# Preconcentration using deep eutectic solvents and high performance liquid chromatographic analysis of neonicotinoid insecticides

Rawikan Kachangoon

A Thesis Submitted in Partial Fulfillment of Requirements for
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# การเพิ่มความเข้มข้นโดยใช้ตัวทำละลายดีพยูเทกติกและการวิเคราะห์ด้วยโครมาโทกราฟีของเหลวสมรรถนะสูงของสารเคมีกำจัดแมลงกลุ่มนีโอนิโคตินอยด์

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The examining committee has unanimously approved this Thesis, submitted by Miss Rawikan Kachangoon , as a partial fulfillment of the requirements for the Master of Science Chemistry at Mahasarakham University

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**TITLE** Preconcentration using deep eutectic solvents and high performance liquid chromatographic analysis of neonicotinoid insecticides

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## ABSTRACT

Two different sample preconcentration methods were proposed including (i) in-situ metathesis reaction of deep eutectic solvent (DES) and (ii) dispersive liquid-liquid microextraction (DLLME) using hydrophobic DESs. In the first part, a simple two-step microextraction method followed by high-performance liquid chromatography for rapid determination of neonicotinoid insecticide residues was investigated. Thiamethoxam, clothianidin, imidacloprid, and thiacloprid were chosen as the target analytes. The method used with a cloud-point extraction based on in-situ metathesis reaction of deep eutectic solvents in order to achieve high extraction efficiency. Ultrasonic energy was used to provide mechanical shaking to accelerate the in-situ metathesis reaction. Various experimental parameters affecting the extraction efficiency such as salts addition, types and concentrations of surfactant, concentrations of hydrogen bond donor and hydrogen bond acceptor were investigated and optimized. Four neonicotinoid insecticides were separated within 10 min using monolithic column, with a mobile phase of 26% (v/v) acetonitrile in water, at a flow rate of 0.5 mL $min^{-1}$, and photodiode array detection at 254 nm. Under the optimum conditions, high enrichment factors (EF) (50-250) and low limits of detection (0.0003-0.001 µg $mL^{-1}$) were obtained. The proposed method was successfully applied to determine neonicotinoid insecticide residues in surface water, soil and human urine samples and high recoveries were obtained.

In second part, a sensitive hydrophobic DES-based dispersive liquid-liquid microextraction for the determination of neonicotinoid insecticide residues (thiamethoxam, clothianidin, acetamiprid, and thiacloprid) in various samples were established using high-performance liquid chromatography. A hydrophobic deep eutectic solvent was synthesized using decanoic acid as hydrogen bond donor and tetrabutylammonium bromide as hydrogen bond acceptor. The hydrophobic DES extraction phase was rigid after DLLME, being easy to be collected for analysis. Several key parameters were optimized such as salts addition, types of disperser solvent, molar ratio and concentration of hydrophobic DES and extraction times. Four neonicotinoids were separated within 13 min using a Purosphere® STAR RP-18 endcapped column, with a mobile phase of 25 %(v/v) acetonitrile in water at a

flow rate of 1 mL $min^{-1}$, and photo diode array detection at 254 nm. Under the optimum conditions, good precision, high enrichment factor and low limit of detection were obtained. The method is simple, green and practical, and could be applied to the extraction to neonicotinoid insecticide residues in surface water, soil and egg yolk samples and high recoveries within the range of 70-115% were obtained.

Keyword : in-situ metathesis, deep eutectic solvent, hydrophobic deep eutectic solvent, neonicotinoid insecticides, HPLC, extraction

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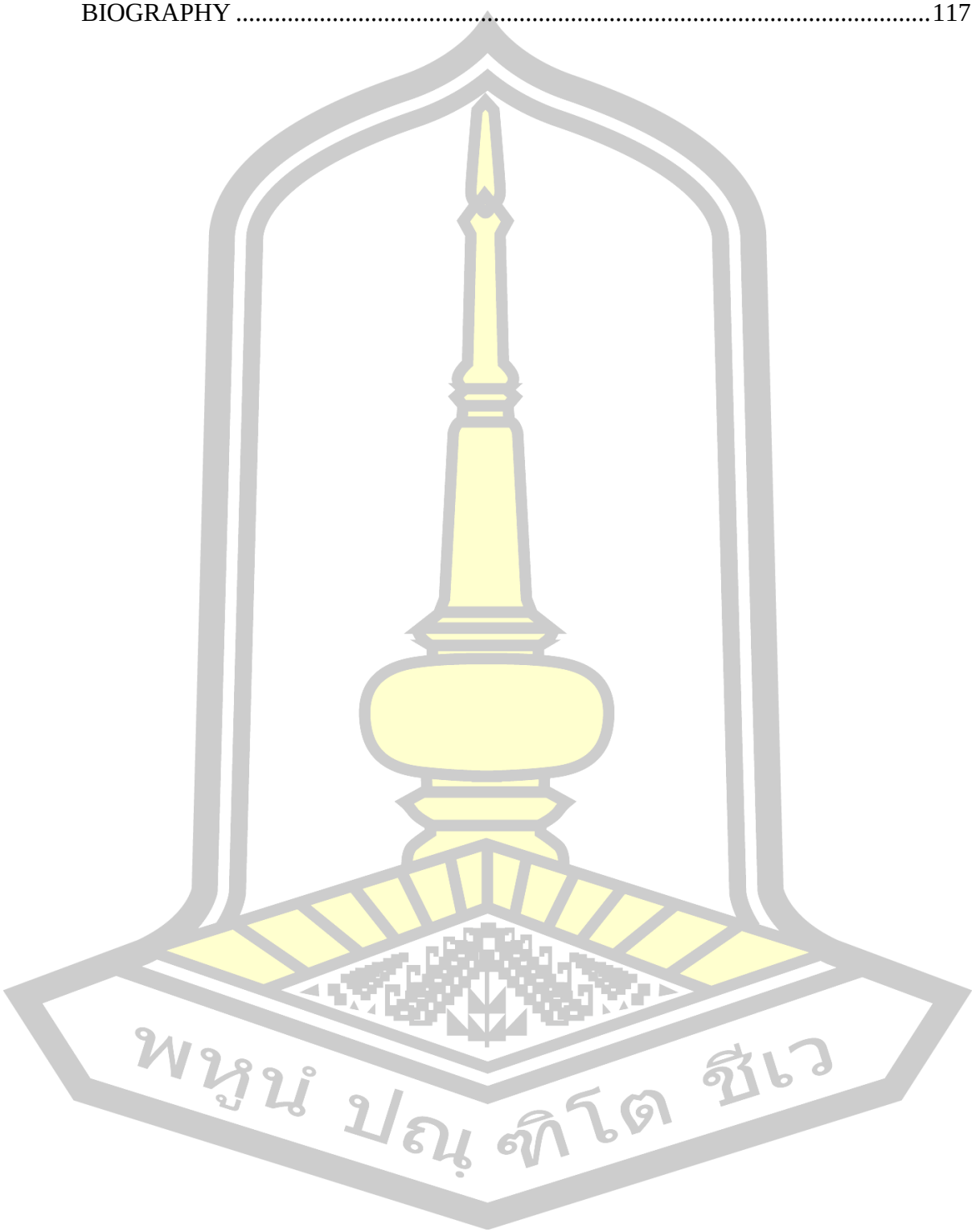

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# CHAPTER 1
# INTRODUCTION

## 1.1 Background and rational

Despite substantial technological advances in the field of analytical chemistry, most instruments are still not capable of directly handling relatively complex samples [1]. Therefore, sample preparation is an important and a preliminary step before analysis. Traditional sample preparation methods such as liquid-liquid extraction (LLE) [2] and solid-phase extraction (SPE) [3] are still being used in many laboratories. However, traditional LLE and SPE are time consuming, labor intensive and require large volume of toxic organic solvents [4]. New trends in sample preparation are exemplified by miniaturization of the extraction apparatus, reduction of pretreatment steps and time, and improvement of selectively to reduce matrix effect [5]. To solve these problems, another microextraction modes were introduced known as solid-phase microextraction (SPME) [6] and liquid-phase microextraction (LPME) [7]. SPME integrates sampling, extraction, concentration and sample introduction into a single solvent-free step. However, SPME has some limitation with regards to the fiber used such as high cost, carry-over effect, fragility and limited lifetime [8]. LPME approaches are much more cost-effective, and can be used in many different modes, such as single drop microextraction (SDME) [9], hollow fiber liquid-phase microextraction (HF-LPME) [10], ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME) [11] and dispersive liquid-liquid microextraction (DLLME) [12]. In 2006, a new liquid-liquid micro-extraction method named dispersive liquid–liquid micro-extraction (DLLME) was developed. It is based on the formation of the fine droplets of an extractant in an aqueous sample solution when a water-immiscible extraction solvent (extractant) dissolved in a water-miscible organic dispersive solvent is rapidly injected into a sample solution. [13]. The analytes in sample solution are extracted into the fine droplets, which are further separated by centrifugation. However, one of the limitations of DLLME is related to the requirement of a high density, but hazardous extraction solvent i.e. chlorobenzene, chloroform and carbon tetrachloride [14]. The extraction is then evaporated to dryness before analysis by high performance

liquid chromatography (HPLC), because these halogenated solvents are not compatible with the mobile phase of reversed-phase HPLC.

