การสกัดสารชาแลนทินจากผลของมะระขึ้นกด้วยตัวทำละลายที่ความดันสูง

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EXTRACTION OF CHARANTIN FROM THE FRUITS OF *MOMORDICA CHARANTIA*
USING HIGH PRESSURE SOLVENT

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ชาแลนทินเป็นสารผสมของสเตอรอยด์ ที่มีฤทธิ์ลดน้ำตาลในเลือด พบในผลของมะระขึ้นก ซึ่งเป็นพืชที่มีการ
ปลูกอย่างแพร่หลายในประเทศไทย และ ประเทศในแถบเอเชีย โดยทั่วไปสารชาแลนทินถูกสกัดด้วยวิธีซอคเลทโดยใช้ตัว
ทำละลาย คือ คลอโรฟอร์ม วิธีนี้เป็นวิธีที่ง่าย แต่อาจมีสารละลายอินทรีย์หลงเหลืออยู่ในสารสกัด ซึ่งจะก่อให้เกิดอันตราย
ต่อผู้บริโภคได้ ในการศึกษาสำหรับการสกัดสารชาแลนทินด้วยทางเลือกใหม่ ก็คือ การสกัดด้วยตัวทำละลายที่ความดันสูง
ซึ่งเป็นวิธีที่ให้ประสิทธิภาพการสกัดสูง โดยใช้เวลาการสกัดน้อย ทำให้ใช้ปริมาณตัวทำละลายน้อย ในงานวิจัยครั้งนี้จะ
ทำการศึกษาผลกระทบของตัวแปรหลายตัวแปร คือ ชนิดของตัวทำละลาย อัตราส่วนของตัวทำละลายกับน้ำ อัตราการไหล
ของตัวทำละลาย (2-6 มิลลิลิตรต่อนาที) และ อุณหภูมิ (50-150 องศาเซลเซียส) ที่ความดัน 10 เมกกะปาสคาล ในขั้นตอน
การวิเคราะห์หาปริมาณสารชาแลนทินจะใช้วิธีโครมาโทกราฟีของเหลวสมรรถนะสูงซึ่งวิธีนี้มีความจำเป็นที่จะต้องมีการ
กำจัดคลอโรฟิล และ น้ำตาลที่อยู่ในสารสกัด ซึ่งจะรบกวนการวิเคราะห์ ด้วยการล้างด้วย อัตราส่วน 50 ต่อ 50 และ 70 ต่อ
30 ของเมทานอลกับน้ำ และ เฮกเซน ตามลำดับ จากผลการทดลอง อัตราการสกัด ขึ้นอยู่กับ ชนิดของตัวทำลาย โดย พบว่า
อะซีโตน กับ เอทานอล ให้อัตราการสกัดที่สูงกว่า เอทิล อะซีเตด ไดคลอโรมีเทน และ น้ำ โดยอัตราการสกัดจะสูงขึ้นเมื่อ
อุณหภูมิสูงขึ้น และพบว่า การสกัดด้วยตัวทำละลายที่ความดันสูง ด้วยตัวทำละลาย คือ อัตราส่วน 50 ต่อ 50 ของเอทานอล
กับน้ำ ที่อุณหภูมิ 100 องศาเซลเซียส และ อัตราการไหลของตัวทำละลาย 2 มิลลิลิตรต่อนาที ให้ประสิทธิภาพที่สูงกว่า
สกัดด้วยวิธีซอคเลท โดยพบว่า ใช้เวลาการสกัด และ ปริมาณตัวทำละลายน้อยกว่าวิธีซอคเลท

ภาควิชา	วิศวกรรมเคมี	ลายมือชื่อนิสิต..
สาขาวิชา	วิศวกรรมเคมี	ลายมือชื่ออาจารย์ที่ปรึกษา..
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Charantin is a mixture two steroid compounds, which has been shown to possess antidiabetic activity. Charantin can be to found in fruits of *Momordica charantia* (bitter melon), prevalently grown in Thailand and other Asian countries. The compound is highly soluble in chloroform thus extraction of such compound was conventionally carried out in the solvent using a Soxhlet apparatus. This process is simple however, the product may be toxic if chloroform residue is left in the extract. In this study, a more benign alternative for charantin extraction accelerated solvent extraction (ASE) with ethyl alcohol was proposed. Experiments were conducted to determine the effects of several factors including type of solvent (water, ethanol, acetone, dichloromethane, and ethyl acetate), solvent composition, flow rate (2-6 ml/min), temperature (50-150 ^{o}C), while the pressure was maintained at 10 MPa. The quantitative analysis of charantin was performed with high performance liquid chromatography (HPLC). Prior to the analysis, however a purification step was required to remove chlorophylls and sugars that could interfere with the analysis. This was achieved by washing the crude extract with 50% methanol, followed by 70% methanol, and hexane, respectively. The results from this study reveal that the efficiency of accelerated solvent extraction depends on the type of solvent used. Acetone and ethanol gave higher efficiency than ethyl acetate, dichloromethane, and water. Extraction yield was also found to increase as temperature increased and similar yields could be achieved using either pure ethanol or a 50% ethanol solution in water. Compared with Soxhlet extraction, ASE was shown to permit high extraction efficiency as it requires low solvent volume and short extraction time.

Department   Chemical Engineering   Student's signature…………………….

Field of study   Chemical Engineering   Advisor's signature…………………….

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CHAPTER I

INTRODUCTION

1.1 Rationale

Bitter melon *(Momordica charantia)*, a vegetable indigenous to tropical regions of Asia, belonging to the Cucurbitaceae family, contains an array of biologically active phytochemicals. These include triterpenes, proteins and steroids. Fruit and seeds of bitter melon are traditionally used as medicinal herbs as, anti-HIV, anti-ulcer, anti-viral, anti-asthenic, anti-inflammatory, anti-leukemic, anti-microbial, anti-mutagenic, anti-mycobacterial, anti-osteoporotic, anti-diabetic, anti-tumor, aperitive and aphrodisiac agents (Taylor., 2002). Of particular interest, unripe fruit of the bitter melon have been found to have blood sugar lowering capacity, similar to that of insulin. The component from *Momordica charantia* that is responsible for this action is charantin, which is a mixture of two compounds, namely, sitosteryl glucoside and stigmasteryl glucoside.

Charantin has been conventionally extracted with chloroform using Soxhlet apparatus. This process must then be followed by evaporation to separate chloroform from the product. This process is simple however the product may be toxic if chloroform residue is left in the extract. Alternatively, more acceptable organic solvent such as ethanol can be used, but this requires longer extraction time as ethanol is an inferior solubilizing agent to chloroform. Recently, other benign solvents have been the focus of investigation in extraction of active compounds from natural products. Supercritical carbon dioxide is an interesting new solvent for extraction because the operating temperatures for supercritical carbon dioxide extraction are generally low (about 40-60 $^{\circ}$C), thus the method is appropriate for extraction of many thermally labile compounds. Moreover, the method leaves no solvent residue in the product. Despite these advantages, it has been found that large compounds with high molecular weight such as charantin (MW=578) is only slightly soluble in supercritical carbon dioxide. Alternatively, pressurized hot water (PHW) has been shown to be

another promising benign solvent for extraction of natural compounds. In pressurized hot water extraction, water is maintained in the liquid state even at the temperature higher than the boiling point temperature. The high temperature enables high solubility of solutes in the solvent as water polarity can be lowered as the temperature increases to the values close to those of organic solvents. Extraction with PHW has been shown to be effective for several medicinal compounds, specifically for essential oils from majoram (Jimenez-Carmona et al., 1999), savory and peppermint (Kubatova et al., 2001), and oregano (Ayala, et al., 2001). Other than essential oils, other bioactive compounds have been extracted by this technique. They are hypericin and pseudohypericin from St. John's wort (Mannila et al., 2002), iridoid glycosides from *Veronica lonifolia* (Suomi et al, 2000), and kava lactones from kava roots (Kubatova et al., 2001). The temperatures for PHW extraction used in these investigations were between 100 and 250 °C. However, based on our preliminary experiments for extraction of charantin, to obtain the solute solubility in pressurized hot water close to that of chloroform at room temperature, the water temperature must be as high as 400 ^{o} C. At such temperature, water is highly corrosive and is not appropriate for extraction of natural compounds.

In this study, we therefore propose to investigate charantin extraction from *Momordica charantia* with pressurized hot organic solvents that are regarded as safe. This method was sometimes called Accelerated Solvent Extraction (ASE) or Pressurized Solvent Extraction (PSE). Compared to PHWE, PSE would require lower temperatures to establish a solvent polarity similar that of chloroform. The method has been shown to permit high extraction efficiency with low solvent volume and short extraction time because the solvents are used near their critical region where they have high extraction properties. Successful cases have been reported for fatty acids from wheat germ oil (Dunford et al., 2003), furanocoumarins from *Pastinaca sativa* (Waksmundzka-Hajnos et al., 2004) and zearalenone and α-zearalenol from wheat (Urraca et al., 2004). The objective of this study is to investigate the effect of solvent types, composition of solvents, temperature, and flow rate on ASE of charantin. This fundamental information is necessary for selecting the optimal operational conditions. The yield of the product will be compared with that obtained by Soxhlet extraction.

1.2 Objectives

1.2.1 To determine the feasibility of using pressurized solvent to extract charantin from the fruits of *Momordica charantia*.

1.2.2 To establish appropriate protocol for the analysis of charantin extracted with high performance liquid chromatography.

1.2.3 To compare the efficiency of accelerated solvent extraction with that of conventional Soxhlet extraction.

1.2.4 To determine the appropriate temperature, type of solvents, flow rate solvent and composition of solvent mixtures for accelerated solvent extraction of charantin from *Momordica charantia*.

1.3 Expected benefits

1.3.1 This study provides a new benign alternative for extraction of high quality charantin.

1.3.2 This study provides fundamental information useful for large scale industrial processes.

1.4 Working scopes

1.4.1 Establishment of an appropriate method for the analysis of charantin with high performance liquid chromatography.

1.4.2 Investigation of the effect of types of solvents, flow rate solvent, composition of solvent mixtures and the operating temperatures on accelerated solvent extraction of charantin from *Momordica charantia*.

CHAPTER II

BACKGROUNDS AND LITERATURE REVIEWS

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels. Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas, which help the body convert sugar, starches and other compounds into necessary energy. In diabetic patients, the pancreases undergo auto-immune attack, thus either stop producing insulin or still do so but inadequately. As a result, the patients blood sugar rises. This causes several chronic symptoms such as non-congenital blindness, kidney failure, and the risk of heart disease.

The common treatment for diabetes is injection of insulin (generally synthetic), which helps maintain blood sugar within a normal range. However, the pancreas of an insulin injected patient cannot simulate the pancreas of a healthy individual (Belinda, 2000). In some cases, the injected patient shows signs of side effects. As a result, plant drugs are frequently considered as less toxic alternative medicines which are free from side effects.

Some medicinal plants which have been reported to be beneficial to patients with diabetes mellitus are *Aegle marmelos L.* (Sharma et al.,1996), *Camellia sinensis L.* (Gomes et al.,1995), *Lythrum salicaria L.* (Lamela et al.,1985), *Achyranthes aspera L.* (Akhtar et al.,1991), *Momordica charantia L.* (Ahmed et al.,2001). This research concerns the extraction of anti-diabetic compound, charantin, from *Momordica charantia L.*, due to its local abundance.

2.1 *Momordica charantia*

2.1.1 Botanical description *Momordica charantia*

Momordica charantia commonly known as bitter gourd, Karela, Balsam pear, bitter cucumber and bitter melon (Belinda., 2000), is a medicinal plant belonging to the Cucurbitaceae family. *Momordica charantia* grows in tropical areas, including parts of Asia. It's a slender, high-climbing vine, with slightly fuzzy dark green stems.

