3:1 (8).This suggested that Phikud Navakot dosing in one day is approximately 150-300 mg.

Figure 1 Phikud Navakot 80% ethanolic extract
(Reproduced from (8))

2. Orodispersible tablets

Tablets are the most popular pharmaceutical dosage form. They are dispensed for 70% of the total medicine. Tablets obtain adventages to both manufacturers and pateints. For manufacturers, they are simple and economical for manufacturing. They also provide a preferable stability and they are convinient for packaging, shipping and storage. For patients, they are unit dosage form which provide the accurate dosing unit and they are convenient in administration and storage (10). However, there are disadvantages over the tablet formulation especially for patients with the swollowing disorders such as disable or mentally ill patients as well as pediatric and elderly patients. Hence, Orodispersible tablets (ODTs) are developed to overcome the disadvantage of tablets considering on the ease of medication. Orodispersible tablets are tablets that quickly disintegrate or dissolve in the mouth without the presence of water or required chewing. Orodispersible tablets

have advantages over the conventional tablets dosage form. They can be easily administered especially for the patients who undergo the swollowing disorders such as disable, dysphagia or mentally ill patients, the patients who cannot swallow or refuse to swallow such as pediatric patients, geriatric patients and psychiatric patients (5). Orodispersible tablets are also known as melt in mouth tablets, orally disintegrating tablets, fast disintegrating tablets or mouth dissolving tablets. British Pharmacopeia defines orodispersible tablets as "An uncoated tablets intended to be place in the mouth where they dispersed rapidly before being swallow" and the orodispersible tablets should perform the disintegration time of not more than 3 minutes (11). The United States Pharmacopeia defines "Orally disintegrating tablets are intended to disintegrate rapidly within the mouth to provide a fine dispersion before the patient swallows the resulting suspension where the API is intended for gastrointestinal delivery and/or absorption" and the orally disintegrating tablets should perform the disintegration time of not more than 30 seconds (12). The United States Pharmacopeia also recommends that the weight of tablets should not exceed 500 mg to perform an effective disintegration.

In recent years, there are variety of orodispersible tablets launched in the market containing various group of medicine including anti-histamine drugs such as loratadine and desloratadine, nonsteroidal anti-inflammatory drugs such as piroxicam, ibuprofen, rofecoxib and nimesulide, anti-depressant drugs such as clonazepam, anti-migrane drugs such as zolmitriptan and rizatriptan, anti-ulcer drugs such as famotidine, anti-emetic drugs such as ondansetron, analgesic drugs such as paracetamol and anti-Alzheimer drug such as donepezil (13).

2.1 Orodispersible tablets manufacturing technologies

2.1.1 Conventional Technologies

Various techniques are used to develop the orodispersible tablets including cotton candy process, wet granulation method, melt extrusion method, tablet molding, sublimation process, freeze drying, effervescent system and direct compression (14). The cotton candy process, wet granulation method, melt extrusion method, tablet molding, sublimation process are the methods that employed

heating process. They are not suitable for the active ingredients that susceptible to heat. The freeze drying, effervescent system and direct compression are the alternative methods for susceptible to heat active ingredients. The preparation process of orodispersible tablets are shown in Figure 2. There are advantages and disadvantages of these techniques for the manufacturing of the orodispersible tablets. The suitable method should be selected upon the consideration of the property of active ingredients, the availability of excipients and the desired disintegration time.

Direct compression is one of the most simple and economical method in the manufacturing of orodispersible tablets. It require two simple steps of mixing the active ingredients with other excipients and compression into the tablets. It is preferable for thermolabile and moisture sensitive active ingredients because it requires no heating or wetting during the manufacturing process. This method is applied to manufacture the orodispersible tablets by the addition of superdisintegrants such as crospovidone (Polyplasdone®), sodium starch glycolate (Explotab®) and croscamellose sodium (Ac-Di-Sol®) and sugar based excipients such as mannitol, maltose and maltilol (15). Superdisintegrants aid in tablet disintegrating by swelling, wicking, repulsion and deformation mechanism (16). While, sugar based excipients exhibit high aqueous solubility which aid in the disintegration of the tablets (13).

2.1.2 Patented technologies

In the recent year, the patented technologies are developed such as Flashtab Technology, Durasolv Technology and Zydis Technology (14). These technologies are designed to produce a preferable orodipersible tablets property.

2.2 Orodispersible tablets formulation

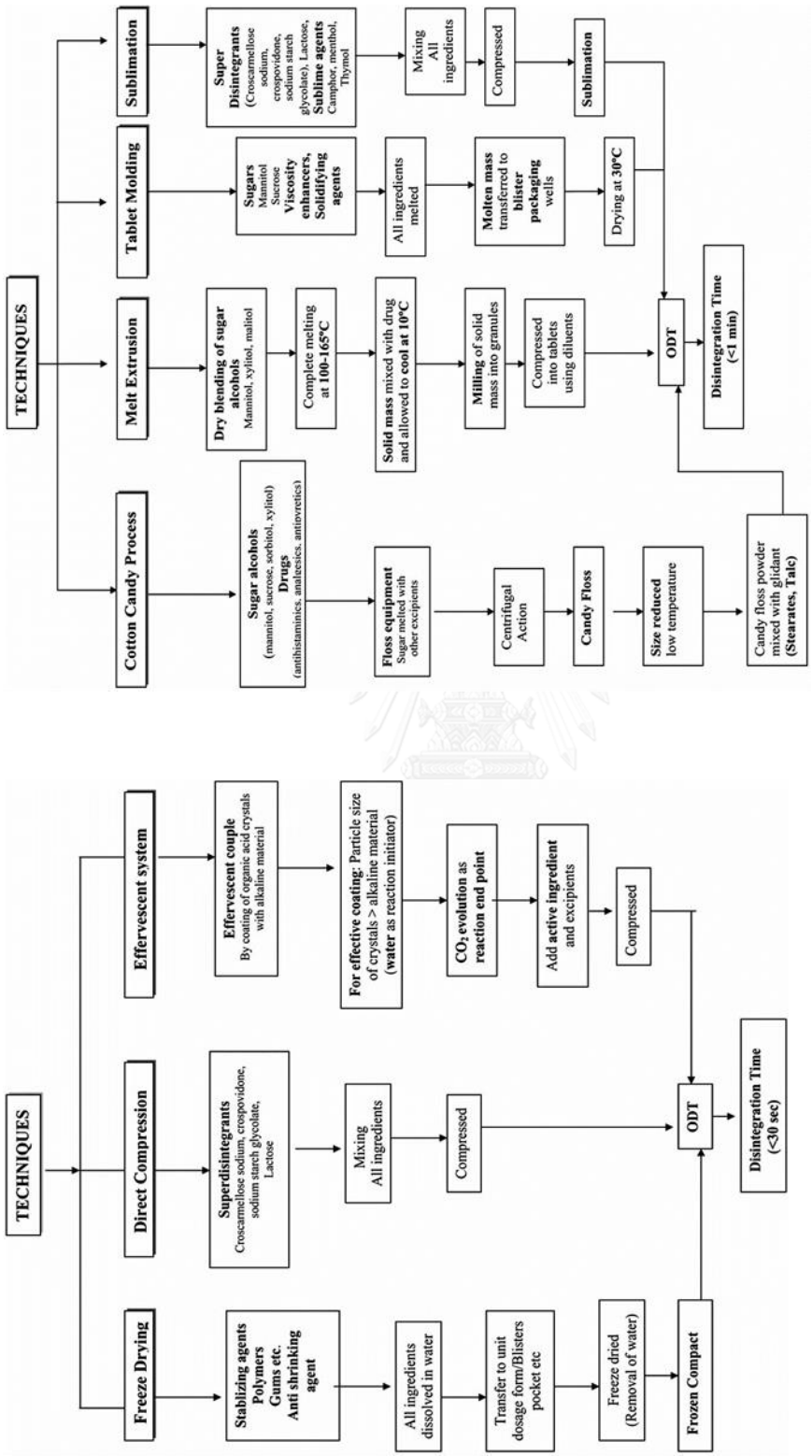
The desired characteristics for orodispersible tablets are that they should perform a fast disintegration time as well as exhibit a good mouth feel. The orodispersible tablets are purposed to put on the tongue and dissolve or disperse in the saliva (15). Hence, the developed orodispersible tablets should perform a pleasant mouth feel to achieve patient's compliance. The suitable excipients should

be selected in the formulation to exhibit preferable orodispersible tablets characteristics. The commonly used excipients in orodipersible tablets are diluents especially sweet diluents such as sugar based excipients to exhibit a good mouth feel. Disintegrants such as croscamellose sodium, crospovidone and sodium starch glycolate are the important composition of compressed orodispersible tablets to aid in the disintegration of the tablets. Flavoring agents such as sucralose are also used in the formulation to aid in a good mouth feel. Glidants such as colloidal silicon dioxide are used to aid in the flow of the formulation and lubricants such as magnesium stearate and stearic acid are used to prevent sticking of the formulations to the punches and help ejecting of the tablets from dies (17).

2.2.1 Diluents

Sugar based excipients are the commonly used as diluents in the orodispersible tablets formulation because they display high aqueous solubility property and sweetness resulting in a pleasing mouth feel. Sugar based excipients used in the orodispersible tablets are dextrose, amorphous sucrose, mannitol, maltitol and xylitol. Sugar alcohols such as xylitol, maltitol and mannitol are containing fewer calories compared to sucrose and they do not promote tooth decay. Mannitol and xylitol obtain negative heats of solution and exhibit a cooling sensation in the mouth which leads to a pleasant mouth feel (17). Sugar base excipients were classified into two types. Type 1 is the saccharides that exhibits low mouldability and high dissolution rate such as lactose and mannitol. Type 2 is the saccharide that exhibits high mouldability and low dissolution rate such as maltose and maltilol (15).

Figure 2 The preparation process of orodispersible tablets (Reprodecued from (14))



2.2.2 Disintegrants

Disintegrants are the most important composition of compressed orodispersible tablets because of the purpose of orodispersible tablets which require the fast disintegration time. Disintegrants used in orodispersible tablets are starch, pregelatinized starch, modifies starch, cellulose and its derivatives, microcrystalline cellulose, alginates, ion-exchange resin, gums and agar. However, there are the miscellaneous groups of disintegrant that are developed for the faster disintegration which are described as superdisintegrants. The superdisintegrants are the disintegrants that are effective at low concentration and provide greater disintegration efficiency than the conventional disintegrants. They are strongly hygroscopic which aid in absorbtion of water into the tablets. Therefore, they may affect the stability of the moisture sensitive active ingredients. In conventional tablets, superdisintegrants such as sodium starch glycolate, crospovidone and croscamellose sodium are used at the concentration of 2-5% in the formulation. In orodispersible tablets, up to 15% of superdisintegrants may be beneficial (15, 17) . Patil et al. evaluated orodispersible tablets of genisetron hydrochloride using direct compression method. Three superdisintegrants, i.e. crospovidone, croscamellose sodium and sodium starch glycolate at 3%, 6%, 9% and 12% were used in the formulations. The results showed the shorter disintegration time of the tablets with the elevated concentration of croscamellose sodium and sodium starch glycolate while crospovidone exhibited different results. They exhibited the disintegration time of 30, 16, 20 and 28 seconds at the concentration of 3%, 6%, 9% and 12%, respectively. At 6% concentration, the tablets with crospovidone exhibited the shortest disintegration time. However, crospovidone performed shortest disintegration time compared to other superdisintegrants (18). Deshmukh, Zade and Sakarkar developed ondansetron orodispersible tablets by direct compression method with the addition of croscamellose sodium, sodium starch glycolate and crospovidone at 20%, 25%, and 30% in the formulation (19). The similar result was obtained to the previous study of Patil et al. that the shorter disintegration time of the tablets with the elevated concentration of croscamellose sodium and sodium

starch glycolate while crospovidone exhibited the shortest disintegration time at the lowest concentration and it also performed shortest disintegration time compare to other superdisintegrants (18, 19). The result suggested that the higher concentration of superdisintegrant did not always produce faster disintegration time (17).

Three major groups of disintegrants have been developed including midified starches; sodium carboxymethyl starch and sodium starch glycolate, cross linked polyvinylpyrrolidone; crospovidone and modified cellulose; sodium carboxymethyl cellulose. These superdisintegrants are widely used in the orodispersible tablets dosage form to perform fast disintegration time. The first group of superdisintegrant exhibits the swelling mechanism. They rapidly absorb the medium and swell leading to the disintegration of tablets. The use of high concentration may cause gelling on exposure to water and retard the disintegration. The second group of superdisintegrant exhibits the wicking as well as minor swelling mechanism. They disintegrate by the capillary action where the medium penetrates into tablets and replaces absorbed air on the particles and weakens the intermolecular bond leading to the breaking of the tablets. The third group of superdisintegrant exhibits both wicking and swelling mechanism (20). The swelling and wicking mechanism of superdisintegrants are show in Figure 3.

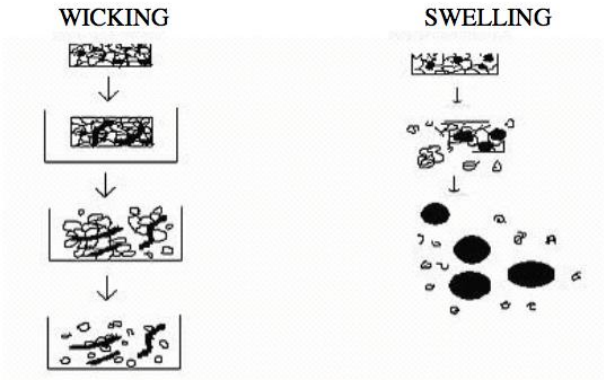


Figure 3 Swelling and wicking mechanism of superdisintegrants
(Reproduced from (20))

3. Quality control

3.1 Quality control of herbs

Quality control is an important part of pharmaceutical product development to assure the safety, quality and efficacy of the herbal product. The compound approch and fingerprint approach are the principle method to control the quality of herbs. The compound approach is the method to control the quality of herbs by the identification and quantification of chemical markers while the fingerprint approach is the method to control the quality of herbs by the chromatographic fingerprint which refer to the chromatographic pattern of herbs which exhibit chemical components of pharmacologically active or chemically characteristics (7).

Chemical markers are the constituents or groups of constituents of a herbal medicinal product which are of interest for quality control purposes regardless of whether they possess any therapeutic activity (21). Accoding to The European Medicines Agency (EMEA), "the chemical markers that provide the therapeutic activity but are not responsible for the full therapeutic effect are defined as active markers and constituents or groups of constituents that serve solely for analytical purposes are defined as analytical markers"(22).

Phikud Navokot consisted of nine herbs including: *Angelica sinensis* (Kot-Chieng), *Angelica dahurica* (Kot-Sor), *Ligusticum sinense* (Kot-Hua-Bua), *Atractylodes lancea* (Kot-Khe-Ma), *Artemisia annua* (Kot-Chu-La-Lum-Pha), *Picrorhiza kurrooa* (Kot-Garn-Prow), *Saussurea lappa* (Kot-Gra-Duk), *Nardostachys grandiflora* (Kot-Cha-Da-Mung-Sri) and *Terminalia chebula* (Kot-Poong-Pla). Previous studies showed the chemical constituents in each Phikud Navakot herbs using various analytical techniques such as HPLC, GC and LC-MS. The constituents in each Phikud Navakot herbs are shown in Table 1.

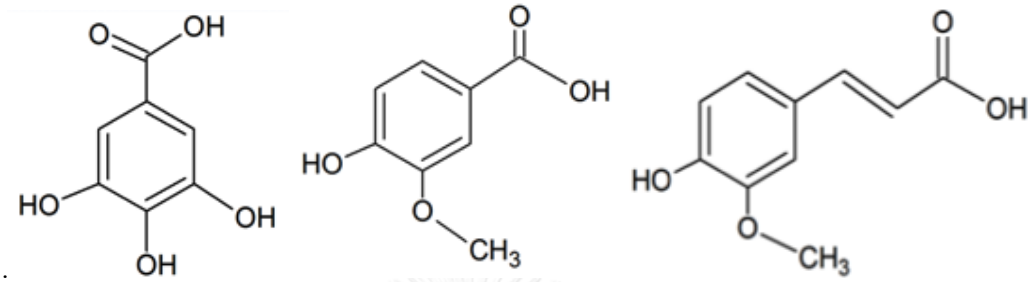
Table 1 Phikud Navakot herbs and their constituents

Herbs	Constituents	References
Angelica sinensis (Kot-Chieng)	Ferulic acid, Z-ligustilide, Butylidenephthalid, Polysaccharides Ferulic acid, Senkyunolide I, Senkyunolide H, Coniferyl ferulate, E-Ligustilide, E-Butylidenephthalide, Z-Ligustilide Z-Butylidenephthalide	(23) (24)
Angelica dahurica (Kot-Sor)	Imperatorin, Isoimperatorin	(25)
Ligusticum sinense (Kot-Hua-Bua)	Levistolide A, (Z)-3-butylidene-7-hydroxyphthalide, Senkyunolide B, 3-butylphthalide, (Z)-ligustilide, Riligustilide, Neocnidilide, Senkyunolide A, Beta-sitostesol	(26)
Atractylodes lancea (Kot-Khe-Ma)	Beta-eudesmo Atractylone, Hinesol, Beta-eudesmol, Atrctylodin	(27) (28)
Artemisia annua (Kot-Chu-La-Lum-Pha)	1-6, alpha-amyrenone, Alpha-amyrin, Beta-amyrin, Taraxasterone, Oleanolic acid, Stigmasterol, Sitosterol, Baurenol	(29)
Picrorhiza kurrooa (Kot-Garn-Prow)	Vanillic acid, Apocyanin, Veronicoside, Minecoside, Picein, 6-feruloylcatalpol	(30)

Herbs	Constituents	References
Saussurea lappa (Kot-Gra-Duk)	Costunolide, Dehydrocostus lactone, Cynaropicrin, Lappadilactone, Germacrenes	(31) (32)
	Dehydrocostus lactone, Santamarine Beta-cyclocostunolide, 10-alpha-hydroxyl-artemisinic acid	
Nardostachys grandiflora (Kot-Cha-Da-Mung-Sri)	Alpha-patcho-ulense, Angelicin, Beta-eudesemo, Beta-atchoulense, Beta-sitosterol, Calarene, Elemol, Jatamansin, Jatamansinol	(33)
Terminalia chebula (Kot-Poong-Pla)	Gallic acid, Chebulic acid, Punicalagin, Chebulanin, Corilagin, Neochebulinic, Ellagic acid, Chebulegic acid, Chebulinic acid	(34)

Sotanaphun et al. developed the gradient HPLC method developed using acetonitrile and 1% v/v acetic acid as solvent to determine the compositions of Phikud Navakot extract. Various standards were used to compare chromatograms and spectra of NVK-E based on the previous studies of the presence of components in each Phikud Navakot herb. The standards used in this study were gallic acid, chlorogenic acid, vanillic acid, caffeic acid, umbelliferone, coumaric acid, ferulic acid, rutin and imperatorin. The standards which matched the chromatograms and spectra with NVK-E were gallic acid and vanillic acid (8). Nalinratana et al. sucessfully developed the gradient HPLC method to monitor gallic acid, vanilic acid, feulic acid and rutin in NVK-E. The results suggested that there were 0.143%, 0.396%, 0.004% and 0.024% of gallic acid, vanillic acid, rutin and ferulic acid, respectively in the hydroethanolic extract of Phikud Navakot (%w/w) and 0.136%, 0.362%, 0.002% and

0.003% of gallic acid, vanillic acid, rutin and ferulic acid, respectively in the water extract of Phikud Navakot (%w/w) (4). Chemical structures of gallic acid, vanillic acid and ferulic acid as analytical markers as shown in Figure 4 and rutin in Figure 5, respectively.



A B C

Figure 4 Chemical structures of (A) gallic acid (B) vanillic acid, and (C) ferulic acid

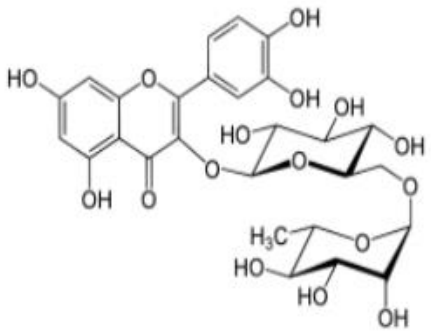


Figure 5 Chemical structure of Rutin

(Reproduced from (35))

The quantitative analysis of substances using HPLC method should perform the validation of the analytical method to ascertain the performance characteristic of the procedure which should meet the requirements for the analytical application. The analytical performance characteristics such as accuracy, precision, specificity, detection limit, quantitation limit, linearity, range and robustness were performed in the analytical method validation procedure (12). AOAC Guideline for Dietary Supplements and Botanicals stated the validation of chemical methods for dietary

supplements and botanical to perform the performance characteristics which were applicability, selectivity, calibration and reliability characteristic such as accuracy, repeatability precision, measurement uncertainty and intermediate precision (36).

3.2 Quality control of orodispersible tablets formulation

3.2.1 Preformulation study

Preformulation studies of orodispersible tablets including bulk density, tapped density, compressibility index and Hausner's ratio are performed to determine the density and the flow property of the formulation (5).

3.2.2 Orodispersible tablets evaluation

Tablet thickness, tablet hardness, Uniformity of weight, disintegration time, in-vitro drug release, friability test, in-vitro dispersion time test, wetting time, water absorption ratio and stability study are the methods to evaluate the quality of the orodispersible tablets (5). The quality control of the active ingredients, excipients as well as the orodispersible tablets formulation should be performed to obtain the preferable orodispersible characteristics.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Absolute ethanol, AR grade (Lab Scan Co., Ltd., Thailand)
2. Acetic acid glacial, ACS (CARLO ERBA Reagents S.A.S., France)
3. Chloroform, ACS (CARLO ERBA Reagents S.A.S., France)
4. Citric acid monohydrate (Batch No. 1302135643, ThermoFischer Scientific Pty Ltd., Austalia)
5. Colloidal silicon dioxide (Aerosil® 200, Lot No. 154080414, Jebsen & Jessen Chemicals Ltd., Thailand
6. Croscamellose sodium (Ac-Di-Sol®, Lot No. IR 704/30, Rama Production Co., Ltd., Thailand)
7. Crospovidone (Polyplasdone® XL-10, Batch No. 032301814, Maxway Co., Ltd., Thailand)
8. Crude Phikud Navakot herbal materials (Vejpong Pharmacy Co., Ltd., Thailand)

- Angelica dahurica
- Angelica sinensis
- Artemisia annua
- Atractylodes lancea
- Ligusticum sinense
- Nardostachys grandiflora
- Picrorhiza kurrooa
- Saussurea lappa
- Terminalia chebula

9. Ferulic acid (99.8% purity, Lot: STBB8393V, Sigma-Aldrich Co., LLC, USA)

10. Folin & Ciocalteu's phenol reagent (Lot No. HC263137, Merck Ltd., Germany)
11. Gallic acid (Gallic acid monohydrate, 99.4% purity, Lot: 392513, Fluka, Sigma-Aldrich Co., LLC, USA)
12. Gallic acid (Gallic acid monohydrate, 99.7% purity, Batch No. MKBP6646V, SIAL, Sigma-Aldrich Co., LLC, USA)
13. Hydrochloric acid, 37% (CARLO ERBA Reagents S.A.S., France)
14. Kaolin (light, Lot No. H5114, S. Tong Chemical Co., Ltd., Thailand)
15. Karl fischer reagent (Lot No. HX44043405, Merck Ltd., Germany)
16. Ludiflash® (Lot No. 44075288Q0, BASF, Germany)
17. Magnesium carbonate (light, Lot No. 60100, Sigma-Aldrich Co., LLC, USA)
18. Mannitol (Batch No. 1305140152, ThermoFischer Scientific Pty Ltd., Austalia)
19. Methanol, ACS grade (Merck Ltd., Germany)
20. Methanol, HPLC grade (Merck Ltd., Germany)
21. Microcrystalline cellulose (Avicel® PH102, Lot No. P213825304, FMC BioPolymer, USA)
22. Monobasic potassium phosphate (Batch No. 1401251756, Ajax Finechem Pty Ltd., Australia)
23. Pectin (Lot No. GR04678, Srichan United Dispesary Co., Ltd., Thailand)
24. Sodium chloride (Batch No. 1403164044, ThermoFischer Scientific Pty Ltd., Austalia)
25. Sodium hydroxide pellets AR (Lot No. 2244910514, Thermo Fisher Pvt Ltd., India)
26. Sodium starch glycolate (Explotab®, Batch No. 6111410070, Gujarat Coporate, India)
27. Starch, Pregelatinized (Starch 1500, Lot No. IN505698, Colorcon Inc., USA)
28. Strearic acid (Batch No. 0000434409, Pancreac Quimica S.L.U., Spain)
29. Sucralose (Batch No. 113061004, Chemipan Corporation Co., Ltd., Thailand)

30. Talcum (Lot No. 45GHM1408113, Guangxi Lonsheng Huamei Talc Development Co., Ltd., China)
31. Vanillic acid (98.7% purity, Lot: BCBH4868V, Sigma-Aldrich Co., LLC, USA

Apparatus

1. Analyical balance (PB3002, Mettler-Toledo Ltd., Switzerland)
2. Analytical Balance (ML303, Mettler-Toledo Ltd., Switzerland
3. Analytical Balance (XP205, Mettler-Toledo Ltd., Switzerland)
4. Differential scanning calorimeter (DSC 822e, Mettler-Toledo Ltd., Switzerland)
    - Aluminium pan 40 µl with lid (Mettler-Toledo Ltd., Thailand)
5. Disintegration apparatus (ZT31, Erweka GmbH, Germany)
6. Dissolution apparatus (Vankel VK7000, Varian Inc., USA)
7. Friability tester (TAR10, Erweka GmbH, Germany)
8. Hardness tester (Monsanto type)
9. High performance liquid chromatographic system
    - 0.22 µm nylon membrane filter (Bonna-Agela Technologies Inc., USA)
    - 0.45 µm nylon syringe filter (Vertical Chromatography Co.,Ltd., Thailand)
    - Auto-sampler (SIL-20AC, Shimadzu, Japan)
    - Column oven (CTO-20A, Shimadzu, Japan)
    - HPLC column (Shiseido MGII C18, 4.6 x 250 mm, 5 µm, Shiseido Co., Ltd., Japan)
    - Photodiode array detector (SPD-M20A, Shimadzu, Japan)
    - Quaternary pump (LC-20AD, Shimadzu, Japan)
10. Karl Fischer Titrator (Metrohm 785 DMP, Metrohm Ltd., Switzerland)
11. Microplate reader (Spectramax M5, Molecular Devices, LLC., USA)

- 96-well micro plates (Lot No. BA1I02A, SPL Life Science, Ltd., Korea)

12. Plenary mixer (AR4005, Erweka GmbH, Germany)
13. Sonicator (Elmasonic S70H, Elma Electric Inc., USA)
14. Stability cabinet (KBF 720- ICH, Binder, Germany)
15. Tabletting machine, automatic (single punch, Viuhang Engineering, Thailand)
16. Tabletting machine, mechanical (single punch, BS215B, Kasuga Electric Works Ltd., Japan)

Methods

1. Extraction of NVK-E from crude Phikud Navakot

Equal quantities of 9 crude materials consisted of *Angelica sinensis* (Kot-Chieng), *Angelica dahurica* (Kot-Sor), *Ligusticum sinense* (Kot-Hua-Bua), *Atractylodes lancea* (Kot-Khe-Ma), *Artemisia annua* (Kot-Chu-La-Lum-Pha), *Picrorhiza kurrooa* (Kot-Garn-Prow), *Saussurea lappa* (Kot-Gra-Duk), *Nardostachys grandiflora* (Kot-Cha-Da-Mung-Sri) and *Terminalia chebula* (Kot-Poong-Pla) were ground and macerated in 10 folds by weight of 80% ethanol with for 24 hours and extracted at 100°C for 3 hours. The extract was then filtered. The remaining residue was repeatedly extracted at 100°C for an additional 3 hours. The extract from the first and the second extraction were pooled together and evaporated until obtaining herb to extract ratio (HER) of 3.3:1. Details on the extraction process of NVK-E are shown in Figure 6. The extraction process was in accordance with Sotanaphun et al. (8).