Recently, Abbot *et al*. [15] investigated a new class of extraction solvent, namely deep eutectic solvent (DES). They are considered as "green solvent" due to easy synthesis, structural designability and environmental friendliness. DESs are composed of a mixture of safe, cheap, renewable, and biodegradable organic compounds that are capable of associating of each other through hydrogen bonding and forming a compound that has a melting point far below that of either component. DESs are formed by complexation of quaternary ammonium salt (usually chlorine chloride (ChCl)) as the hydrogen bond acceptor (HBA) together with urea, organic acids, alcohols, amines, and amides as the hydrogen bond donor (HBD) [16–18]. The eutectic mixture was prepared by stirring the two components at a proper temperature until a homogeneous transparent liquid was formed [19]. An example, the synthetic route of ChCl-Glycerol is presented in Figure 1 [20].

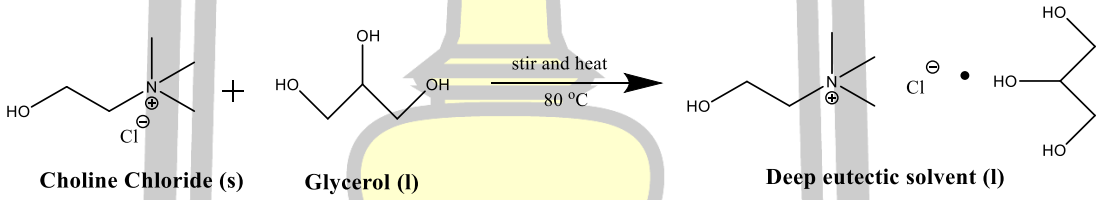



**Figure 1.** The synthetic route of ChCl-Glycerol.

The formation of hydrogen bonding between the halide anion of chlorine chloride and functional groups of hydrogen bond donor agent is responsible for the decrease in the freezing point of DESs in relation to the melting point of the individual components [21]. DESs have unique properties such as high purity and environmental friendliness [18]. DESs as an extraction solvent must have some characteristics such as high extraction affinity to the analytes, low solubility in aqueous solution and easy dispersion into water [22]. Over the year, in situ two phase extraction was investigated. It is the combination of growth and product formation with simultaneous product extraction [23]. In 2008, the metathesis reaction has been applied to develop a green solvent such as IL based in situ dispersive liquid-liquid microextraction method (DLLME), but these drawbacks limit of IL such as relatively expensive [24], complicated synthetic process, high price, and potential toxicity obstruct their use in DLLME [25]. Therefore, the search of non-toxic,

low cost, and environmentally friendly solvents, namely DES is still a challenging task in development and application of in-situ metathesis reaction for the used as extraction solvent. The applications of DESs to extraction processes involved in analytical method development, the isolation of bioactive compounds, and the removal of pollutants have been previously reviewed [26,27]. More specific reviews on the analytical applications of DESs as extraction solvents in liquid and solid samples have also been published [28]. A subclass of DESs called natural deep eutectic solvents (NADESs), which are formed from cellular primary metabolites, has been applied to similar analytical methods and reviewed in detail [29]. In most of the studies mentioned in the aforementioned reviews, the DESs used were hydrophilic and water-miscible, and the analytes of interest were extracted from solid or non-aqueous liquid samples that could be phase separated from the DESs. The hydrophilic DES property is anticipated because of their innate hydrogen-bonding ability [30]. Thus, hydrophobic DESs are required.

Hydrophobic DESs have been reported in 2015 [31] and could be excellent solutes for extraction technique instead of traditional water-immiscible toxic organic solvents and ILs [32]. Hydrophobic DESs cloud be obtained by combining decanoic acid and quaternary ammonium salts [33], menthol or quaternary ammonium salts with carboxylic acids [32], or DESs obtained solely from carboxylic acids [34].

Neonicotinoids were first developed and registered in the early 1990s, and have currently become the world's most widely used insecticides [35]. They are a class of insecticides chemically similar to nicotine and have strong selective neurotoxicity effect to insects [36]. Consequently, the occurrence and fate of neonicotinoids in environment have become an important global issue. This class of insecticides are small molecules with high solubility and low volatility [37]. Currently-seven neonicotinoids, thiamethoxam, clothianidin, imidacloprid, acetamiprid, thiacloprid, dinotefuran and nitepyram are used in agricultural production [38]. In fact, neonicotinoids have been widely detected in the environment with concentrations of parts per billion (ppb)-parts per million (ppm) in soil and parts per trillion (ppt)-ppb in water and ppb-ppm in plant [39]. In 2013, the European Union (EU) announced the restriction on the use of imidacloprid, thiamethoxam, and clothianidin following an assessment of their risk to bees by the European Food

Safety Authority (EFSA) [40–42]. Therefore, a simple and selective method for monitoring of neonicotinoid insecticide residues at low concentration levels is required to secure food quality and to protect hazard for consumer [43].

In this work, we proposed simple preconcentration methods for the sensitive determination of neonicotinoid insecticide residues. Two different simple microextraction methods for enrichment of neonicotinoid were investigated including (i) in-situ metathesis reaction of DES and (ii) DLLME using hydrophobic DES. The experimental parameters affecting the extraction efficiency is evaluated systematically such as salts addition, extraction time, and selection of concentration of extraction solvent. Applicability of the proposed methodologies was investigated in various samples.

**1.2 Purposes of the research**

1. To develop in-situ metathesis reaction using DES for preconcentration of some neonicotinoid insecticide residues.

2. To develop dispersive liquid-liquid microextraction using hydrophobic DES of some neonicotinoid insecticide residues.

3. To explore the feasibility of the developed methodologies for application in real samples.

**1.3 Scope of research**

1. The method was validated by the following parameters: calibration curves, limits of detection (LOD), limits of quantitation (LOQ), and reproducibility.