The flowers have 5 petals and are about an inch in diameter. The fruit appears as a warty gourd, usually oblong and resembling a small cucumber. The young fruit is emerald green, turning to orange-yellow when ripe and then becoming soft and opening to reveal pendulous red seeds. All parts of the plant, including the fruit, taste very bitter.

In Thailand, there are two different types of *Momordica charantin*: Ma-ra-chinese and Ma-ra-khee-nok. The first type has smooth light green fruit with average length of 15-25 cm. And the second type has rough dark green fruit with average length of 5-8 cm.
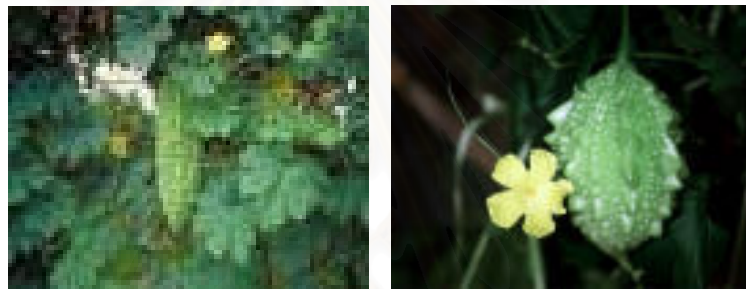
Figure 2.1 Types of *Momordica charantia*

2.1.2 Biological activities of *Momordica charantia*

Momordica charantia contains three different groups of biologically active phytochemicals such as triterpenes, proteins, and steroids. In various studies, *Momordica charantia* fruit has been shown to reduce the total cholesterol and triglycerides both in the presence and absence of dietary cholesterol (Jayasooriya et al., 2000; Ahmed et al., 2001). Furthermore, protein found in *Momordica charantia*, namely MAP-30, was proven useful for treating HIV infections. Other biological activities in *Momordica charantia* are summarized in Table 2.1. Of a particular interest in this study, the hypoglycemic chemicals in *Momordica charantia*, charantin, a mixture of two steroids, contained mainly in the fruit, has been proven for its ability to promote insulin release and to potentiate the effect of insulin (Ali et al., 1993; Viklant et al., 2001).

Table 2.1 Biological activities of extracts from various parts of *M. charantia*.

Part	Activities		Type extract	
Fruit	1.	Antimutagenic	1.	ETOH(80%),CHCl$_3$, Hexane,MEOH Extract
	2.	DNA Adduct Formation inhibition	2.	ETOH (80%) Extract
	3.	Antihyperglycemic	3.	Hot H$_2$O Extract
	4.	Hypoglycemic	4.	Hot H$_2$O Extract
	5.	Cytochrome P450	5.	Fruit
	6.	Glutathione-s-transferase induction	6.	Fruit
	7.	Aminopyrine-de-methylase Inhibition	7.	Fruit
	8.	AnilineHydroxylase Inhibition	8.	Fruit
	9.	BenzopyreneMetabolism Inhibition	9.	Fruit
	10.	Aflatoxin Inactivation	10.	Fruit
	11.	Chondrogenesis Inhibition	11.	MEOH Extract
	12.	Antimicrobial	12.	Hot H$_2$O Extract
	13.	Antibacterial	13.	Solvent Extract
	14.	Antimycobacterial	14.	Solvent Extract
	15.	Antiyeast	15.	Solvent Extract
	16	Insecticide	16.	Solvent Extract

Source : Taylor, 2002

2.2 Charantin

Charantin is hypoglycemic compound which is a mixture of two compounds namely, sitosteryl glucoside ($C_{35}H_{60}O_6$) and stigmasteryl glucoside ($C_{35}H_{58}O_6$). The chemical structures of these compounds are shown in Figure 2.2 and Figure 2.3. The molecular weight of charantin is 578.434 and the compound is slightly polar, and the melting point is about 243.5°C.
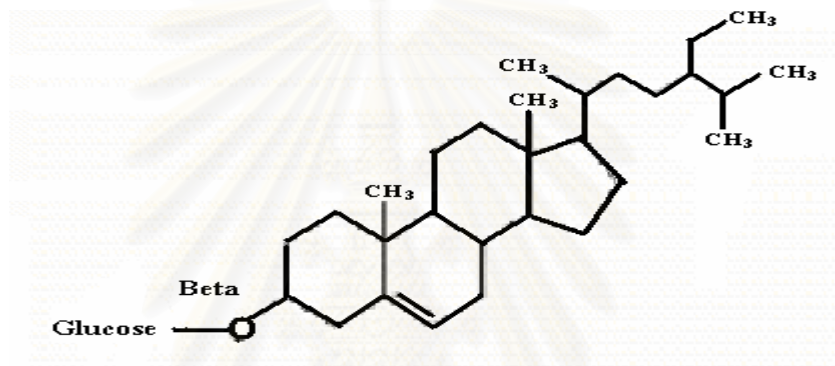
Figure 2.2 Chemical structure of sitosteryl glucoside.
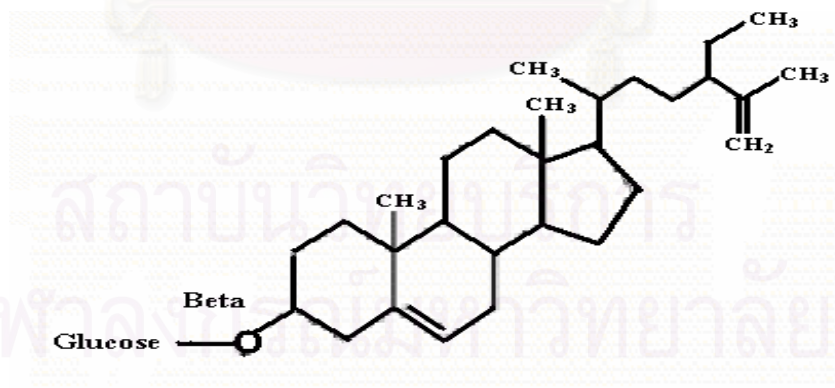
Figure 2.3 Chemical structure of stigmasteryl glucoside.

2.3 Mass transfer mechanism

The mechanism of mass transfer in solvent extraction of natural materials involves 5 steps (Figure 2.4).
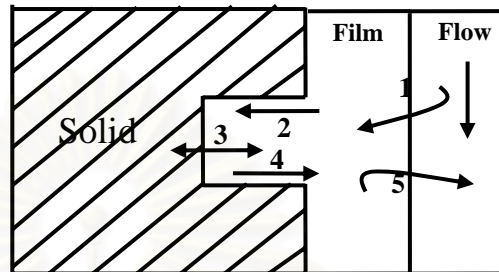
Figure 2.4 Mass transfer mechanisms

Step 1: Solvent molecularly transfers from bulk fluid through thin layer of the fluid that covers the surface of solid materials. The driving force of this molecular transfer is the difference in solvent concentrations between the bulk fluid and the thin fluid film. This film acts as resistance to the molecular transfer of solvent, thus the transfer rate is affected by the film thickness. The thickness of the fluid film depends on the velocity of bulk liquid solvent. For example, higher solvent velocity gives thinner film, and thus, less resistance.

Step 2: Solvent molecularly transfers from the surface of materials through interconnected voids or pores in the solid materials. The transfer rate in this step is affected by the pore-size, porosity, and tortuosity of the solid matrix.

Step 3: Solvent dissolves the solute inside the porous solid materials. This step is sometime called solubilization step or solubility step, and the degree to which this step affect the overall extraction depends on chemical properties of the solute and the solvent.

Step 4: The solution of the desired solute molecularly diffuses out of the solid materials through the porous matrix to the surface of solid materials.

Step 5: Dissolved solute transfers from the surface of the solid through the boundary layer of fluid to the bulk fluid.

2.4 Solubility

The solubility of solute depends on the interaction between the molecules of the solute and the solvent, which is dictated by the molecular structures and the activity coefficient of the solution. Generally, the solubility of solid/solvent system increases with an increase in temperature and can be approximated according to the following equation.

$$ln \ X^{SAT} = \frac{\Delta_{fus} S}{R} \left(1 - \frac{T_m}{T} \right) - ln \ \gamma^{SAT} \qquad (2.4.1)$$

where X is solubility parameter (mole fraction of the solute)

$\Delta_{fus}S$ is entropy of fusion

T_m is normal melting temperature

T is absolute temperature

R is gas constant

γ is activity coefficient

This equation shows that solute solubility depends on the temperature and the intermolecular forces between the solute and the solvent as represented by the activity coefficient. For an ideal solution, the activity coefficient is equal to 1. For non-ideal solution, activities coefficient cannot be neglected. Many solubility estimation methods can be used to estimate solubility such as Robbins chart, UNIFAC model, Hansen solubility parameter, and Margules equation. This estimation of activity coefficient can be used as a guide for solvent selection in many situations. The detailed derivation of the above equation can be found in Azevedo, 1999.

2.5 Accelerated solvent extraction

Accelerated Solvent Extraction (ASE) or Pressurized Solvent Extraction (PSE) is an extraction procedure that uses organic solvents under high pressure and high temperature in an automated system. The solid sample is placed in a stainless steel vessel into which the solvent is pumped and brought to the desired operating pressure (between 10-20 MPa) by an HPLC pump and a back-pressure regulator. The system is heated to 80-200 ^{o}C which is near the critical region of the solvent as highlighted in Figure 2.5, where the solvent has high extraction properties.

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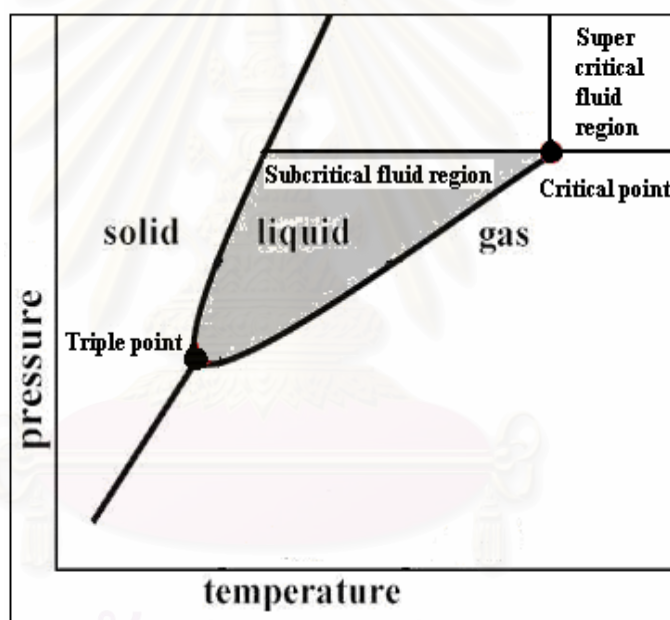
Figure 2.5 Schematic pressure-temperature diagram of critical fluid region

In that physical region, the high temperature enables high solubility and high diffusion rate of solutes in the solvent. At the pressure high enough for solvent to be maintained in the liquid state, solvent can penetrate effectively into the sample. As a result, the solubility of organic compounds in pressurized solvent increases. The critical properties for some common fluids are shown in Table 2.2.

Table 2.2 Critical properties for common fluids (Kirk-Othmer., 1997)

Solvent	T_c, °C	P_c, MPa	ρ_c, g/cm^3
Ethylene	9.3	5.04	0.22
Carbon dioxide	31.1	7.38	0.47
Ethane	32.2	4.88	0.20
Propane	96.7	4.25	0.22
Ammonia	132.5	11.28	0.24
Acetone	234.9	4.70	0.26
Methanol	239.5	8.10	0.27
Ethanol	240.8	6.14	0.28
Ethyl Acetate	250.1	3.88	0.31
Chloroform	263.2	5.47	0.50
Toluene	318.6	4.11	0.29
Dichloromethane	236.8	6.08	0.46
Water	374.2	22.05	0.32

When the temperature of a liquid increases, the density decreases, and the diffusivity increases. Both parameters are directly associated with the solvating power. Due to the lower viscosity and higher diffusivity of the solvent, mass transfer into the extraction solvent is faster. The higher temperatures also make it easier for the solvent to overcome intermolecular interactions of the solute and matrix effects. All are important factors which affect efficiency of extraction.