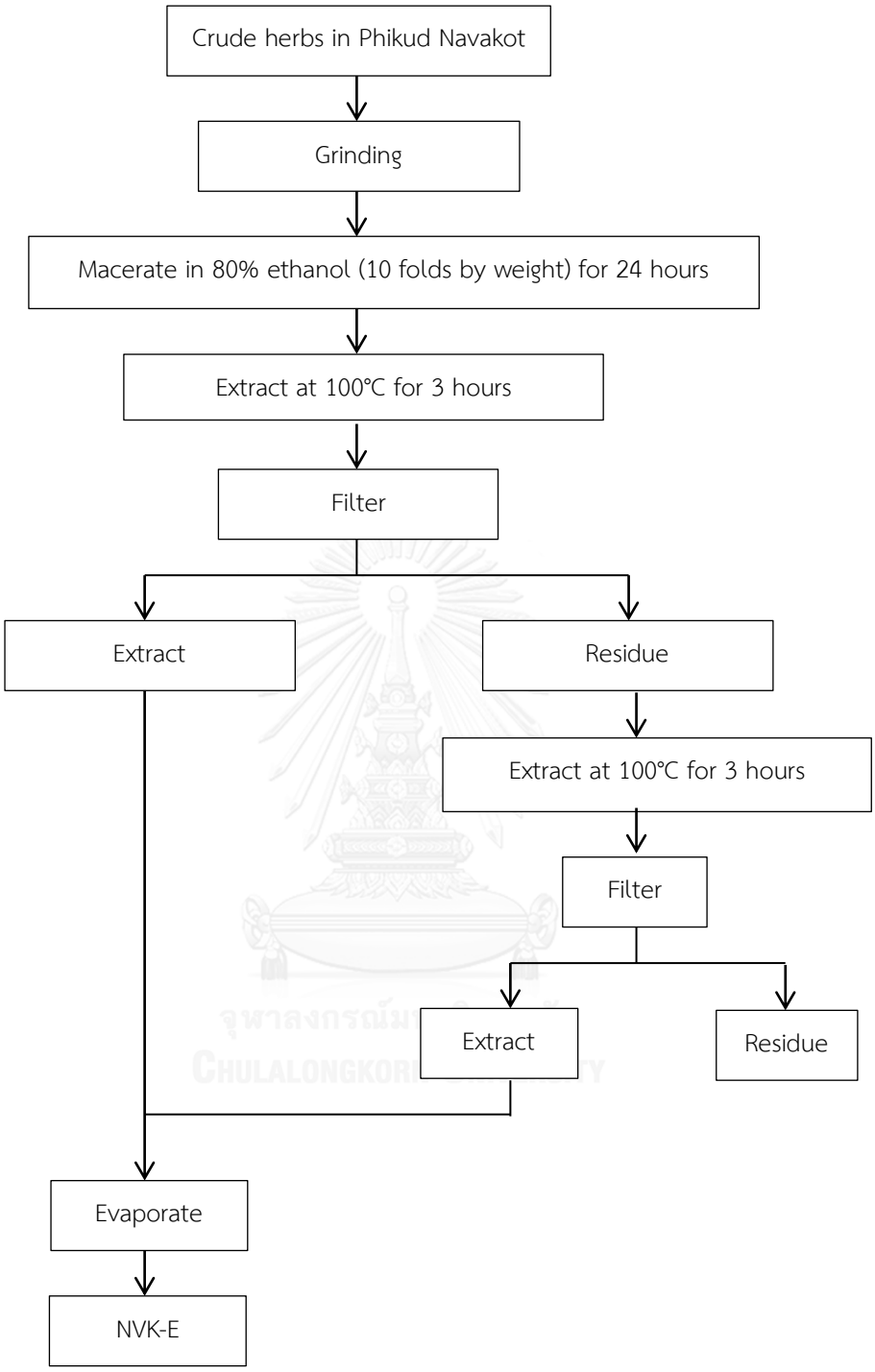


Figure 6 Extraction process diagram of NVK-E

2. Quality control of NVK-E

2.1 Total phenolic content determination (%Gallic Acid Equivalent, %GAE)

Folin-Ciocalteau method was used to test for total phenolic content (%Gallic Acid Equivalent, %GAE) in NVK-E. The method was modified from Sotanaphun et al. (8).

Standard preparation

Standard gallic acid was dissolved in ethanol to obtain standard solutions at the concentrations of 0.0025, 0.005, 0.01, 0.02, 0.04, 0.06 and 0.08 mg/ml.

Sample preparation

NVK-E was dissolved in ethanol to make solution with concentrations of 0.6, 0.7 and 0.8 mg/ml.

Procedure

Transfer 20 µl of standard solutions and 20 µl of sample solutions to a 96-well plate. Add 100 µl of 10 %v/v Folin-Ciocalteau reagent to each solution, incubated for 10 min. Then add 80 µl of 7.5 %w/v sodium bicarbonate solution to each solution, incubated for 60 min. Determine the absorbances of the test solutions at the wavelength of 765 nm using microplate reader. A calibration curve was constructed by plotting absorbances against concentrations. The slope, y-intercept and r^2 were calculated using the Least Squares Regression method. Calculate the total phenolic content in NVK-E (mg/ml) from the acquired least squares regression equation and then calculate the total phenolic content in term of %Gallic Acid Equivalence (%GAE) using the following equation;

$$\%\text{Gallic Acid Equivalence (\%GAE)} = \frac{\text{Calculated total phenolic content (mg/ml)}}{\text{Concentration of NVK-E (mg/ml)}} \times 100 \qquad \textbf{Equation 1}$$

2.2 DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) Assay

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution. This free

radical is reduced in the presence of an antioxidant molecule, giving rise to colourless solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry (37). The method was modified from Sotanaphun et al. (8).

Control solution preparation

Ascorbic acid was used as positve control. Standard ascorbic acid was dissolved in water to obtain standard solutions at the concentration of 25 µM.

Sample preparation

NVK-E was dissolved in DMSO to obtain solution at the concentrations of 1.95, 3.91, 7.81, 31.25, 62.50 and 125 µg/ml.

Vehicle control preparation

DI Water was used as vehicle control for ascorbic acid and DMSO for sample solution.

Procedure

Transfer 5 µl of control solutions, 5 µl of sample solutions, 5 µl of water and 5 µl of DMSO to a 96-well plate. Add 195 µl of 80 µM DPPH solution to each solution, incubate for 30 min. Determine the absorbances of the test solutions at the wavelength of 510 nm using microplate reader. Calculate % DPPH scavenging using the following equation;

$$\% \text{ DPPH scavenging} = \frac{A_{\text{vehicle control}} - A_{\text{sample}}}{A_{\text{vehicle control}}} \times 100 \qquad \textbf{Equation 2}$$

2.3 Analytical method development for the determination of analytical markers in NVK-E

2.3.1 Chromatagraphic condition

HPLC method was developed to monitor three phenolic compounds, i.e. gallic acid, vanillic acid and ferulic acid, in NVK-E. The chromatographic condition was modified from Nalinratana et al. as stated below (4):

Column: Shisedo MGII® C18 (5 µm, 4.6 x 250 mm)

Mobile phase: gradient system, solvent A (methanol) and solvent B (1% v/v acetic acid, pH 2.7)

Injection volume: 100 µL

Flow rate: 1.0 mL/min

Detector: PDA detector at 190-400 nm, monitored wavelength 270 nm

Temperature: 27°C

Run time: 70 minutes

Table 2 The gradient condition in HPLC system

Time (min)	% Solvent A	% Solvent B
0	0	100
5	0	100
45	40	60
55	80	20
60	80	20
65	0	100
70	0	100

Solvent A (methanol) and solvent B (1% v/v acetic acid, pH 2.7) were filtered through 0.22 µm membrane filter and then degassed by sonication for 30 minutes prior to use.

2.3.2 Analytical method validation

HPLC system was validated by using standard addition method. Selectivity, linearity, accuracy and precision were performed in accordance with the AOAC Guidelines for Dietary Supplements and Botanicals.

Standard preparation

Standard gallic acid, vanillic acid and ferulic acid were individually dissolved in methanol to prepare a stock solution for each referred. Each stock solution was used to prepare standard mixture solutions of gallic acid, vanillic acid and ferulic acid by diluting with water to concentrations of about 880, 690 and 200 µg/ml, respectively. The standard mixture solutions were further diluted with water to obtain final concentrations of about 1.60, 1.40 and 0.40 µg/ml, respectively. The solutions were filtered through 0.45 µm syringe filter prior to use.

Sample preparation

NVK-E of 50 mg was accurately weighed and placed in a well-sealed glass container. Water of 80 ml was added and the solution was sonicated for 1 h. The solution was further diluted with water to 100 ml and filtered through a 0.45 μm syringe filter prior to use.

System suitability

System suitability was performed using RP-HPLC-DAD by injecting six replicates of NVK-E with standard mixture solution to obtain HPLC peak responses.

Selectivity

Selectivity was examined by separately injecting standard mixture solution of the three analytical markers, NVK-E, and NVK-E with standard mixture solutions using RP-HPLC-DAD to obtain HPLC peak responses. Peak purity index was determined by matching each spectrum under the chromatographic peak to the spectrum at the peak apex. Peak purity index was obtained from the LC solution program. It can be calculated from the following equation:

$$\text{Peak purity index} = \frac{S}{S_{apex}} = \frac{A_{1j}(\lambda_1 t_j)\ A_{2j}(\lambda_2 t_j) \ldots\ldots A_i(\lambda_i t_j)}{A_{1apex}(\lambda_1 t_{apex})\ A_{2apex}(\lambda_2 t_{apex}) \ldots\ldots A_{iapex}(\lambda_i t_{apex})}$$

Equation 3

S is the test spectrum.

S_{apex} is the spectrum at apex.

A_{1j} is the absorbance of test peak at the time interval$_j$

$\lambda_1 t_j$ is the wavelength of test peak at the time interval$_j$

A_{1apex} is the absorbance at peak at apex.

$\lambda_1 t_{apex}$ is the wavelength at peak apex.

Linearity

Linearity of the system was examined using the standard addition method. Five different concentrations of standard mixture solution were used to construct the

calibration curve. NVK-E of 50 mg was accurately weighed and placed in well-sealed glass containers. Water of 80 ml was added and the solution was sonicated for 1 h. From the preliminary study, 50 mg of NVK-E was found to contain gallic acid, vanillic acid and ferulic acid of about 0.16, 0.14 and 0.04 mg, respectively. Hence, the solution of 50 mg NVK-E in 100 ml of water contained gallic acid, vanillic acid and ferulic acid of about 1.6, 1.4 and 0.4 mg/ml, respectively. A standard mixture solution at 50%, 75%, 100%, 125% and 150% content of each marker in NVK-E was added to NVK-E as shown in Figure 6. A standard mixture solution was added to each NVK-E solution to obtain final concentrations of about 2.40, 2.80, 3.20, 3.60 and 4.00 µg/ml for gallic acid; 2.10, 2.45, 2.80, 3.15 and 3.50 µg/ml for vanillic acid; and 0.60, 0.70, 0.80, 0.90 and 1.00 µg/ml for ferulic acid. Each solution was then diluted with water to a volume of 100 ml. The solution was filtered through a 0.45 µm syringe filter prior to use. Each concentration was analyzed in triplicate. A calibration curve was constructed by plotting peak areas against concentrations. The slope, y-intercept and correlation coefficient (r) were calculated using the Least Squares Regression method.

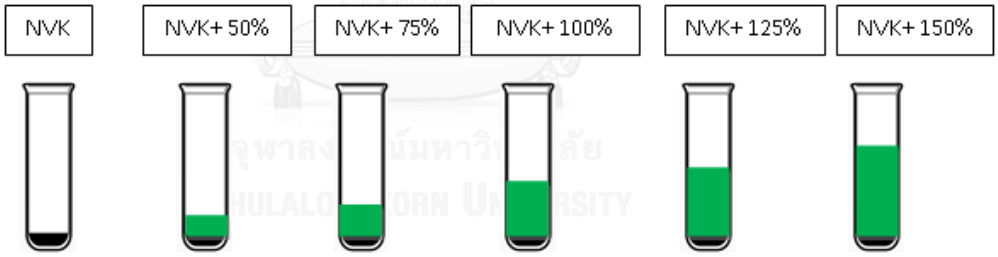


Figure 7 NVK-E and NVK-E with 50%, 75%, 100%, 125% and 150% standard mixture solution added

Accuracy and Precision

Accuracy and precision of the system were evaluated using the standard addition method. Three different concentrations were prepared by accurately weighing 50 mg of NVK-E into well-sealed glass containers, after which 80 ml of water was added and the solution was sonicated for 1 h. A standard mixture solution at 50%, 100% and 150% content of each marker in NVK-E was added to each NVK-E

sample to obtain final concentrations of 2.40, 3.20 and 3.60 µg/ml for gallic acid; 2.10, 2.80 and 3.15 µg/ml for vanillic acid; and 0.60, 0.80 and 1.00 µg/ml for ferulic acid. Water was then added to obtain a volume of 100 ml. The solution was filtered through a 0.45 µm syringe filter prior to use. Each concentration was analyzed in triplicates. Inter-day precision and accuracy were performed on three different days. Average %recovery, %relative standard deviation (%RSD), and the Horwitz ($HorRat_r$) values were calculated to determine the accuracy and precision of the system. % recovery, $HorRat_r$ and RSD_r (calculated, %) are calculated from the following equations:

% Recovery = (amount found/ amount added) x 100 **Equation 4**

$HorRat_r$ = RSD_r (found, %) / RSD_r (calculated, %) **Equation 5**

RSD_r (calculated, %) = $C^{-0.15}$ **Equation 6**

C expressed as a mass fraction of gallic acid , vanillic acid or ferulic acid in NVK-E.

2.3.3 Chemical analysis of NVK-E

Standard preparation

Standard gallic acid, vanillic acid and ferulic acid were individually dissolved in methanol to prepare a stock solution for each standard. Each stock solution was used to prepare standard mixture solutions of gallic acid, vanillic acid and ferulic acid by diluting with water to concentrations of about 880, 690 and 200 µg/ml, respectively. The standard mixture solutions were further diluted with water to obtain final concentrations of about 1.60, 1.40 and 0.40 µg/ml, respectively. The solutions were filtered through a 0.45 µm syringe filter prior to use.

Sample preparation

Accurately weighed 50 mg of NVK-E and placed in a well-sealed glass container. Added 80 ml of water into the container and sonicated for 1 h. The solution was further diluted with water to 100 ml and filtered through a 0.45 µm syringe filter prior to use. Calculated the quantity of each marker according to the following equation:

$$\text{Quantity of marker in NVK-E (mg)} = \frac{A_{NVK\text{-}E}}{A_{standard\ marker}} \times C_{standard\ marker}\ (\mu g/ml) \times \text{dilution factor}/1000$$

Equation 7

$A_{NVK\text{-}E}$ is the peak area response of marker in NVK-E.

$A_{standard\ marker}$ is the peak area response of standard marker.

$C_{standard\ marker}$ is the concentration of standard marker (µg/ml).

3. Preformulation Studies

3.1 Physicochemical characterization of NVK-E

3.1.1 The dissolving and dispersing ability of NVK-E in solvents

The method was modified from Sotanaphun et al. (8). Determined the dissolving ability of NVK-E in various solvents by dissolving 0.1 g of NVK-E with 0.1 ml of each solvent. If NVK-E did not dissolve, gradually increased solvent volume by 0.1 ml until the volume of 1 ml. If NVK-E did not dissolve, gradually increased solvent volume by 1 ml until the volume of 10 ml. If NVK-E did not dissolve, gradually increased solvent volume by 10 ml until the volume of to 100 ml. The dispersing ability of NVK-E in the solvents was simultaneously investigated. In this study, the usage of solvent volume that exceed than 1000 parts (100 ml) was described as not dissolved. Water and chloroform were selected as a representative of polar and non polar solvents. One percent hydrochloric acid, 1% sodium hydroxide and phosphate buffer pH 6.8 were selected as acid, base and buffer solvents, respectively. Methanol, ethanol and acetronitrile were selected to represent organic solvents.

3.1.2 Thermal properties of NVK-E

Determine the thermal properties of NVK-E using differential scanning calorimeter (DSC 822e , Mettler Toledo, USA). Accurately weighed 4-10 mg of NVK-E in a 40-µl standard aluminium pan and sealed for analysis. An empty pan was used as a reference. The DSC runs were conducted over a temperature range of 25-300ºC at rate of 10ºC/min. Ultra high pure nitrogen was used at a flow rate of 60 mL/min.

The obtained DSC thermogram was used to determine the thermal properties of NVK-E.

3.2 Physicochemical characterization of excipients

3.2.1 Thermal properties of excipients

The excipients were selected from commonly used excipients in orodispersible tablet formulations including diluent, binder, disintegrant, lubricant, glidant and flavoring agent (17). Adsorbents were also used in this formulation to convert NVK-E, an extremely viscous liquid, into dry powder (NVK-EP). Adsorbents were utilized to covert liquid drug into dry powder which was applicable for the solid dosage form such as tablets and capsules (38). Avicel® PH102 (microcrystalline cellulose), magnesium carbonate, Aerosil®200 (colloidal silicon dioxide), starch 1500, talcum, kaolin, aluminium hydroxide and pectin were selected as adsorbents (39). Mannitol and Ludiflash®, a combination of 90% mannitol, 5% polyvinyl acetate and 5% crospovidone, were selected as diluent. These sugar based excipients showed high aqueous solubility property and sweetness, resulting in a pleasing mouth-feel which was preferable for orodispersible tablets formulation (15). Polyvinyl acetate in Ludiflash® functioned as a tablet binder. Polyplasdone® (Crospovidone), Explotab® (sodium starch glycolate) and Ac-Di-Sol® (croscamellose sodium) were selected as disintegrants. These three disintegrants are also known as superdisintegrants. The superdisintegrants were more effective than the traditional disintegrants due to their lower concentrations with greater disintegrating efficiency (20). Stearic acid was selected as lubricant because of its acidic nature which was preferable for the acidic active ingredients. Aerosil® (colloidal silicon dioxide) was selected as glidant. (39). Sodium chloride, citric acid and sucralose were selected as flavoring agents to mask the bitter taste of NVK-E. Sucralose, synthetic sweetener, showed the prominence in taste masking than the natural sweetener. Nevertheless, it provided the after-taste perception. It was commonly used in combination with sugar alcohols such as mannitol to decrease its after-taste perception (40). Citric acid, an acidulant sourness flavoring agent, was selected. The combination of citric acid and sucralose increased the taste masking efficiency of sucralose. (41). Citric acid itself stimulated saliva

production that would aid in the faster disintegration of the orodisperible tablet formulation (42). Excipients and their function in the formulation were shown in Table 3.

Thermal properties of the selected excipients were conducted using differential scanning calorimeter. Accurately weighed 4-10 mg of each excipient in a 40-µl standard aluminium pan and sealed for analysis. An empty pan was used as a reference. The DSC runs were conducted as described in Section 3.1.2. The obtained DSC thermograms were used to determine the thermal properties of each selected excipients.

3.3 Incompatibility Study

NVK-E and each of the selected excipients were physically mixed 1:1 by mortar and pestle (43). Accurately weighed the mixture of approximately 4-10 mg in a 40-µl standard aluminium pan and sealed for analysis. An empty pan was used as reference. The DSC runs were conducted as described in Section 3.1.2. Obtained DSC thermograms were used to determine the compatibility of NVK-E and each selected excipients.

4. Formulation Development

4.1 Deveopment of intermediate NVK-E dry powder (NVK-EP)

NVK-E was extremely viscous liquid which was difficult to manipulate. Adsorbents were also used to adsorb NVK-E into dry powder (NVK-EP). Adsorbents were utilized to covert liquid drug into dry powder which was applicable for the solid dosage form such as tablets and capsules (38). Avicel® PH102 (microcrystalline cellulose), magnesium carbonate, magnesium oxide, starch 1500, talcum, kaolin, aluminium hydroxide, pectin and Aerosil® 200 (colloidal silicon dioxide) were selected to adsorb NVK-E into dry powder. NVK-E of 100 mg was transferred to the mortar. Afterward, 10 mg of each selected adsorbent was transferred to the mortar and mixed. If NVK-E did not become dry, gradually added another 10 mg of the excipient and mixed until NVK-E become dry or until the limited of 100 mg (1:1 ratio). The adsorbent with least quanity to adsorb NVK-E into dry powder (NVK-EP) was selected.

Table 3 Excipients and their function in tablet formulation

Excipients	Function of excipients
Avicel® PH 102 (microcrystalline cellulose)	Adsorbents
Magnesium carbonate	
Aerosil®200 (colloidal silicon dioxide)	
Starch 1500	
Talcum	
Kaolin	
Aluminium hydroxide	
Petin	
Ludiflash®(consisted of mannitol, crospovidone and polyvinyl acetate)	Diluent, disintegrant and binder
Mannitol	Diluent
Polyplasdone® (crospovidone)	Disintegrants
Explotab® (sodium starch glycolate)	
Ac-Di-Sol® (croscamellose sodium)	
Aerosil® 200 (silicon dioxide)	Glidant
Stearic acid	Lubricant
Citric acid	Flavoring agents
Sucralose	
Sodium chloride	

4.2 Chemical analysis of intermediate NVK-E dry powder (NVK-EP)

NVK-EP was then assayed for three phenolic compound, gallic acid, vanillic acid and ferulic acid. The chromatographic condition was performed as described in Section 2.3.1.

Standard preparation

Gallic acid, vanillic acid and ferulic acid were individually dissolved in methanol to prepare a stock solution for each standard. Each stock solution was

used to prepare standard mixture solutions of gallic acid, vanillic acid and ferulic acid by diluting with water to concentrations of about 880, 690 and 200 µg/ml, respectively. The standard mixtures solutions were further diluted with water to obtain final concentrations of 1.60, 1.40 and 0.40 µg/ml, respectively. The solutions were filtered through a 0.45 µm syringe filter prior to use.

Sample preparation

Accurately weighed NVK-EP equivalent to NVK-E 50 mg and placed in a well-sealed glass container. 80 ml of water was added and the solution was sonicated for 1 h. The solution was further diluted with water to 100 ml and filtered through a 0.45 µm syringe filter prior to use. Calculated the quantity of each marker according to the following equation:

$$\text{Quantity of marker in NVK-EP (mg)} = \frac{A_{NVK\text{-}EP}}{A_{standard\ marker}} \times C_{standard\ marker}\ (\mu g/ml) \times \text{dilution factor} / 1000$$

Equation 8

$A_{NVK\text{-}EP}$ is the peak area response of marker in NVK-EP.

$A_{standard\ marker}$ is the peak area response of standard marker.

$C_{standard\ marker}$ is the concentration of standard marker (µg/ml).

4.3 Orodispersible tablets formulation development

4.3.1 Orodispersible tablets preformaulation and formulation

NVK-EP, NVK-E dry powder, was used as an active ingredient in the formulation. Mannitol and Ludiflash® were used as a diluent. Polyplasdone®, Explotab® and Ac-Di-Sol® were used as disintegrants. Aerosil® 200 was used as a glidant and stearic acid was used as a lubricant. Citric acid, sucralose and sodium chloride were used as flavoring agents. The weight per tablet of the orodispersible tablet was 606.80 mg. The amount of the ingredients in the formulation is shown in Table 4.

In this study, disintegrant type and amount were varied. Three super-disintegrants, i.e. Polyplasdone®, Explotab® and Ac-Di-Sol® were used and the amount was varied from 4%, 6% and 10% in the formulation. In orodispersible tablets formulation, a superdisintegrant could be used alone or in combination and could be used up to 15% in the formulation to perform a satisfactory disintegration time (17). The study from Prajapati and Patal evaluated the orally disintegrating tablets containing piroxicam (44). The result suggested that different type and amount of disintegrants exhibited in the different of tablet properties such as disintegration time, wetting time or %friability.

Table 4 Amount of ingredients in the formulation

Ingredients		%	mg/tab.
NVK-E Extract	NVK-EP	8.24	50.00
Avicel® PH 102	NVK-EP	8.24	50.00
Ludiflash®		65.92	400.00
Citric acid		3.00	18.20
Sucralose		0.80	4.85
Sodium chloride		0.50	3.03
Disintegrant		*	*
Aerosil® 200		0.50	3.03
Stearic acid		2.80	16.99
Mannitol		**	**
Total		100	606.80

Powder flow was performed to test the flow property of formulations prior to the tableting process. The flow property of formulations were determined by compressibility index (Carr's index), Hausner's ratio and angle of repose. Angle of repose method was determined using 10 mm orifice funnel. The funnel was fixed with the holder at the height of about 5 cm from the base. Weigh approximately 4 g of the formulation and placed into the funnel. Let the formulation powder flow

through the funnel. Measure the powder pile at the top of the cone (height, cm) and measure the diameter of the powder pile (diameter, cm). Calculate the angle of repose as the following equation:

$$\tan(\alpha) = \text{height}/0.5 \text{ base} \qquad \textbf{Equation 9}$$

The flow property of formulations are described in Table 4. Literally, the formulations with an angle of repose of not exceeding 50° was manufactured satisfactorily (12).

Table 5 Flow Property and corresponding Angle of Repose

Flow Property	Angle of Repose (degree)
Excellent	25-30
Good	31-35
Fair-aid not needed	36-40
Passable-may hang up	41-45
Poor-must agitate, vibrate	46-55
Very poor	56-65
Very, very poor	>66

Compressbility index (Carr's index) and Hausner's ratio were determined by evaluating bulk density and tapped density of powders using 25 ml graduate cylinder. Accurately weighed about 10 mg of the sample and placed to the graduate cylinder. Calculate the bulk density according to the following equation;

$$\text{Bulk Density } (\rho_{bulk}) \text{ (g/ml)} = \text{Weight of powders/ Initial volume} \qquad \textbf{Equation 10}$$

Then determine the tapped density which was obtained by mechanically tapping a graduate measuring cylinder containing a powder sample. After observing the initial powder volume, the measuring cylinder was mechanically tapped, and volume readings are taken until no volume change was observed. The mechanical

tapping was achieved by raising the cylinder and allowing it to drop under its own weight. Calculate the bulk density according to the following equation:

Tapped Density (ρ_{tapped}) (g/ml) = Weight of powders/ Tapped volume **Equation 11**

Calculate the flow property of the formulation by calculating compressibility index and Hausner's ratio according to the following equation:

Compressibility Index = $100 \times [(\rho_{tapped} - \rho_{bulk})/\rho_{tapped}]$ **Equation 12**

Hausner's Ratio = $(\rho_{tapped}/\rho_{bulk})$ **Equation 13**

The flow properties of the formulations are described in Table 6 (12).

Table 6 Scale of flowability

Compressibility Index (%)	Flow Character	Hausner's Ratio
≤10	Excellent	1.00–1.11
11–15	Good	1.12–1.18
16–20	Fair	1.19–1.25
21–25	Passable	1.26–1.34
26–31	Poor	1.35–1.45
32–37	Very poor	1.46–1.59
>38	Very, very poor	>1.60

The tableting process was performed by direct compression method using an automatic tableting machine (Viuhang Engineering, Thailand) with 13 mm diameter single punch. Diagram of tableting process by direct compression method is shown in Figure 8.