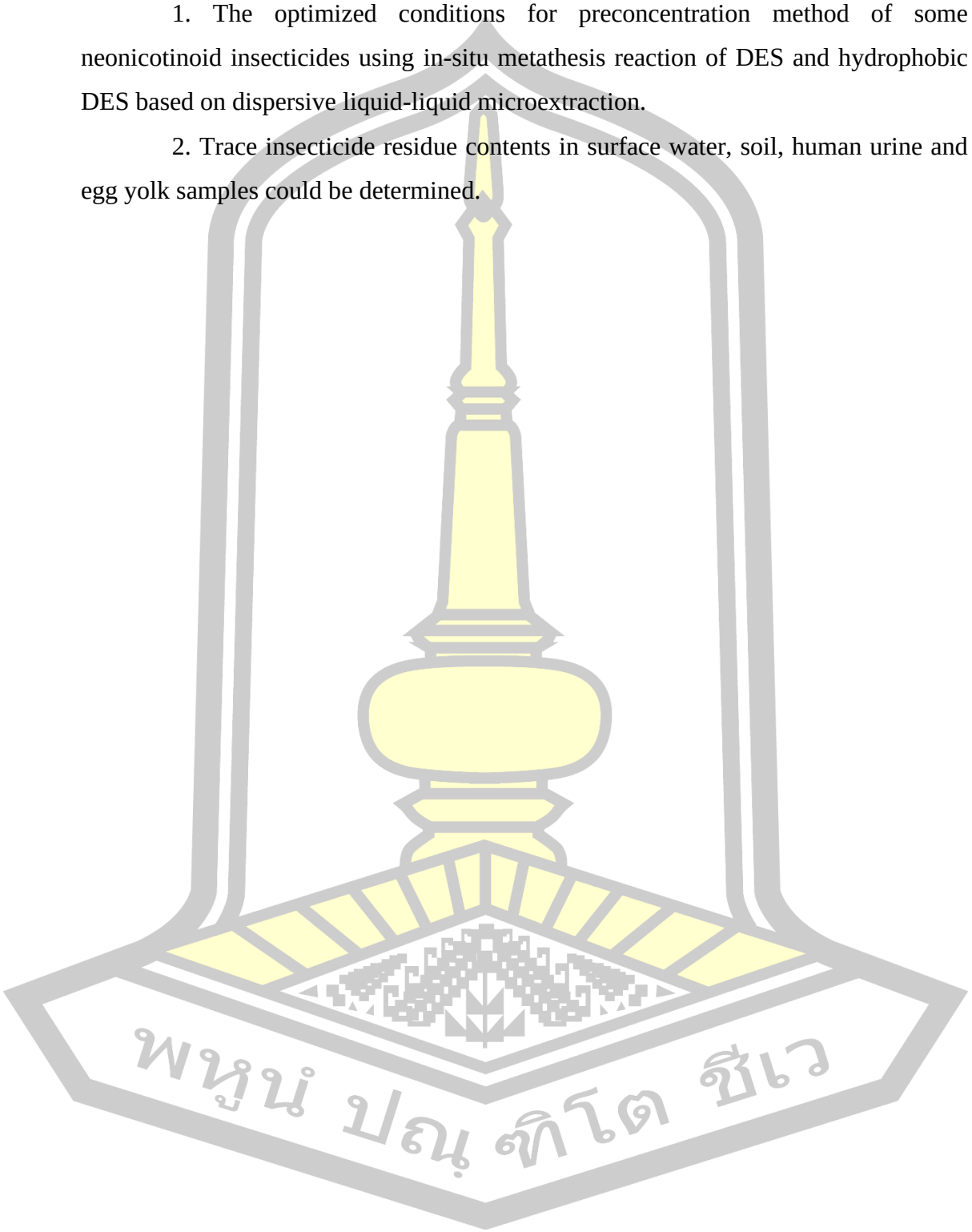
2. The preconcentration method was studied for determination of neonicotinoid insecticide residues in surface water, soil, urine and egg yolk samples.

3. Developed method was applied to analysis of neonicotinoid residues in surface water, soil, human urine and egg yolk samples.

**1.4 Benefit of research**

1. The optimized conditions for preconcentration method of some neonicotinoid insecticides using in-situ metathesis reaction of DES and hydrophobic DES based on dispersive liquid-liquid microextraction.

2. Trace insecticide residue contents in surface water, soil, human urine and egg yolk samples could be determined.



# CHAPTER 2
# LITERATURES REVIEW

## 2.1 Neonicotinoid insecticides

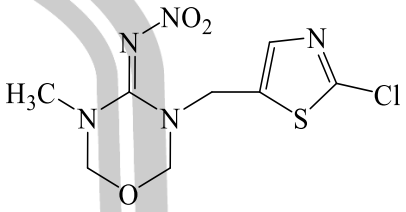
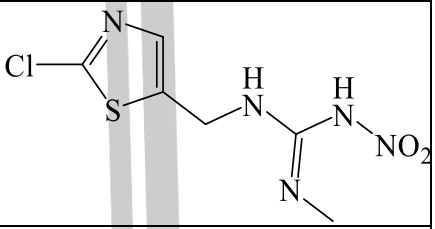
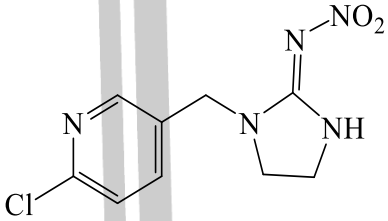
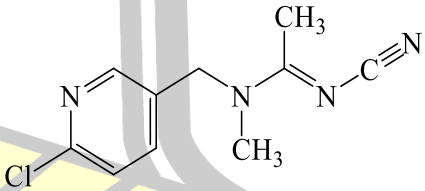
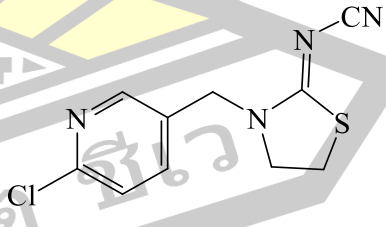
Neonicotinoids are one of the new major classes of insecticides, derived synthetically from nicotinoids. These compounds are the first new class of insecticides introduced in the last 50 years [44], following the discovery of nithiazine in 1985, numerous modifications have been made in the chloropyridyl nucleus to improve insecticidal spectrum and selective affinity to insect nicotinic acetylcholine receptor (nAChRs) and they were active against many sucking and biting pet insects, such as aphids, whiteflies, some lepidopteron and coleopteran species [45]. There are seven commercial neonicotinoids such as dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid [46]. Imidacloprid is currently the most widely used insecticide in the word [47]. Nowadays, the use of neonicotinoids was a matter of concern due to their high mobility in plants and environmental matrices, having been detected in surface water samples, obtained in the region near agriculture areas, from different regions of the planet. When introduced in agriculture fields (soil, foliar, and seed treatments) [48], they are commonly used on rice, maize, sunflowers, rapeseed, potatoes, sugar beets, vegetables, and fruits crop [21]. Consequently, restriction in their agriculture uses and maximum residue limits (MRLs) in some food commodities have been established [49]. Although, the environmental risks associated to neonicotinoids, and/or their primary transformation products, are still under evaluation [50], the EU has already included five neonicotinoids (four active ingredients and the main degradation product of thiamethoxam) in the watch list of emerging pollutants to be monitored in continental waters [42].

The European union (EU) commission in adopting a proposal for a two-year restriction use (applied from 1 December 2013) on three pesticides belonging to the neonicotinoid family (clothianidin, imidacloprid and thiamethoxam), the EU has established maximum permitted residue limits (MRLs) for the neonicotinoid insecticides, including some neonicotinoid metabolites (acetamiprid metabolite IM-2-1, thiamethoxam metabolite clothianidin) in honey and pollen intended for

human use ranging from 10-200 μg $kg^{-1}$, referring to the sum of the parent compound and its metabolites [51]. Although, the EU has established MRLs for the neonicotinoids in honey and pollen, honey liqueur, vegetables, fruits, and nature products which appears on the market as a traditional alcoholic drink is loosely controlled [52].

The physical and chemical properties of neonicotinoids are shown in Table 1 [53] molecular weights range from 160 to 292. Neonicotinoids have higher water solubility than other insecticides. The water solubility of neonicotinoids can also be altered by commercial formulations of the insecticides.