Literature review

Pressurized solvent extraction (PSE) or Accelerated solvent extraction (ASE) is initially used for extraction of environmental contaminants (herbicides, pesticides, hydrocarbons) from soils, sediments and animal tissues. The reviews of related literature on extraction of environmental samples are summarized in Table 2.4. The technique is now more frequently used for food (meat, seeds, feeds), pharmaceutical products, and several other biological samples. This technique has been shown to be suitable to replace the Folch extraction (solid-liquid extraction) for oxysterols in food (Boselli et al., 2001). The efficiency of extractions with pressurized solvents (hexane, methylene chloride, isopropanol and ethanol) of polar and nonpolar lipids was examined in corn and oat kernels. Several studies reported the effects of solvent polarity and temperature on the recovery of total lipids, fatty acids, phenolic, glycolipids, and phytosterol from various plants. These are summarized in Table 2.5.

Table 2.3 Review studies on accelerated solvent extraction of environmental soil sample.

Author	Products	Types of solvent	Temperature (^{o}C)	Pressure (MPa)	Analysis	Objective
1. Li et al., 2003	Polycyclic aromatic hydrocarbons (PAHs), Phenols and Polychlorinated biphenyls (PCBs)	1. Methanol 2. Acetone	50-150	10	GC	To study effects of temperature, types of solvent and compare PSE with sonication extraction and MAP
2. Campbell et al., 2001	Bentazone and Chloroarylaliphatic acids	Acetone	100	10	GC-MS	To study effects of Na$_4$EDTA and PFBBr in PSE
3. Abrha et al., 2000	Polychlorinated biphenyls(PCBs)	Acetone / Hexane	50, 75, 125	14	GC	To study effects of temperature and compare PSE with Soxhlet extraction

Author	Products	Types of solvent	Temperature (^{o}C)	Pressure (MPa)	Analysis	Objective
4. Richter et al., 2000	Hydrocarbon contamination	1. Dichloromethane 2. Hexane 3. Heptane 4. Dichloromethane/Acetone 5. Hexane /Acetone 6. Heptane /Acetone	125-200	10	GC	To study optimum condition for clean up with PSE and compare PSE with sonication and Soxhet extraction
5. Bjorklund et al., 1999	Polychlorinated biphenyls(PCBs)	n-Hexane/Acetone	100	10	GC	To compare PSE with SFE
6. Berset et al., 1999	Polycyclic aromatic hydrocarbons (PAHs)	Hexane/Acetone/Toluene (10:5:1, v/v/v)	100	13.8	HRGC-MS and LC-FD	To compare PSE with Soxhlet extraction, sonication, Saponification, Shaking and SFE

Table 2.4 Review studies on accelerated solvent extraction of natural products.

Author	Products / Plants	Types of solvent	Temperature (^{o}C)	Pressure (MPa)	Analysis	Objective
1.Waksmundzka-Hajnos et al., 2004	Furanocoumarins / Pastinaca sativa	1. Petroleum ether 2. Methanol	100	6	HPLC	To compare PSE with Soxhlet extraction, USAE and MASE
2. Urraca et al., 2004	Zearalenone, α-Zearalenol / Wheat	Acetonitrile/Methanol compositions	50, 70	10	LC-FD	To study effects of temperature and compositions of solvent
3. Bonoli et al., 2004	Phenolic compounds / Barley	1. Ethanol/Water (4:1, v/v) 2. Methanol/Water (4:1, v/v) 3. Acetone/Water (4:1, v/v)	60, 90, 120	20	MEC	To study optimum condition for PSE and compare with Solid liquid extraction

Author	Products / Plants	Types of solvent	Temperature (^{o}C)	Pressure (MPa)	Analysis	Objective
4. Dunford et al., 2003	Fatty acids / Wheat germ oil	1. Ethanol 2. Iso-propanal 3. Acetone 4. High purity hexane 5. Iso-hexane 6. n-hexane	45 -135	10	GC	To study effects of temperature, time, types of solvent and compare PSE with Soxhlet extraction
5. Ong et al., 2003	Glycyrrhizin, Berberine, Baicalien / Glycyrrhizae, Coptidis rhizoma, Scutlellaiae radix	Methanol	100	1-3	HPLC	To compare PSE with solvent extraction, Soxhlet extraction and PHWE
6. Bjorklund et al., 2002	Polychlorinated biphenyls(PCBs)/ Oysters Mussels, Carp	Acetone / Hexane	100	14	GC	To compare PSE with Soxhlet extraction, MAE and SFE

Author	Products / Plants	Types of solvent	Temperature (°C)	Pressure (MPa)	Analysis	Objective
7. Kubatova et al., 2001	Dihydrokawain, Kawain, Desmethoxyyangonin, Tetrahydroyangonin, Dihydromethysticin, Yangonin, Methysticin / Piper methysticum root	Water	100, 150, 175, 200	6-7	GC-MS	To study effects of temperature, time and compare PSE with solvent extraction
8. Schafer et al., 1997	1. Fatty acids / cereal lipids 2. Lipids / Egg yolk, chicken breast muscle	1. Chloroform/Methanol 2. Isopropanol/Hexane	100, 120, 150	0.8	GC	To compare PSE with sonication extraction

CHAPTER III

MATERIALS AND METHODS

3.1 Experiment

3.1.1 Chemicals

Standard charantin was isolated from the aerial part of *Momordica charantia* by Miss Monraudee Chanchai, Department of Pharmaceutical Chemistry and Phytochemistry, Mahidol University. Ethanol and methanol were purchased from Fisher Scientific, UK.

3.1.2 Sample preparation

The Fruits of *Momordica charantia* were cleaned and cut into small pieces, and then oven dried at $50\,^{\circ}\text{C}$ for a day. The dried sample was then ground in a motar, and the particle size was measured by a particle size analyzer (Coulter, Model LS230). The mean particle size was 0.3 mm. The dried sample was then stored in dry place until use.

Figure 3.1 Powder of *Momordica charantia* fruits

3.1.3 Soxhlet extraction

1.0 g of raw materials was extracted with 120 ml of solvent for 2 hr. The amount of compound remained in the sample residue was extracted repeatedly in 30 ml volumes of methanol using ultrasonication. The extract was filtered and evaporated to obtain viscous crude extract and purified before the analysis with HPLC. All extractions were performed in triplicate.
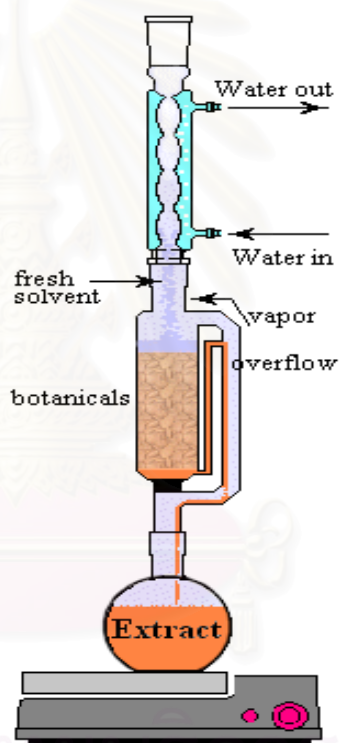
Figure 3.2 Component of Soxhlet apparatus.

3.1.4 Accelerated solvent extraction

Accelerated solvent extraction was performed using an apparatus shown in Figure 3.3. The extraction system consisted of an HPLC pump (PU 980, JASCO, Japan), an oven (HARAEUS D63450) where the extraction vessel (10 ml, Thar Design, USA) was mounted, a pressure gauge and a back pressure regulator (AKICO, Japan). All connections were made with stainless steel

capillaries (1/16 inch inside diameter). The pump was used to deliver the extraction solvent into system. The solvent was then preheated to the required temperature in a 3-m preheating coil installed in the oven before the extraction vessel, which was preloaded with 1.0 g of sample. The back pressure regulator placed at the outlet of the extraction system was used to maintain the system pressure between 10 MPa to ensure that solvent was in liquid state at all temperatures tested. After being cooled in a coil immersed in a water bath to prevent possible product degradation, the extract was collected in sample vials every 10 to 20 minutes. The amount of compound remained in the sample residue was extracted repeatedly in 30 ml volumes of methanol using ultrasonication. Each sample vial was then evaporated under vacuum to remove all the solvent and methanol and was then added to the crude extract, which was then purified before the analysis with HPLC. In this study, all experiments were performed in triplicate.

The experimental variables to be studied and their ranges were listed in Table 3.1.

Table 3.1 Condition for experiment

Variables	Conditions
Types of solvent	Acetone, Dichloromethane Ethyl Acetate, Ethanol, Water
Temperature	50, 80, 100, 120,150 ^{o}C
Pressure	10 MPa
Flow rate	2, 4, 6 ml/min
Composition (% organic solvent)	0, 20, 50, 80, 100 %
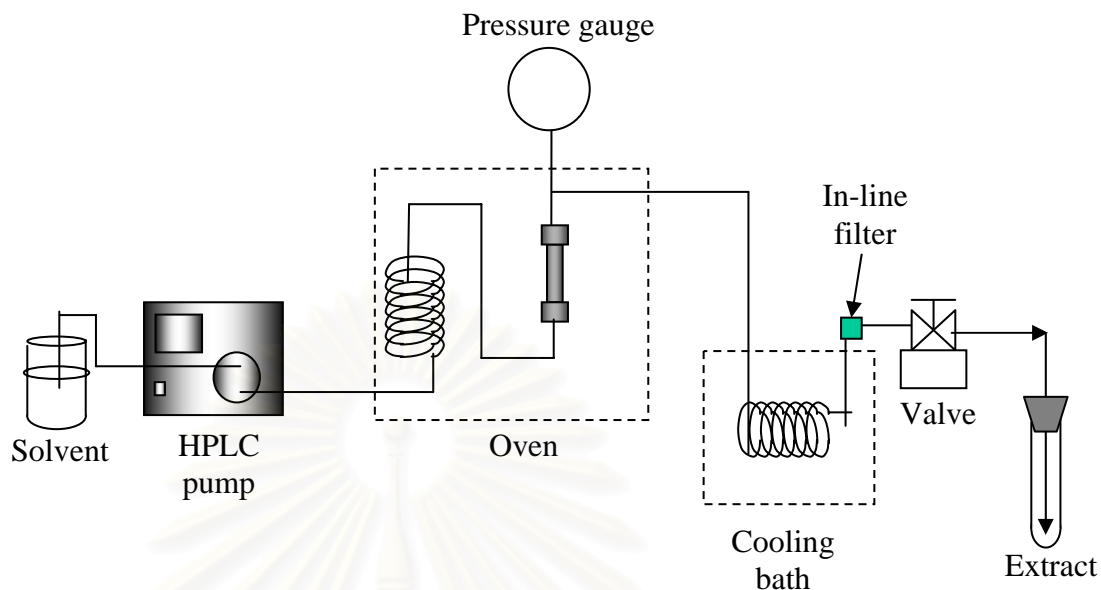
Figure 3.3 Schematic diagrams of ASE Apparatus