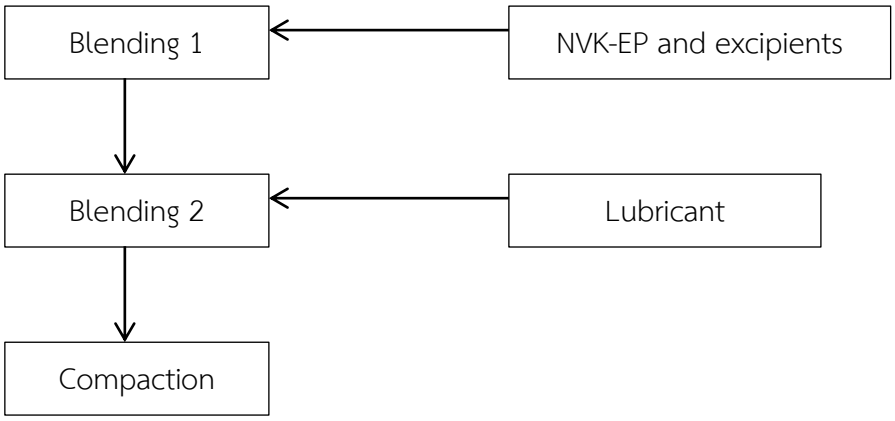


Figure 8 Diagram of tableting process by direct compression method

The formulation with uniformity of mass within ±5% (11), %friability less than 1.0% (12), tablet hardness of about 5-10 kg.force and disintegration time not more than 180 seconds as described in British Pharmacopoeia were selected (11). The acceptance criteria of the selected formulation are shown in Table 7.

Uniformity of mass

20 tablets were randomly selected and weighed individually. Determine the average mass of 20 tablets. Not more than 2 of the individual weight deviates more than 5% of the average mass and none deviates by more 10% (11).

Friability

11 tablets (approximately 6.5 g) were randomly selected to perform friability using tablet friability tester. The apparatus was set to 25 rounds/min for 4 minutes (total 100 rounds). The tablets were weighed before and after the test. %Friability of the tablet should not exceed 1.0% (12). Calculated %friability according to the following equation:

$$\%\text{Friability} = \{[\text{Weight}_{\text{Before test}} - \text{Weight}_{\text{After test}}]/\text{Weight}_{\text{Before test}}\} \times 100 \qquad \textbf{Equation 14}$$

Tablet hardness

6 tablets were randomly selected to perform tablet hardness using tablet hardness tester. Each tablet was measured for the hardness. The average hardness of 6 tablets (kg.force) was determined (12).

Disintegration time

6 tablets were randomly selected to perform disintegration time using disintegration apparatus. Tablets were placed individually into each disintegration basket to determine the disintegration time. Water at 37°C was used as the immersion fluid. The disintegration time of orodispersible tablets should not be more than 3 minutes (11).

Table 7 The acceptance criteria of the selected formulation

Test	Acceptance criteria
Uniformity of Mass	±5% average weight
Friability (%)	< 1.0%
Tablet Hardness (kg.force)	5 – 10 kg.force
Disintegration Time (sec)	< 180 sec

4.3.2 Chemical analysis of orodispersible tablets formulation

Assay for three phenolic compound, gallic acid, vanillic acid and ferulic acid. The chromatographic condition was performed as described in Section 2.3.1.

4.3.2.1 Selection of solvent

As the tablet contained various excipients, suitable solvents should be used to extract or dissolve the markers from the formulation. Water, the solvent used in NVK-E analysis were selected. The amount of water was varied from 100 ml, 200 ml, 300 ml and 500 ml, respectively.

Standard preparation

Gallic acid, vanillic acid and ferulic acid were individually dissolved in methanol to prepare a stock solution for each standard. Each stock solution was

used to prepare standard mixture solutions of gallic acid, vanillic acid and ferulic acid by diluting with water to concentrations of about 480, 420 and 126 µg/ml, respectively. The standard mixtures solutions were further diluted with water to obtain final concentrations of 0.9, 0.7 and 0.2 µg/ml, respectively. The solutions were filtered through a 0.45 µm syringe filter prior to use.

Sample preparation

NVK-E was used as a standard to compare the quantity of the three markers extracted or dissolved from the formulation. Twenty-five milligram, 50 mg and 75 mg of NVK-E was accurately weighed and separately placed in a well-sealed glass container. Then, dissolved each NVK-E with water 100 ml, 200 ml, 300 ml and 500 ml, respectively, and sonicated for 1 hour and filtered through a 0.45 µm syringe filter prior to use.

NVK-E in formulation was prepared by separately weighed four of 25 mg, 50 mg and 75 mg of NVK-E and the amount of placebo equivalent to 1 tablet (556.7 mg), placed in a well-sealed glass containers. 80 ml, 160 ml, 240 ml and 400 ml of water was separately added to each container and the solution was sonicated for 1 h. The solution was further diluted with water to 100 ml, 200 ml, 300 ml and 500 ml, respectively and filtered through a 0.45 µm syringe filter prior to use. Compare the quantity of all markers in the formulation with those in NVK-E. Calculated the quantity of the each marker according to the following equation:

$$\text{Quantity of marker in NVK-E} = \frac{A_{NVK\text{-}E}}{A_{\text{standard marker}}} \times C_{\text{standard marker}}(\mu g/ml) \times \text{dilution factor} \times \text{solvent added (ml)}/1000$$

Equation 15

$A_{NVK\text{-}E}$ is the peak area response of marker in NVK-E.

$A_{\text{standard marker}}$ is the peak area response of standard marker.

$C_{\text{standard marker}}$ is the concentration of standard marker (µg/ml).

4.3.2.2 Analysis of markers in the formulations

The selected formulation was then assay for three phenolic compound, gallic acid, vanillic acid and ferulic acid. The chromatographic condition was performed as described in Section 2.3.1.

Standard preparation

Gallic acid, vanillic acid and ferulic acid were individually dissolved in methanol to prepare a stock solution for each standard. Each stock solution was used to prepare standard mixture solutions of gallic acid, vanillic acid and ferulic acid by diluting with water to concentrations of 480, 420 and 126 µg/ml, respectively. The standard mixtures solutions were further diluted with water to obtain final concentrations of 0.4, 0.3 and 0.08 µg/ml, respectively. The solutions were filtered through a 0.45 µm syringe filter prior to use.

Sample preparation

NVK-E was used as a standard to compare the quantity of the three markers extracted or dissolved from the formulation. Fifty milligram of NVK-E was accurately weighed and separately placed in a well-sealed glass container. Then, dissolve each NVK-E with 500 ml of water, sonicate for 1 hour and filtered through a 0.45 µm syringe filter prior to use.

Accurately weighed 50 mg of NVK-E and 556.7 mg of placebo and placed in a well-sealed glass container. 400 ml of water was added, and the solution was sonicated for 1 h. The solution was further diluted with water to 500 ml and filtered through a 0.45 µm syringe filter prior to use. Compare the quantity of all markers in the formulation with those in NVK-E. Calculated the quantity of the each marker according to the following equation:

$$\text{Quantity of marker in the formulation} = \frac{A_{formulation}}{A_{standard\ marker}} \times C_{standard\ marker}\ (\mu g/ml) \times \text{dilution factor}/1000$$

Equation 16

$A_{formulation}$ is the peak area response of marker in the formulation.

$A_{standard\ marker}$ is the peak area response of standard marker.

$C_{standard\ marker}$ is the concentration of standard marker (µg/ml).

4.4 Orodispersible tablets evaluation

The selected formulation was manufactured by direct compression method using an automatic tableting machine (single punch, Viuhang Engineering, Thailand) with 13 mm diameter single punch. The quality control was performed in accordance with the United States Pharmacopeia and British Pharmacopoeia (11, 12).

4.4.1 Chemical analysis of orodispersible tablets

4.4.1.1 Analytical method validation

The method validation was performed using HPLC. The chromatographic condition was performed as described in Section 2.3.1. The quantity of three phenolic compound, gallic acid, vanillic acid and ferulic acid in the formulation were determined. Selectivity, linearity, accuracy and precision were performed in accordance with the AOAC Guidelines for Dietary Supplements and Botanicals.

Standard preparation

Gallic acid, vanillic acid and ferulic acid were individually dissolved in methanol to prepare a stock solution for each standard. Each stock solution was used to prepare standard mixture solutions of gallic acid, vanillic acid and ferulic acid by diluting with water to concentrations of 480, 420 and 126 µg/ml, respectively. The standard mixtures solutions were further diluted with water to obtain final concentrations of 0.4, 0.3 and 0.08 µg/ml, respectively. The solutions were filtered through a 0.45 µm syringe filter prior to use.

System suitability

System suitability was performed by RP-HPLC-DAD by injecting six replicates of standard mixture solution to obtain HPLC peak responses.

Selectivity

Selectivity was examined by separately injecting standard mixture solutions of the three analytical markers, NVK-E, and NVK-E-EP, placebo and NVK-E with placebo using RP-HPLC-DAD to obtain HPLC peak responses.

Linearity

Five different concentrations of standard mixture solution were used to construct the calibration curve. Gallic acid, vanillic acid and ferulic acid were individually dissolved in methanol to prepare a stock solution for each standard. Each stock solution was used to prepare standard mixture solutions of gallic acid, vanillic acid and ferulic acid by diluting with water to concentrations of about 480, 420 and 126 µg/ml, respectively. The standard mixtures solutions were further diluted with water to obtain final concentrations of 0.15, 0.25, 0.35, 0.45 and 0.55 µg/ml for gallic acid; 0.13, 0.21, 0.29, 0.38 and 0.46 µg/ml for vanillic acid; and 0.04, 0.06, 0.08, 0.10 and 0.014 µg/ml for ferulic acid (equivalent to about 40%, 70%, 100%, 130% and 160% content of each marker in NVK-E in the formulation). The solutions were filtered through a 0.45 µm syringe filter prior to use. Each concentration was analyzed in triplicate. A calibration curve was constructed by plotting peak areas against concentrations. The slope, y-intercept and correlation coefficient (r) were calculated using the least squares regression method.

Accuracy and Precision

Three different concentrations were prepared by accurately weighing 50 mg of NVK-E and the amount of placebo equivalent to 1 tablet into well-sealed glass containers, after which 400 ml of water was added and the solution was sonicated for 1 h. Water was then added to obtain a volume of 500 ml. The solutions were filtered through a 0.45 µm syringe filter prior to use. Each concentration was analyzed in triplicate. Inter-day precision and accuracy were performed on three different days. Average % recovery, % relative standard deviation (%RSD), and $HorRat_r$ values were calculated as in Section 2.3.2 to determine the accuracy and precision of the system.

4.4.1.2 Analysis of orodispesible tablets formulation

Assay for three phenolic compound, gallic acid, vanillic acid and ferulic acid. The chromatographic condition was performed as described in Section 2.3.1.

Standard preparation

Gallic acid, vanillic acid and ferulic acid were individually dissolved in methanol to prepare a stock solution for each standard. Each stock solution was used to prepare standard mixture solutions of gallic acid, vanillic acid and ferulic acid by diluting with water to concentrations of about 480, 420 and 126 µg/ml, respectively. The standard mixtures solutions were further diluted with water to obtain final concentrations of 0.4, 0.3 and 0.08 µg/ml, respectively. The solutions were filtered through a 0.45 µm syringe filter prior to use.

Sample preparation

Randomly selected and finely powder of 20 Tablets. Accurately weighed the formulations equivalent to NVK-E 50 mg and placed in a well-sealed glass container. 400 ml of water was added, and the solution was sonicated for 1 h. The solution was further diluted with water to 500 ml and filtered through a 0.45 µm syringe filter prior to use. Calculated the quantity of the each marker according to the following equation:

$$\text{Quantity of marker in the formulation} = \frac{A_{formulation}}{A_{standard\ marker}} \times C_{standard\ marker}\ (\mu g/ml) \times \text{dilution factor}/1000$$

Equation 17

$A_{formulation}$ is the peak area response of marker in the formulation.

$A_{standard\ marker}$ is the peak area response of standard marker.

$C_{standard\ marker}$ is the concentration of standard marker (µg/ml).

4.4.2 Tablet hardness

Tablet hardness was tested as described in Section 4.3.1.

4.4.3 Friability

Friability of the tablet was tested as described in Section 4.3.1.

4.4.4 Total phenolic content determination

Standard preparation

Standard gallic acid was dissolved in ethanol to make standard solutions at the concentration of 0.0025, 0.005, 0.01, 0.02, 0.04, 0.06 and 0.08 mg/ml.

Sample preparation

Dissolved an accurately weighed tablet formulation equivalent to NVK-E 50 mg and placed in 500 ml of water and sonicated for 1 hour. Transfer 20 µl of standard solutions and 20 µl of sample solution to a 96-well plate. Added 100 µl of 10 %v/v Folin-Ciocalteau solution to each solution, incubated for 10 min. Then added 80 µl of 7.5 %w/v sodium bicarbonate solution to each solution, incubated for 60 min. Determine the absorbances of the test solutions at the wavelengths 765 nm using microplate reader (Spectramax M5). A calibration curve was constructed by plotting absorbances against concentrations. The slope, y-intercept and r^2 were calculated using the least squares regression method. Calculate the total phenolic content in NVK-E from the acquired least squares regression equation and then calculated the total phenolic content in terms of %Gallic Acid Equivalence (%GAE) using the following equation:

$$\%\text{Gallic Acid Equivalence (\%GAE)} = \frac{\text{Calculated total phenolic content (mg/ml)}}{\text{Concentration of NVK-E (mg/ml)}} \times 100$$

Equation 18

4.4.5 Disintegration time

Disintegration time of the tablet was tested as described in Section 4.3.1.

4.4.6 Dissolution testing

The dissolution procedure was developed according to the United States Pharmacopeia. 6 tablets were randomly selected to perform dissolution using dissolution apparatus. Tablets were placed individually into each dissolution vessel. Apparatus 2 (paddle) was used at the rotation speed of 75 rpm with 900 ml of water as a dissolution medium. After 60 minutes, the sample from each vessel was drawn

using a suitable syringe and filtered through 0.45 µm syringe filter prior to use. Each sample solution was then determined for the total phenolic content using Folin-Ciocalteau method as described in Section 4.4.4.

4.4.7 Water determination

The water determination was performed using Karl Fischer Titrator. The tablet samples were analyzed in duplicate. The tablet samples were ground before use. Accurately weight about 1 gram of the ground tablet samples and placed into Karl Fischer Tritrator. The water content of the tablet samples were then determined. %Water content was obtained from Karl Fischer Tritrator.

4.4.8 Uniformity of mass

Uniformity of mass was tested as described in Section 4.3.1.

4.4.9 Content uniformity

10 tablets were randomly selected and determined the content of NVK-E with the same method as describe in Section 4.3.1.2. The acceptance value should be not more than 15.0 (12). Calculate the acceptance value using the following equation:

$$AV = IM\text{-}XI + ks \qquad \textbf{Equation 19}$$

AV is the acceptance value.

M is the reference value if $98.0\% \leq X \leq 101.5\%$, then M = X. If $X < 98.5\%$, then M = 98.5%. If $X \geq 101.5\%$ then M = 101.5%.

X is mean of the individual content.

k is acceptability constant which is equal to 2.4.

s is the standard deviation of the sample.

5. Stability study

The stability study was performed in accordance with the ASEAN Guideline on Stability Study of Drug Product. The developed tablets were individually packed in a

well-sealed aluminium bags. The stability was performed under the accelerated storage condition (at 40°C±2°C/75%RH±5%RH) and long term storage condition (at 30°C±2°C/75%RH±5%RH) of 6 months. The samples were determined the quality as in Section 4.4.1.2 and 4.4.2-4.4.8 at 0, 3 and 6 months.

CHAPTER IV
RESULTS AND DISCUSSION

1. Extraction of NVK-E from crude Phikud Navakot

Phikud Navakot consisted of equal quantity of nine herbs including *Angelica sinensis* (Kot-Chieng), *Angelica dahurica* (Kot-Sor), *Ligusticum sinense* (Kot-Hua-Bua), *Atractylodes lancea* (Kot-Khe-Ma), *Artemisia annua* (Kot-Chu-La-Lum-Pha), *Picrorhiza kurrooa* (Kot-Garn-Prow), *Saussurea lappa* (Kot-Gra-Duk), *Nardostachys grandiflora* (Kot-Cha-Da-Mung-Sri) and *Terminalia chebula* (Kot-Poong-Pla). Crude herb in Phikud Navakot were ground and extracted according to the extraction process in Section 1, Chapter 3 to obtain Herb to Extract Ratio (HER) of 3.3:1. The Phikud Navakot extract (NVK-E) obtained was highly viscous with dark-brown color. NVK-E exhibited unique aroma and very bitter taste. The appearance of NVK-E is shown in Figure 10.

Angelica dahurica

(Kot-Sor)

Angelica sinensis

(Kot-Chieng)

Artemisia annua

(Kot-Chu-La-Lum-Pha)

Atractylodes lancea

(Kot-Khe-Ma)

Ligusticum sinense

(Kot-Hua-Bua)

Nardostachys grandiflora

(Kot-Cha-Da-Mung-Sri)

Picrorhiza kurrooa

(Kot-Garn-Prow)

Saussurea lappa

(Kot-Gra-Duk)

Terminalia chebula

(Kot-Poong-Pla)

Figure 9 Nine herbs which are constituents in Phikud Navakot

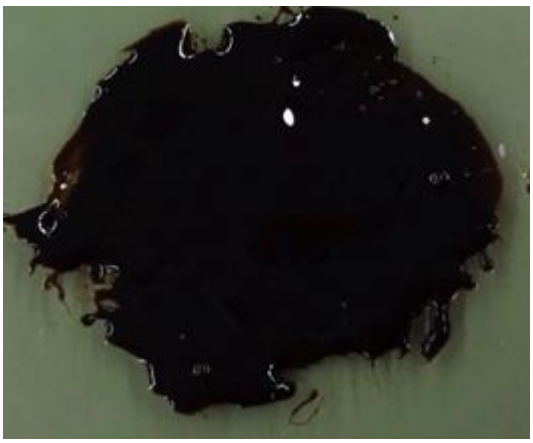


Figure 10 Visual appearance of Phikud Navakot extract (NVK-E)

2. Quality control of NVK-E

2.1 Total phenolic content determination (%Gallic Acid Equivalent, %GAE)

Folin-Ciocalteau method was used to test for total phenolic content in NVK-E. A calibration curve of standard gallic acid was constructed by plotting absorbance against concentrations as shown in Figure 11. Total phenolic content of NVK-E (%GAE) was determined in triplicate at three concentrations of 0.6, 0.7 and 0.8 mg/ml of NVK-E. The result showed that total phenolic content of NVK-E (%GAE) was 11.73± 0.63% as shown in Table 8.

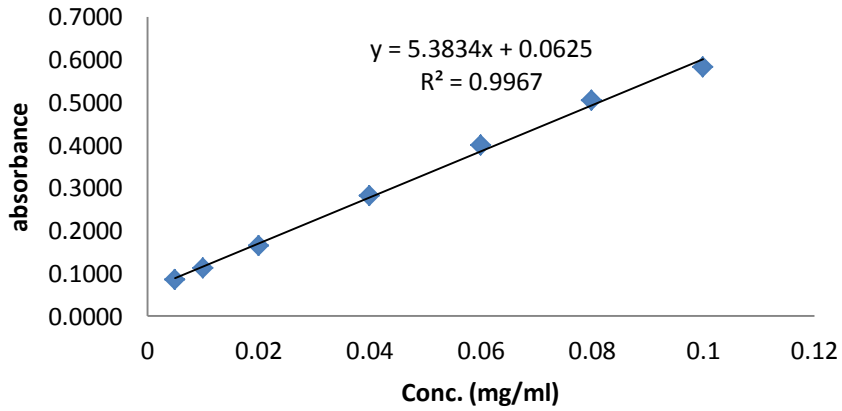


Figure 11 Calibration curve of standard gallic acid

Table 8 %GAE of NVK-E

Conc. (mg/mL)	Absorbance			mean±S.D.	GAE	%GAE	Average
NVK-E	1	2	3		(mg/ml)		%GAE
0.60	0.4319	0.4650	0.4794	0.4588±3.51	0.0736	12.27	
0.70	0.4984	0.5005	0.5334	0.5108±2.48	0.0833	11.90	11.73±0.63
0.80	0.5389	0.5385	0.5380	0.5380±2.28	0.0883	11.04	

2.2 DPPH Assay

DPPH assay was used to determine an anti-oxidant activity in NVK-E. The result showed that NVK-E exhibited anti-oxidant activity against DPPH free redical which is shown in Table 9 and Figure 12. The EC_{50} (The half maximal effective concentration) value , NVK-E exhibited the EC_{50} value of 19.56 ± 0.09 µg/mL.

Table 9 DPPH Scavenging activity of NVK-E

Concentrtion of NVK-E (µg /mL)	%Scavenging
1.95	6.01±3.51
3.91	14.78±2.48
7.81	24.69±2.28
15.63	39.53±0.99
31.25	66.19±0.74
62.50	90.18±1.45
125.00	93.24±0.90

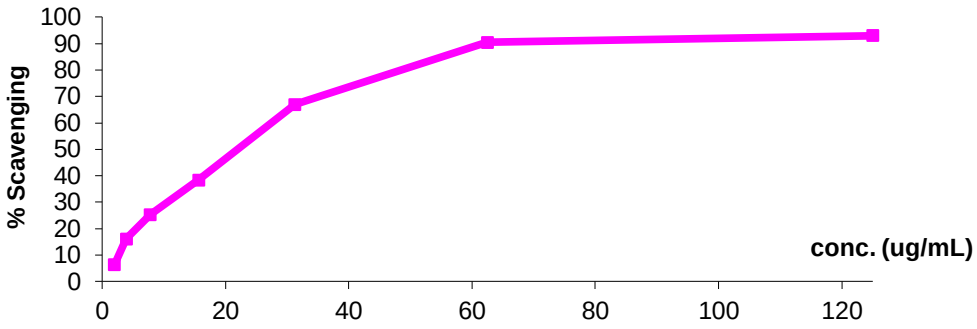


Figure 12 DPPH Scavenging activity of NVK-E

2.3 Analytical method Development for the determination of analytical markers in NVK-E

HPLC method was developed to monitor three phenolic compounds, i.e. gallic acid, vanillic acid and ferulic acid in NVK-E. NVK-E was highly viscous liquid with a complex matrix composition, hence, "standard addition method" was chosen to analyze the three analytical markers in NVK-E. The "standard addition method" is particularly useful for the analysis of analytes when the matrix effect within the sample is unknown or varied (36).

2.3.1 Analytical method validation

The analytical method was validated by using standard addition. In this study, a standard mixture solution was added into the analyte to compensate for the matrix effect of the sample. Selectivity, linearity, accuracy and precision were performed in accordance with the AOAC Guidelines for Dietary Supplements and Botanicals.

System suitability

System suitability tests were an integral part of HPLC methods. It was used to verify that the chromatographic system is adequate for the intended analysis. The system suitability test was performed by collecting data from six successive replicates injections of standard (12). The %RSD of peak areas, resolution, tailing factor and theoretical plates were evaluated which is shown in Table 10.

Table 10 System suitability of the HPLC system

Analytical markers	Concentration (µg/ml)	%RSD[a]	Tailing factor (T_f)	Resolution (R_s)	Number of theoretical plates (N)
Gallic acid	3.6	0.6	1.1	-	13866
Vanillic acid	2.8	1.1	1.1	56	124578
Ferulic acid	0.8	1.4	1.0	29	249135

[a] n=6

Selectivity

Peak responses of the three markers in standard mixture solutions (Figure 16) were clearly separated from each other, with retention times of 12.1, 34.9 and 46.1 minutes similar to standard gallic acid (Figure 13), vanillic acid (Figure 14) and ferulic acid (Figure 15), respectively. Peak responses of the three markers in NVK-E were also well separated from each other and exhibited retention times identical to the results for standard solution (Figure 17). For NVK-E with standard mixture solutions, peak response of the three markers corresponded with the retention times of gallic acid, vanillic acid and ferulic acid and their areas increased in direct correlation with concentrations of the added standard mixture solution (Figure 18). The standard mixture solution exhibited peak purity index of 1.000, 1.000 and 1.000 for gallic acid, vanillic acid and ferulic acid, respectively. Meanwhile, NVK-E exhibited peak purity index of 1.000, 1.000 and 0.997 for gallic acid, vanillic acid and ferulic acid, respectively. The results suggested there was no interference with the peak responses of all three analytical markers.

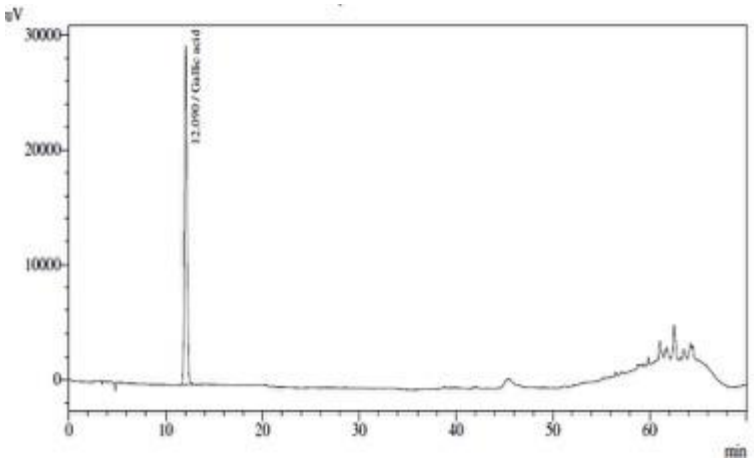


Figure 13 Chromatogram of 1.60 µg/ml standard gallic acid (12.1 min)

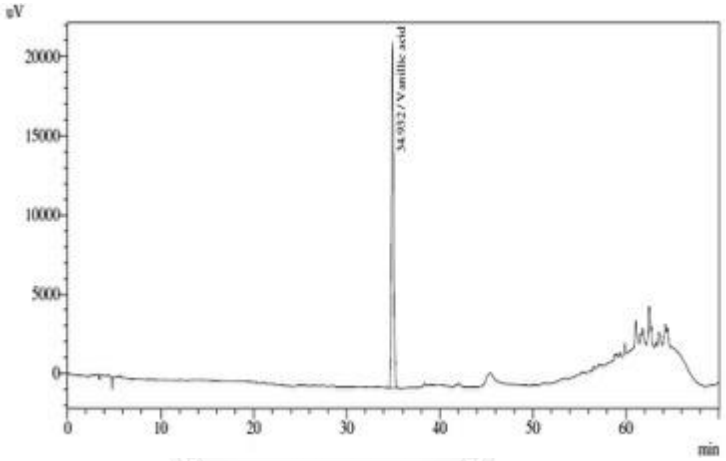


Figure 14 Chromatogram of 1.40 µg/ml standard vanillic acid (34.9 min)

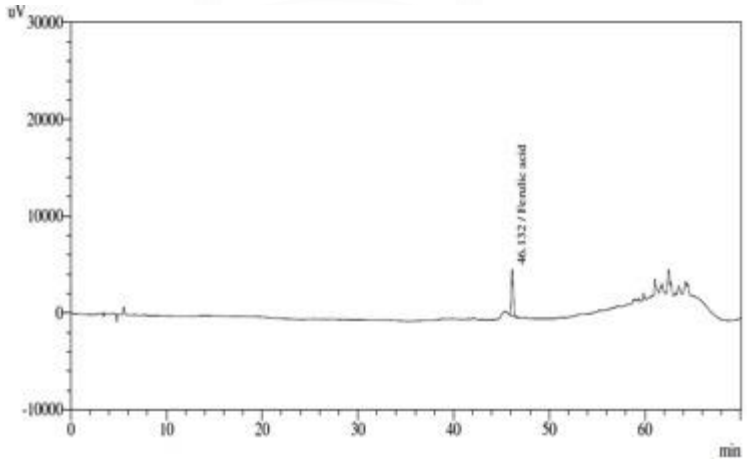


Figure 15 Chromatogram of 0.40 µg/ml standard ferulic acid (46.1 min)

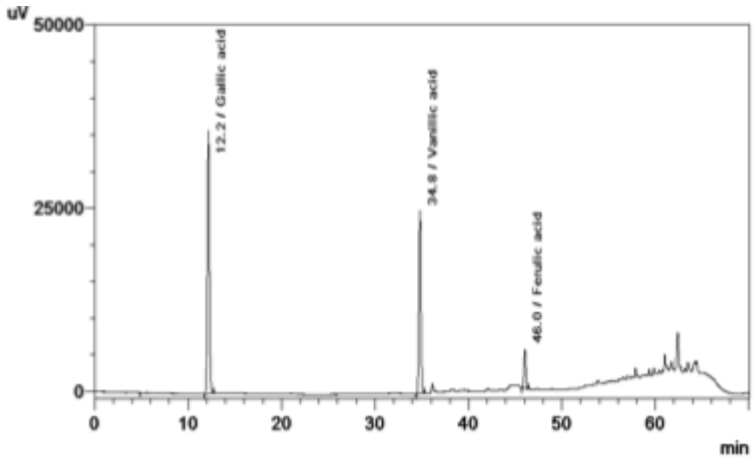


Figure 16 Chromatogram of a standard solution of 1.60 µg/ml gallic acid (12.2 min), 1.40 µg/ml vanillic acid (34.8 min) and 0.40 µg/ml ferulic acid (46.0 min)

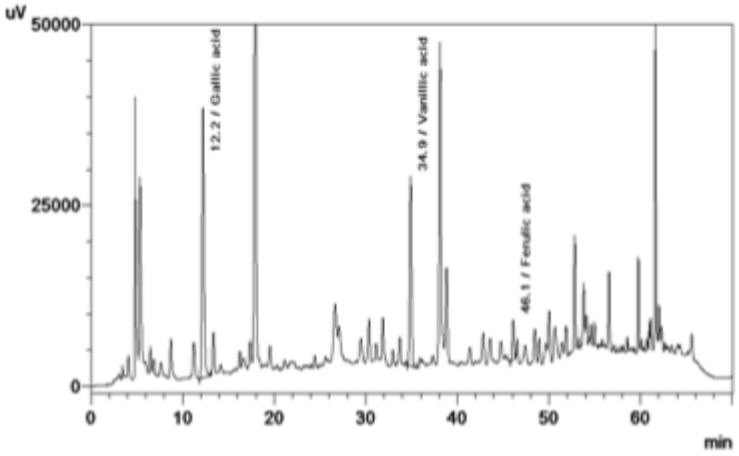


Figure 17 Chromatogram of 50 mg NVK-E where gallic acid, vanillic acid and ferulic acid were found to elute at 12.2, 34.9 and 46.1 min, respectively

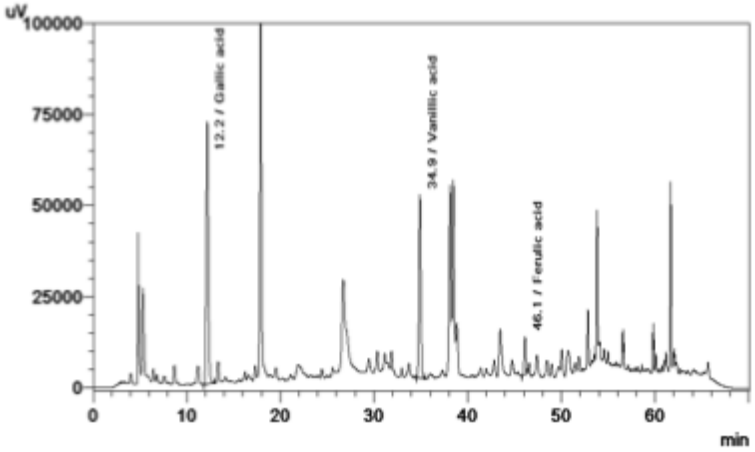


Figure 18 Chromatogram of 50 mg NVK-E combined with standard mixture solution at concentrations of 1.60 µg/ml gallic acid (12.2 min), 1.40 µg/ml vanillic acid (34.9 min), and 0.40 µg/ml ferulic acid (46.1 min)

Linearity

Linearity parameters of the HPLC system are shown in Table 11 . The results show that all three analytical markers exhibited good linearity within specified ranges with correlation coefficient (r) of 0.9995, 0.9999 and 0.9983 for gallic acid, vanillic acid and ferulic acid, respectively. These results complied with the AOAC Guidelines for Dietary Supplements and Botanicals which stated that the correlation coefficient (r) should be >0.99 for acceptable linearity. The linearity of gallic acid, vanillic acid and ferulic acid are shown in Figure 19, 20 and 21, respectively. Chromatograms of Linearity with standard addition at 50%, 75%, 100%, 125% and 150% are shown in Figure 22, 23, 24, 25 and 26, respectively.