**Table 1.** Properties of the studied neonicotinoid insecticides from other chemical classes.

| **Neonicotinoid Insecticide** | **Water Solubility (mg $L^{-1}$) at 20 ˚C** | **Log Kow** | **Structure** |
|---|---|---|---|
| *N*-nitro-guanidines | | | |
| Thiamethoxam | 4100 | -0.13 |  |
| Clothianidin | 340 | 0.91 |  |
| Imidacloprid | 610 | 0.57 |  |
| *N*-cyano-amidines | | | |
| Acetamiprid | 4250 | 0.8 |  |
| Thiacloprid | 184 | 1.26 |  |

**2.2 Extraction based on DES**

The solvents are used extensively for dissolving reactants, affecting chemical reactivity, extracting and washing the products and for separating the mixtures [54]. Traditional organic solvents, in spite of a large number of self-evident advantages, are generally volatile, flammable, explosive and toxic for human beings, animals and even plants. Conventional organic solvents are not only hazardous to the environment but also show acute and chronic toxicity, carcinogenicity, and ecological toxicity and non-biodegradability [55]. To improve the protection of human health and the environment from the risks associated with the use of hazardous organic volatile solvents, tremendous efforts have been devoted for the development of alternative green reaction media [18]. DESs show similar physicochemical properties to ionic liquids, but they are much cheaper and safer for their use as solvents as compared to ionic liquids in synthetic transformations. As compared to RTILs, DESs have, however, notable advantages such as (i) their convenient synthesis (100% atom economy), (ii) their very low price since most of DESs can be prepared from readily accessible chemicals and (iii) their low toxicity, especially DESs derived from choline chloride (ChCl) and renewable chemicals [56]. Choline chloride is a commonly used organic salt for DESs, since it is biocompatible and most of the HBDs are cheap and environmentally benign such as urea, glycerol or carboxylic acids. Moreover, DESs do not produce toxic metabolites and are biodegradable. Additionally, the synthesis of RTILs is not environmentally friendly and generally requires a large amount of salts and solvents in order to completely exchange the anions. These drawbacks together with the high price of common ILs unfortunately restrict their industrial emergence such as metal electroplating, electro deposition and biocatalysts [21]. DESs have emerged as an interesting type of RTILs and have shown their usefulness as environmentally benign sustainable alternative to the conventional organic solvents in synthetic chemistry to increase efficiency of organic transformations. DESs are low melting mixtures based on a combination of readily available, biodegradable, recyclable and inexpensive components that are formed by mixing a quaternary ammonium or metal salt with a simple hydrogen bond donor (HBD), such as acids, amides, amines and alcohols and mostly exist as liquid at or below 100 °C because the melting point is drastically reduced after mixing two components as compared to

the melting points of the original two components. The charge delocalization occurring through hydrogen bond formation between the halide anion and the hydrogen donor moiety is responsible for the decrease in the freezing point of the mixture relative to the melting points of its individual component. Like RTILs, one of the most promising advantages of DESs is their extremely low vapor pressure i.e. low volatility, which is very attractive for their use in greener catalytic technologies [18,55]. Li *et al.* [57], synthesized the six kinds of new type of green betaine-based deep eutectic solvent (DESs) and applied the DESs aqueous two-phase system (DES-ATPs) for the extraction of protein followed by spectrometry and Fourier Transform Infrared (FTIR) spectrometer. Six kinds of DESs were synthesized by a heating method, eutectic mixtures consisted of hydrogen-bond acceptor (Betaine) and hydrogen-bond donor (urea, methylurea, D-(+)-glucose, D-sorbitol, ethylene glycol, and glycerol) with accurate calculated amounts of water were stirred at 100 ºC until an evenly, colorless liquid was formed such as the synthetic route of betaine-urea DES was shown in Figure 2 [57].

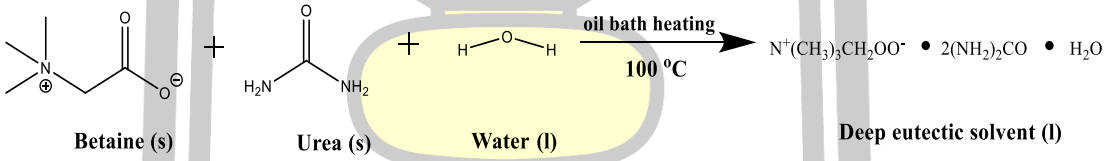



**Figure 2.** The synthetic route of betaine-urea to obtain deep eutectic solvent.

Since, the main physicochemical properties of DESs which are responsible for their use as green solvents at room temperature are: freezing points, density, viscosity, polarity, ionic conductivity and acidity/ alkalinity [15]. Nowadays, DESs have attracted a great deal of attention as promising green extraction media. Therefore, DESs are widely used in analytical chemistry and thus the process of extraction or separation [58]. Applications of DESs are growing fast as alternatives in research, industry and new process developments because of their potential as environmentally benign solvents and advantages over traditional ionic liquids [59]. Recently, various other applications of DESs have been reported. DESs indicate high solubilities for a wide range of solutes including metal oxides. The dissolution of metal oxides in a eutectic mixture of urea/choline chloride is quantified and plays

a key role in few processes such as metal winning, corrosion remediation, and catalyst preparation. Due to solvent properties of DESs which enable them to dissolve metal oxides electro polishing of stainless steel also have been proposed [16]. There were also investigated possible uses of DESs for increasing bioavailability in early drug development such as toxicology [18]. DESs can be also used in biocatalysis [19], pharmaceuticals [60] and in many others various chemical and industrial applications. Information involving applications of deep eutectic solvents in extraction process are summarized in Table 2.