3.2 Sample purification

To purify the crude extract, four steps were taken as schematically shown in the diagram in Figure 3.4 (Chanchai, 2002). In the first step five milliliters of 50:50 (v/v) methanol-water was added. The mixture was then sonicated for 15 min and then centrifuged at 3500 rpm for 15 min to separate the supernatant from the precipitate. In the second step, five milliliters of 70:30 (v/v) methanol-water was then added to the precipitate from the previous step, and the mixture was then sonicated for 15 min and centrifuged at 3500 rpm for 15 min. In the third step, the precipitate from the previous step was then added with three milliliters of hexane, and the mixture was sonicated for 15 min and centrifuged at 3500 rpm for 15 min. In the last step, the precipitate from step 4 was re-dissolved in 200 μl of 1:1 (v/v) chloroform-methanol and then adjusted to volume with 800 μl of methanol (for PSE and with 1800 μl for Soxhlet). The purified solution was then filtered through a Millipore membrane filter (0.45 μm) before being analyzed by an HPLC. The supernatant fraction from each step was checked using HPLC and verified with HPLC that it does not contain any charantin.

```
┌─────────────────┐
│  Crude extracts │
└────────┬────────┘
         │ add 5 ml of 50% MeOH,
         │ sonicate for 15 min,
         │ centrifuge at 3,500 rpm
    ┌────┴────┐
┌───────────────┐  ┌───────────────┐
│ Supernatant-1 │  │ Precipitate-1 │
└───────────────┘  └───────┬───────┘
                           │ add 5 ml of 70% MeOH,
                           │ sonicate for 15 min,
                           │ centrifuge at 3,500 rpm
                      ┌────┴────┐
            ┌───────────────┐  ┌───────────────┐
            │ Supernatant-2 │  │ Precipitate-2 │
            └───────────────┘  └───────┬───────┘
                                       │ add 3 ml of hexane,
                                       │ sonicate for 15 min,
                                       │ centrifuge at 3,500 rpm
                                  ┌────┴────┐
                        ┌───────────────┐  ┌───────────────┐
                        │ Supernatant-3 │  │ Precipitate-3 │
                        └───────────────┘  └───────┬───────┘
                                                   │ Add 200 µl CHCl₃: MeOH
                                                   │ (1:1), sonicate for 15 min
                                                   │ adjust volume with 800 µl
                                          ┌────────────────────┐
                                          │ Sample for analysis│
                                          └────────────────────┘
```

Figure 3.4 Diagram for purification preparation for HPLC analysis

3.3 **HPLC Analysis**

HPLC were performed with a C-18 Inertsil ODS-3 column (5 μm particle, 4.6x250 mm ID). The mobile phase was 100:2 (v/v) methanol/water, which was run at a flow rate of 1 ml/min. The UV detection wavelength was 204 nm. The sample injection volume was 20 μl. A standard calibration curve was made from a plot of peak areas versus concentrations for a series of standard solutions in methanol, whose concentrations were 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10 mg/ml.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Preliminary results

In our preliminary investigation, 1.0 g of bitter melon fruit powder whose average size of particle was 400 μm (shown in Appendix A, Figure A-1.2) was extracted with pressurized solvent in a continous flow system at the temperature range of 50-150 °C and the pressure of 10 MPa, using the apparatus shown in Figure 3.1. The extracts were collected at every 10 minute intervals and are shown in Figure 4.1. Generally, the color of the extracts initially obtained was dark green and that of the extracts obtained toward the end of extraction run became paler. The dark green color was due to the chlorophylls nonselectively extracted with other compounds including charantin.

Figure 4.1 Comparison of extracts obtained ASE with different times.

To correctly analyze the amount of charantin extracted with HPLC, a purification step was necessary to remove the chlorophylls and sugars from the crude extracts that may interfere the charantin peak. To do this, the extracts were evaporated to dry under vacuum. The sugars were then removed by extracting the dried residue

with 50% and then again with 70% methanol as described in chapter 3. Chlorophylls and other impurities were then removed by extracting the remaining sample with hexane. The chromatograms of different extracted fractions were checked to ensure no charantin was extracted and lost into these fractions during purification as (Figure 4.2). As seen from the figure, the retention time of charantin was 13 min.

The purified sample was then analyzed for the concentration of charantin by HPLC analysis using C-18 reversed phase (5 μm particle, 4.6x250 mm ID.), Inertsil ODS-3 Column. The Isocratic elution was used with methanol and water (100:2) as the mobile phase. The spectroscopic scan of the standard charantin as shown in Appendix A (Figure A-1.3) suggested that appropriate detection wavelength was at 204 nm. The chromatograms of the crude and purified sample shown in Figure 4.3 illustrate that the purification method employed was suitable for the determination of amount charantin in the *Momordica charantia* fruits.
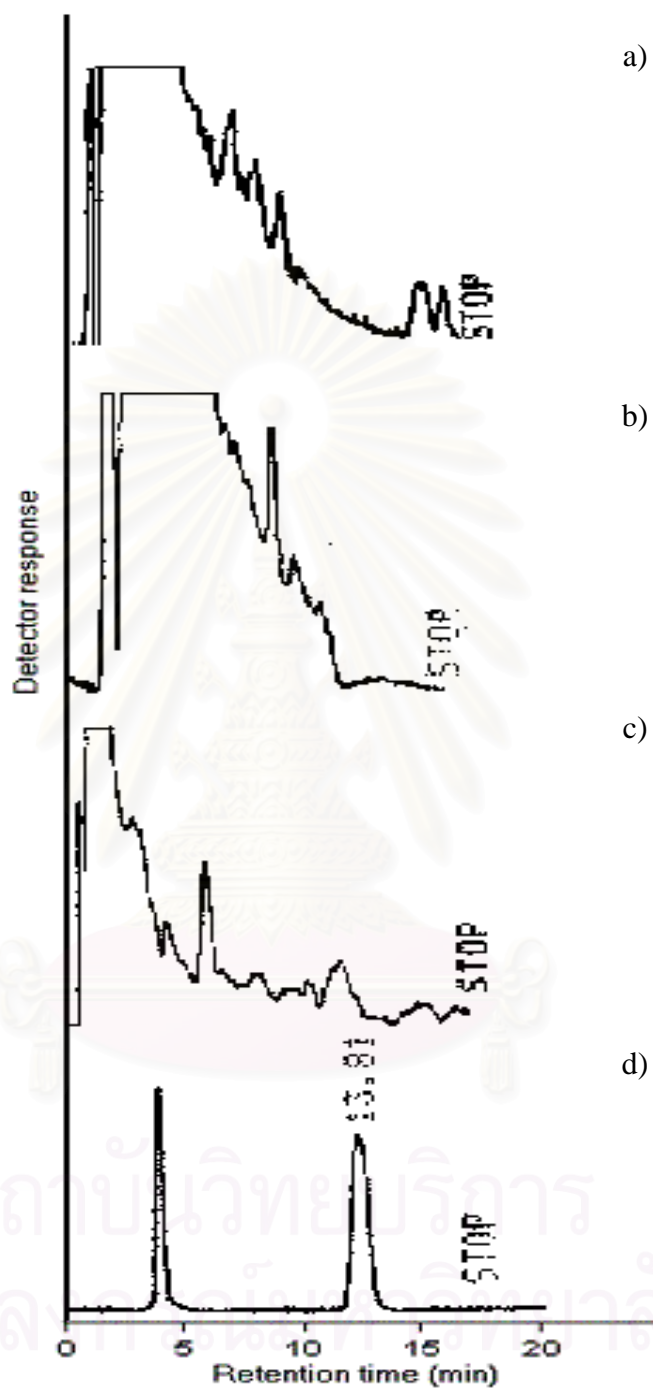
Figure 4.2 Chromatograms of each extracted fractions a) Supernatant fraction after extraction of sample with 50% methanol, b) Supernatant fraction after extraction of sample 70%, c) Supernatant fraction after extraction with hexane, d) Standard charantin.
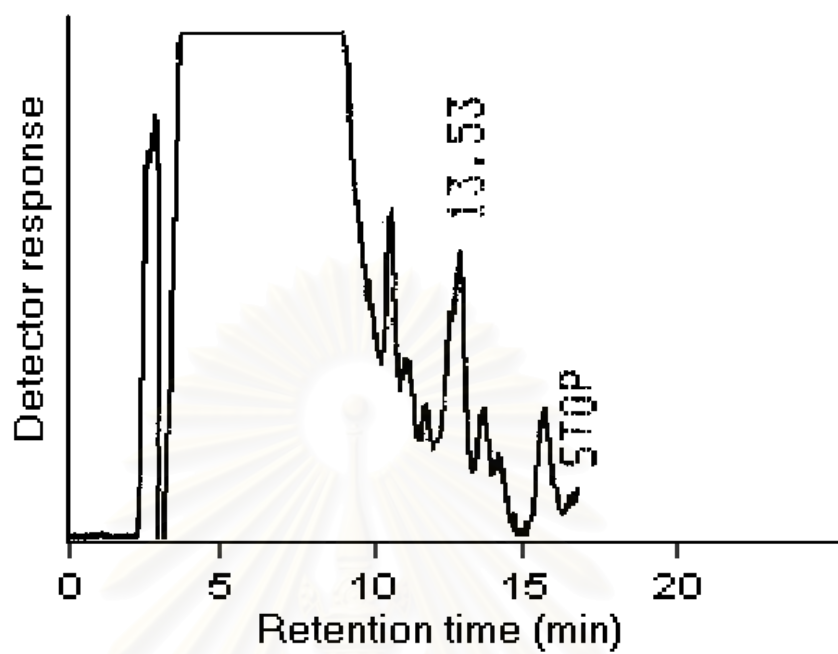
a)
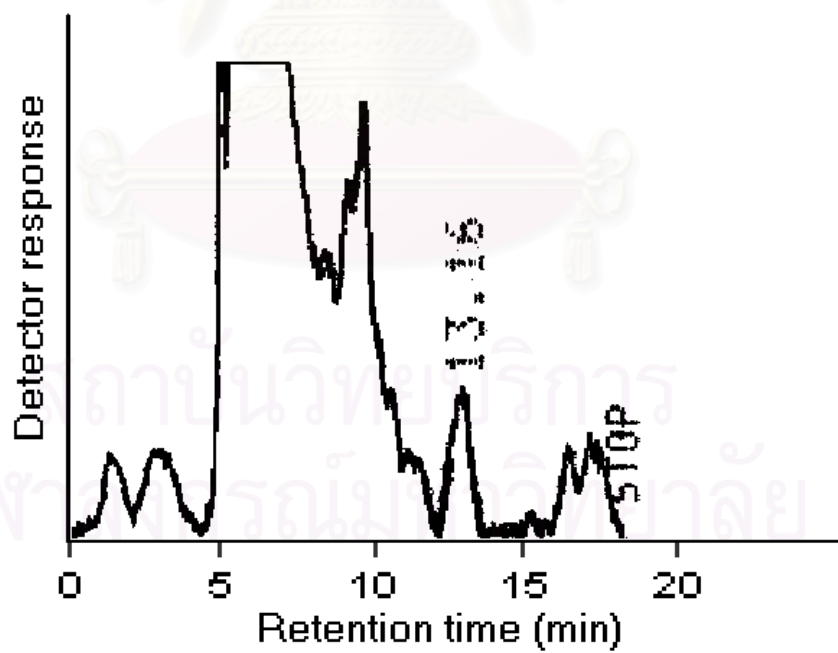
b)

Figure 4.3 Chromatogram of charantin in fruits *Momordica charantia* a) Crude extraction (before purified) b) Accelerated solvent extraction (after purified).

In the following sections, the effects of variables such as, type of solvent, temperature, solvent flow rate, and the composition of solvent mixture on the percent recovery of the product by accelerated solvent extraction are presented and discussed. The total amount of charantin in the fruit samples was determined to be approximately 0.126 ± 0.018 mg/g dried fruit. Despite the same lot of samples, some variation in the total amount of charantin was present. The extraction efficiency was therefore expressed in terms of percent charantin recovery, which was determined for each run from the amount of charantin extracted divided by the total amount of charantin in the fruit sample. The total amount present in the fruit sample was the sum of the total amount extracted and the amount remained in the sample residue recovered by repeated ultrasonic extractions. The recovery defined this way was appropriate as all the experiments were conducted under 200 $^{\circ}$C, the temperature at which charantin degradation occurs (Chanchai, 2002). This was also verified experimentally in this study.