Table 11 Linearity parameters of the HPLC system

Analytical markers	Linearity range (µg/ml)	Slope	Y-intercept	r
Gallic acid	2.40-4.00	329847.54	-1247.9003	0.9999
Vanillic acid	2.10-3.50	271915.75	15220.3255	0.9999
Ferulic acid	0.60-1.00	187276.71	-5495.9840	0.9982

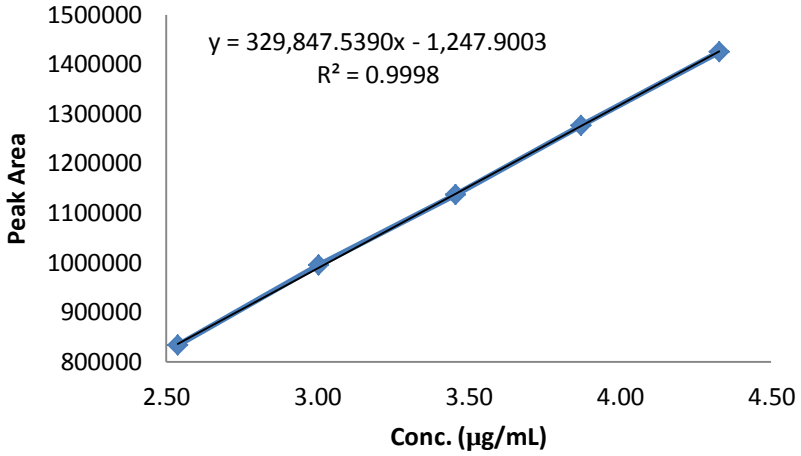


Figure 19 Linearity evaluation of various concentrations of gallic acid

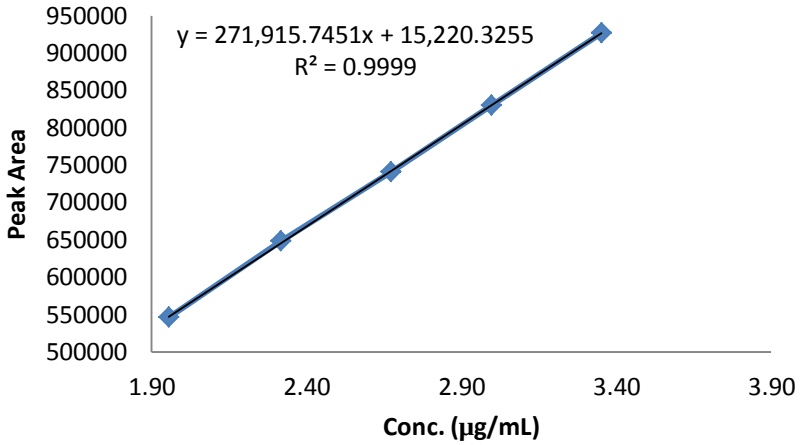


Figure 20 Linearity evaluation of various concentrations of vanillic acid

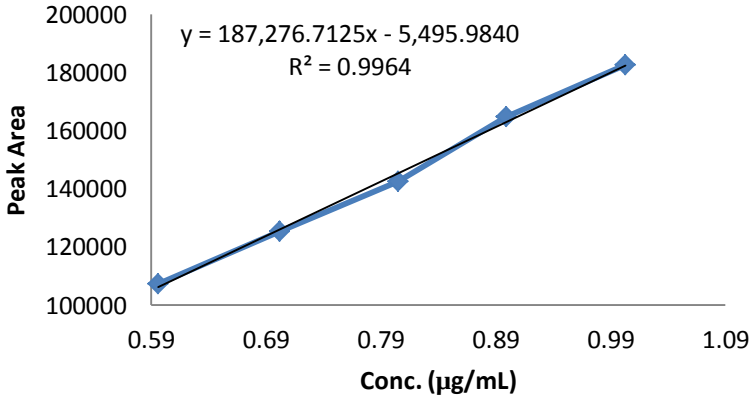


Figure 21 Linearity evaluation of various concentrations of ferulic acid

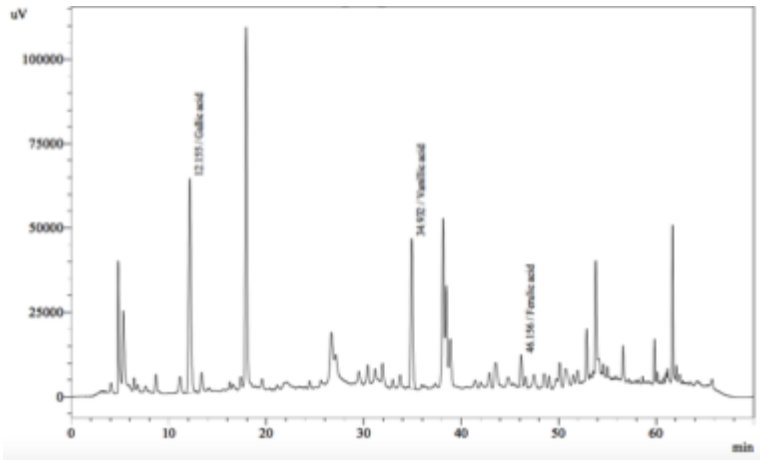


Figure 22 Chromatogram of 50 mg NVK-E with standard mixture solutions at 50% concentrations of 0.80 µg/ml gallic acid (12.2 min), 0.70 µg/ml vanillic acid (34.9 min), and 0.20 µg/ml ferulic acid (46.1 min).

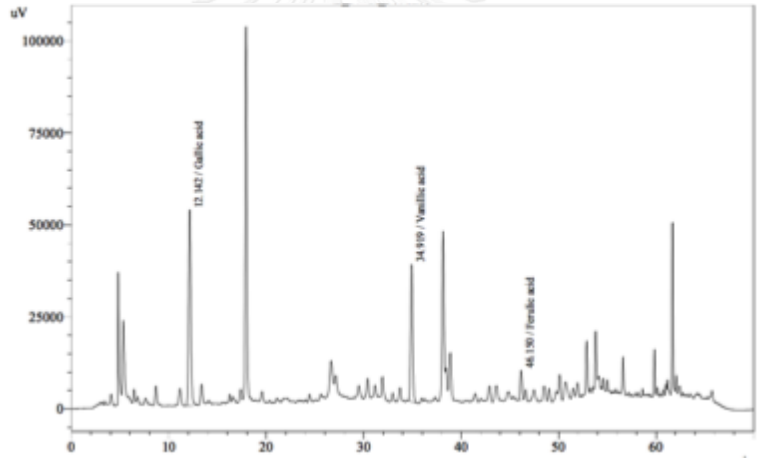


Figure 23 Chromatogram of 50 mg NVK-E with standard mixture solutions at 75% concentrations of 1.20 µg/ml gallic acid (12.2 min), 1.05 µg/ml vanillic acid (34.9 min), and 0.30 µg/ml ferulic acid (46.1 min).

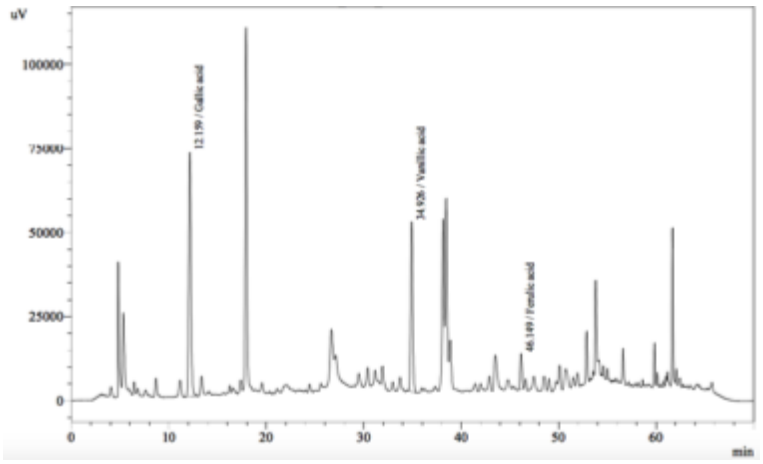


Figure 24 Chromatogram of 50 mg NVK-E with standard mixture solutions at 100% concentrations of 1.60 µg/ml gallic acid (12.2 min), 1.40 µg/ml vanillic acid (34.9 min), and 0.40 µg/ml ferulic acid (46.1 min)

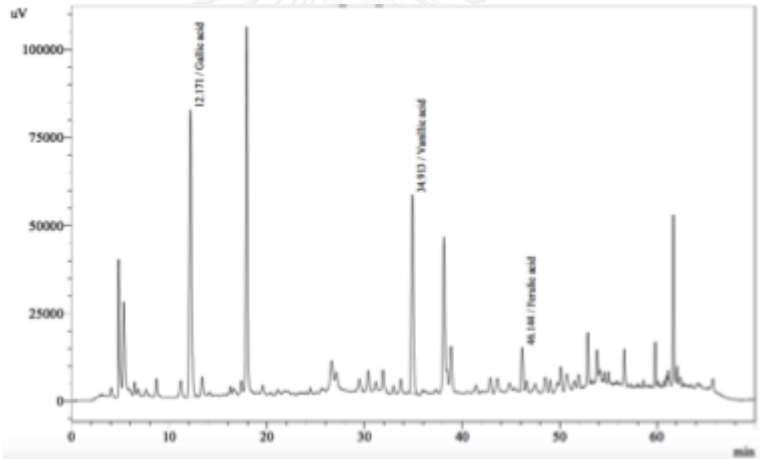


Figure 25 Chromatogram of 50 mg NVK-E with standard mixture solutions at 125% concentrations of 2.00 µg/ml gallic acid (12.2 min), 1.75 µg/ml vanillic acid (34.9 min), and 0.50 µg/ml ferulic acid (46.1 min)

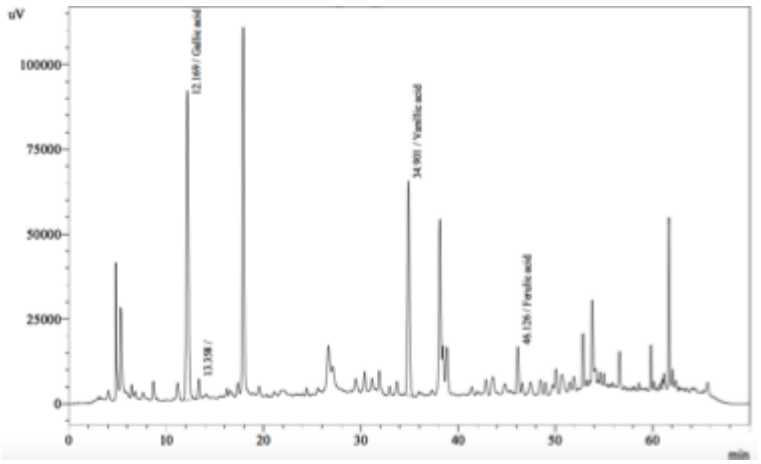


Figure 26 Chromatogram of 50 mg NVK-E with standard mixture solutions at 150% concentrations of 2.40 µg/ml gallic acid (12.2 min), 2.10 µg/ml vanillic acid (34.9 min), and 0.60 µg/ml ferulic acid (46.1 min)

Accuracy and Precision

The accuracy of the system is described as the average %recovery, which are found to be 98-102, 99-102 and 98-101 for gallic acid, vanillic acid and ferulic acid, respectively. The average %recovery of all three markers comply with the AOAC Guidelines for Dietary Supplements and Botanicals which stated that this value should be between 92-105%. The precision of the system is exhibited as The Horwitz ratio ($HorRat_r$) values which are shown in Table 12. The AOAC Guidelines for Dietary Supplements and Botanicals suggested that $HorRat_r$ values of < 2 indicate good repeatability. The repeatability results for three markers show $HorRat_r$ values of < 2, which complied with the AOAC Guidelines for Dietary Supplements and Botanicals for acceptable repeatability (36).

Table 12 Accuracy and Precision of the HPLC system determined by $HorRat_r$

	Average Recovery (%) ± SD[a]	Amount added (µg/ml)	Amount found (µg/ml) ± S.D.[b]	%RSD (found)	$HorRat_r$
Gallic acid					
50% Day 1	101.22±0.30	2.51	2.54±0.02	0.77	0.34
Day 2	101.76±0.36	2.72	2.77±0.02	0.59	0.26
Day 3	100.49±0.17	2.82	2.84±0.01	0.20	0.09
100% Day 1	99.56±0.65	3.37	3.36±0.01	0.44	0.19
Day 2	100.21±0.35	3.58	3.59±0.00	0.13	0.06
Day 3	101.39±1.86	3.69	3.74±0.06	1.70	0.75
150% Day 1	98.41±0.52	4.28	4.21±0.04	1.02	0.45
Day 2	100.41±1.74	4.44	4.50±0.01	2.22	0.97
Day 3	100.38±1.45	4.57	4.58±0.06	1.28	0.56
Vanillic acid					
50% Day 1	102.11±0.90	1.94	1.98±0.02	1.08	0.45
Day 2	101.64±0.69	2.10	2.13±0.01	0.44	0.18
Day 3	100.52±0.31	2.24	2.25±0.01	0.27	0.11
100% Day 1	99.73±0.96	2.61	2.60±0.02	0.76	0.32
Day 2	100.56±1.15	2.78	2.80±0.03	1.02	0.43
Day 3	100.40±1.24	2.93	2.94±0.03	1.09	0.46
150% Day 1	98.99±0.48	3.32	3.28±0.03	0.95	0.40
Day 2	101.58±1.41	3.46	3.51±0.06	1.81	0.76
Day 3	100.76±1.09	3.62	3.65±0.04	1.23	0.51
Ferulic acid					
50% Day 1	101.36±0.86	0.59	0.60±0.01	1.10	0.39
Day 2	99.29±1.12	0.59	0.59±0.01	0.99	0.35
Day 3	98.90±0.63	0.75	0.74±0.00	0.63	0.22
100% Day 1	98.30±0.80	0.79	0.77±0.00	0.50	0.18
Day 2	98.59±0.91	0.79	0.78±0.01	1.11	0.40
Day 3	99.95±1.05	0.96	0.96±0.01	1.06	0.38
150% Day 1	98.39±0.98	0.99	0.98±0.01	1.43	0.51
Day 2	99.28±1.02	0.98	0.98±0.01	1.43	0.51
Day 3	99.80±2.01	1.17	1.17±0.03	2.18	0.78

[a] n=3 [b] n=3

2.3.2 Chemical analysis of NVK-E

After the previous analytical method validation, NVK-E matrix was proven not to interfere with the analysis and the HPLC system was also suitable for the analysis of the three markers, gallic acid, vanillic acid and ferulic acid. NVK-E was analyzed in six replicates. The average quantities of gallic acid, vanillic acid and ferulic acid in 50 mg of NVK-E were 0.1589, 0.1417 and 0.0412 mg, respectively which are shown in Table 13.

Table 13 Average quantities of the analytical markers in 50 mg of NVK-E.

Analytical marker	**mg/50mg NVK (±S.D.)**	**%(w/w) (±S.D.)**
gallic acid	0.1589±0.0026	3.2±0.05
vanillic acid	0.1417±0.0035	2.8±0.06
ferulic acid	0.0412±0.0007	0.8±0.01

3. Preformulation Studies

3.1 Physicochemical characterization of NVK-E

3.1.1 The dissolving and dispersing ability of NVK-E in solvents

Solvents selected for this study were water, chloroform, 1% hydrochloric acid, 1% sodium hydroxide, phosphate buffer pH 6.8, methanol, absolute ethanol and acetronitrile. Water and chloroform were selected as a representative of polar and non-polar solvents. One percent hydrochloric acid, 1% sodium hydroxide and phosphate buffer pH 6.8 were selected as acid, base and buffer solvents, respectively. Methanol, ethanol and acetronitrile were selected to represent organic solvents. The results showed that NVK-E was not dissolved in both polar solvent (water) and non-polar solvent (chloroform). In addition, NVK-E was also not dissolved in acidic solvent (1% hydrochloric acid), buffer solution (phosphate buffer pH 6.8) and organic solvents (methanol, ethanol and acetronitrile). However, NVK-E was found to dissolve in basic solvent (1% sodium hydroxide, 1:80 parts)

which might due to its acidic phenolic constituents. The observed dissolving and dispersing ability of NVK-E are shown in Figure 27. Apparently, NVK-E was able to disperse in water, 1% hydrochloric acid, buffer pH 6.8, methanol, ethanol and acetronitrile but it was unable to disperse in chloroform. NVK-E was found to disperse in water more quickly than other solvents. The dissolving and dispersing ability of NVK-E in various solvents is shown in Table 14.

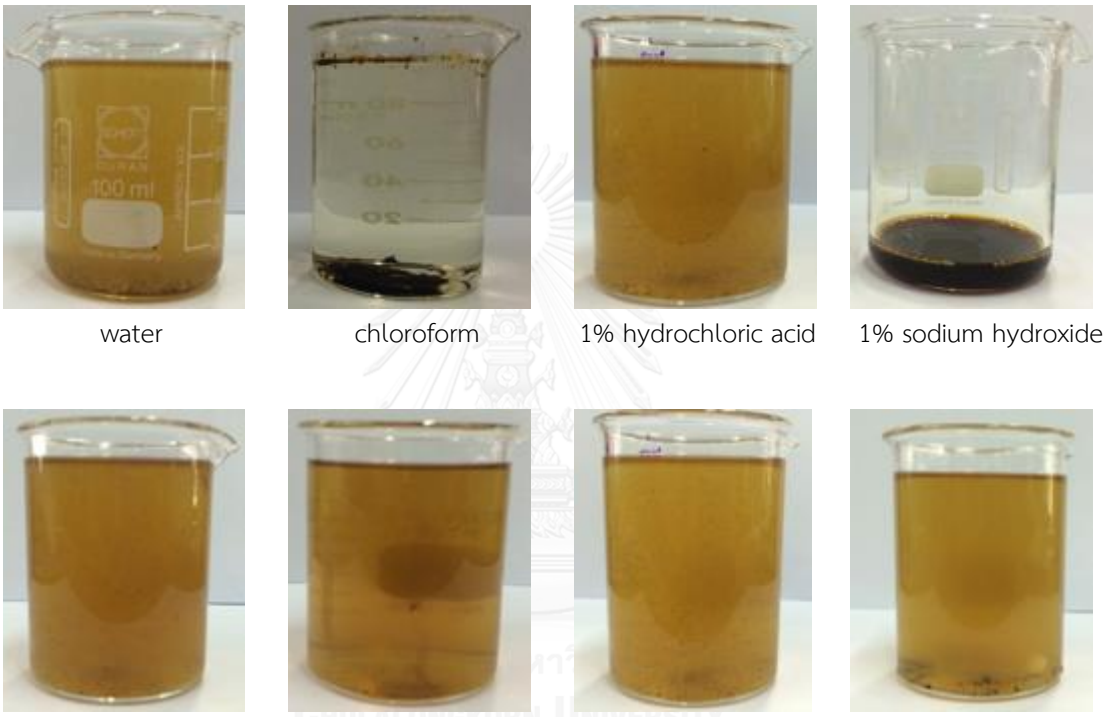


Figure 27 Observed dissolving and dispersing ability of NVK-E in various solvents

Table 14 The dissolving and dispersing ability of NVK-E in various solvents

Solvents	The dissolving ability of NVK-E	The dispersing ability of NVK-E
Water	Not dissolved	Dispersed
Chloroform	Not dissolved	Not dispersed
1% Hydrochloric acid	Not dissolved	Dispersed
1% Sodium hydroxide	Dissolved (1:80 parts)	Dispersed
Phosphate buffer pH 6.8	Not dissolved	Dispersed
Methanol	Not dissolved	Dispersed
Ethanol	Not dissolved	Dispersed
Acetronitrile	Not dissolved	Dispersed

3.1.2 Thermal properties of NVK-E

Thermal properties of NVK-E was determined using differential scanning calorimeter. The DSC runs were conducted over a temperature range of 25-300ºC at rate of 10ºC/min. DSC thermogram of NVK-E was observed to dehydrate/desolvate starting at 80-160ºC (peak 122.23ºC) and degraded at the temperatures higher than 170 ºC. The DSC thermogram of NVK-E at scanning rate of of 10ºC/min from 25-300ºC is shown in Figure 28.

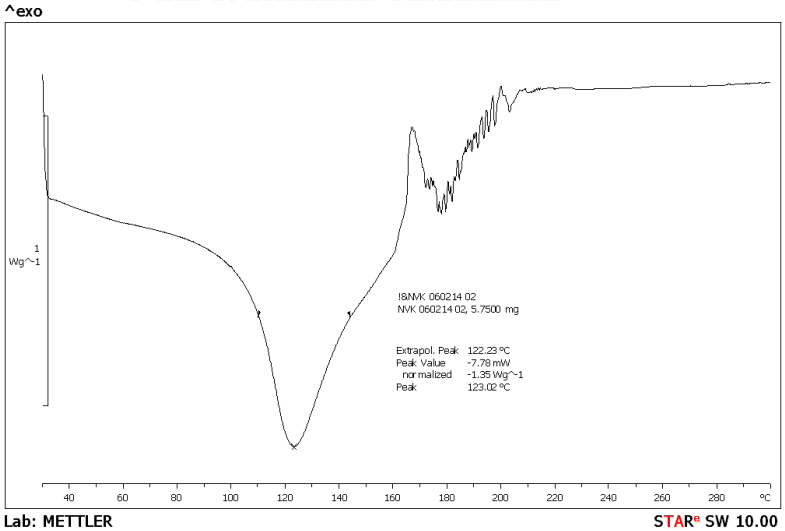


Figure 28 DSC thermogram of NVK-E at scanning rate of of 10ºC/min from 25-300ºC.

3.2 Physicochemical characterization of excipients

3.2.1 Thermal properties of excipients

In this study, Avicel® PH102 (microcrystalline cellulose), magnesium carbonate, Aerosil®200 (colloidal silicon dioxide), starch 1500, talcum, kaolin, aluminium hydroxide and pectin were selected as adsorbents to adsorb NVK-E and convert it to dry powder. Mannitol and Ludiflash® were selected as diluents. Polyvinyl acetate in Ludiflash® functioned as a tablet binder. Polyplasdone® (crospovidone), Explotab® (sodium starch glycolate) and Ac-Di-Sol® (croscamellose sodium), were selected as disintegrants. Stearic acid was selected as lubricant. Aerosil®200 (colloidal silicon dioxide) was selected as glidant. Sodium chloride, citric acid and sucralose were selected as flavoring agents. The thermal properties of all the excipients were performed using differential scanning calorimeter (DSC) at scanning rate of of 10ºC/min from 25-300ºC. Records of endothermic/exothermic events of excipients are compared and DSC thermograms of every excipient are presented in Appendix A.

Avicel® PH 102 was observed to dehydrate/desolvate at the temperature of 40-160°C (peak=87.23°C). Starch 1500 was observed to dehydrate/desolvate at 35-180°C (peak=95.00 °C). Talcum, kaolin and aluminium hydroxide were observed for melting temperatures at 173.64°C, 189.79°C and 298.59°C, respectively. Pectin was observed the melting temperature at 182.63°C. It was also observed to dehydrate/desolvate at 195-245°C (peak=210.00°C) and the decomposition occurred at the temperature higher than 240ºC. Ludiflash® was observed to have the melting temperature at 166.31°C. Citric acid was observed to have the melting temperature at 55.99°C and 155.44°C. Dehydration/desolvation temperature was observed to be at 65-100°C (peak 91.35°C) and the decomposition at the temperatures higher than 170ºC. Sucralose was observed to have two melting peaks at 122.67°C and 123.09°C. Polyplasdone® XL-10, Explotab® and Ac-Di-Sol® were observed to dehydrate/desolvate at temperatures of 30-160ºC (peak 93.35ºC), 25-200ºC (peak 104.78ºC) and 40-200ºC (peak 102.97ºC), respectively. Stearic acid and mannitol were observed to exhibit the melting temperature at 58.12°C and 176.92°C,

respectively. No peak was observed in the range of 25-300ºC for magnesium carbonate, Aerosil® 200 and sodium chloride because these inorganic compounds melt at temperatures higher than 300ºC.