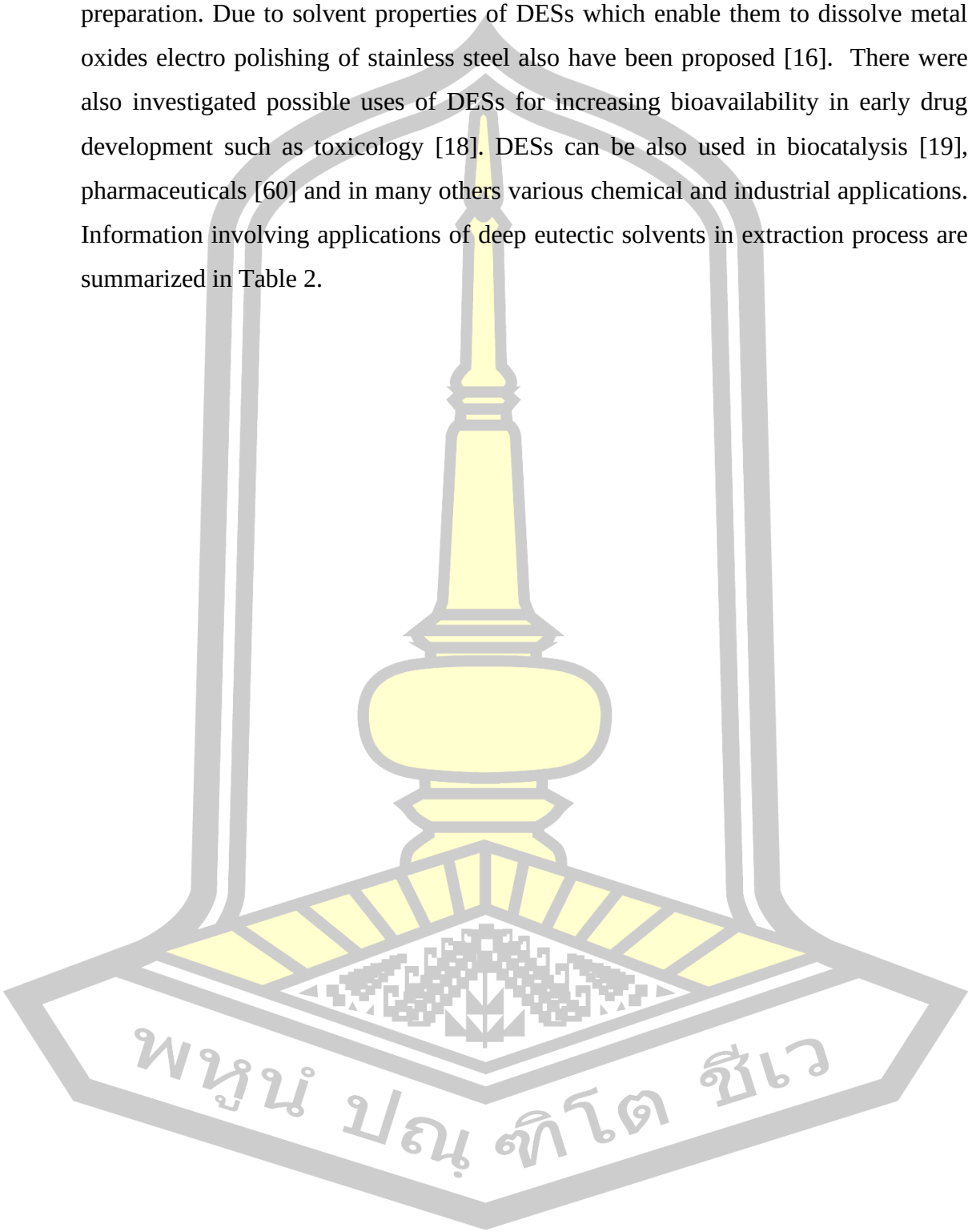


**Table 2.** Literatures on extraction method using deep eutectic solvent.

| Author (year) | Analytes / Samples | Extraction method | Detection | LODs |
|---|---|---|---|---|
| Solaesa *et al.* (2019) [61] | Brominated flame retardants and organochloride pollutants/ fish oils | DES-VALLME | GC-MS/MS | 0.2-0.7 ng $g^{-1}$ |
| Wang *et al.* (2019) [62] | Triazine and herbicides/ edible vegetable oils | VA-DES-LLME | HPLC-UV | 0.60-1.50 µg $L^{-1}$ |
| Farajzadeh *et al.* (2018) [63] | Pesticides/ fruit juice and vegetable samples<br>- Diazinon<br>- Metalaxyl<br>- Bromopropylate<br>- Oxadiazon<br>- Fenazaquin | Temperature-controlled LPME | GC-FID | 0.13-0.31 ng $mL^{-1}$ |
| Lamei *et al.* (2017) [64] | Methadone/ water and biological samples | Air assisted emulsification LLME | GC-FID | 0.7 µg $L^{-1}$ |
| Yousefi *et al.* (2017) [65] | Organochlorine pesticides/ water samples | DES magnetic bucky gels in developing dSPE | GC-µECD | 0.0004-0.0027 µg $L^{-1}$ |
| Farajzadeh *et al.* (2017) [66] | Some pesticide residues/ fruit and vegetable samples | Gas assisted DLLME | GC-FID | 0.24-1.4 µg $L^{-1}$ |

**2.3 Hydrophobic deep eutectic solvent extraction**

Another type of novel alternative solvents that have gained considerable attention within the past few years is deep eutectic solvents (DESs) [67]. DESs have attracted great interest in many fields as a new generation of green and sustainable solvents [68]. DESs are formed as a result of specific interactions, mostly hydrogen bonding, between two compounds, one of which is a hydrogen bond donor (HBD) and the other one is a hydrogen bond acceptor (HBA) [34]. The eutectic mixture obtained is characterized by a much lower melting point than either of the two components [18]. DESs could be easily prepared from more available and greener (natural) materials without need to further purification [69]. However, most DESs reported so far are miscible with water and solid at room temperature [70], and cannot be used as extraction agents in DLLME for the extraction of analytes from aqueous samples [68]. Since, in some case such as DES of ChCl-Ph, this restriction has been resolved by using an emulsifier such as tetrahydrofuran (THF) [71]. Therefore, in order to extend liquid phase microextraction (LPME) applications of DESs in water-based samples as well as increase eco-environmentally of analytical procedures, development of hydrophobic DESs could be resulting in less DES volume usage as well as elimination of disperser or emulsifier organic solvents [72].

In 2015, Kroon et al. [32] first reported hydrophobic DESs consisting of decanoic acid and various quaternary ammonium salts and used them to extract volatile fatty acids from diluted aqueous solutions. Later, they further applied decanoic acid-lidocaine based hydrophobic DESs to the removal of alkali and transition metal ions from water [73]. Recently, the preparation and application of hydrophobic DESs was reported, such as using decanoic acid and various quaternary ammonium salts [32], menthol-based hydrophobic low viscosity solvents [74], indium extraction from hydrochloric and oxalic acids using hydrophobic deep eutectic and low-transition-temperature mixtures [75], thus greatly expanding the possibilities of DES. Most importantly, the merit of hydrophobic DESs lies in that they can be used as extraction solvents to construct DLLME methods for the extraction of analytes from water samples [66], thus greatly expanding the possibilities of DES. Information on another application of hydrophobic deep eutectic solvents in extraction process are summarized in Table 3.

**Table 3.** Literatures on extraction method using hydrophobic deep eutectic.

| Author (year) | Analytes / Samples | Extraction method | Detection | LODs |
|---|---|---|---|---|
| Deng *et al.* (2019) [68] | Pyrethroid insecticides/ tea beverage and fruit juice samples | DLLME-HFIP-DESs | HPLC-DAD | 0.06-0.17 ng $mL^{-1}$ |
| Liu *et al.* (2019) [76] | Pyrethroid insecticides/ water samples<br>- Deltamethrin<br>- Fenvalerate<br>- Permethrin<br>- Etofenprox<br>- Bifenthrin | UA-DLLME based hydrophobic DES | HPLC-UV | 0.30-0.60 µg $L^{-1}$ |
| Makoś *et al.* (2018) [34] | Polycyclic aromatic hydrocarbons/ aqueous samples | UA-DLLME based hydrophobic DES | GC-MS | 0.0039-0.0098 µg $L^{-1}$ |
| Zhu *et al.* (2018) [69] | Synthetic pigments/ beverage samples<br>- Tebuconazole<br>- Diazinon<br>- Fenazaquin<br>- Clodinafop-propargyl<br>- Haloxyfop-R-methyl | LLME-hydrophobic DES | HPLC-UV | 0.016-1.12 ng $mL^{-1}$ |

**Table 3.** Literatures on extraction method using hydrophobic deep eutectic (cont.).

| Author (year) | Analytes / Samples | Extraction method | Detection | LODs |
|---|---|---|---|---|
| van Osch *et al.* (2016) [73] | Alkali and transition metal ions/ water samples | Hydrophobic DES extraction via ion exchange mechanism | NMR, FTIR and TGA | - |
| Ribero *et al.* (2015) [74] | - | Synthesized hydrophobic low viscosity DES based DL-methol | NMR, FTIR, DSC and TGA | - |

*-: no reported

**2.4 In-situ metathesis reaction**

Among of extractions and preconcentration techniques, DLLME is the most widely used for the separation and preconcentration of pesticides from different matrices. DLLME was introduced by Rezaee *et al.* in 2006 [77]. In traditional DLLME, dispersion solvent is required to dissipate the extraction solvents in an aqueous solution. However, the dispersion solvents may simultaneously increase the solubility of the analytes in water samples, thereby decreasing the recovery [68-69]. To avoid the use of these dispersion solvents, in situ DLLME was developed. In chemistry, in situ typically means “in the reaction mixture”. There are numerous situations in which chemical intermediates are synthesized in situ in various processes. This may be done because the species is unstable, and cannot be isolated, or simply out of convenience [18]. Over the year, in situ two-phase extraction was investigated. It is the combination of growth and product formation with simultaneous product extraction [23]. In addition to being used in situ extraction, two-phase are applied as downstream processes to selectively extract and preconcentrate the target analyte and thus ease further downstream processes. However, there are few reports on the use of in situ extraction with ionic liquid [78–84] for various target analytes. To achieve this goal, we employed deep eutectic solvents (DESs) as the extraction solvent, since these solvents can efficiently extract a various sample. The applications of in-situ metathesis reaction in extraction process are summarized in Table 4.

**Table 4.** Literatures on in-situ metathesis reaction in extraction method.

| Author (year) | Analytes/ Samples | Extraction conditions | Detection | LODs |
|---|---|---|---|---|
| Cacho *et al.* (2018) [80] | Organo-phosphorous pesticides/ environmental water | Sample volume: 10 mL<br>Internal standard: 1 ng $L^{-1}$ TBP<br>Extraction solvent: $[C_4MIm][NTf_2]$<br>240 µL of $Li[NTf_2]$ mixed with 200 µL of 1 mol $L^{-1}$ ([C4MIm]Cl)<br>Agitation: centrifuged at 3000 rpm for 2 min | GC-MS | 4.1-9.7 ng $L^{-1}$ |
| Fan *et al.* (2017) [85] | Pyrethroid/ water samples | Sample volume: 10 mL<br>Extraction solvent: $[P_{4448}][N(CN)_2]$<br>200 µL of $[P_{4448}][Br]$ mixed with 300 µL of 0.2 mmol $L^{-1}$ $Na[N(CN)_2]$<br>Agitation: manual shaken | HPLC | 0.16-0.21 µg $L^{-1}$ |
| Fan *et al.* (2015) [86] | Chlorophenols/ honey samples | Sample volume: 5 mL<br>Extraction solvent:<br>100 µL $[C_4MIM][BF_4]$ mixed with 300 µL $LiNTf_2$<br>Agitation: centrifuged at 4000 rpm for 4min | HPLC-UV-Vis-PDA | 0.8-3.2 µg $L^{-1}$ |