4.2 Effect of solvent type

The effect of solvent type on extraction efficiency was determined for the following solvents: ethanol, acetone, ethyl acetate, dichloromethane, and water. Ethyl acetate and dichloromethane are relatively non-polar organic solvents, while ethanol and acetone are relatively polar, and water is very polar. The extractions were carried out at 100°C, 10 MPa, and at a flow rate of 2 ml/min. As shown in Figure 4.4, acetone and ethanol gave the highest efficiency and the efficiencies were significantly higher than those of the other solvents.
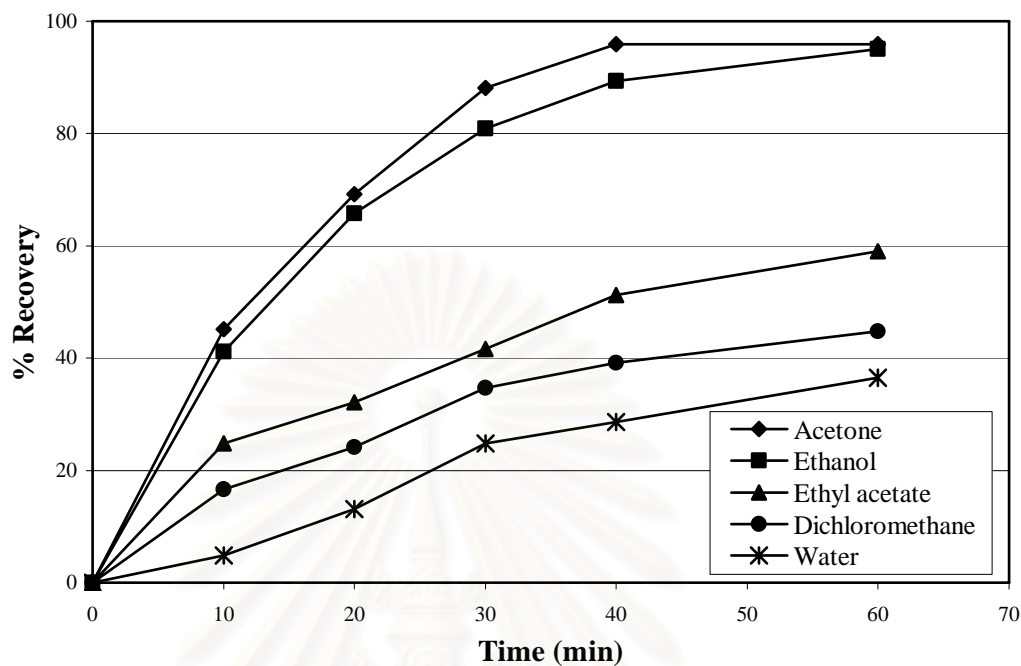
Figure 4.4 Effect of solvent type on the extraction efficiency. Extraction conditions for all the runs were as follows: temperature 100°C and flow rate 2 ml/min at 10 MPa.

It is worth noting from the purification procedure that at ambient temperature, charantin was not extracted with either 50% or 70% methanol solutions, or pure hexane. Based on the basic principle of "like dissolves like", this finding suggests that at ambient temperature, the polarity of the methanol solutions is too high to extract the compound, whereas the polarity of hexane is too low. Because charantin is a mixture of two steroidal glycosides, the compound can be classified as polar lipid, and thus it is insoluble in either very polar solvent or very non-polar solvent. Generally the molecules of solvents such as water and alcohol contain a hydrogen atom attached to an oxygen electronegative atom. The polarity of these solvents stems from the bond dipole of the O-H bond, or hydrogen bond. Due to the large difference in electronegativities of the oxygen and the hydrogen atom, combined with the small size of the hydrogen atom, these solvents tend to separate themselves from other compounds that do not contain the hydrogen bond. Although charantin molecule contains a hydrogen bond containing glucose unit which makes the compound slightly

polar, the lipid portion of the molecule is too large for the compound to be well dissolved in these solvents at ambient temperature. The compound on the other hand due to this same glucose unit, does not as well dissolve in very non-polar solvent like hexane. However, charantin has been shown to have higher solubility in solvents such as chloroform and dichloromethane which do not contain O-H bonds. These solvents are less polar than water and alcohols due to the lack of hydrogen bond, but are more polar than hexane, due to the asymmetric molecular arrangement of Cl and H atoms around the carbon atom. This makes the solvents suitable for dissolving polar lipids like charantin. Generally, extraction of charantin is not performed near ambient temperature, but at the temperature close to the solvent boiling temperature using a Soxhlet apparatus as higher temperature causes intermolecular interactions within the solvent to decrease, causing higher molecular motion, and making the solute to be more easily dissolved in the solvent. At the solvent boiling temperatures, chloroform and dichloromethane are still superior solvents to ethanol as was shown in our experiment extraction in ethanol using Soxhlet apparatus requires significantly longer period than in the other two solvents to recover all charantin in the plant material.

When the temperature of the solvent increased to 100°C as in the ASE, the results in Figure 4.4 demonstrate that ethanol was more effective than dichloromethane. As the temperature of the solvents increased above the boiling temperatures, the dipole-dipole forces and the hydrogen bonds in the solvents are further broken down (Stengele et al., 2001), the liquid polarity further decreases. In the case of acetone and ethanol, the polarity decreased to the values that are more favorable for charantin extraction, while the polarity of dichloromethane and ethyl acetate were decreased to the degree that become less favorable. In the case of water, increasing the temperature also decreases the polarity, and thus the extraction efficiency. However, water is and extremely polar liquid (i.e. $\varepsilon=80$ at ambient temperature), at 100 °C, water polarity still remains high, making it not an effective solvent for charantin compared to acetone and ethanol. Due to several advantages of ethanol over acetone, such as being naturally derived, having low cost, and being more widely used as processing solvent in food and pharmaceutical industries, it was chosen as extraction solvent for further investigations.

4.3 Effect of temperature

Temperature is expected to have a significant effect on the extraction efficiency extraction, due to the importance of this factor on the analyte solubility. The effect of extraction temperature on *Momordica charantia* extraction efficiency was examined over the range of 50-150 °C, the temperature range sufficiently low to avoid charantin decomposition. Each experiment was operated with ethanol as extraction solvent at flow rate of 2 ml/min and the pressure of 10 MPa.
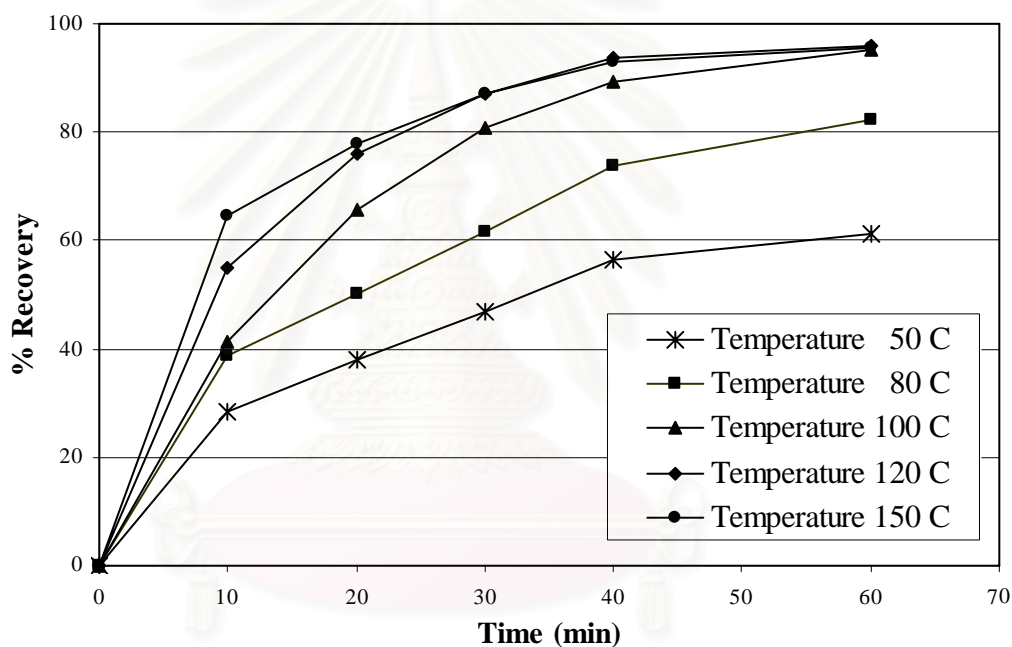
Figure 4.5 Effect of temperature on the extraction efficiency. Extraction conditions for all the runs were as follows: solvent ethanol and flow rate 2 ml/min at 10 MPa.

The results are shown in Figure 4.5 which reveals that extraction efficiency was greatly influenced by temperature. As described earlier, the increase in temperature decreases solvent polarity as a result of reduced polar forces and hydrogen bonding, making ethanol more suitable for extraction of charantin. Moreover, at elevated temperature, the solvent density and viscosity decrease, resulting in increased mass transfer of the solvent into the matrix of plant sample.

When temperature increases from 120°C to 150 ºC however, the percent charantin extracted did not change. This suggests that there appears to be an optimal temperature between 100°C and 150 ºC. It is possible that beyond this temperature, further decrease in ethanol polarity may be disadvantageous for extraction of charantin. Although theory of the effect of temperature on solvent polarity, and thus solubility has been extensively studied and documented, the experimental data near critical temperature are not available. Particularly, polarity is a complex property which can not be easily measured either by dipole moment or dielectric constant. More detailed study is needed to completely understand the behavior of solvent and the solute solubility under the subcritical conditions.

4.4 Effect of solvent flow rate

The effect of solvent flow rate was studied in the range of 2-6 ml/min at the pressure of 10 MPa for two limits of extraction temperatures: 100 ºC and 150 ºC. The results are shown in Figures 4.6 and 4.7.
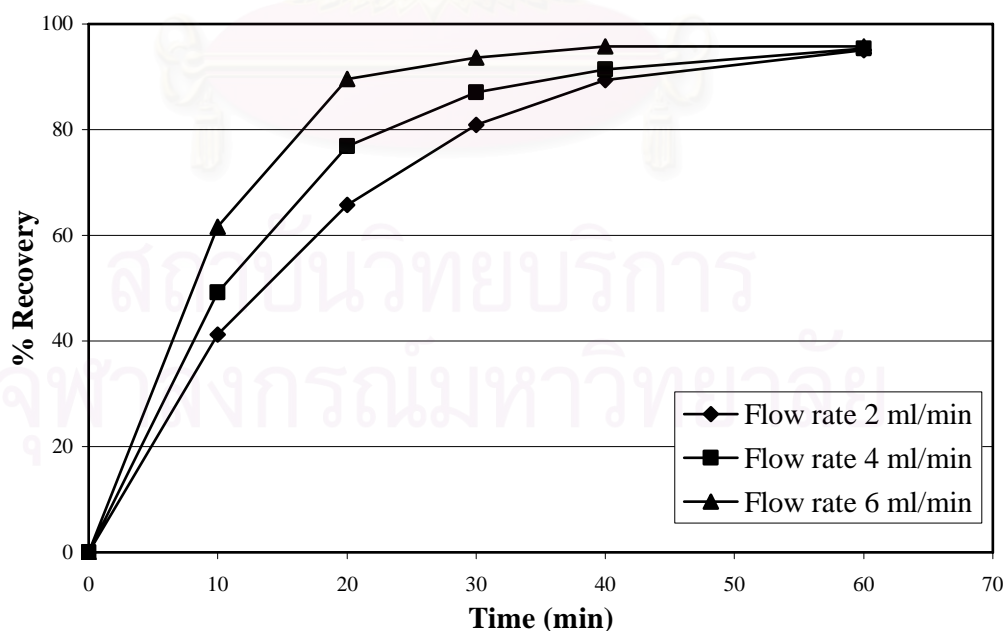
a)
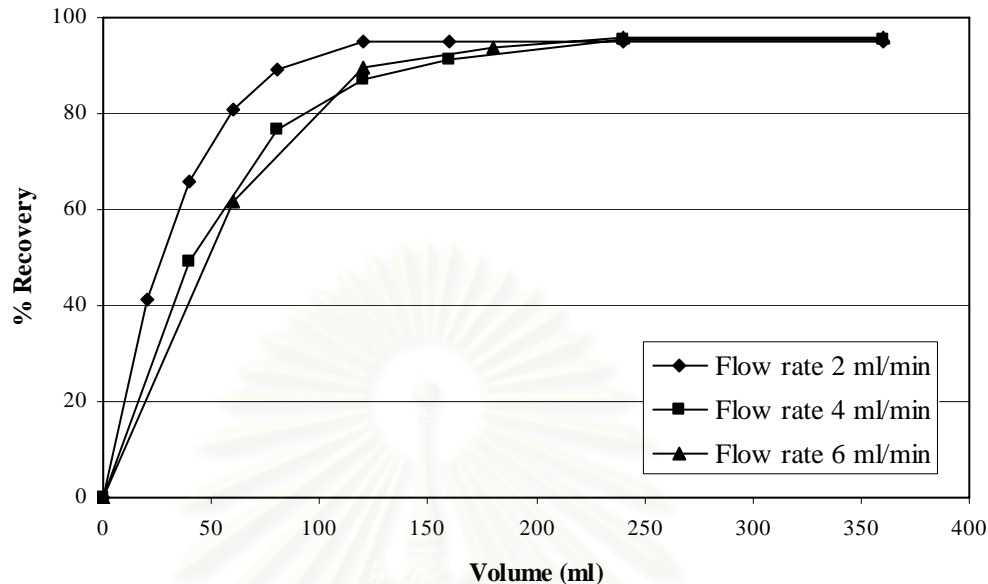
b)

Figure 4.6 Effect of flow rate on the extraction efficiency of pressurized ethanol extraction at temperature 100°C at 10 MPa, a) Percent recovery versus time, b) Percent recovery versus volume of solvent.