Table 15 Thermal events of various excipeints

Excipients	Desolvation/ Dehydration	Melting 1	Melting 2	Remark
Avicel® PH 102	87.23 °C	-	-	-
Magnesium carbonate	-	-	-	Ref. Melting 350°C
Aerosil® 200	-	-	-	Ref. Melting 1700°C
Starch 1500	95.00 °C	-	-	-
Talcum	-	173.64 °C	-	-
Kaolin	-	189.79 °C	-	-
Aluminium hydroxide	-	298.59 °C	-	Ref. Melting at 300 °C
Pectin	210.00 °C	182.63 °C	-	Decompose at 204.67°C
Ludiflash®	-	166.31°C	-	-
Citric acid (monohydrate form)	91.35°C	55.99°C	155.44°C	Decompose at 214.74 °C
Sucralose	-	122.67°C	126.93°C	-
Sodium chloride	-	-	-	Ref. Melting 801°C
Polyplasdone® XL-10	101.36°C	-	-	-
Explotab®	104.78°C	-	-	-
Ac-Di-Sol®	102.97°C	-	-	-
Stearic acid	-	58.12°C	-	-
Mannitol	-	176.92°C	-	-

3.3 Incompatibility Study

Incompatibility was evaluated by DSC thermal analysis by comparing DSC thermograms of pure components with 1:1 physical mixtures. In general, DSC thermograms of solid drug and solid excipient mixtures were assumed to be

compatible if the thermal properties of mixtures were the sum of the individual components (43). However, the active ingredient in this study was in liquid form, meanwhile all of the excipients were in solid form, so the interpretation of DSC thermograms of the mixtures might not be straightforward. In this study, DSC thermograms of the mixtures were classified into 2 groups. The first group was excipients which could not dissolve in NVK-E. The mixtures of this group were visually dry. DSC thermograms of this group of mixtures were obviously similar to the excipients. This group consisted of Avicel® PH102, magnesium carbonate, Aerosil® 200, kaolin, Polyplasdone® XL10, stearic acid. The second group was excipients which could dissolve in NVK-E. The mixtures of this group were visually glutinous. DSC thermograms of this group of mixtures were mostly similar to NVK-E but some of them were slightly changed due to the solubility and crystallization of excipients in NVK-E. This group consisted of starch 1500, talcum, aluminium hydroxide, pectin, Ludiflash®, citric acid, sucralose, sodium chloride, Explotab®, Ac-Di-Sol® and mannitol. The summary of compatibility screening of NVK-E and excipients are shown in Table 16. DSC thermograms of the mixture are included in Appendix B. The physical appearances of NVK-E and excipients were additionally observed. The mixtures of NVK-E and Avicel® PH102, magnesium carbonate, Aerosil® 200, kaolin, Polyplasdone® XL10 and stearic acid were dry. Simultaneously, the mixtures of NVK-E and starch 1500, talcum, aluminium hydroxide, kaolin, Ludiflash®, citric acid, sucralose, sodium chloride, Explotab®, Ac-Di-Sol® and mannitol were glutinous which are shown in Table 17. The study suggested that DSC thermal analysis might not be a suitable method to determine incompatibility between liquid drugs and solid excipients because the thermograms of the mixtures were difficult to interpretate. Moreover, the exposure of drug-excipient mixtures with the high temperature performed using DSC thermal analysis did not occur under the actual storage condition. Hence, the observed drug-excipient interaction from the DSC thermal analysis might not be relevant under ambient conditions (43). Other combination technique such as HPLC should be utilized to determine the incompatibility study.

Table 16 Summary of compatibility screening of NVK-E and excipient

Characteristic of Excipients	Characteristic of DSC Thermograms	Excipients
1. Excipients that could not dissolve in NVK-E	Similar to the excipients	Avicel® PH102 magnesium carbonate Aerosil® 200 kaolin Polyplasdone® XL10 stearic acid
2. Excipients that could dissolve in NVK-E	Similar to NVK-E but slighly changed due to solubility and crystallization	starch 1500 talcum aluminium hydroxide pectin Ludiflash® citric acid sucralose sodium chloride Explotab® Ac-Di-Sol® mannitol

Table 17 Physical characteristic of the 1:1 physical mixture of NVK-E and excipients

Mixture	Color	Odor	Attribute
NVK-E and Avicel® PH 102	dark brown	herbal odor of NVK-E	dry
NVK-E and magnesium carbonate	light brown	pungent odor	dry
NVK-E and Aerosil® 200	dark brown	herbal odor of NVK-E	dry
NVK-E and starch 1500	dark brown	herbal odor of NVK-E	glutinous
NVK-E and talcum	dark brown	herbal odor of NVK-E	glutinous
NVK-E and kaolin	light brown	herbal odor of NVK-E	dry
NVK-E and aluminium hydroxide	dark brown	herbal odor of NVK-E	glutinous
NVK-E and pectin	dark brown	herbal odor of NVK-E	glutinous
NVK-E and Ludiflash®	dark brown	herbal odor of NVK-E	glutinous
NVK-E and citric acid	dark brown	herbal odor of NVK-E	glutinous
NVK-E and sucralose	dark brown	herbal odor of NVK-E	glutinous
NVK-E and sodium chloride	dark brown	herbal odor of NVK-E	glutinous
NVK-E and Polyplasdone® XL-10	light brown	herbal odor of NVK-E	glutinous
NVK-E and Explotab®	dark brown	herbal odor of NVK-E	glutinous
NVK-E and Ac-Di-Sol®	light brown	herbal odor of NVK-E	glutinous
NVK-E and stearic acid	dark brown	herbal odor of NVK-E	dry
NVK-E and mannitol	dark brown	herbal odor of NVK-E	glutinous

4. Formulation Development

4.1 Development of intermediate NVK-E dry powder (NVK-EP)

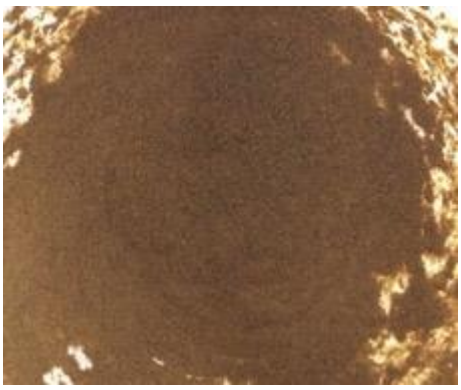
NVK-E was extremely viscous liquid which was difficult to manage. Adsorbents were utilized to convert NVK-E into dry power (NVK-EP) that was easy to formulate in the solid dosage form such as tablets and capsules (38). Avicel® PH102, magnesium carbonate, Aerosil® 200, starch 1500, talcum, kaolin, aluminium hydroxide and pectin were selected to adsorb NVK-E into dry powder. The result showed that magnesium carbonate and Aerosil® 200 were the most suitable excipients with the least amount to incorporate NVK-E into dry powder (1:0.3). Avicel® PH102 was the second most

capable excipient with 1:0.5 ratio to incorporate NVK-E into dry powder. Kaolin with 1:0.8 ratio was used to incorporate NVK-E into dry powder. Talcum, starch 1500, aluminium hydroxide and pectin with 1:1 ratio were unable to incorporate NVK-E into dry powder as shown in Figure 29. However, the pungent odor was present after mixing magnesium carbonate with NVK-E. A change in physical characteristics such as odor might lead to incompatibility between substances. In addition, the presence of pungent odor in the drug substances or excipients did not prefer for the orodispersible tablets formulation. Meanwhile, Aerosil® 200 was a very bulky and light substance. Its particles were easily disseminated during the mixing process, so it was difficult to incorporate with NVK-E. Therefore, this study selected Avicel® PH102, the second most capable excipient to adsorb NVK-E, as adsorbent to adsorb NVK-E into dry powder.

Table 18 Characteristics of mixtures between NVK-E and adsorbents after mixing at the different ratios

Mixtures	Ratio	Characteristic of the mixture	Remark
NVK-E and Avicel® PH 102	1 : 0.5	dry powder	-
NVK-E and Magnesium carbonate	1 : 0.3	dry powder	pungent odor
NVK-E and Aerosil® 200	1 : 0.3	dry powder	-
NVK-E and Kaolin	1 : 0.8	dry powder	-
NVK-E and Starch 1500	1 : 1	sticky lump	-
NVK-E and Talcum	1 : 1	sticky lump	-
NVK-E and Aluminium hydroxide	1 : 1	sticky lump	-
NVK-E and Pectin	1 : 1	sticky lump	-

NVK-E and Avicel® PH 102 at 1:0.5 ratio



NVK-E and magnesium carbonate at 1 : 0.3 ratio

NVK-E and Aerosil® 200 at 1: 0.3 ratio

NVK-E and kaolin 1:0.8 at 1 : 1 ratio

NVK-E and talcum at 1 : 1 ratio

NVK-E and starch 1500 at 1 : 1 ratio

NVK-E and $Al(OH)_3$ 1 : 1 ratio

NVK-E and pectin at 1 : 1 ratio

Figure 29 Characteristic of NVK-E mixed with adsorbents at indicated ratios

The use of Avicel® PH 102 at the ratio of 1:0.5 was suitable to convert NVK-E into dry powder. However, the mixture of NVK-E and Avicel® PH 102 at the ratio of 1:0.5 was hygroscopic when exposed to air. Hence, the used of Avicel® PH 102 at the ratio of 1:1 was selected in this study to convert NVK-E into dry powder. The use of Avicel® PH 102 at the ratio of 1:1 was more stable to humidity and easier to incorporate than those of 1:0.5 ratio.

The NVK-E dry powder (NVK-EP) was prepared by gradually mixing NVK-E and Avicel® PH 102 at the ratio of 1:1. Ethanol of about 2% (w/w) was added during the mixing to disperse the highly viscous NVK-E. After the mixing, the mixture was dried at the temperature of 60°C to remove the residual solvent. The mixture was then sieved through 40 mesh sieve and kept in a well-closed container to avoid exposure to humidity prior to further formulation process. Diagram of the semi-finished intermediate NVK-EP preparation process is shown in Figure 30. The physical appearance of NVK-EP is shown in Figure 31.

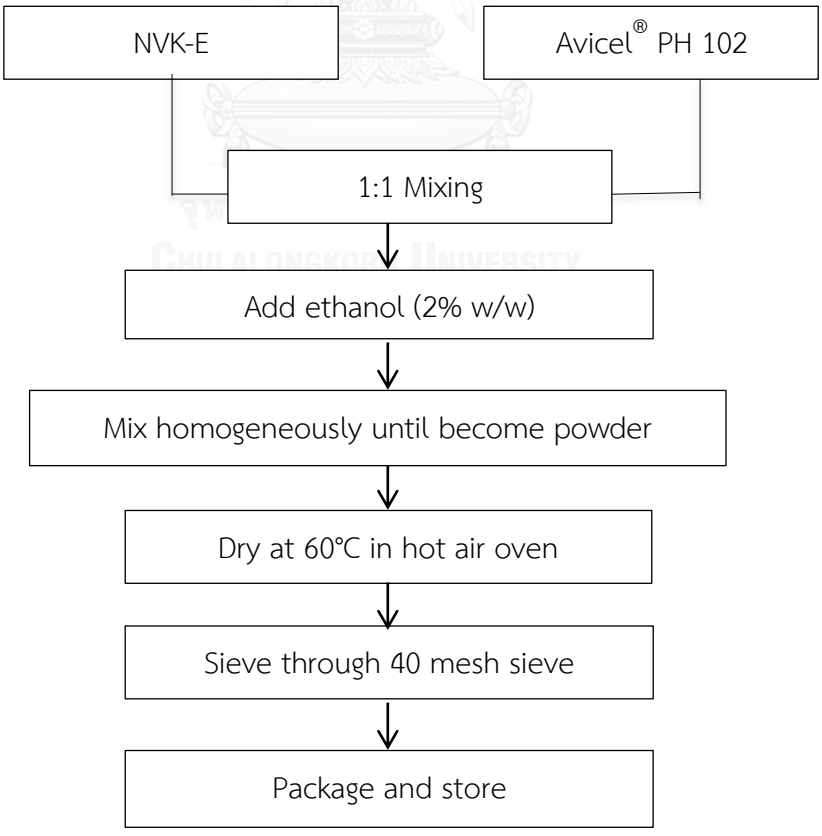


Figure 30 Diagram of the intermediate NVK-EP preparation process

Figure 31 NVK-E dry powder (NVK-EP)

4.2 Chemical analysis of intermediate NVK-E dry powder (NVK-EP)

The analysis of the three markers, gallic acid, vanillic acid and ferulic acid in NVK-EP was performed in six replicates. The quantity of gallic acid, vanillic acid and ferulic acid in 100 mg of NVK-EP were 0.1677, 0.1412 and 0.0394 mg, respectively (Table 19).

Table 19 Quantity of the analytical markers in NVK-EP

Analytical marker	**mg/100mg NVK-EP ±S.D.**	**%(w/w) ±S.D.**
gallic acid	0.1677±0.0017	3.35±0.03
vanillic acid	0.1412±0.0035	2.82±0.07
ferulic acid	0.0394±0.0026	0.79±0.05

4.3 Orodispersible tablets formulation development

4.3.1 Orodispersible tablets preformulation and formulation

In this study, disintegrant type and amount were varied. Three super-disinegrants, i.e. Polyplasdone®, Explotab® and Ac-Di-Sol® were used and the amount was varied from 4%, 6% and 10% in the formulation (Table 20). The flow properties of formulation F1-F10 were determined by Compressibility Index (Carr's Index), Hausner's Ratio and angle of repose. Flow properties of the formulation are shown in Table 21. The result showed that formulations F1 and F2 presented passable flow property meanwhile formulations F3-F10 presented poor flow property determined by Carr's Index and Hausner's Ratio. However, with angle of repose method,

formulations F1-F10 exhibited excellent flow property. The results showed poor correlation between two methods because variations between each method (12). Carr's Index and Hausner's Ratio were indirect methods for determining flow property. Bulk density, size, shape, surface area, moisture content and cohesiveness of materials could influence the observed Carr's Index and Hausner's Ratio while angle of repose was a method determined the flow property by characterizing the interparticulate friction between particles in the formulations or resistance to movement between particles. Therefore, angle of repose method was a better method to represent the flow of drug powder from the hopper to the die during the tableting process. In this study, flow property obtained from angle of repose was selected to represent the flow property of the formulation. However, angle of repose also provided some variations such as accuracy of measurements between each attempt (12). According to the United States Pharmacopeia, angle of repose not exceeding 50° was satisfied for further manufacturing procedure.

Formulations F1-F10 were compressed into tablets by direct compression method using a mechanical tableting machine (single punch, BS215B, Kasuga Electric Works Ltd., Japan) with 13 mm diameter punch. Picture of tablets from formulations F1-F10 are shown in Figure 32. The orodispersible tablet formulation F1-F10 were evaluated for the uniformity of mass, %friability, tablet hardness and disintegration time. The test results are shown in Table 22.

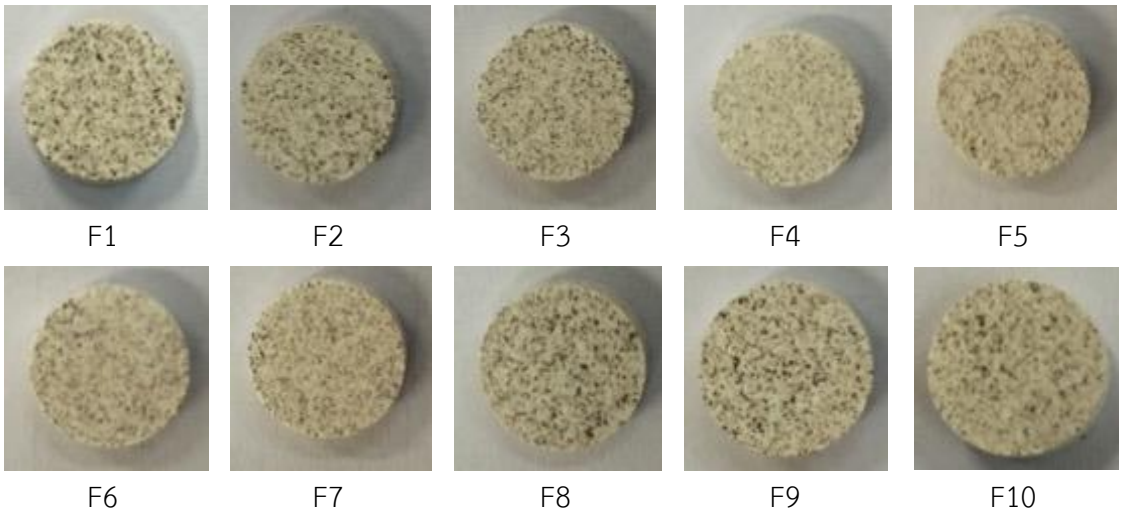


Figure 32 Orodispersible tablets formulation F1-F10

Table 20 The amount in percentage of ingredients in formulations (F1-F10)

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
NVK-E	8.24	8.24	8.24	8.24	8.24	8.24	8.24	8.24	8.24	8.24
Avicel® PH 102	8.24	8.24	8.24	8.24	8.24	8.24	8.24	8.24	8.24	8.24
Ludiflash®	65.92	65.92	65.92	65.92	65.92	65.92	65.92	65.92	65.92	65.92
Citric acid	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Sucralose	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Polyplasdone®	-	2.00	6.00	10.00	-	-	-	-	-	-
Explotab®	-	-	-	-	2.00	6.00	10.00	-	-	-
Ac-Di-Sol®	-	-	-	-	-	-	-	2.00	6.00	10.00
Aerosil® 200	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Stearic acid	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80
Mannitol	10.00	8.00	4.00	0.00	8.00	4.00	0.00	8.00	4.00	0.00
Total	**100.00%**									

Table 21 Flow properties of formulations (F1-F10)

Formulation	Bulk density ±S.D.	Tapped density ±S.D.	Hausner's ratio ±S.D.	Carr's index ±S.D.	Flow	Angle of repose ±S.D.	Flow
F1	0.6033±0.02	0.7702±0.01	1.28±0.02	22±1.44	Passable	26±2	Excellent
F2	0.5660±0.02	0.7333±0.01	1.30±0.03	23±1.53	Passable	25±1	Excellent
F3	0.5295±0.01	0.7481±0.03	1.41±0.04	29±2.02	Poor	24±1	Excellent
F4	0.5225±0.01	0.7241±0.02	1.39±0.02	28±1.01	Poor	25±1	Excellent
F5	0.5400±0.01	0.7537±0.01	1.40±0.05	28±2.89	Poor	26±1	Excellent
F6	0.5457±0.01	0.7527±0.00	1.38±0.02	28±1.00	Poor	26±0	Excellent
F7	0.5365±0.01	0.7419±0.01	1.38±0.04	28±2.25	Poor	26±1	Excellent
F8	0.5570±0.01	0.7510±0.01	1.35±0.02	26±1.04	Poor	26±1	Excellent
F9	0.5122±0.01	0.7007±0.03	1.37±0.04	27±2.02	Poor	28±1	Excellent
F10	0.5166±0.01	0.7329±0.01	1.42±0.04	30±1.80	Poor	27±2	Excellent

The average weight of formulation F1-F10 were 596-607 mg and the uniformity of weight were within the limit of ±5% the average weight. Percent friability of formulations F1-F10 were not more than 1.0%. Tablet hardness were varied within the formulation F1-10 to obtain %friability of not more than 1%. The presence of mannitol in the formulation exhibited low mouldability resulting in the brittleness of the tablet (15). Therefore, tablet formulation F1 with 10% mannitol required a high compacting pressure during the tableting process. The presence of Polyplasdone® XL-10, instead of mannitol in formulations F2, F3 and F4 resulted in good compressibility. Explotab® in formulations F5, F6 and F7 and Ac-Di-Sol® in formulations F8, F9 and F10 also performed good compressibility. The result suggested that with the lower concentration of superdisintegrant in the formulation, the higher %friability was observed. Disintegration time was performed with the formulations F1-F10. The result showed that formulation F1 with the high tablet hardness and the absent of a superdisintegrant exhibited the disintegration time of more than 180 seconds. Formulations F2, F3 and F4 with Polyplasdone® XL-10 as a superdisintegrant (2%, 6% and 10%, respectively) exhibited disintegration times of 46, 26 and 22 seconds, respectively. The result suggested that the higher concentration of Polyplasdone® XL-10, the shorter disintegration times were obtained from the formulation. Formulations F5, F6 and F7 were formulations with Explotab® as a superdisintegrant (2%, 6% and 10%, respectively) exhibited disintegration time of 44, 55 and 55 seconds. The higher concentration of Explotab® was resulting in the longer disintegration times of formulations. Explotab® performed a disintegrant property by rapidly absorbed water resulting in swelling which led to the rapid disintegration of the tablets. High concentration of Explotab® may cause gelling which created a barrier to the penetration of water into the tablet and retarded the disintegration (16). F8, F9 and F10 were formulations with Ac-Di-Sol® as a superdisintegrant (2%, 6% and 10%, respectively) exhibited disintegration time of 135, 55 and 60 seconds. It exhibited the same disintegration effect as Explotab® because the disintegration mechanism of Ac-Di-Sol® was by swelling and wicking. It could form gels at the high concentration resulting in retard disintegration. The higher concentration of Ac-Di-Sol® was also resulted in the longer disintegration time. Except for the

formulation F8, it exhibited a disintegration time of 135 seconds because higher tablet hardness was used during the tableting process to obtained %friability of not more than 1%. The diagram of the disintegration time and hardness of orodispersible tablets formulations F1-F10 are shown in Figure 33.

Table 22 Quality control of orodisperible tablet formulations (F1-F10)

Formulation	Average weight of tablets ±S.D. (mg/tab)	Uniformity of weight (±5%)[a]	Friability (%)	Tablet hardness ±S.D. (kg-force)[b]	Disintegration Time (sec)
F1	607±8	Pass	0.82	10.7±0.8	> 180
F2	597±5	Pass	0.63	7.2±0.4	46
F3	613±6	Pass	0.87	7.2±0.9	26
F4	607±7	Pass	0.62	9.3±0.6	22
F5	604±8	Pass	0.95	6.1±0.5	44
F6	607±7	Pass	0.73	6.5±0.4	55
F7	599±7	Pass	0.75	6.6±0.8	55
F8	606±9	Pass	0.65	9.6±0.5	135
F9	597±8	Pass	0.62	5.8±0.7	55
F10	598±10	Pass	0.78	5.8±0.6	60

a (n=20), b (n= 6)

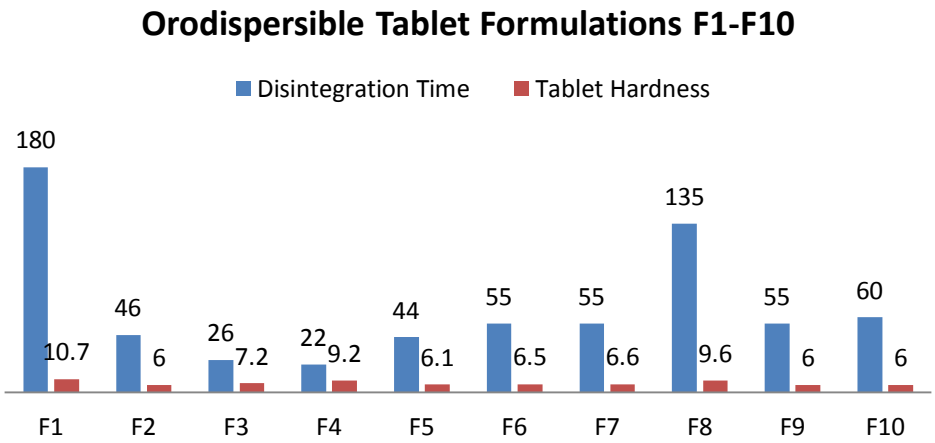


Figure 33 The disintegration time and hardness of orodispersible tablet formulations F1-F10 formulation F8, F9 and F10 also performed good compressibility

4.3.2 Chemical analysis of orodispersible tablet formulations

4.3.2.1 Selection of solvent

As the tablets contained about 50 mg of NVK-E and 556.7 mg of excipients, suitable solvents should be selected to extract or dissolve the markers from the formulation. Water, solvent used in NVK-E extraction was selected. The amounts of water were varied from 100 ml, 200 ml, 300 ml and 500 ml, respectively. NVK-E 25 mg, 50 mg and 100 mg (50%, 100% and 150% in the formulation) with the additional of 556.7 mg placebo formulation (F1) was used as a representative of the placebo from the formulations. The result showed that with the higher volume of water used to extract the sample, the higher amount of markers dissolved. The used of 500 ml water to extract 50 mg of NVK-E in the formulation F1 met the requirement of the AOAC Guidelines for Dietary Supplements and Botanicals that %recovery of gallic acid and vanillic acid should be within 90-108% and ferulic acid should be within 85-110%. The %recovery of NVK-E in formulation F1 extracted by water at different volume is shown in Table 23.

Table 23 Percent recovery of NVK-E in formulation F1 extracted by water at different volume

%Recovery (gallic acid)	**Water (ml)**			
	100	200	300	500
NVK50%	59.29	73.02	91.32	96.47
NVK100%	63.38	88.49	97.79	94.67
NVK150%	65.36	91.52	100.07	99.95
%Recovery (vanillic acid)	**Water (ml)**			
	100	200	300	500
NVK50%	92.24	93.27	95.28	96.11
NVK100%	91.90	98.47	99.59	96.40
NVK150%	91.77	97.40	99.59	98.52
%Recovery (ferulic acid)	**Water (ml)**			
	100	200	300	500
NVK50%	78.85	78.77	71.54	83.79
NVK100%	80.76	93.53	90.48	85.57
NVK150%	81.96	93.88	98.30	90.23

4.3.2.2 Analysis of markers in the formulations

For this study, formulation F4 was selected because it showed good compressibility, uniformity of weight and it also obtained the shortest disintegration time than other formulations. The analysis showed that %recovery of markers in formulaion F4 were 86.15% for gallic acid, 98.03% for vanilic acid and 84.15% for ferulic acid. The initial %recovery of gallic acid and ferulic acid obtained from the formultion F4 fail to meet the requirement of of the AOAC Guidelines for Dietary Supplements and Botanicals that %recovery of gallic acid and vanillic acid should be within 90-108% and ferulic acid should be within 85-110% (Table 24).

Table 24 Percent recovery of analytical markers in tablets formulation F4

Analytical markers	%Recovery
Gallic acid	86.15±0.68
Vanillic acid	98.03±0.68
Ferulic acid	84.15±0.95

Further attempt was performed with the formulation F1 (as representative of formulations without superdisintegrant), F4, F7 and F10 as representative of formulations with Polyplasdone® XL-10, Explotab® and Ac-Di-Sol® as superdisintegrants, respectively. Results showed that formulation F1, without superdisintegrant exhibited the highest %recovery of 101.09% for gallic acid, 98.76% for vanillic acid and 97.77% for ferulic acid. Meanwhile formulations F4, F7 and F10 with the presence of Polyplasdone® XL-10, Explotab® and Ac-Di-Sol® exhibited lower %recovery as shown in Table 25. This suggested that superdisintegrants in the formulation interfered with the extraction of markers from NVK-E. Polyplasdone® XL-10 seemed to have the most effect than other superdisintegrants. Therefore, formulation F7 with Explotab® as a superdisintegrant was selected for further study due to its good compressibility, uniformity of weight, disintegration time and %recovery of gallic acid, vanillic acid and ferulic acid. This formulation met the requirement in AOAC Guidelines for Dietary Supplements and Botanicals which stated that %recovery of gallic acid and vanillic acid should be within 90-108% and ferulic acid should be within 85-110%.