**Table 4.** Literatures on in-situ metathesis reaction in extraction method (cont.).

| Author (year) | Analytes/ Samples | Extraction conditions | Detection | LODs |
|---|---|---|---|---|
| Hu *et al.* (2015) [83] | Pyrethroid insecticides/ water samples | Sample volume: 10 mL<br>Extraction solvent: $[P_{44412}]PF_6$<br>35 g $[P_{44412}]Br$ mixed with 21.4 mg $KPF_6$<br>Agitation: centrifuged at 10,000 rpm for 10 min | HPLC-MS-ESI | 0.71-1.54 µg $L^{-1}$ |
| Galán-Cano *et al.* (2012) [87] | Chlorophenols/ water samples | Sample volume: 50 mL<br>Internal standard: 5 ng $mL^{-1}$ 4-bromophenol<br>Extraction solvent:<br>50 mg $[Omim][PF_6]$ mixed with 50 mg $KPF_6$<br>Agitation: vortex 1 min and centrifuged at 5000 rpm for 5 min | GC-MS | 60-440 ng $L^{-1}$ |
| Zhang *et al.* (2012) [82] | Phenylurea pesticides/ water samples | Sample volume: 10 mL<br>Extraction solvent: $[C_6MIM]NTf_2$<br>360 µL $LiNTf_2$ mixed with 0.034 g $[C_6MIM]Cl$<br>Agitation: ultrasound for 4 min and centrifuged at 3500 rpm for 10 min | HPLC-VWD | 0.06-0.08 µg $L^{-1}$ |

**2.5 Sample preparation and chromatographic determination of neonicotinoids**

There are a number of reports for determination of neonicotinoid insecticide residues in different samples by various sample preparation and preconcentration techniques followed by instrumental analysis such as LC-MS, GC, and HPLC. After extraction, the DES phase containing the target analytes can be directly introduced into the HPLC-UV [88], HPLC-MS [89], GC-FID [13] and GC-MS [90] systems without extract pre-dilution. UV-Vis and spectrofluorimetric [3] detection can also be implemented. In part of extraction, new techniques miniaturizing solid-phase extraction (SPE) or liquid-liquid extraction (LLE) have appeared such as solid phase microextraction (SPME) and liquid phase microextraction (LPME), respectively. SPME as a solventless extraction is very popular technique in recent years that use different fiber materials in various configurations for the extraction of a wide range of volatile analytes [58]. LPME comprises a range of slightly different techniques characterized by using low amounts of sample matrices and small volumes of organic solvents [91]. Usually, the LPME process based on DES assumes preliminary DES synthesis followed by DES mixing with sample resulting target analytes extraction. Recently, new approach for extraction of phenolic compounds from organic phase based on in situ deep eutectic mixtures formation has been proposed [27]. The extraction solvent is formed in situ in relatively fast chemical reaction in a sample solution and no dispersion solvent is required. Only a few examples of that procedure can be found in the literatures. Information on sample preparation and preconcentration techniques combined with various instruments are summarized in Table 5.

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids

| **Author (year)** | **Analytes / Samples** | **Chromatographic condition/ Preconcentration technique** |
|---|---|---|
| Xiao *et al.* (2011) [92] | Seven neonicotinoid insecticide residues/ bovine tissues<br>- Nitenpyram<br>- Thiamethoxam<br>- Imidacloprid<br>- Imidacloprid-$d_4$<br>- Clothianidin<br>- Acetamiprid<br>- Thiacloprid | LC-MS/MS:<br>Column: Waters Symmetry Shield™ RP-C18 column (150 mm × 2.1 mm, 3 µm)<br>Mobile phase:<br>- (A) acetonitrile<br>- (B) water<br>Both acidified with 0.1% formic acid<br>Flow rate: 0.2 mL $min^{-1}$<br>Injection volume: 20 µL<br>Detector: Quattro LC triple quadrupole-MS/MS-ESI interface<br>PSE:<br>Sample: 2.5 g<br>Agitation: vortex for 30s, centrifugated at 3800 rpm for 5 min<br>SPE cartridge: Oasis HLB cartridge conditioned with 5 mL MeOH and 5 mL water<br>Eluent: 3 mL of MeOH, eluent evaporated to dryness under a gentle stream of $N_2$ at 40 °C. |

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids (cont.).

| Author (year) | Analytes / Samples | Chromatographic condition/ Preconcentration technique |
|---|---|---|
| Zhang *et al.* (2012) [2] | Some neonicotinoid insecticides/ cucumber<br>- Thiacloprid<br>- Acetamiprid<br>- Imidacloprid | MEKC:<br>Column: Beckman P/ACE MDQ Capillary Electrophoresis, uncoated fused-silica capillary (50 cm × 75 μm i.d.)<br>Detector: diode array detector<br>DLLME:<br>Sample: 20.0 g, diluted to 20.0 mL with double-distilled water<br>Agitation: centrifuged at 3500 rpm for 10 min<br>Disperser solvent: ACN<br>Extraction solvent: $CH_3Cl$<br>Agitation: vortexed for 1 min, centrifuged at 3500 rpm for 5 min |
| Yáñez *et al.* (2013) [93] | Seven neonicotinoid insecticides/ beeswax<br>- Acetamiprid<br>- Clothianidin<br>- Dinotefuran<br>- Imidacloprid<br>- Nitenpyram<br>- Thiacloprid<br>- Thiamethoxam | LC-ESI-MS:<br>Column: fused-core type column (Kinetex® $C_{18}$, 150 mm × 4.6 mm, 2.6 μm)<br>Temperature of column: at 35 °C<br>Mobile phase:<br>- (A) 0.1% formic acid in water<br>- (B) acetonitrile<br>Flow rate: 0.5 mL $min^{-1}$<br>Detector: a single quadrupole MS analyzer-ESI interface |

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids (cont.).

| Author (year) | Analytes / Samples | Chromatographic condition/ Preconcentration technique |
|---|---|---|
| Giroud *et al.* (2013) [94] | Pyrethroid and neonicotinoid insecticides/ cucumber<br>- 6-Chloronicotinic acid<br>- Thiamethoxam<br>- Olefin<br>- 5-Hydroxy-imidacloprid<br>- Clothianidine<br>- Imidacloprid<br>- Acetamiprid<br>- Thiacloprid<br>- Lambda-cyhalothrine<br>- Cypermethrine<br>- Deltametrine<br>- Esfenvalerate<br>- Bifenthrine | UPLC-MS/MS:<br>Column: Kinetex Phenyl-Hexyl column (100 mm × 2.1 mm, 2.6 µm)<br>Mobile phase:<br>- (A) 0.01% HOAc with 0.04 mmol $L^{-1}$ ammonium acetate in MilliQ water<br>- (B) MeOH-water<br>Flow rate: 0.4 mL $min^{-1}$<br>Oven temperature: 60 °C<br>Injection volume: 2 µL<br>Detector: a Xevo TQ-S triple quadrupole-ESI<br>QuEChERS:<br>Sample: 2 g of sample<br>Extraction solvent: 5 mL of pure water, 5 mL of heptane and 10 mL of ACN with 2% TEA<br>Agitation: ceramic bar, vortexed, manually shaken for 10s, centrifuged at 5000 rpm for 2 min<br>Buffer: acetate buffer<br>Sorbents: $MgSO_4$, PSA |

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids (cont.).

| **Author (year)** | **Analytes / Samples** | **Chromatographic condition/ Preconcentration technique** |
|---|---|---|
| Dankyi *et al.* (2014) [45] | Neonicotinoid insecticide residues/ soils from cocoa plantations<br>- Imidacloprid<br>- Acetamiprid<br>- Thiacloprid<br>- Thiamethoxam<br>- Clothianidin | LC-MS/MS:<br>Column: BDS Hypersil reversed-phase C-18 column (250 mm × 2.1 mm, 5 µm)<br>Column temperature: 30 ºC<br>Mobile phase:<br>- (A) 99% of 10 nmol $L^{-1}$ ammonium acetate, with 1% methanol<br>- (B) 90% methanol with 10% of 10 nM ammonium acetate<br>Flow rate: 200 µL $min^{-1}$<br>Detector: MS-ESI in positive mode<br>QuEChERS:<br>Sample: 5 g<br>Extraction solvent: 10 ml of ACN in 1% HOAc<br>Sorbents:<br>(I) 4.0 g $MgSO_4$, 1.5 g NaOAc<br>(II) 4.0 g $MgSO_4$, 1.0 g NaCl<br>(III) 4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, and 0.5 g SCDS |