For extraction at 100 °C as shown in Figure 4.6 a), the extraction rate was higher at higher flow rate. This is because, at high flow rate, higher amount of solvent entered the reactor, increasing the chance for solvent-solute contact. When consider the data plotted as the percent recovery versus volume of solvent shown in Figure 4.6 b), for the same volume of solvent passed, the slower flow rate resulted in higher extraction efficiency. At lower flow rate, solvent residence time was higher, allowing it to be in closer contact with the solute within the matrix. This result demonstrates that internal diffusion plays a role in ASE extraction of charantin from the fruits of *Momordica charantia*. Similar trends were observed for the effect of solvent flow rate at higher extraction temperature (150°C), whose results are shown in Figure 4.7 a) and b). The plots for percent charantin extracted versus volumes for various flow rates lined more closely to each other however for extraction at 150°C than at 100°C, indicating that, at higher temperature, ASE was to the lesser extent influenced by internal diffusion.
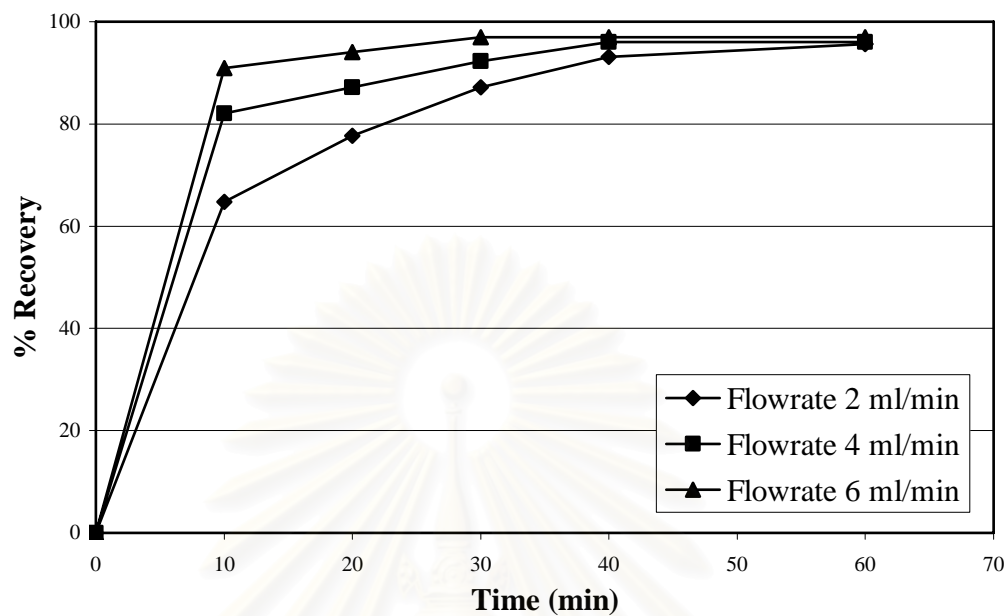
a)
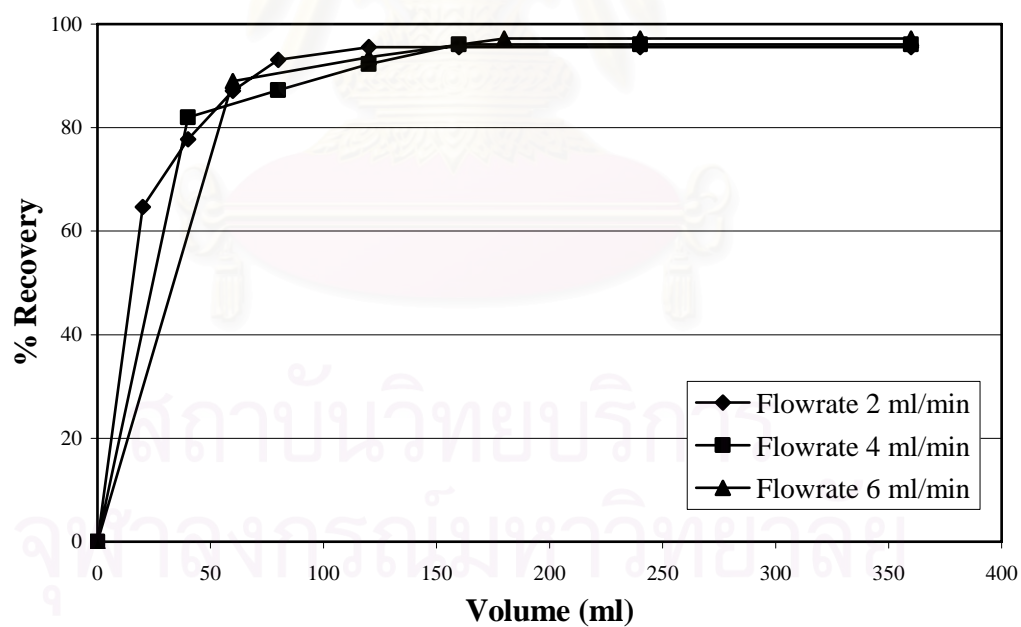
b)

Figure 4.7 Effect of flow rate on the extraction efficiency for pressurized ethanol extraction at 150°C and 10 MPa, a) Percent recovery versus time, b) Percent recovery versus volume of solvent.

4.5 Effect of solvent composition

The mixtures of ethanol and water were tested to determine the effect of mixture composition on pressurized solvent extraction efficiency. The composition of 0%, 20%, 50%, 80%, and 100% ethanol were tested. The conditions used in each experiment were 10 MPa, at the flow rate of 2 ml/min. The experiment results for extraction at two temperatures of 100°C and 150°C are shown in Figure 4.8.

a)
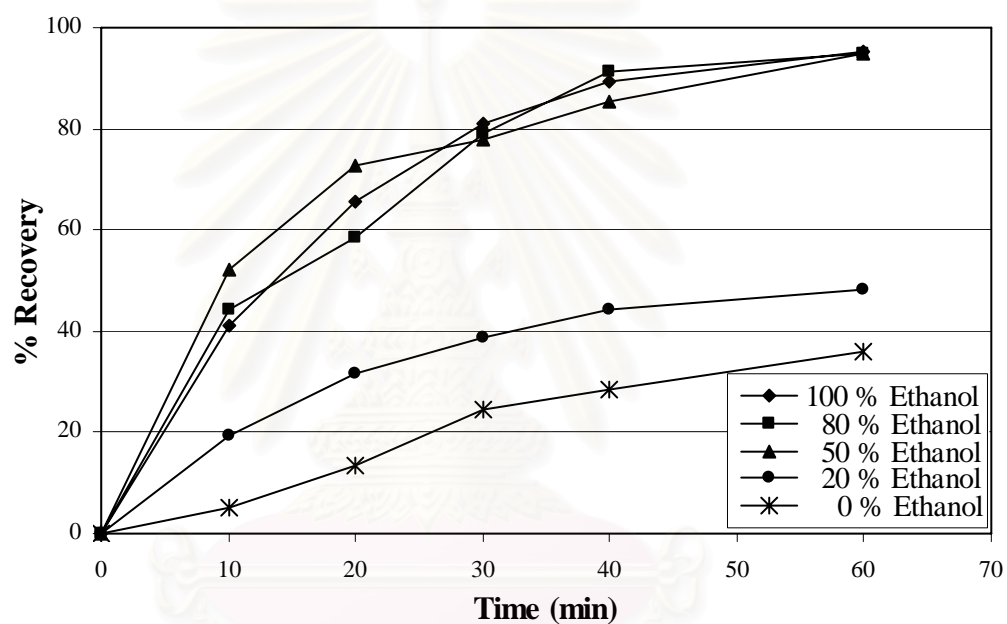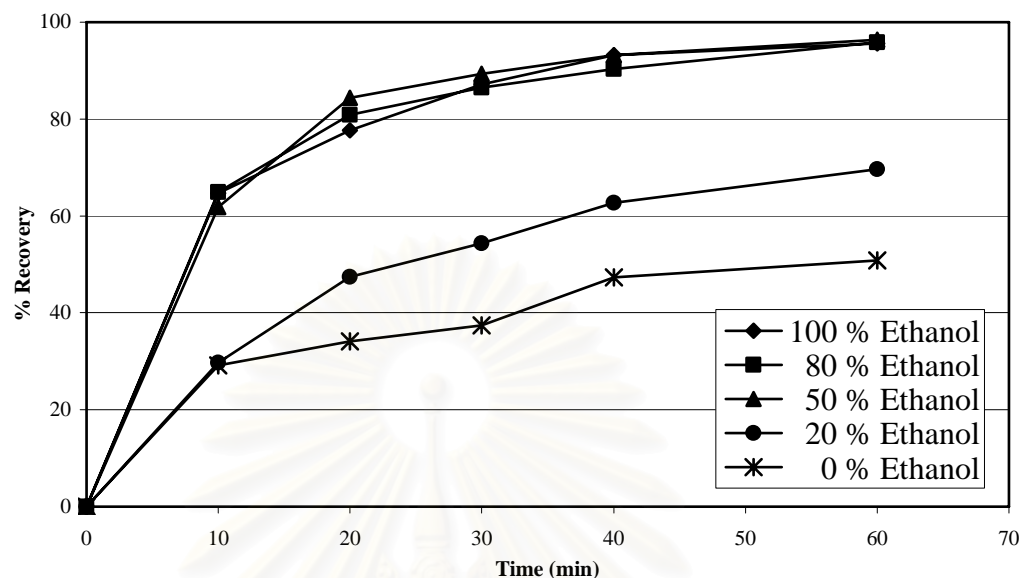
b)

Figure 4.8 Effect of percent ethanol on the efficiency for pressurized solvent extraction at 10 MPa and solvent flow rate of 2 ml/min, a) 100°C and b) 150 °C.