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Table 25 Percent recovery of analytical markers in tablets formulations F1, F4, F7 and F10

	%recovery±S.D.
Gallic acid	
F1	101.09±0.40
F4	86.15±0.68
F7	101.88±0.01
F10	101.07±3.30
Vanillic Acid	
F1	98.76±0.72
F4	98.03±0.68
F7	98.21±0.05
F10	96.96±1.58
Ferulic acid	
F1	97.77±2.14
F4	84.15±0.95
F7	88.97±1.73
F10	88.45±0.03

4.4 Orodispersible tablet evaluations

4.4.1 Analysis of orodispersible tablets

4.4.1.1 Analytical method validation

The method validation was performed using HPLC method as described in Section 2.3.1. The quantity of three phenolic compounds, gallic acid, vanillic acid and ferulic acid in the formulation F7 were determined. Selectivity, linearity, accuracy and precision were performed in accordance with the AOAC Guidelines for Dietary Supplements and Botanicals.

System suitability

System suitability tests were an integral part of HPLC methods. It was used to verify that the chromatographic system is adequate for the intended analysis. The

system suitability test was performed by collecting data from six successive replicates injections of standard. The %RSD of peak areas, resolution, tailing factor and theoretical plates were evaluated (Table 26).

Table 26 System suitability of the HPLC system

Analytical markers	Concentration (µg/ml)	%RSD[a]	Tailing factor (T_f)	Resolution (R_s)	Number of theoretical plates (N)
Gallic acid	0.34	1.8	1.1	-	8609
Vanillic acid	0.29	2.4	1.2	56	78706
Ferulic acid	0.08	2.2	1.1	29	16615

[a] n=6

Selectivity

Peak responses of the three markers in standard mixture solutions were clearly separated from each other, with retention times of 12.3, 34.9 and 46.2, respectively (Figure 34). Peak responses of the three markers in the formulation F7 were also well separated from each other and exhibited retention times identical to the results for standard solution (Figure 35). The results suggested that there was no interference with the peak responses of all three analytical markers. However, the peak purity could not be evaluated in this study because the concentration of the three markers were too low.

Linearity

Linearity parameters of the HPLC system are shown in Table 27. The results showed that all three analytical markers exhibited good linearity within specified ranges with correlation coefficients (r) of 0.9998, 0.9999 and 0.9992 for gallic acid, vanillic acid and ferulic acid, respectively. These results complied with the AOAC Guidelines for Dietary Supplements and Botanicals which stated that the correlation coefficient (r) should be > 0.99 for acceptable linearity. The linearity evaluation of gallic acid, vanillic acid and ferulic acid are shown in Figures 36, 37 and 38,

respectively Chromatograms for the linearity study at the concentrations of 40%, 70%, 100%, 130% and 160% are shown in Figures 39, 40, 41, 42 and 43, respectively.

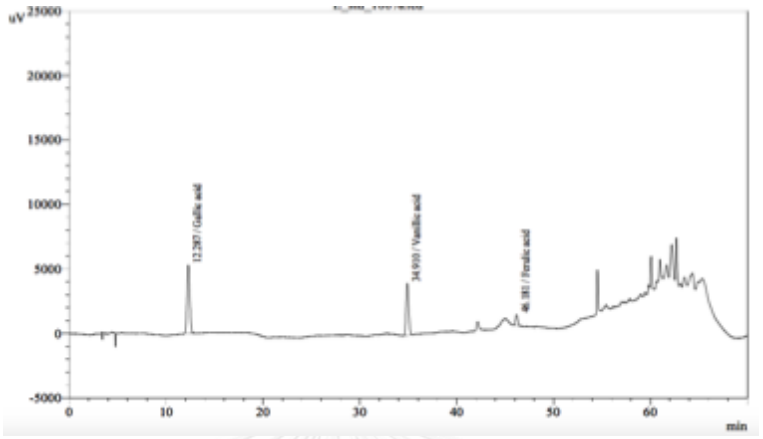


Figure 34 Chromatogram of a standard mixture solution of 0.34 µg/ml gallic acid (12.3 min), 0.29 µg/ml vanillic acid (34.9 min) and 0.08 µg/ml ferulic acid (46.2 min)

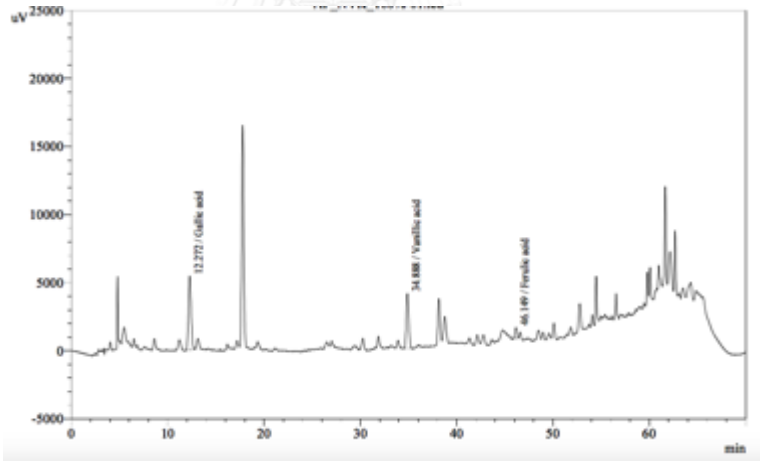


Figure 35 Chromatogram of formulation F7 where gallic acid, vanillic acid and ferulic acid were found to elute at 12.3, 34.9 and 46.1 min, respectively

Table 27 Linearity parameters of the HPLC system

Analytical markers	Linearity range (µg/ml)	Slope	Y-intercept	r
Gallic acid	0.15-0.54	325643.26	-6595.6000	0.9998
Vanillic acid	0.13-0.46	261719.16	-2605.4000	0.9999
Ferulic acid	0.04-1.40	187188.3891	-2354.5500	0.9992

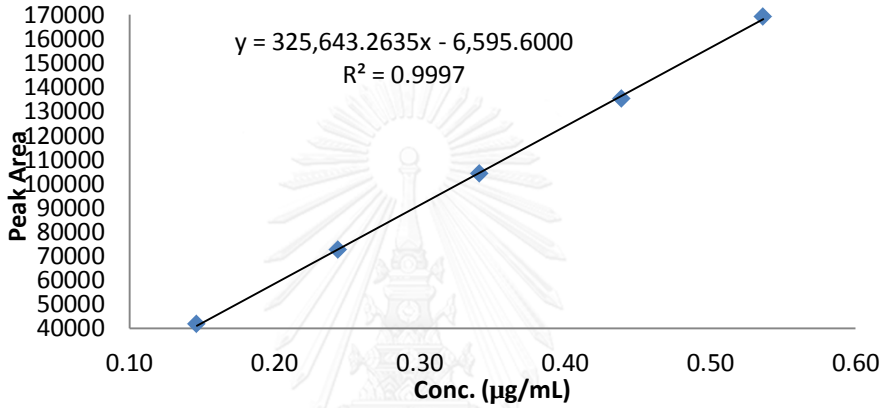


Figure 36 Linearity evaluation of various concentrations of gallic acid

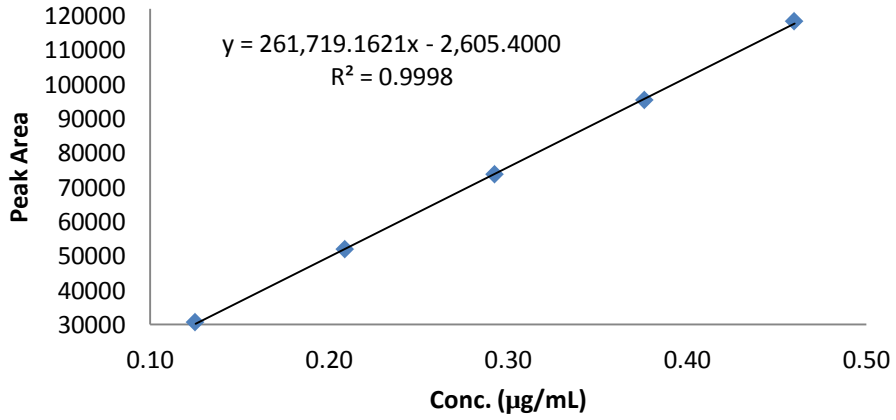


Figure 37 Linearity evaluation of various concentrations of vanillic acid

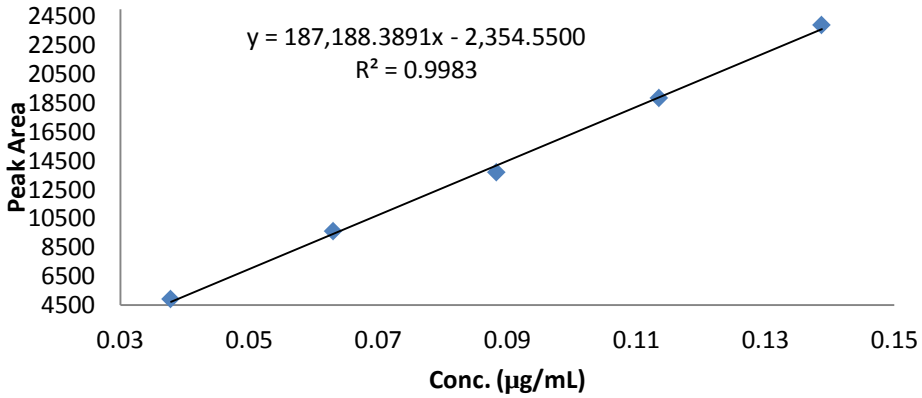


Figure 38 Linearity evaluation of various concentrations of ferulic acid

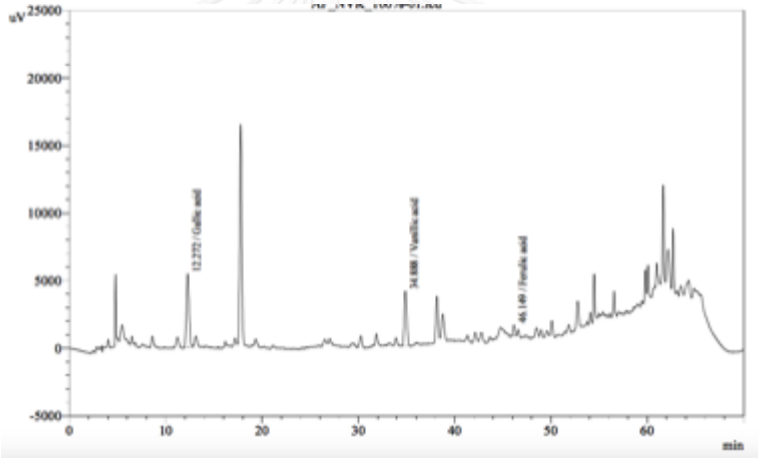


Figure 39 Chromatogram of standard mixture solution at 40% concentration showing 0.15 µg/ml gallic acid (12.3 min), 0.13 µg/ml vanillic acid (34.9 min), and 0.04 µg/ml ferulic acid (46.2 min)

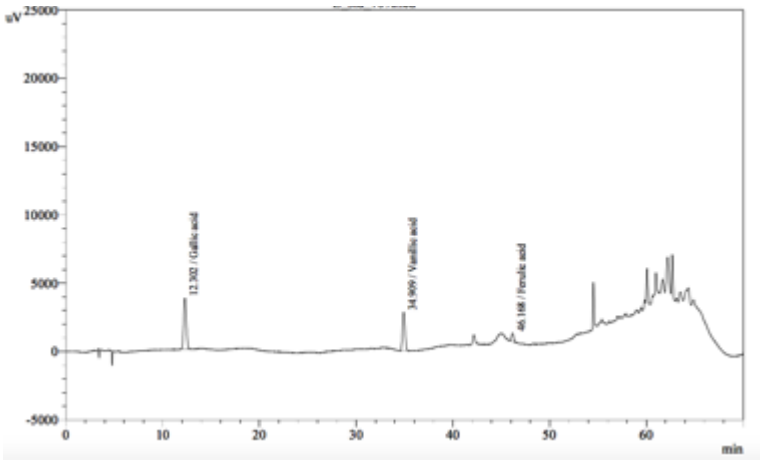


Figure 40 Chromatogram of 50 mg NVK-E with standard mixture solution at 70% concentration showing 0.24 µg/ml gallic acid (12.3 min), 0.20 µg/ml vanillic acid (34.9 min), and 0.06 µg/ml ferulic acid (46.2 min)

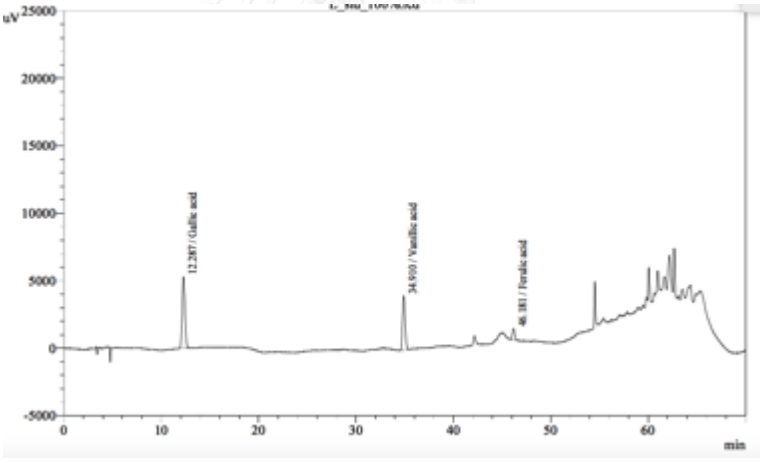


Figure 41 Chromatogram of 50 mg NVK-E with standard mixture solution at 100% concentration showing 0.34 µg/ml gallic acid (12.3 min), 0.29 µg/ml vanillic acid (34.9 min), and 0.08 µg/ml ferulic acid (46.2 min)

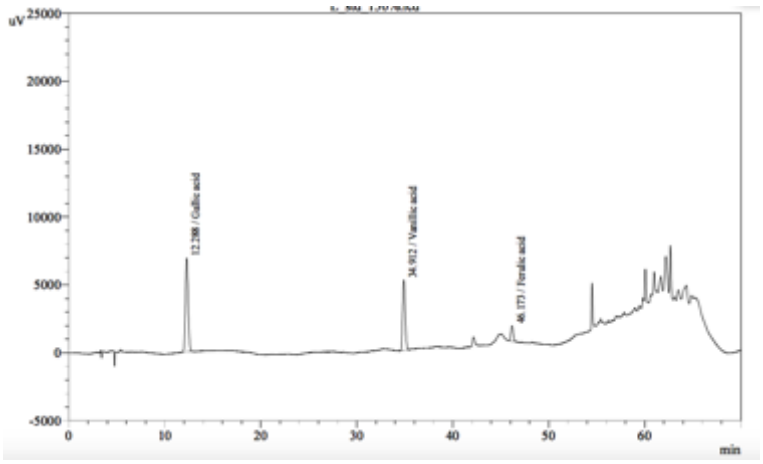
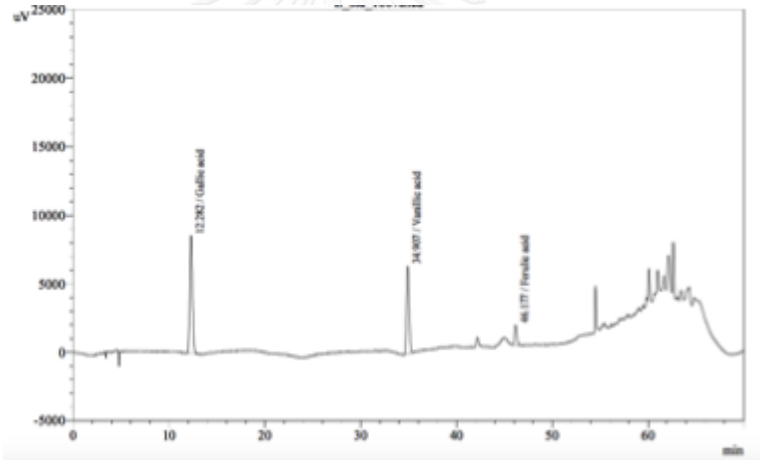


Figure 42 Chromatogram of 50 mg NVK-E with standard mixture solution at 130% concentration showing 0.44 µg/ml gallic acid (12.3 min), 0.38 µg/ml vanillic acid (34.9 min), and 0.11 µg/ml ferulic acid (46.2 min)



.Figure 43 Chromatogram of 50 mg NVK-E with standard mixture solution at 160% concentration showing 0.54 µg/ml gallic acid (12.3 min), 0.46 µg/ml vanillic acid (34.9 min), and 0.14 µg/ml ferulic acid (46.2 min)

Accuracy and Precision

The accuracy of the system is described as the average %recovery, which were found to be 95-106, 97-105 and 89-102 for gallic acid, vanillic acid and ferulic acid, respectively. The average %recovery of all three markers (Table 28) complied with the AOAC Guidelines for Dietary Supplements and Botanicals which stated that this value should be between 90-108% for gallic acid and vanillic acid, 85-110% for ferulic acid. The precision of the system is exhibited as the Horwitz Ratio ($HorRat_r$)

values are shown in Table 28. The AOAC Guidelines for Dietary Supplements and Botanicals suggested that $HorRat_r$ values of < 2 indicate good repeatability. The repeatability results for all three markers showed $HorRat_r$ values of < 2, which complied with the AOAC Guidelines for Dietary Supplements and Botanicals for acceptable repeatability.

4.4.1.2 Analysis of orodispesible tablet formulations

The analysis of the three markers, gallic acid, vanillic acid and ferulic acid in orodispersible tablet formulation F7 was performed in two replicates. The quantity of gallic acid, vanillic acid and ferulic acid in in orodispersible tablet formulation F7 were 0.1598, 0.1395 and 0.0326 mg respectively which were equivalent to percent labeled amount (%LA) of 95.1%, 98.6% and 97.4% for gallic acid, vanillic acid and ferulic acid, respectively (Table 29). The quantity of markers in 100 mg NVK-EP added in tablet formulation F7 was set as 100% labeled amount which were 0.1677 mg, 0.1412 mg and 0.0394 mg for gallic acid, vanillic acid and ferulic acid, respectively. However, the average of 85% of ferulic acid was extracted from the formulation according to the content uniformity study. Therefore, the average amount of 0.0333 mg ferulic acid extracted from formulation F7 was instead set as 100% labeled amount in this study (Table 30). The average amount of ferulic acid is shown in Section 4.4.9.

Table 28 Quantity of the analytical markers in orodispersible tablet formulation F7

Analytical markers	mg/tab	%LA
gallic acid	0.1598	95.1
vanillic acid	0.1395	98.6
ferulic acid	0.0326	97.4

Table 29 Accuracy and Precision of the HPLC system determined by $HorRat_r$

	Average %Recovery	Amount added (µg/ml)	Amount found (µg/ml)	%RSD (found)	$HORRAT_r$
Gallic acid					
50% DAY 1	100.84±2.59	0.18	0.18±0.00	2.56	0.76
DAY 2	95.12±2.59	0.17	0.16±0.00	2.72	0.80
DAY 3	106.09±4.94	0.17	0.18±0.01	4.65	1.38
100% DAY 1	96.32±0.45	0.35	0.34±0.00	0.47	0.14
DAY 2	98.32±1.35	0.34	0.33±0.00	1.37	0.41
Day 3	99.40±0.89	0.34	0.34±0.00	0.90	0.27
150% DAY 1	94.86±1.36	0.53	0.50±0.01	1.43	0.42
DAY 2	98.84±1.05	0.51	0.50±0.01	1.07	0.32
DAY 3	98.05±1.49	0.51	0.50±0.01	1.52	0.45
Vanillic acid					
50% DAY 1	105.19±1.52	0.14	0.15±0.00	1.45	0.41
DAY 2	96.88±3.96	0.14	0.13±0.01	4.09	1.17
DAY 3	98.18±4.64	0.14	0.14±0.01	4.72	1.34
100% DAY 1	101.04±0.82	0.28	0.29±0.00	0.81	0.23
DAY 2	100.78±1.51	0.27	0.27±0.00	1.50	0.43
Day 3	98.99±1.20	0.28	0.28±0.00	1.21	0.34
150% DAY 1	100.21±0.85	0.43	0.43±0.00	0.85	0.24
DAY 2	100.11±2.45	0.41	0.41±0.01	2.44	0.70
DAY 3	99.64±1.16	0.42	0.42±0.00	1.16	0.33
Ferulic acid					
50% DAY 1	101.79±2.52	0.04	0.04±0.00	2.48	0.59
DAY 2	91.74±1.90	0.04	0.03±0.00	2.07	0.49
DAY 3	96.58±5.69	0.04	0.04±0.00	5.89	1.40
100% DAY 1	93.22±3.16	0.08	0.08±0.00	3.39	0.81
DAY 2	100.39±4.03	0.08	0.08±0.00	4.02	0.96
Day 3	89.97±4.61	0.08	0.07±0.00	5.13	1.22
150% DAY 1	89.26±2.20	0.12	0.11±0.00	2.46	0.59
DAY 2	102.25±3.52	0.11	0.12±0.00	3.44	0.82
DAY 3	89.19±1.51	0.12	0.10±0.00	1.69	0.40

Table 30 Quantity of the analytical markers equivalent to 100% labeled amount

Analytical markers	mg of markers equal to 100% labeled amount
gallic acid	0.1677
vanillic acid	0.1412
ferulic acid	0.0333*

*mg of ferulic acid (100% la.) was obtained from the analysis of content uniformity in formulation F7 as shown in Section 4.4.9

4.4.2 Tablet hardness

Tablet hardness was determined using tablet hardness tester. The average of six tablets obtained the hardness of 5.4±0.4 Kg.force which was satisfactory for orodispersible tablets.

4.4.3 Friability

Friability was determined using tablet friability tester. Percent friability obtained from the formulation was 0.09% which complied with the United States Pharmacopeia that %Friability of the tablets should not exceed 1.0% (12).

4.4.4 Total phenolic content determination

Total phenolic content determination of tablet formulation F7 was performed in two replicates. Total phenolic content (%GAE) of formulation F7 was 8.19%. The result showed that total phenolic content in formulation F7 was less than NVK-E (11.73%). This suggested that some of the phenolic compound might not be effectively extracted from the formulation. However, Total phenolic content (%GAE) obtained from this study was considered as the total phenolic content of formulation F7.

4.4.5 Disintegration time

Disintegration time was performed using disintegration apparatus. The tablet formulation F7 exhibited a disintegration time of 50 seconds which complied with British Pharmacopoeia that the disintegration time of orodispersible tablets should not be more than 3 minutes (11).

4.4.6 Dissolution testing

The dissolution testing was performed using dissolution apparatus. Apparatus 2 (paddle) was used at the rotation speed of 75 rpm for 60 minutes with 900 ml of water as the dissolution medium. Each sample solution was then determined for the total phenolic content using Folin-Ciocalteau method. The %dissolved was compared from the total phenolic content (%GAE) of formulation F7 obtained from Section 4.4.4. The result showed that more than 86% total phenolic acid dissolved in 60 minutes as shown in Table 31.

Table 31 Total phenolic content (%GAE) and %dissolved of formulation F7

Formulation F7	%GAE	%dissolved
1	7.83	95.62
2	7.34	89.64
3	8.60	105.02
4	7.27	88.73
5	7.09	86.56
6	8.39	102.51
Av	7.75	94.69
SD	0.63	7.69
%RSD	8.10	8.12

4.4.7 Water determination

Water determination was performed using Karl Fischer Titrator. The average water determination of formulation F7 was 2.00% (n=2).

4.4.8 Uniformity of mass

The average weight of 20 tablets was 0.6068 mg and there was no tablet deviated for more than 5%. It complied with British Pharmacopoeia on Uniformity of Mass which stated that not more than 2 of the individual tablet deviates more than 5% of the average mass and none deviates by more than 10%(11).

4.4.9 Content uniformity

Content uniformity of tablets formulation F7 was performed using HPLC method. The content of markers in the individual tablet are shown in Table 32. The Acceptance Value (AV) obtained from formulation F7 were 6.0, 6.0 and 11.8 for gallic acid, vanillic acid and ferulic acid, respectively. It complied with the United States Pharmacopeia that the Acceptance Value should not be more than 15.0 (12).

5. Stability study

The stability study was performed in accordance with the ASEAN Guideline on Stability Study of Drug Product. The developed tablets were individually packed in well-sealed aluminium bags (Figure 44). The stability studies were performed under the accelerated and long term storage condition of 0, 3 and 6 months. The samples were evaluated for the quality as in Section 4.4.1.2 and Sections 4.4.2-4.4.8 at 0, 3 and 6 months.

Table 32 Content uniformity of formulation F7

	Gallic acid		Vanillic acid		Ferulic acid	
	mg/tab	**%la**	**mg/tab**	**%la**	**mg/tab**	**%la**
1	0.1652	103.9	0.1516	103.9	0.0325	97.7
2	0.1673	105.3	0.1538	105.4	0.0344	103.5
3	0.1616	101.7	0.1487	101.9	0.0321	96.6
4	0.1564	98.4	0.1444	99.0	0.0311	93.6
5	0.1603	100.9	0.1471	100.8	0.0316	94.9
6	0.1543	97.1	0.1452	99.5	0.0335	100.8
7	0.1637	103.0	0.1532	105.0	0.0319	96.0
8	0.1584	99.7	0.1481	101.5	0.0346	104.0
9	0.1594	100.3	0.1498	10.6	0.0348	104.6
10	0.1590	100.1	0.1497	102.6	0.0360	108.3
Av	0.1606	101.0	0.1492	102.2	0.0333	100.00
SD	0.0040	2.5	0.0031	2.2	0.0016	4.93
%RSD	2.48	2.48	2.10	2.10	4.93	4.98
AV	-	6.00	-	5.98	-	11.83

Figure 44 Tablets formulation F7 in well-sealed aluminium bags

5.1 Analysis of orodispesible tablet formulation

The quantity of gallic acid, vanillic acid and ferulic acid were analyzed at the initial period, 3 months storage and 6 months storage. The result showed that at 3 months under accelerated storage condition, gallic acid, vanillic acid and ferulic acid were reduced -0.4%, -6.5% and -19.0%, respectively (Table 33). The tablets formulation F7 failed to comply with ASEAN Guideline on Stability Study of Drug Product under accelerated storage condition, which stated that not more than 5% change in assay from its initial value. According to ASEAN Guideline on Stability Study of Drug Product, the stability study under the accelerated condition should not be further evaluated. The stability study should be further performed under long term storage condition of 30°C±2°C/75%RH±5%RH. The result showed that under long term storage condition, quantity of all three markers changed not more than 5% at 3 and 6 months storage condition (Table 34) which complied with ASEAN Guideline on Stability Study of Drug Product.

Table 33 Quantity of the analytical markers in tablet formulation F7 at initial and 3 months under accelerated storage condition

Analytical marker	initial		3 months	
	mg/tab	% change	mg/tab	%change
gallic acid	0.1598	-	0.1592	-0.4
vanillic acid	0.1395	-	0.1304	-6.5
ferulic acid	0.0326	-	0.0264	-19.0

Table 34 Quantity of the analytical markers in tablet formulation F7 at initial, 3 months and 6 months under long term storage condition

Analytical marker	initial		3 months		6months	
	mg/tab	% change	mg/tab	%change	mg/tab	%change
gallic acid	0.1598	-	0.1622	+1.5	0.1537	-3.8
vanillic acid	0.1395	-	0.1394	-0.7	0.1458	+4.5
ferulic acid	0.0326	-	0.0329	+0.9	0.0330	+1.2

5.2 Tablet hardness

At the initial period, formulation F7 obtained tablet hardness of 5.4±0.4 Kg-force. The tablet hardness were 5.2±0.4 Kg-force at 3 months under accelerated storage condition and 5.1±0.2 Kg-force and 5.1±0.4 Kg-force at 3 months and 6 months under long term storage condition, respectively. The change was not significant and could be conducted that the hardness remained constant up to 6 months in designed package.

5.3 Friability

At the initial period, formulation F7 obtained %friability of 0.09%. The %friability were 0.15% at 3 months under accelerated storage condition and 0.09% and 0.10% at 3 months and 6 months under long term storage condition, respectively. It complied with the United States Pharmacopeia that %Friability of the tablet should not exceed 1.0% (12).