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids (cont.).

| Author (year) | Analytes / Samples | Chromatographic condition/ Preconcentration technique |
|---|---|---|
| Jovanov *et al.* (2015) [95] | Seven neonicotinoid insecticides/ honey samples<br>- Nitenpyram<br>- Thiamethoxam<br>- Acetamiprid<br>- Thiacloprid<br>- Clothianidin<br>- Dinotefuran<br>- Imidacloprid | HPLC-DAD:<br>Column: ZORBAX Eclipse XDB-C18 column (50 mm × 4.6 mm, 1.8 µm)<br>Column temperature: 30 °C<br>Mobile phase:<br>- (A) acetonitrile<br>- (B) ultra-pure water with 0.1% formic acid<br>Flow rate: 0.7 mL $min^{-1}$<br>Detector: a triple quad LC-MS<br>DLLME:<br>Sample: 50 mL<br>Dispersive solvent: ACN<br>Extraction solvent: dichloromethane<br>Agitation: vortex, sonicate, centrifuge<br>QuEChERS:<br>Sample: 15.0 mL<br>Buffering salts:<br>400 mg of $MgSO_4$<br>1000 mg of NaCl<br>500 mg of sodium citrate dibasic sesquihydrate<br>1000 mg of sodium citrate tribasic dehydrate<br>Sample cleanup: 900 mg of $MgSO_4$ and 150 mg of PSA<br>Agitation: vortexed, centrifuged |

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids (cont.).

| **Author (year)** | **Analytes / Samples** | **Chromatographic condition/ Preconcentration technique** |
|---|---|---|
| Abdel-Ghany *et al.* (2016) [46] | Eight neonicotinoid insecticide residues and two primary metabolites/ cucumbers and soil<br>- Acetamiprid<br>- Imidacloprid<br>- Nitenpyram<br>- Thiamethoxam<br>- Flonicamid<br>- Clothianidin<br>- Dinotefuran<br>- Thiacloprid | <u>HPLC–MS/MS:</u><br>Column: Gemini C18 Column (100 mm × 1 mm i.d.)<br>Detector: FTICR-MS-ESI<br><u>QuEChERS:</u><br>Sample: 10 g<br>Extraction solvent: ACN<br>Sorbents: 4 g of anhydrous $MgSO_4$, 1 g of NaCl, C18 and 0.8 g anhydrous $MgSO_4$<br>Agitation: vortex, centrifuge at 3000 rpm for 5 min |
| Pastor-Belda *et al.* (2016) [96] | Spirocyclic tetronic/tetramic acid derivatives and neonicotinoid insecticides/ fruits and vegetables | <u>LC-MS:</u><br>Column: reversed phase ODS2 analytical column (150 mm × 4 mm, 5 µm)<br>Mobile phase:<br>- (A) 0.1 % (v/v) formic acid<br>- (B) acetonitrile<br>Flow rate: 0.7 mL $min^{-1}$<br>Injection volume: 10 µL<br>Detector: triple quadrupole MS-ESI<br><u>SLE:</u><br>Extraction solvent: acetonitrile |

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids (cont.).

| Author (year) | Analytes / Samples | Chromatographic condition/ Preconcentration technique |
|---|---|---|
| | | DLLME:<br>Extraction solvent:<br>- chloroform (extraction of neonicotinoid insecticides)<br>- carbon tetrachloride (extraction Spirocyclic tetronic/tetramic acid derivatives) |
| Farajzadeh *et al.* (2016) [49] | Neonicotinoid insecticides/ fruit juice and vegetable samples | LC-DAD:<br>Column: STR-ODS (II) analytical column (150 mm × 4.6 mm id., 5 µm)<br>Mobile phase: acetonitrile: water (30:70, v/v)<br>Flow rate: 1 mL $min^{-1}$<br>Injection volume: 5 µL<br>Detector: Diode-array detector (DAD)<br>Wavelength:<br>- imidacloprid: 271 nm<br>- thiamethoxam: 253 nm<br>- acetamiprid: 244 nm<br>Ringer tablet-based ionic liquid phase microextraction:<br>Ringer tablet: 0.9 g<br>Extraction solvent: [HMIM][$PF_6$]<br>Disperser solvent: room temperature ionic liquid (RTIL) |

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids (cont.).

| Author (year) | Analytes / Samples | Chromatographic condition/ Preconcentration technique |
|---|---|---|
| Pastor-Belda *et al.* (2017) [96] | Trace neonicotinoids/ water | HPLC-MS/MS:<br>Column: Agilent Zorbax Eclipse Plus C18 column<br>(100 mm × 2.1 mm i.d., 1.8 µm)<br>Column temperature: 40 °C<br>Mobile phase:<br>- (A) 0.1% formic acid<br>- (B) acetonitrile<br>Flow rate: 0.3 mL $min^{-1}$<br>Injection volume: 2 µL<br>Detector: MS-ESI<br>SPE:<br>Extraction device: SPE cartridge<br>- hydrophilic-lipophilic balance (HLB)<br>- graphitized carbon black (GCB)<br>Extraction solvent: acetonitrile: acetone (8:2, v/v) |
| Shi *et al.* (2017) [50] | Neonicotinoid insecticides/ sunflower seeds | UPLC-MS/MS:<br>Column: ACQUTTY UPLC ® BEH C18 column<br>(100 mm × 2.1 mm, 1.7 µm)<br>- Guard column: BEH C18 VanGuard™ pre-column<br>(5 mm × 2.1 mm, 1.7 µm)<br>Column temperature: 30 °C<br>Autosampler temperature: 15 °C |

**Table 5.** Literatures on sample preparation and chromatographic determination of neonicotinoids (cont.).

| Author (year) | Analytes / Samples | Chromatographic condition/ Preconcentration technique |
|---|---|---|
| | | Mobile phase:<br>- (A) 0.1% formic acid<br>- (B) acetonitrile<br>Flow rate: 0.4 mL $min^{-1}$<br>Detector: mass spectrometry was performed on a Waters Acquity Xevo TQ MS-ESI<br>SPE:<br>SPE cartridge: packed 20 mg (dry weight) of $CH_3NH$-G<br>Sample cleanup:<br>- conditioned: 3 mL of methanol, 3 mL acetonitrile, and 3 mL acetone and 9 mL water |

# CHAPTER 3
# METHODOLOGY

## 3.1 Reagents and Standards

All reagents were analytical grade or higher. They were obtained from various suppliers, as summarized in Table 6. Aqueous solution were prepared in deionized water with the resistivity of 18.2 MΩ.cm from a RiOs™ Type I Simplicity 185 (Millipore, USA).