For both temperatures tested, the effect of solvent mixture composition was similar. The extraction efficiency increased with increasing percentage of ethanol in the solvent mixture until up to 50%. Further increase in ethanol composition did not further increase the extraction efficiency. It is worth nothing that when pure water was used, the solubility of charantin was low because water polarity was extremely high. However, the addition of some amount of water to as high as 50% into ethanol was found not to hinder the extraction efficiency. On the other hand, due the lower viscosity of water, it can more easily penetrates into the pores of the sample matrix, thus the presence of some amount of water contributes to swelling the plant materials. This increases the contact volume and area between the solvent and the plant porous matrix, thus the internal mass diffusion is increased (Li et al., 2005). Rostagno et al (2003) has indeed reported that addition of water enhances the solubility of some glucoside compounds, and thus improves the extraction efficiency. In this study, the extraction efficiency of pure ethanol and ethanol-water mixture up to 50% ethanol are comparable. However, the ethanolic mixture with too high water content has lower

dissolving power for charantin as a result of increased polarity. This can be seen from the results in Figure 4.8 that 20% ethanol-water mixture gave relatively low charantin recovery.

4.6 ASE and Soxhlet extraction

In this section, the comparison of several extraction methods are presented in terms of product recovery, solvent volume, and time required for extraction. The results were shown in Table 4.1.

Table 4.1 Comparison the extraction efficiency between ASE and Soxhlet method

	ASE[1]	ASE[2]	ASE[3]	Soxhlet
Sample (g)	1	1	1	1
Extraction solvent	ETOH	ETOH	50%ETOH	ETOH
Pressure (MPa)	10	10	10	ambient
Temperature (°C)	120	100	100	b.p. solvent
Flow rate (ml/min)	2	2	2	-
Time (min)	40	60	60	150
Solvent volume (ml)	80	120	60/60	200
Percent recovery (%)	96.05	95.10	94.89	97.51

As shown in Table 4.1, ASE could be completed within 60 min with the flow rate of 2 ml/min, while Soxhlet extraction requires longer extraction time of 150 min to achieve the same charantin recovery. The amount of solvent required was also significantly smaller. The time and amount of solvent used could further be reduced by increasing the extraction temperature. This clearly demonstrates that the ASE method reduces the amount of solvent used and time required for extraction of charantin from fruits of *Momordica charantia*. Appropriate choice of extraction may be selected based on specific objective of the extraction operation. For example, the use of extraction with 50 % ethanol-water mixture at temperature 100 °C and the flow rate of 2 ml/ min is one of the attractive choices of operation as it results in high extraction efficiency with minimal energy and solvent requirements.

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. A purification step was required prior to the HPLC analysis of charantin in the extracts obtained from conventional and accelerated solvent extraction. This can be achieved by washing the crude extract with 50% methanol, followed by 70% methanol, and hexane, respectively.

2. The yield and the rate of accelerated solvent extraction depend on the type of solvent used. Acetone and ethanol gave higher yield than ethyl acetate, dichloromethane, and water.

3. The amount of charantin in the extract increases as temperature increases.

4. Similar yield could be achieved using 50% ethanol in water solution as extraction solvent in ASE as with pure ethanol due to the fact that swelling of the plant matrix by water increased mass transfer of the solute into the solvent mixture.

5. With 50% ethanol solvent, ASE at the temperature of 100 °C and the flow rate of 2 ml/min could achieve the similar yield but with less time extraction and volume of solvent than Soxhlet extraction.

5.2 Recommendations

1. At elevated temperature, higher yield was generally resulted, thus extraction with pure water at higher temperature up to 200 °C (the temperature at which charantin degrades) would be of interest for the future investigation. This offers an alternative method of extraction in which no toxic organic solvent is used.

2. It would be interesting to determine the fundamental information regarding the solubility of charantin in the solvents as it is one of the factors that determine the success of the extraction process.

3. Mass transfer modeling of ASE based on the experimental data is useful for the future prediction of extraction behavior and the system scale-up.

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APPENDICES

Appendix A

Table A-1 Standard calibration curve of charantin

Concentration of charantin (mg/ml)	Run 1	Run 2	Average
0.000	0	0	0
0.020	6777	6042	6410
0.030	7071	7172	7122
0.040	8482	8599	8541
0.050	9564	11056	10310
0.060	13708	14556	14132
0.070	14258	16552	15405
0.080	16870	19595	18233
0.090	17932	22005	19969
0.100	27082	26432	26757
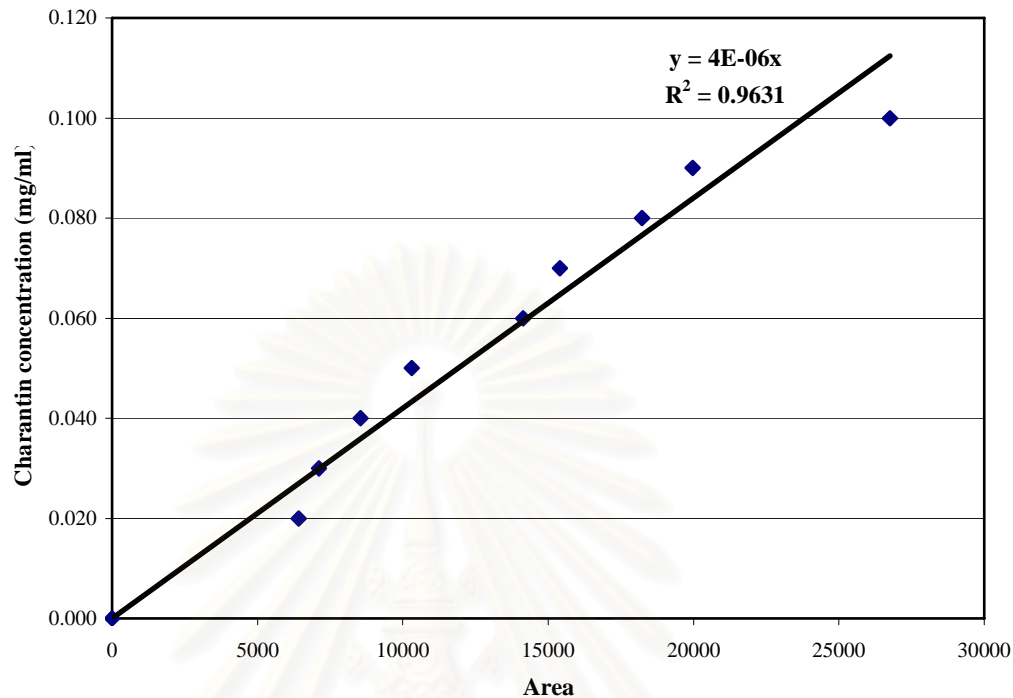
Figure A-1.1 Standard calibration curve of charantin
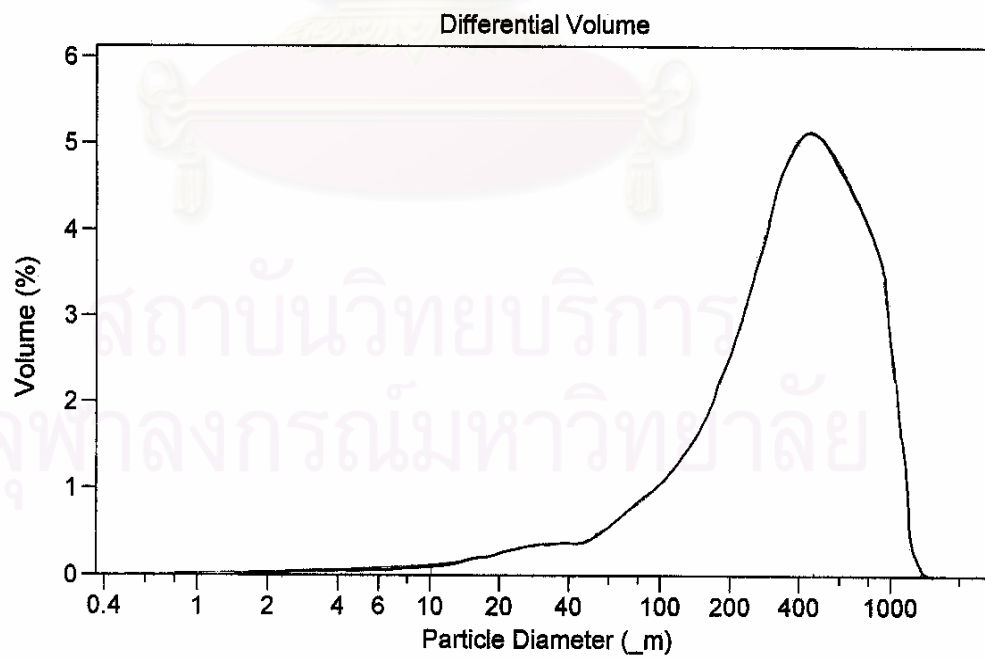
Figure A-1.2 Particle size analyzer
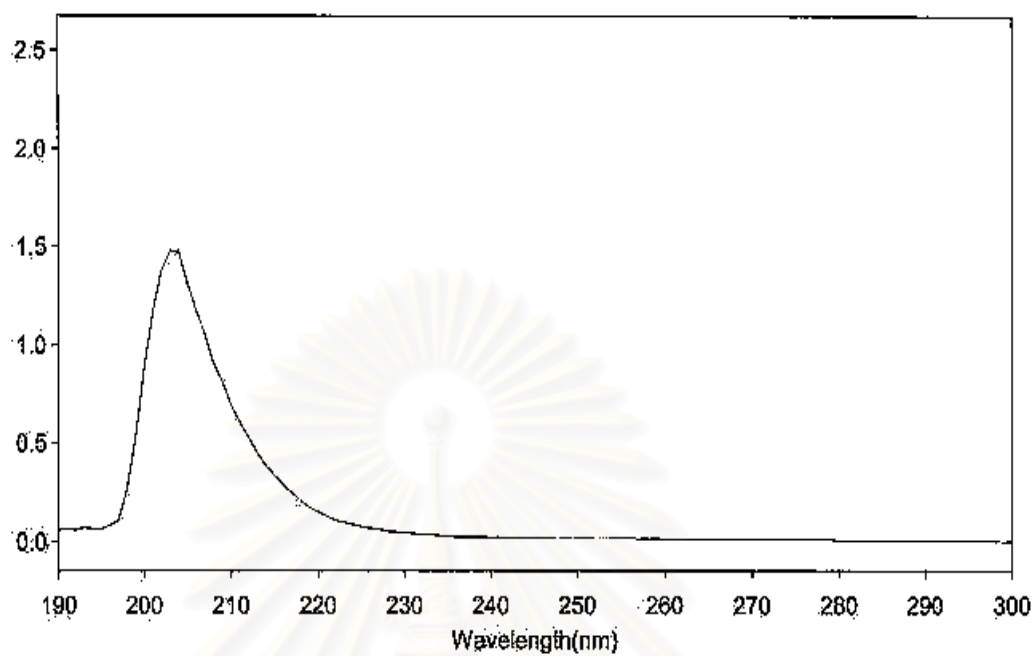
Figure A-1.3 UV-spectrum of standard charantin

Appendix B

Percent recovery

Types of solvent effect experiment

Table B-1.1: Accelerated solvent extraction of acetone temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	41.70	39.88	53.96	45.18	7.660
20	40	70.72	66.18	70.71	69.21	2.617
30	60	92.55	85.52	86.22	88.10	3.870
40	80	96.22	96.14	95.27	95.87	0.524
60	120	96.22	96.14	95.27	95.87	0.524

Table B-1.2: Accelerated solvent extraction of dichloromethane temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	16.60	16.38	17.85	16.94	0.793
20	40	24.10	28.92	30.64	27.89	3.389
30	60	34.74	38.39	40.70	37.94	3.004
40	80	39.21	42.46	45.53	42.40	3.159
60	120	44.79	53.64	51.74	50.05	4.661

Table B-1.3: Accelerated solvent extraction of ethyl acetate temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	25.50	25.45	23.49	24.81	1.148
20	40	32.97	32.25	31.27	32.16	0.853
30	60	41.83	42.48	40.73	41.68	0.885
40	80	50.79	52.67	50.17	51.21	1.304
60	120	57.94	60.53	58.54	59.00	1.352