5.4 Total phenolic content determination

At the initial period, formulation F7 obtained total phenolic content (%GAE) of 8.19%. Total phenolic content (%GAE) changed -16.5% at 3 months under accelerated storage condition and -8.8% and -6.6% at 3 month and 6 month under long term storage condition, respectively. The result showed that total phenolic content (%GAE) of formulation F7 was decreased by the storage time. From the previous study of Sotanaphun et al., the stability of NVK-E was performed under the accelerated storage condition, the result showed that NVK-E at the initial period and under the storage of 4 and 6 months did not change significantly (not more than 5% change) (8). The reduction of total phenolic content (%GAE) during the storage in this study might be because of the variation of the extraction of phenolic compounds from formulation F7 as occurred at initial period or might be because there was an interaction between phenolic compounds and excipients in the formulation. Total phenolic content (%GAE) of formulation F7 at initial, 3 months and 6 months under accelerated condition and long term storage condition are shown in Table 35 and Table 36, respectively.

Table 35 Total phenolic content (%GAE) of formulation F7 at initial and 3 months under accelerated storage condition

Initial		3 months	
%GAE	%changed	%GAE	%changed
8.19	-	6.84	-16.5

Table 36 Total phenolic content (%GAE) of formulation F7 at initial, 3 months and 6 months under long term storage condition

Initial		3 months		6 months	
%GAE	%changed	%GAE	%changed	%GAE	%changed
8.19	-	7.47	-8.8	7.65	-6.6

5.5 Disintegration time

At the initial period, formulation F7 obtained disintegration time of 50 seconds. The disintegration time were 80 seconds at 3 month under accelerated storage condition and 70 seconds and 75 seconds at 3 month and 6 month under long term storage condition, respectively. The disintegration time of formulation F7 increased by the storage time at both accelerated and long term storage condition. However, it complied with the British Pharmacopeia that the disintegration time of orodispersible tablets should not be more than 3 minutes (11).

5.6 Dissolution testing

The average %dissolved of formulation F7 was 94.7% at the initial period. The average %dissolved were 82.05% at 3 months under accelerated storage condition and 81.84% and 86.90% at 3 months and 6 months under long term storage condition, respectively. The result showed that the dissolution of formulation F7 was decreased from the initial period in both accelerated and long term storage condition which might be due to the reduction of total phenolic content (%GAE) determination of formulation F7 as described in Section 5.4. Total phenolic content (%GAE) and %dissolved of formulation F7 at accelerated condition and long term storage condition are shown in Table 37 and Table 38, respectively.

Table 37 Total phenolic content (%GAE) and %dissolved of formulation F7 at initial and 3 months under accelerated storage condition

Formulation F7	initial		3 month	
	%GAE	%dissolved	%GAE	%dissolved
1	7.83	95.62	6.18	75.46
2	7.34	89.64	6.3	76.92
3	8.6	105.02	6.69	81.68
4	7.27	88.73	6.92	84.49
5	7.09	86.56	7.02	85.71
6	8.39	102.51	7.21	88.03
Av	7.75	94.68	6.72	82.05
SD	0.63	7.69	0.41	5.00
%RSD	8.10	8.12	6.10	6.09

Table 38 Total phenolic content (%GAE) and %dissolved of formulation F7 at initial, 3 months and 6 months under long term storage condition

Formulation F7	initial		3 month		6 month	
	%GAE	%dissolved	%GAE	%dissolved	%GAE	%dissolved
1	7.83	95.62	6.24	76.24	6.21	75.84
2	7.34	89.64	6.26	76.47	7.12	86.94
3	8.60	105.02	6.74	82.32	7.07	86.38
4	7.27	88.73	6.84	83.51	7.44	90.81
5	7.09	86.56	7.02	85.67	7.55	92.16
6	8.39	102.51	7.11	86.82	7.31	89.27
Av	7.75	94.68	6.70	81.84	7.12	86.90
SD	0.63	7.69	0.37	4.53	0.48	5.85
%RSD	8.10	8.12	5.57	5.54	6.75	6.73

5.7 Water determination

At the initial period, formulation F7 obtained average water determination of 2.0% (n=2). Average water determination of formulation F7 were 1.99% at 3 months under accelerated storage condition 1.73% and 1.75% at 3 months and 6 months under long term storage condition, respectively. This suggested that the selected packaging in this study was satisfactorily protected the formulation from external humidity.

5.8 Uniformity of mass

At the initial period, formulation F7 obtained average weight of 0.6068 mg and there was no tablet deviated for more than 5%. The average weight of formulation F7 were 0.6078 mg at 3 months under accelerated storage condition and

0.6068 mg and 0.6070 at 3 months and 6 months under long term storage condition, respectively. There was no tablet deviated for more than 5%. They complied with British Pharmacopeia on Uniformity of Mass which stated that not more than 2 of the individual weight deviates more than 5% of the average mass and none deviates by more 10% (11).

CHAPTER V
CONCLUSIONS

This research aimed to develop the orodisperdsible tablets containing Phikud Navakot extract (NVK-E), a traditional Thai herbal medicine using direct compression method. Preformulation study of NVK-E and excipients were evaluated. High performance liquid chromatography (HPLC) method was validated to determined three analytical markers, i.e. gallic acid, vanillic acid and ferulic acid in NVK-E and in the dosage form according to AOAC Guidelines for Dietary Supplements and Botanicals. Selectivity, linearity, precision and accuracy were performed and the result showed that they complied with AOAC Guidelines for Dietary Supplements and Botanicals. The orodispersible tablets containing NVK-E were developed using three superdisintegrant, i.e. Polyplasdone® XL-10, Explotab® and Ac-Di-Sol® at the different concentrations of 2%, 6% and 10% in the formulation. The quality control of orodispersible tablets were determined by monitoring the three analytical markers. The formulation with Polyplasdone® XL-10 as a superdisintegrant exhibited the shortest disintegration time, but it interfered with the analysis of the three analytical markers. Therefore, Explotab® was chosen in the orodispersible tablets formulation to perform further study because it exhibited a preferable disintegration time and did not interfere with the analysis.

The stability study of the orodispersible tablets were evaluated according to the ASEAN Guideline on Stability Study of Drug Product under the accelerated storage condition of 40°C±2°C/75%RH±5% RH. At 3 months, vanillic acid and ferulic acid were changed more than 5% from the initial value which failed to meet the requirement of the ASEAN Guideline on Stability Study of Drug Product. The stability study was then performed under the long term storage condition of 30°C±2°C/75% RH±5% RH. The result showed that there were not more than 5% changed in the amount of three analytical markers at the period of 3 and 6 months which complied with the ASEAN Guideline on Stability Study of Drug Product which stated that there should not be more than 5% changed in the assay from the initial

value. Thus, further stability study should be monitored real-time until 12 months under the long term condition for the conclusion on shelf life of this formulation F7 product.

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APPENDICES

APPENDIX A

DSC thermograms of excipients in the formulation

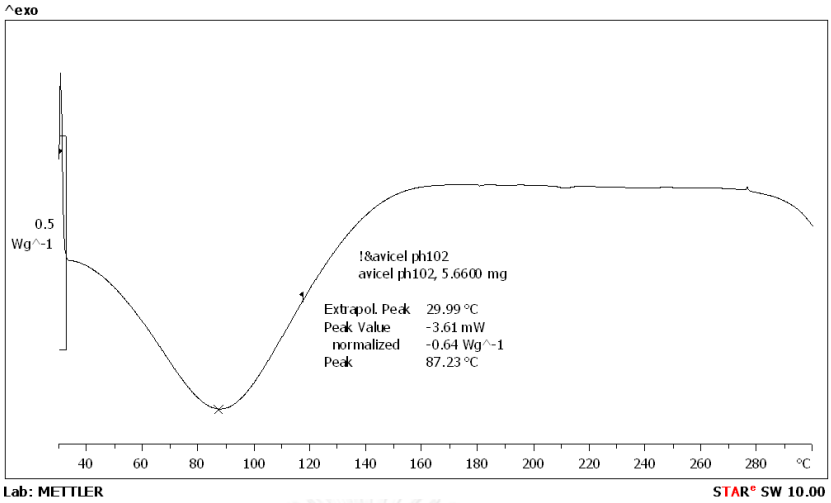

Figure A 1 DSC thermogram of Avicel® PH 102 at scanning rate of 10°C/min from 25-300°C

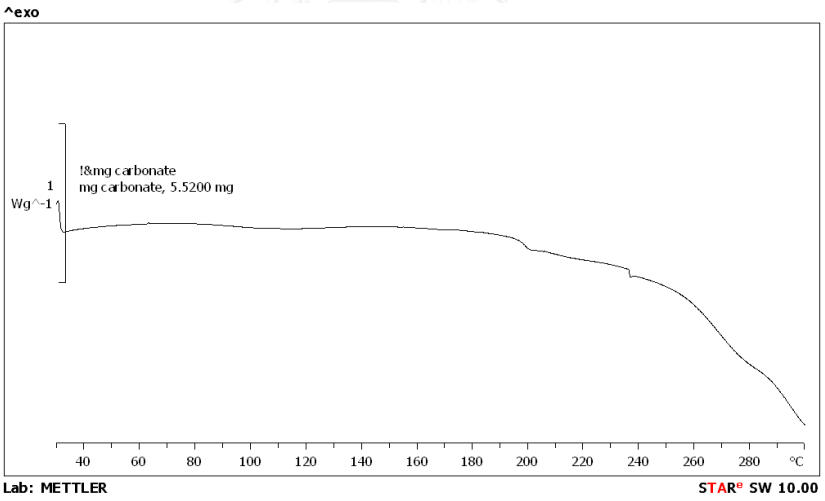

Figure A 2 DSC thermogram of magnesium carbonate at scanning rate of 10°C/min from 25-300°C

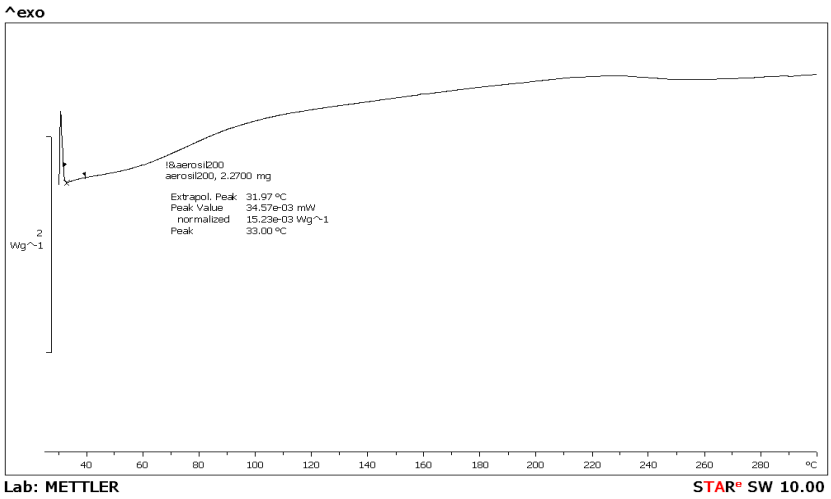

Figure A 3 DSC thermogram of Aerosil® 200 at scanning rate of 10°C/min from 25-300°C

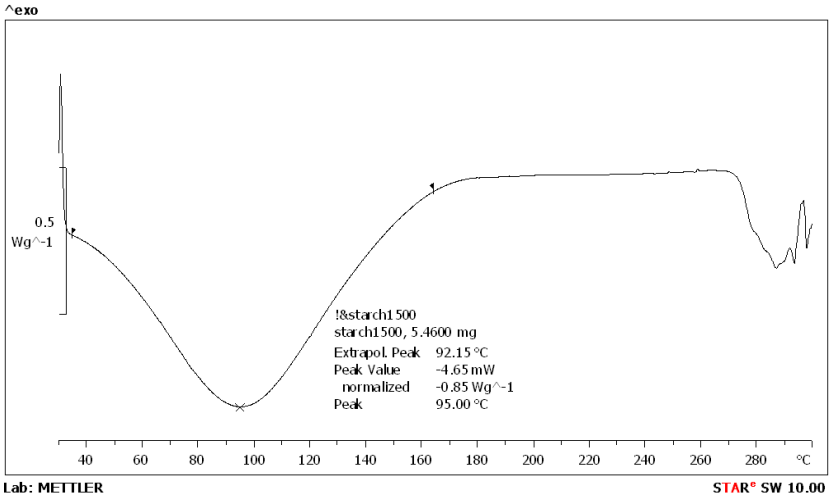

Figure A 4 DSC thermogram of starch 1500 at scanning rate of 10°C/min from 25-300°C

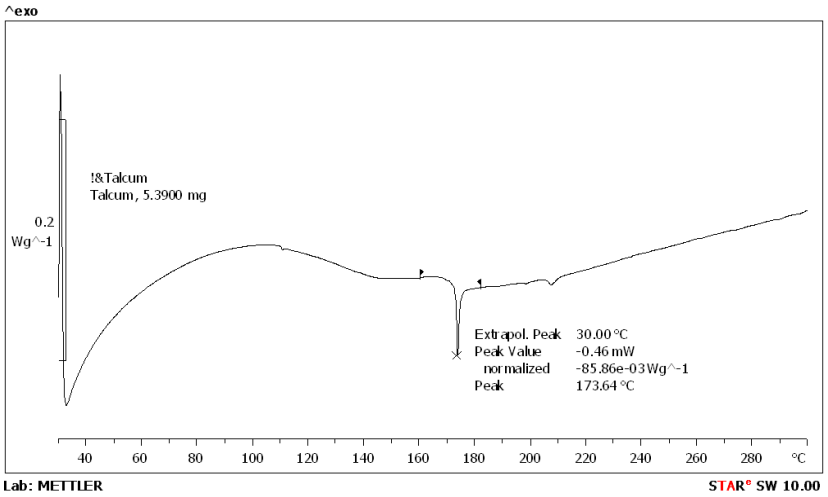

Figure A 5 DSC thermogram of talcum at scanning rate of 10°C/min from 25-300°C

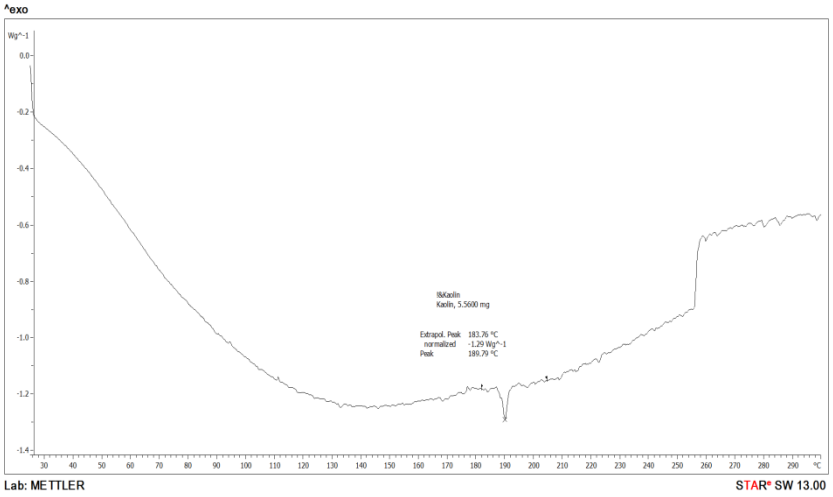

Figure A 6 DSC thermogram of kaolin at scanning rate of 10°C/min from 25-300°C

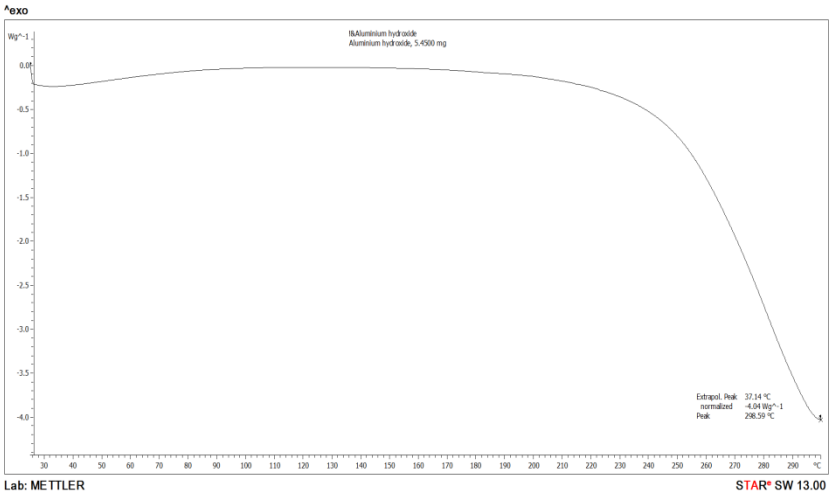

Figure A 7 DSC thermogram of aluminium hydroxide at scanning rate of 10°C/min from 25-300°C

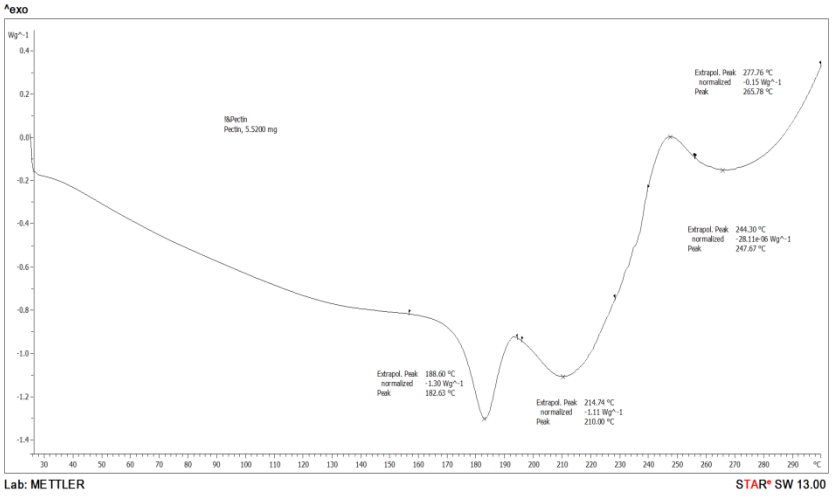

Figure A 8 DSC thermogram of pectin at scanning rate of 10°C/min from 25-300°C

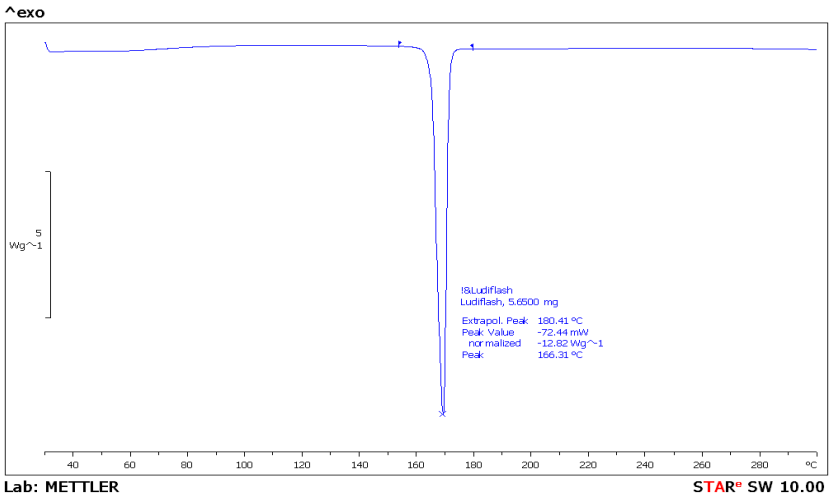

Figure A 9 DSC thermogram of Ludiflash® at scanning rate of 10°C/min from 25-300°C

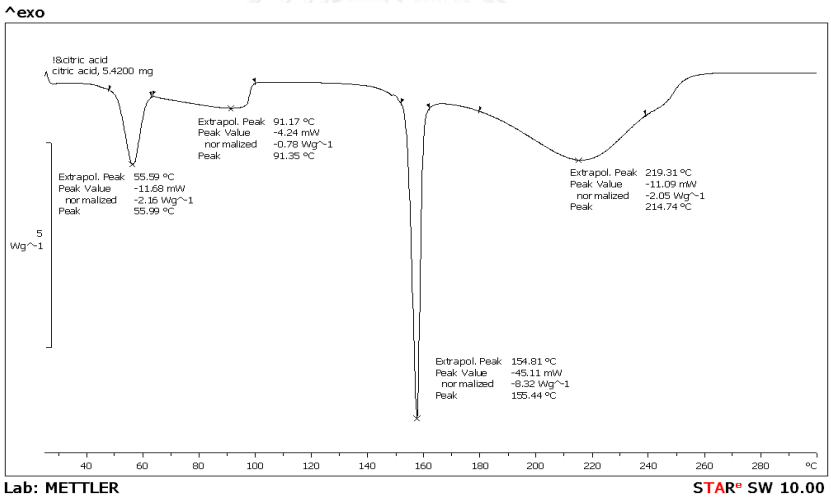

Figure A 10 DSC thermogram of citric acid at scanning rate of 10°C/min from 25-300°C

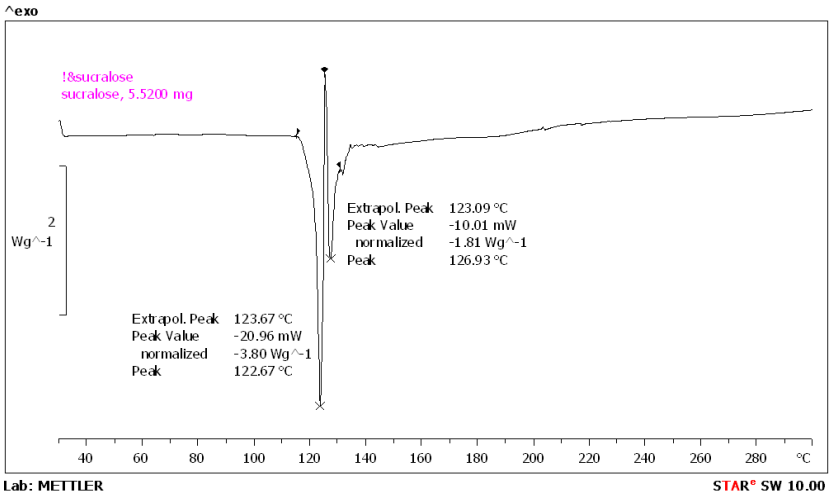

Figure A 11 DSC thermogram of sucralose at scanning rate of 10°C/min from 25-300°C

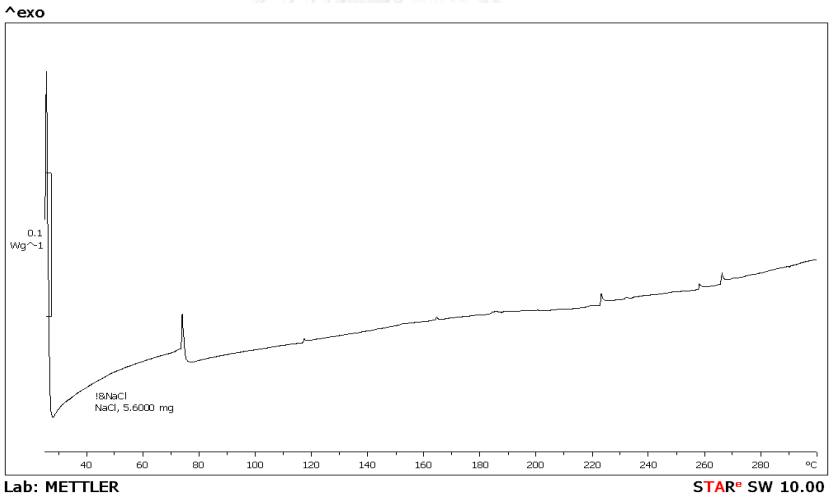

Figure A 12 DSC thermogram of sodium chloride at scanning rate of 10°C/min from 25-300°C

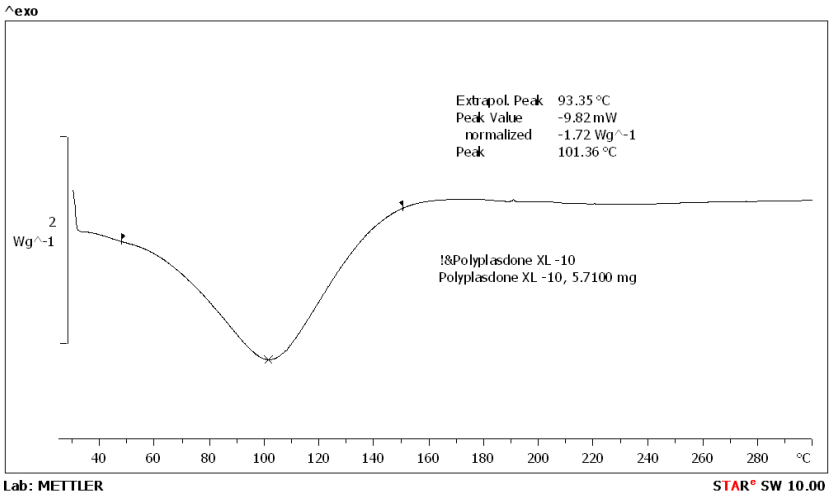

Figure A 13 DSC thermogram of Polyplasdone® XL-10 at scanning rate of 10°C/min from 25-300°C

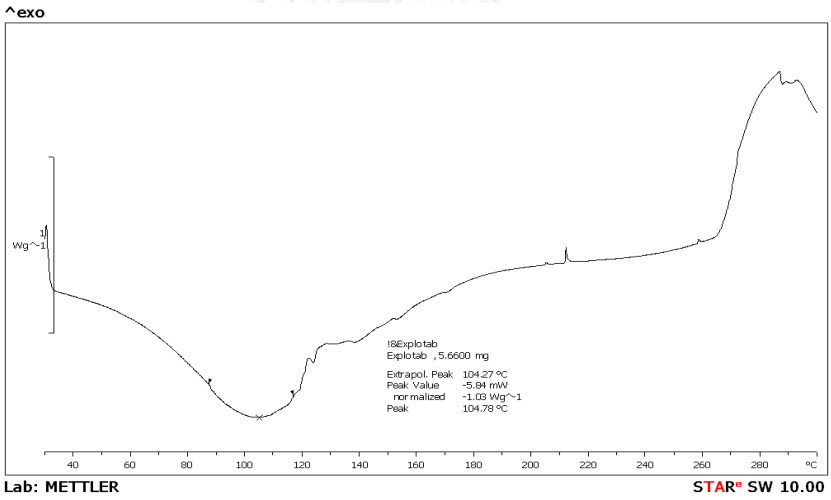

Figure A 14 DSC thermogram of Explotab® at scanning rate of 10°C/min from 25-300°C

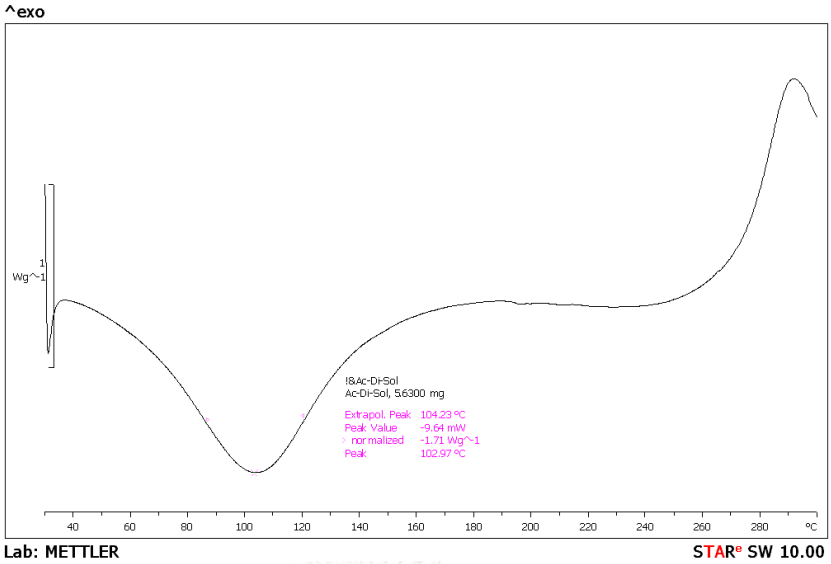

Figure A 15 DSC thermogram of Ac-Di-Sol® at scanning rate of 10°C/min from 25-300°C

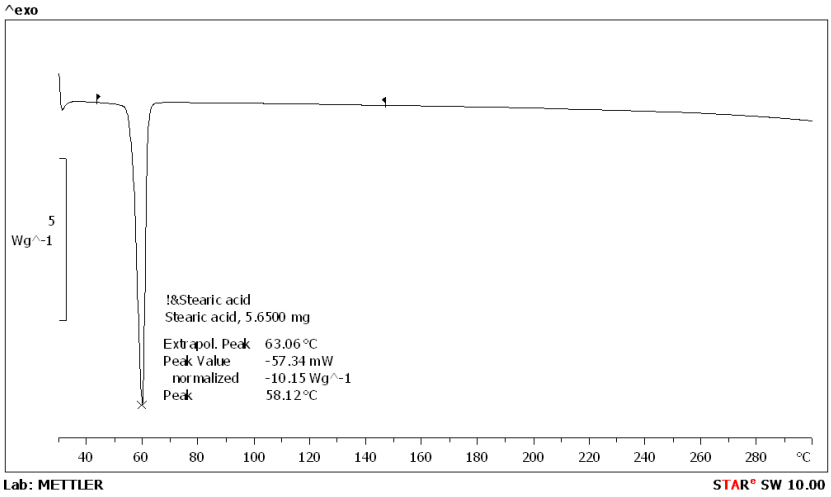

Figure A 16 DSC thermogram of stearic acid at scanning rate of 10°C/min from 25-300°C

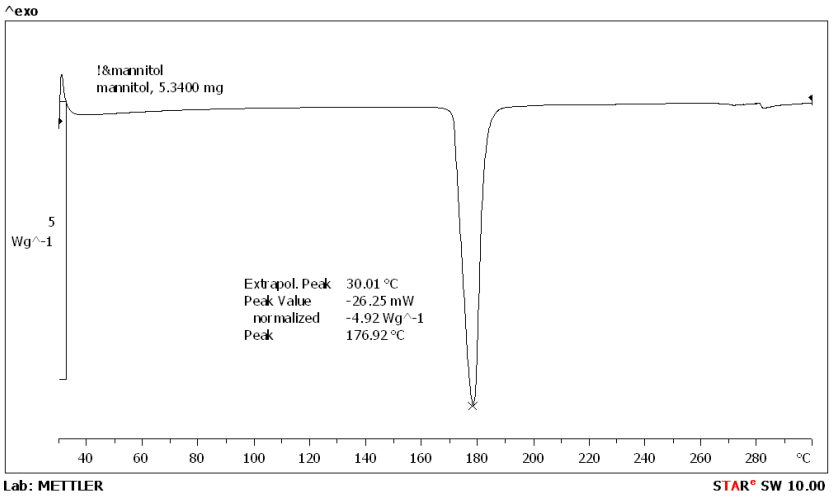

Figure A 17 DSC thermogram of mannitol at scanning rate of 10°C/min from 25-300°C

APPENDIX B

DSC thermograms of NVK-E, excipients and its mixtures

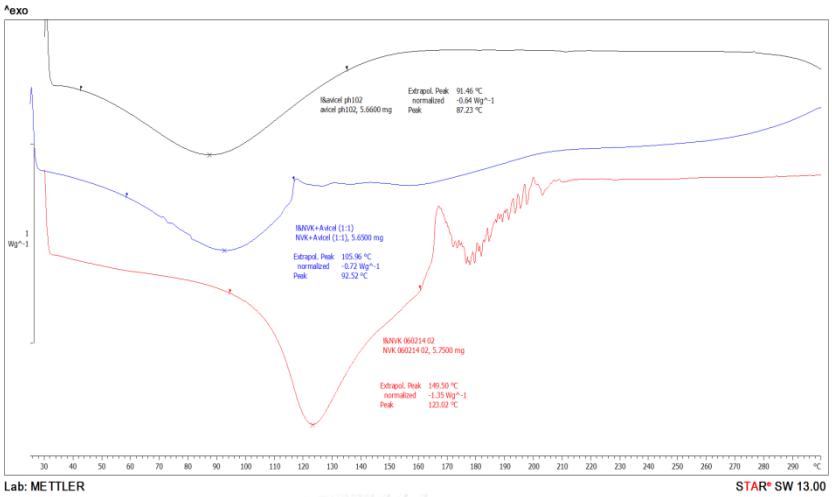

Figure B 1 DSC thermograms of NVK-E, Avicel® PH 102 and its mixture at scanning rate of 10°C/min from 25-300°C

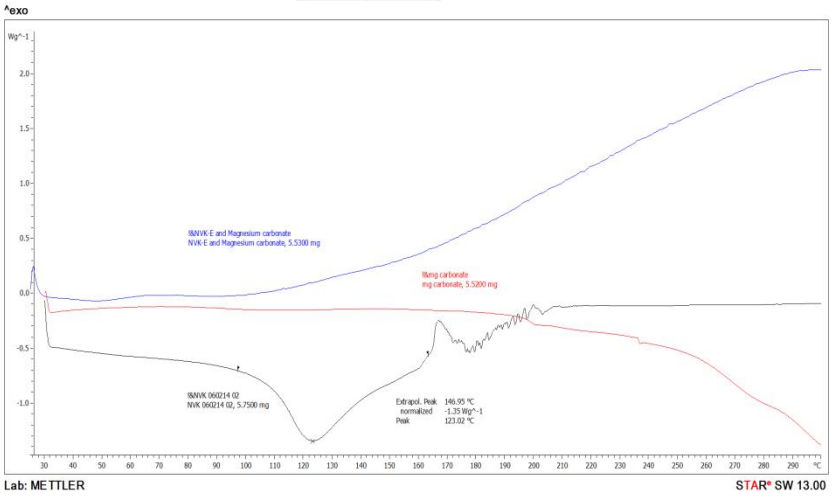

Figure B 2 DSC thermograms of NVK-E, magnesium carbonate and its mixture at scanning rate of 10°C/min from 25-300°C

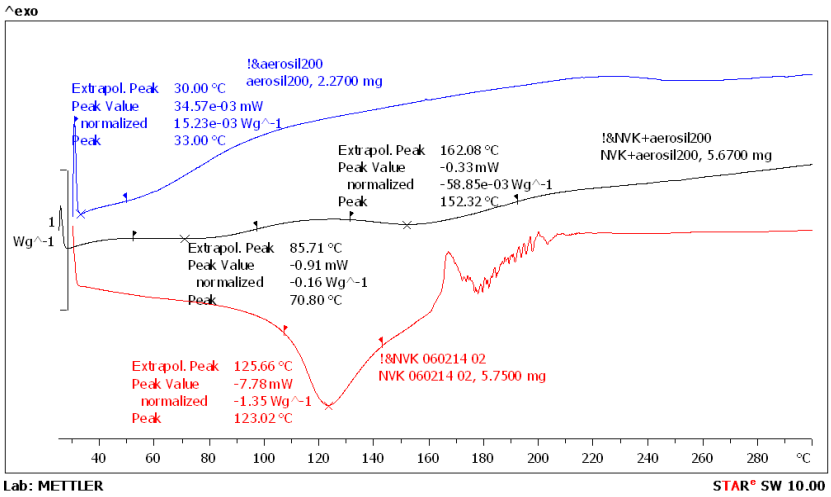

Figure B 3 DSC thermograms of NVK-E, Aerosil® 200 and its mixture at scanning rate of 10°C/min from 25-300°C

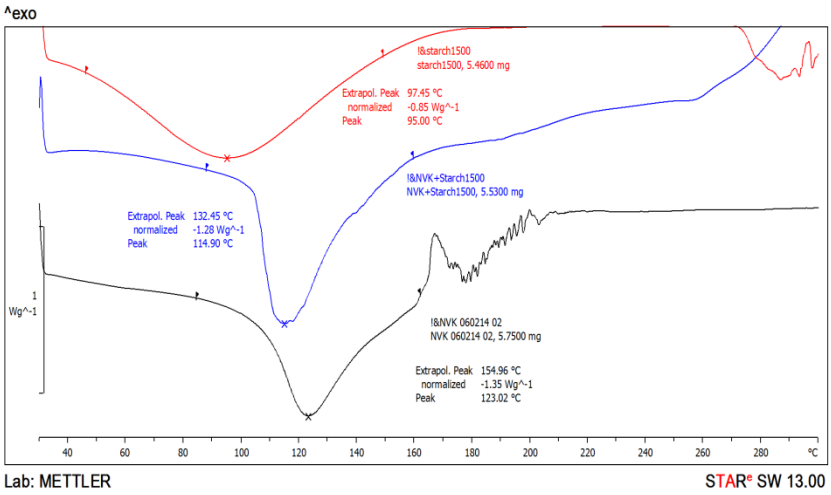

Figure B 4. DSC thermograms of NVK-E, starch 1500 and its mixture at scanning rate of 10°C/min from 25-300°C

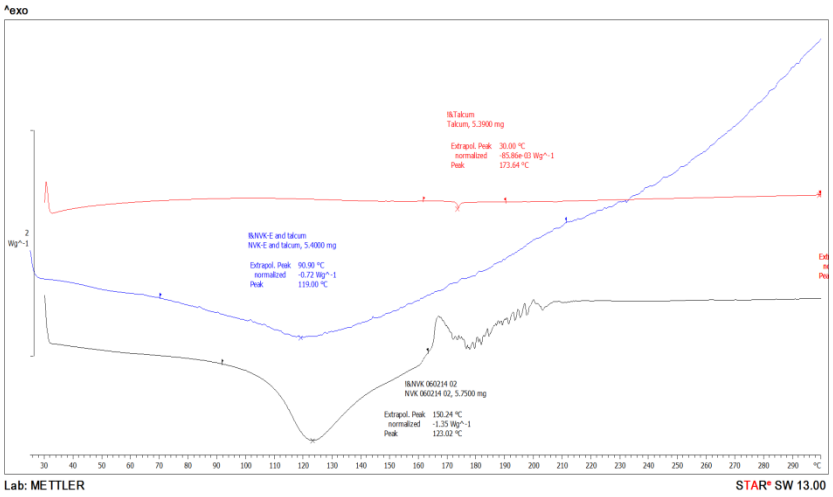

Figure B 5 DSC thermograms of NVK-E, talcum and its mixture at scanning rate of 10°C/min from 25-300°C

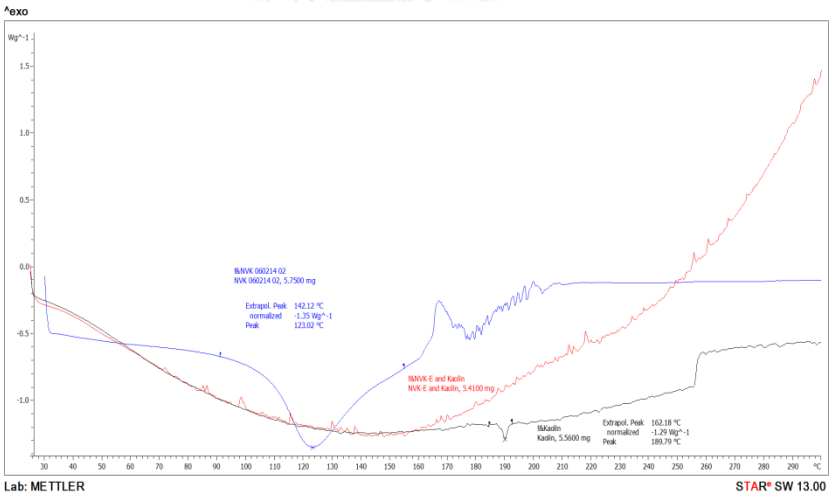

Figure B 6 DSC thermograms of NVK-E, kaolin and its mixture at scanning rate of 10°C/min from 25-300°C

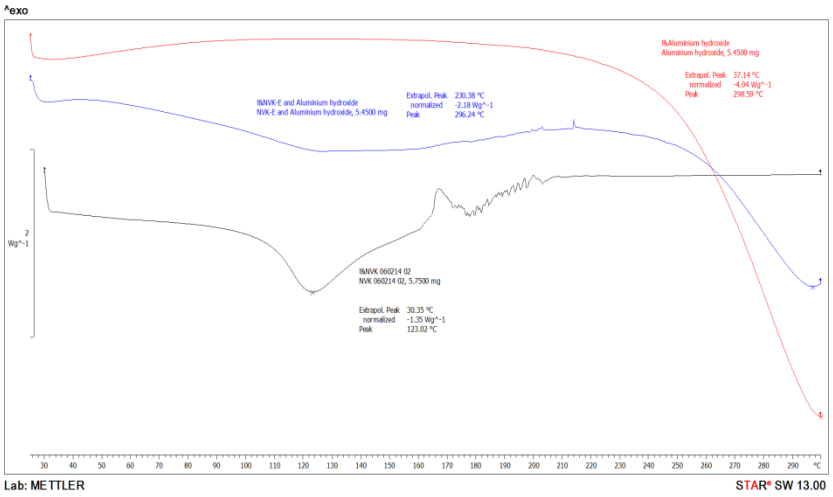

Figure B 7 DSC thermograms of NVK-E, aluminium hydroxide and its mixture at scanning rate of 10°C/min from 25-300°C

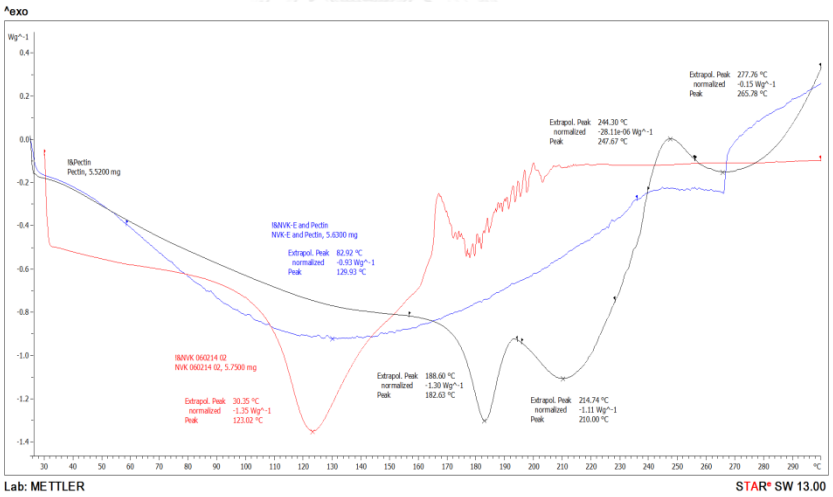

Figure B 8 DSC thermograms of NVK-E, pectin and its mixture at scanning rate of 10°C/min from 25-300°C

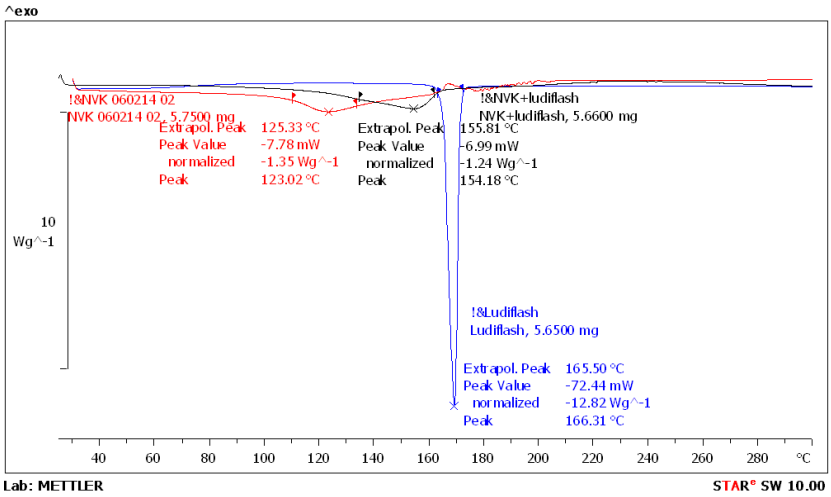

Figure B 9 DSC thermograms of NVK-E, Ludiflash® and its mixture at scanning rate of 10°C/min from 25-300°C

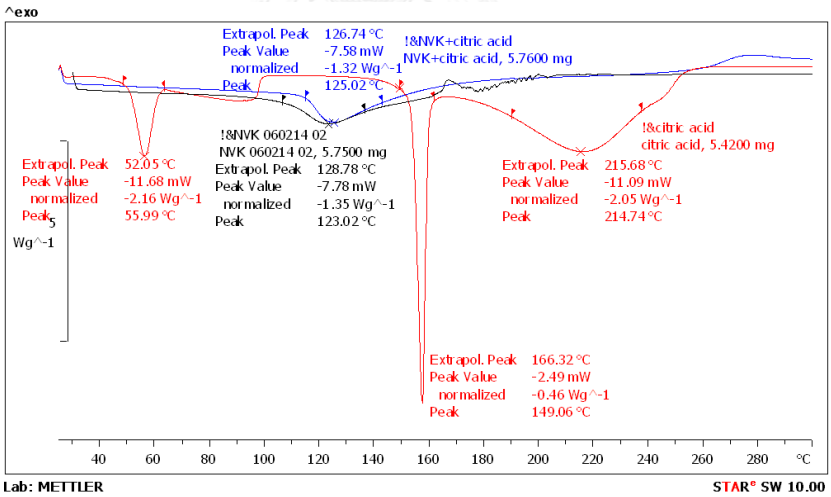

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Figure B 10 DSC thermograms of NVK-E, citric acid and its mixture at scanning rate of 10°C/min from 25-300°C

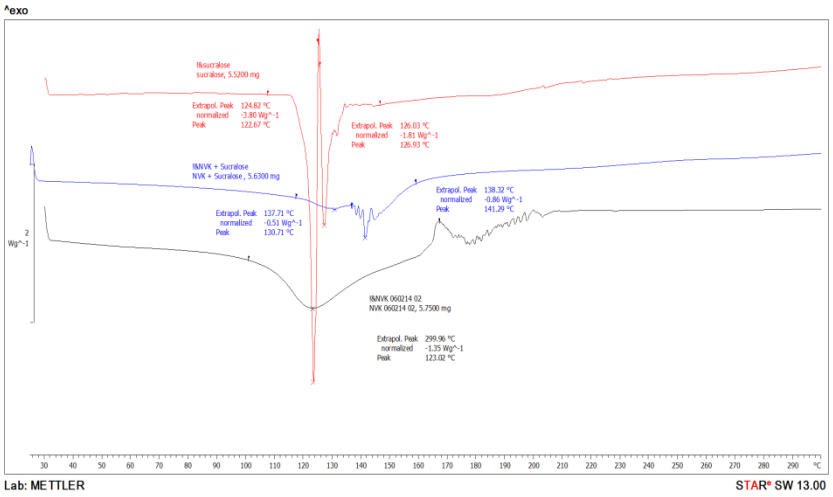

Figure B 11 DSC thermograms of NVK-E, sucralose and its mixture at scanning rate of 10°C/min from 25-300°C

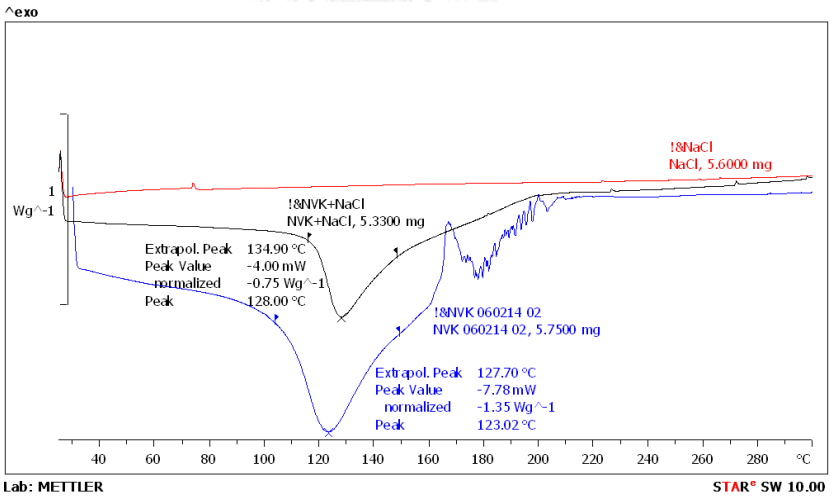

Figure B 12 DSC thermograms of NVK-E, sodium chloride and its mixture at scanning rate of 10°C/min from 25-300°C

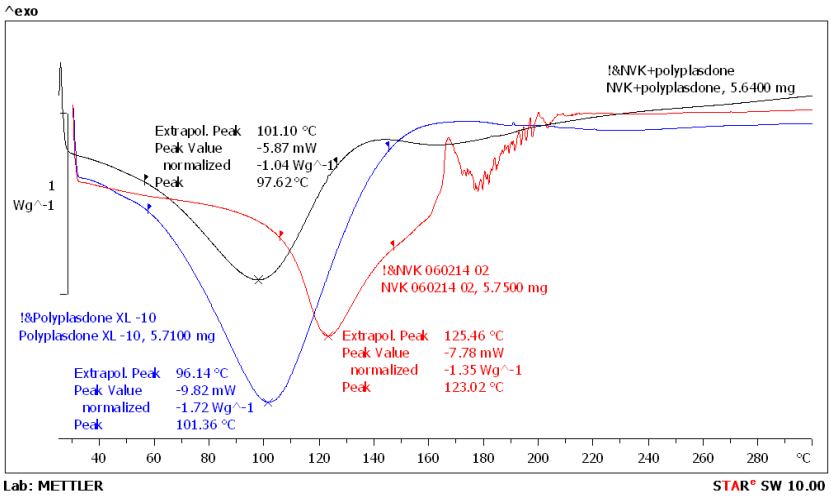

Figure B 13. DSC thermograms of NVK-E, Polyplasdone® XL-10and its mixture at scanning rate of 10°C/min from 25-300°C

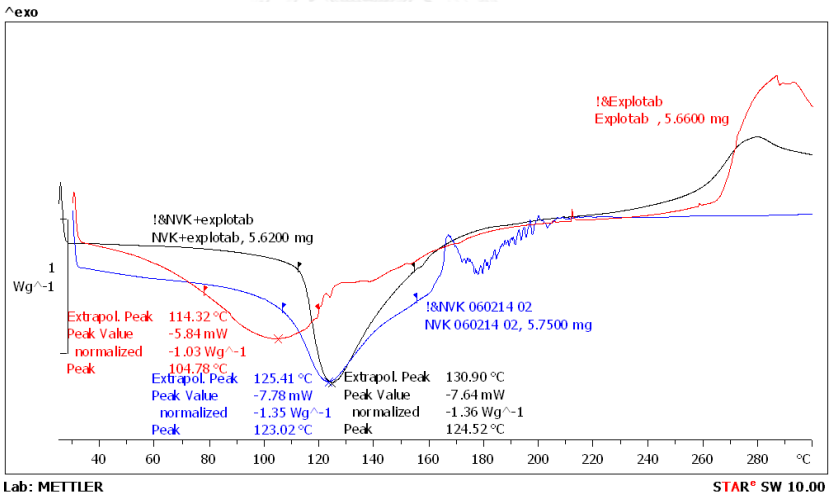

Figure B 14 DSC thermograms of NVK-E, Explotab® XL-10and its mixture at scanning rate of 10°C/min from 25-300°C

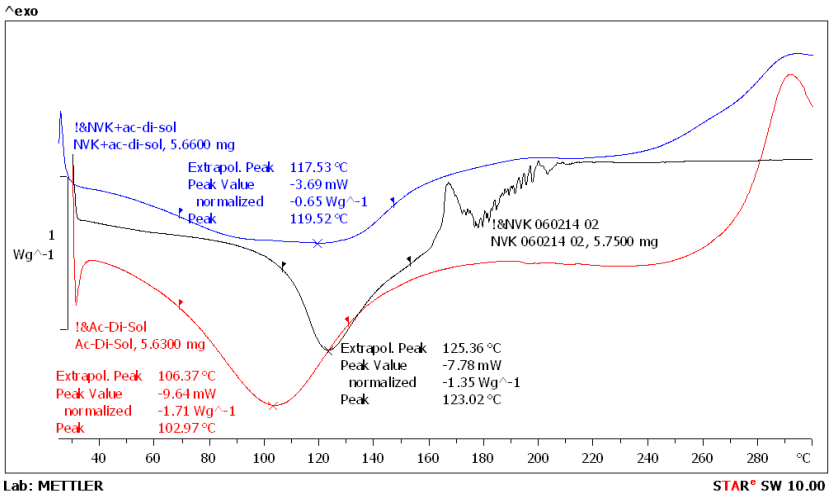

Figure B 15 DSC thermograms of NVK-E, Ac-Di-Sol® and its mixture at scanning rate of 10°C/min from 25-300°C

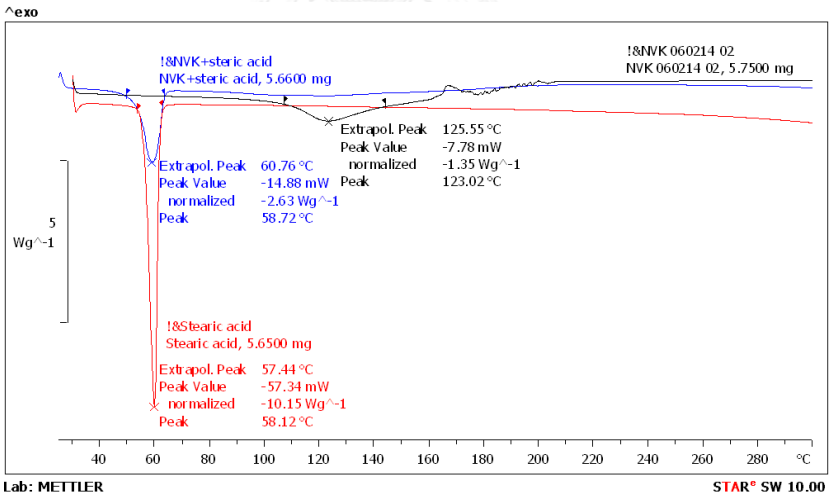

Figure B 16. DSC thermograms of NVK-E, stearic acid and its mixture at scanning rate of 10°C/min from 25-300°C

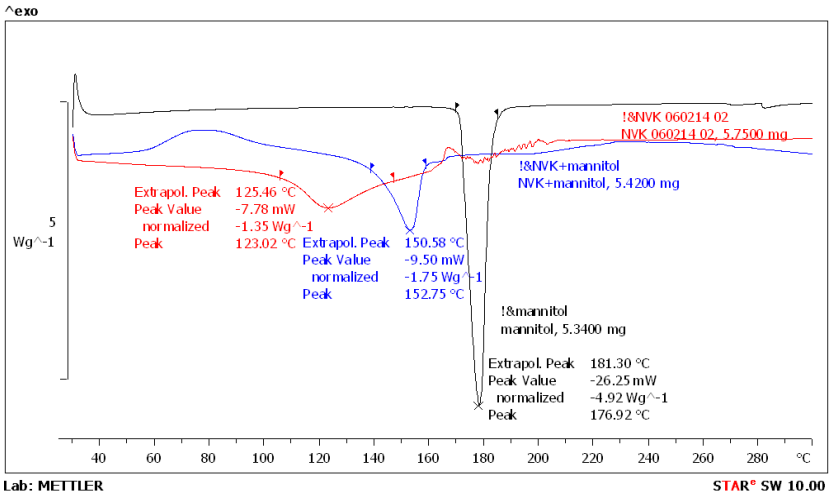

Figure B 17 DSC thermograms of NVK-E, mannitol and its mixture at scanning rate of 10°C/min from 25-300°C

VITA

Miss Pavena Kumnerdnon was born on Mrach 30, 1983 in Nonthaburi, Thailand. She graduated from Ampornpaisarn School and received her Bachelor's degree of Science in from Faculty of Pharmacy, Huachiew Chalermprakiet University in 2007. Now, she works as a pharmacist at Bureau of Drug and Nacrotic, Department of Medical Sciences, Ministry of Public Health.