Temperature effect experiment

Table B-2.1: Accelerated solvent extraction of ethanol temperature 50 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	28.28	31.27	30.87	30.14	1.624
20	40	37.88	39.00	38.97	38.62	0.641
30	60	46.77	47.09	47.17	47.01	0.212
40	80	56.62	54.79	55.16	55.52	0.967
60	120	61.33	61.99	61.74	61.69	0.331

Table B-2.2: Accelerated solvent extraction of ethanol temperature 80 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	42.08	38.91	39.73	40.24	1.647
20	40	55.51	50.11	52.62	52.74	2.702
30	60	62.38	61.74	61.91	62.01	0.331
40	80	70.79	73.93	73.94	72.89	1.816
60	120	83.37	82.40	81.46	82.41	0.951

Table B-2.3: Accelerated solvent extraction of ethanol temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	41.98	44.76	36.77	41.17	4.057
20	40	67.12	67.97	62.21	65.77	3.108
30	60	81.43	82.13	79.18	80.91	1.541
40	80	90.46	88.19	89.40	89.35	1.137
60	120	95.69	92.64	96.96	95.10	2.217

Table B-2.4: Accelerated solvent extraction of ethanol temperature 120 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	52.98	51.90	59.63	54.84	4.188
20	40	73.92	75.59	78.23	75.92	2.173
30	60	86.23	88.27	87.20	87.23	1.017
40	80	92.67	94.23	94.01	93.64	0.843
60	120	96.62	95.93	95.60	96.05	0.519

Table B-2.5: Accelerated solvent extraction of ethanol temperature 150 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	63.33	63.72	67.11	64.72	2.081
20	40	77.35	77.00	78.79	77.71	0.949
30	60	87.41	87.12	86.90	87.14	0.258
40	80	92.69	95.25	91.52	93.15	1.910
60	120	95.25	96.82	94.65	95.57	1.119

Flow rate effect experiment

Table B-3.1: Accelerated solvent extraction of ethanol temperature 100 °C flow rate 4 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	40	47.88	50.48	49.14	49.17	0.949
20	80	78.07	76.93	75.53	76.85	0.988
30	120	88.59	86.55	85.96	87.03	0.418
40	160	92.42	90.27	91.47	91.39	0.845
60	240	96.48	93.94	95.58	95.33	1.163

Table B-3.2: Accelerated solvent extraction of ethanol temperature 100 °C flow rate 6 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	60	63.56	59.36	61.92	61.61	1.805
20	120	88.53	89.71	90.52	89.59	0.576
30	180	94.02	92.92	93.95	93.63	0.731
40	240	96.32	95.17	95.78	95.76	0.426
60	360	96.32	95.17	95.78	95.76	0.426

Table B-3.3: Accelerated solvent extraction of ethanol temperature 150 °C flow rate 4 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	40	81.43	83.26	81.38	82.02	1.328
20	80	87.26	87.38	86.90	87.18	0.335
30	120	92.40	92.56	92.01	92.32	0.384
40	160	95.99	96.23	95.91	96.04	0.229
60	240	95.99	96.23	95.91	96.04	0.229

Table B-3.4: Accelerated solvent extraction of ethanol temperature 150 °C flow rate 6 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	60	87.46	90.94	88.51	88.97	1.718
20	120	92.94	94.10	93.78	93.60	0.226
30	180	97.54	97.01	97.11	97.22	0.073
40	240	97.54	97.01	97.11	97.22	0.073
60	360	97.54	97.01	97.11	97.22	0.073

Composition of solvent effect experiment

Table B-4.1: Accelerated solvent extraction of water temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	5.32	4.40	5.13	4.95	0.487
20	40	15.24	11.19	13.52	13.32	2.033
30	60	29.05	21.01	23.42	24.49	4.122
40	80	32.52	24.74	28.19	28.48	3.899
60	120	36.40	36.82	34.86	36.03	1.028

Table B-4.2: Accelerated solvent extraction of 20 % ethanol temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	22.27	17.65	18.26	19.39	2.510
20	40	31.25	31.38	32.52	31.72	0.699
30	60	37.93	39.12	39.30	38.79	0.744
40	80	41.72	45.81	44.84	44.12	2.137
60	120	45.25	50.74	48.46	48.15	2.760

Table B-4.3: Accelerated solvent extraction of 50 % ethanol temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	54.08	51.01	51.36	52.15	1.681
20	40	76.19	70.83	71.37	72.80	2.952
30	60	81.47	75.96	76.58	78.00	3.020
40	80	89.29	82.57	84.26	85.37	3.495
60	120	94.86	95.07	94.76	94.89	0.157

Table B-4.4: Accelerated solvent extraction of 80% ethanol temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	45.22	42.28	45.80	44.43	1.888
20	40	59.25	54.63	62.00	58.63	3.725
30	60	80.33	74.34	82.69	79.12	4.305
40	80	92.01	88.70	92.88	91.20	2.208
60	120	96.12	92.77	96.07	94.99	1.917

Table B-4.5: Accelerated solvent extraction of water temperature 150 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	27.75	30.37	29.21	29.11	1.315
20	40	32.70	34.88	34.58	34.05	1.181
30	60	35.62	37.81	38.68	37.37	1.579
40	80	45.70	48.55	47.77	47.34	1.470
60	120	49.48	51.68	51.25	50.81	1.171

Table B-4.6: Accelerated solvent extraction of 20 % ethanol temperature 150 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	29.65	31.41	31.08	30.71	0.935
20	40	47.43	51.86	49.91	49.73	2.219
30	60	54.34	56.64	55.05	55.34	1.175
40	80	62.70	65.46	63.69	63.95	1.396
60	120	69.62	71.43	69.81	70.28	0.995

Table B-4.7: Accelerated solvent extraction of 50 % ethanol temperature 150 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	72.58	56.56	60.84	59.72	2.781
20	40	88.78	79.78	82.42	82.19	2.299
30	60	92.39	86.86	89.12	88.45	1.388
40	80	95.09	90.62	92.44	92.07	1.303
60	120	97.43	94.73	96.06	95.74	0.889

Table B-4.8: Accelerated solvent extraction of 80 % ethanol temperature 150 °C flow rate 2 ml/min at pressure 10 MPa

Time	Vol.	Run number			Average	Std.
		1	2	3		
0	0	0.00	0.00	0.00	0.00	0.000
10	20	67.56	62.70	64.58	64.95	2.452
20	40	81.83	80.41	80.25	80.83	0.872
30	60	88.59	84.75	86.02	86.46	1.957
40	80	92.61	88.49	89.85	90.32	2.097
60	120	96.39	95.46	95.65	95.83	0.493

Data of charantin extract with conventional method

Table B-5.1: Soxhlet extraction

Run	Volume (ml)	Time (min)	% Recovery
1	150	150	97.71
2	200	150	97.48
3	200	150	97.35

Mean   97.51

Amount of charantin

Types of solvent effect experiment

Table B-6.1: Accelerated solvent extraction of temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Types of solvent	Run number			Average	% Loss
	1	2	3		
Acetone	0.1415	0.1331	0.1187	0.1311	1.68
Ethyl acetate	0.1711	0.1752	0.1694	0.1719	-28.91
Ethanol	0.1176	0.1232	0.1427	0.1279	4.10
Dichloromethane	0.1057	0.1032	0.1015	0.1035	22.38
Water	0.1241	0.1256	0.1256	0.1251	6.17

Temperature effect experiment

Table B-6.2: Accelerated solvent extraction of ethanol flow rate 2 ml/min at pressure 10 MPa

Temperature (°C)	Run number			Average	% Loss
	1	2	3		
50	0.1009	0.0977	0.0999	0.0995	25.38
80	0.1208	0.1109	0.1110	0.1142	14.32
100	0.1176	0.1232	01427	0.1279	4.10
120	0.1573	0.1544	01407	0.1508	-13.12
150	0.1544	0.1376	0.1439	0.1453	-8.97

Flow rate effect experiment (100 °C)

Table B-6.3: Accelerated solvent extraction of ethanol temperature 100 °C at pressure 10 MPa

Flow rate (ml/min)	Run number			Average	% Loss
	1	2	3		
2	0.1176	0.1232	0.1427	0.1279	4.10
4	0.1278	0.1131	0.1208	0.1206	9.58
6	0.1449	0.1274	0.1324	0.1349	-1.18

Flow rate effect experiment (150 °C)

Table B-6.4: Accelerated solvent extraction of ethanol temperature 150 °C at pressure 10 MPa

Flow rate (ml/min)	Run number			Average	% Loss
	1	2	3		
2	0.1544	0.1376	0.1439	0.1453	-8.97
4	0.1185	0.1117	0.1129	0.1144	14.22
6	0.1474	0.1520	0.1521	0.1505	-12.88

Composition of solvent effect experiment (100 °C)

Table B-6.5: Accelerated solvent extraction of temperature 100 °C flow rate 2 ml/min at pressure 10 MPa

Composition	Run number			Average	% Loss
	1	2	3		
0 % ethanol	0.1241	0.1256	0.1213	0.1237	7.24
20% ethanol	0.1064	0.1145	0.1137	0.1115	16.34
50% ethanol	0.1272	0.1237	0.1211	0.1240	6.99
80% ethanol	0.1132	0.1065	0.1160	0.1119	16.07
100% ethanol	0.1176	0.1232	0.1427	0.1279	4.10

Composition of solvent effect experiment (150 °C)

Table B-6.6: Accelerated solvent extraction of temperature 150 °C flow rate 2 ml/min at pressure 10 MPa

Composition	Run number			Average	% Loss
	1	2	3		
0 % ethanol	0.1309	0.1374	0.1308	0.1330	0.22
20% ethanol	0.0882	0.0862	0.0873	0.0873	34.55
50% ethanol	0.1015	0.0951	0.1028	0.0998	25.16
80% ethanol	0.1081	0.1104	0.1090	0.1092	18.13
100% ethanol	0.1544	0.1376	0.1439	0.1453	-8.97

Data of amount charantin extract with Soxhlet extraction
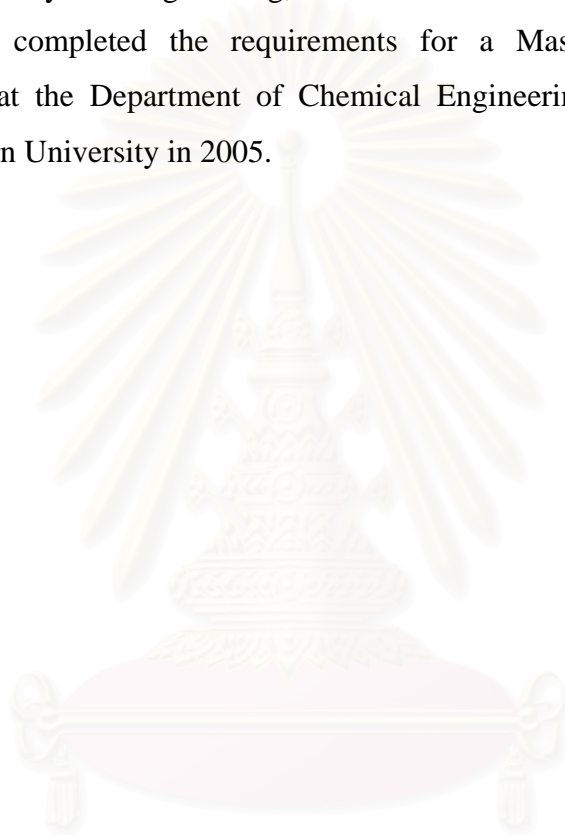
Table B-7.1: Soxhlet extraction

Run	Amount
1	0.1442
2	0.1232
3	0.1325

Average 0.1333 ± 0.018

VITA

Mr. Jesada Pitiphanpong was born on 15 December, 1979 in Nakornphathom, Thailand. He received a Bachelor's Degree of Chemical Engineering from the Faculty of Engineering, Kasetsart University in 2002. After then he subsequently completed the requirements for a Master's Degree in Chemical Engineering at the Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University in 2005.