**Table 6.** Chemical and reagents used in this work.

| **Chemicals** | **Formula** | **Company** | **Country** |
|---|---|---|---|
| Acetamiprid | $C_{10}H_{11}ClN_4$ | Dr. Ehren-storfer | Germany |
| Acetonitrile | $CH_3CN$ | Merck | Germany |
| Ammonium chloride | $NH_4Cl$ | Ajax Finechem | New Zealand |
| Cetyltrimethyl ammonium bromide | $C_{19}H_{42}BrN$ | Calbiochem | Germany |
| Choline chloride | $C_5H_{14}ClNO$ | Sigma-Aldrich | Germany |
| Clothianidin | $C_6H_8ClN_5O_2S$ | Dr. Ehren-storfer | Germany |
| Decanoic acid | $C_{10}H_{20}O_2$ | Sigma-Aldrich | Germany |
| Ethanol | $C_2H_5OH$ | Merck | Germany |
| Imidacloprid | $C_9H_{10}ClN_5O_2$ | Dr. Ehren-storfer | Germany |
| Methanol | $CH_3OH$ | Merck | Germany |
| Phenol | $C_6H_5OH$ | Sigma-Aldrich | Germany |
| Sodium acetate | $CH_3COONa{\cdot}3H_2O$ | CarloErba | France |
| Sodium carbonate | $Na_2CO_3$ | Ajax Finechem | New Zealand |
| Sodium chloride | NaCl | Ajax Finechem | New Zealand |
| Sodium dodecyl sulfate | $C_{12}H_{25}NaO_4S$ | Merck | Germany |

**Table 6.** Chemical and reagents used in this work (cont.).

| Chemicals | Formula | Company | Country |
|---|---|---|---|
| Sodium sulphate | $Na_2SO_4$ | Ajax Finechem | New Zealand |
| Tetrabutylammonium bromide (TBABr) | $(CH_3CH_2CH_2CH_2)_4N(Br)$ | ACROS Organics | USA |
| Thiacloprid | $C_{10}H_9ClN_4S$ | Fluka | Germany |
| Thiamethoxam | $C_8H_{10}ClN_5O_3S$ | Dr. Ehren-storfer | Germany |
| Triton X-100 (TX-100) | $C_{14}H_{22}O(C_2H_4O)_n$, n = 9 or 10 | Merck | Germany |
| Triton X-114 (TX-114) | $(C_2H_4O)_n$, n = 7 or 8 | Merck | Germany |

### 3.2 Instrumentation

The HPLC system consists of a Waters 1525 Binary HPLC pump (USA), and a Photodiode array detector (PDA) operated at 254 nm. Table 7 shows the chromatographic conditions used for separation of neonicotinoid insecticide residues using in-situ metathesis reaction of deep eutectic solvent extraction and Table 8 shows the chromatographic conditions used for separation of neonicotinoid insecticide residues using hydrophobic deep eutectic solvent extraction. Fourier transformed infrared spectra (FTIR) spectra of DES samples were obtained using a Bruker Tensor 27 FT-IR (Bruker corp, Massachusetts, USA). Diamond lens attenuated total resistance (ATR) were used. A centrifuge (Centurion, England) was used for complete phase separation. An ultrasonic bath (Dksh, Germany) and a vortex mixer (Fisher Scientific, USA) were also used.

**Table 7.** The chromatographic conditions used for separation of neonicotinoid insecticide residues using in-situ metathesis reaction of deep eutectic solvent extraction.

| Parameters | Conditions |
|---|---|
| Column | Chromolith® Highresolution RP-18 endcapped column (100 mm × 4.6 mm) |
| Mobile phase | 26 %v/v acetonitrile in water |
| Flow rate (mL $min^{-1}$) | 0.5 |
| Injection volume (µL) | 20 |

**Table 8.** The chromatographic conditions used for separation of neonicotinoid insecticide residues using hydrophobic deep eutectic solvent extraction.

| Parameters | Conditions |
|---|---|
| Column | Purosphere® STAR RP-18 endcapped column (150 mm × 4.6 mm, 5.0 µm) |
| Mobile phase | 25 %v/v acetonitrile in water |
| Flow rate (mL $min^{-1}$) | 1.0 |
| Injection volume (µL) | 20 |

**3.3 Real samples**

3.3.1. Surface water samples

Surface water were collected from the different natural water resources located near agricultural fields in Maha Sarakham province, Northeast Thailand. These samples were filtered through a Whatman No. 1 filter paper before applying to the proposed extraction method.

3.3.2. Soil samples

Soil samples were collected from the different natural located near agricultural fields in Maha Sarakham province, Northeast Thailand. These samples were taken from the surface (0-10 cm depth). Soils were air-dried, ground and sifted through a 2-mm sieve. After that, samples were extracted using the method proposed by Meghesan-Breja *et al.* [97] and Arnnok *et al.* [98]. In detail, the accurately

weighed 20 g soil sample was mixed with 20 mL of water, then 20 mL of acidified acetonitrile (1% acetic acid) was added. The samples were mechanically shaken at 200 rpm for 10 min before adding 24 g of anhydrous sodium sulfate and 6 g of sodium acetate, after which the mixture was shaken by hand for a few minutes. The supernatant was subsequently transferred to a 50 mL centrifuge tube. A 10 mL aliquot of the upper layer was taken and mixed with water up to 125 mL before applying to the proposed extraction method.

3.3.3. Urine samples

Human urine samples from volunteers were asked to donate a spot urine specimen and kept at 4 °C in refrigerator. Amount of people were cultivated in Maha Sarakham province, Northeast Thailand. These samples were filtered through a Whatman No. 1 filter paper before the proposed extraction method.

3.3.4 Egg yolk samples

Chicken, duck, and quail eggs were purchased from local markets in Maha Sarakham province. Before performing each method, it was necessary to separate the yolk from the white, as in the analysis of eggs collected from animals treated with anthelmintic it is known that the concentrations are grater in the yolk [89-90]. Fortification of the samples, when necessary, was performed directly on the yolk once this had been separated from the white, and a period of about 12 h was allowed to elapse before continuing with any of the extraction processes in order to improve the interaction between the analytes and the matrix compounds [101]. In detail, an aliquot of 10.00 g of egg yolk were mixed well with 0.2 g of anhydrous $Na_2SO_4$. 1% (v/v) Acetic acid in acetonitrile (2.00 mL) was added and shaken vigorously by hand for 1 minute, and the homogenized eggs were centrifuged at 3500 rpm for 5 min for complete fat and protein precipitation. The supernatants were collected by micro syringe. The solutions were diluted with deionized water to 10.00 mL, 100 μL of acetic acid was added, and the solutions were centrifuged to ensure complete precipitation of fat and proteins. The samples were spiked with the neonicotinoid insecticides at different concentrations (0.01, 0.05, and 0.10 μg $mL^{-1}$) before fat and protein precipitation. The clear solutions were subjected to dispersive liquid-liquid microextraction using hydrophobic DES as extraction solvent, and the hydrophobic DES rich phase was then analyzed by HPLC.

**3.4 Experimental**

3.4.1 Preparation of standard neonicotinoid insecticides in microextraction methods.

The mixture of standard neonicotinoids solutions such as thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid were prepared in methanol and working solution were diluted in deionized water before injected into HPLC with the optimum conditions. A calibration curve for each analyte was constructed by plotting between the peak areas versus the concentration of mixed standard neonicotinoids solution at eight different concentrations. The linearity range were evaluated by the calibration curve ($y = mx + c$) and the correlation coefficient ($R^2$) value.

The sensitivity of the method were evaluated by limit of detection (LOD) calculated as three times the signal-to-noise ratio 3:1, and limit of quantitation (LOQ) calculated as ten times the signal-to-noise ratio 10:1. Precision of the method were determined by analyzing mixed standard neonicotinoids solution at a concentration of 0.1 µg $mL^{-1}$ for in-situ metathesis reaction of deep eutectic solvent extraction and 0.5 µg $mL^{-1}$ for hydrophobic deep eutectic solvent based on liquid-liquid microextraction in a same day and in three different days, and the repeatability were evaluated in terms of %RSD.

### 3.5 In-situ metathesis reaction of deep eutectic solvent procedure

3.5.1 Synthetic and characterization of DES

The DES was prepared using ChCl and phenol (mole ratio 1:5), the proposed reaction mechanism as shown in Figure 3. The mixture was formed by in-situ metathesis reaction coupled with microextraction method.

DES was made by combining ChCl with phenol, the formation of hydrogen bonds between bond donors and ChCl was monitored using FT-IR spectroscopy.

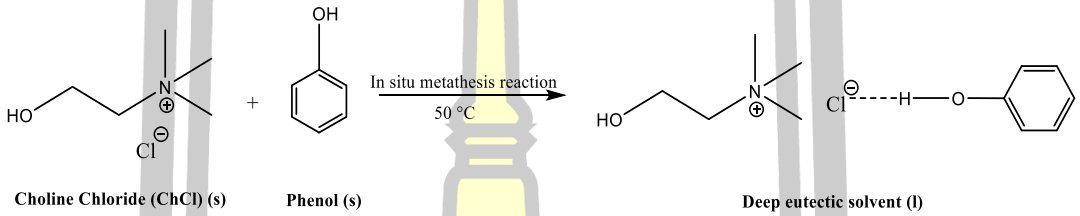



**Figure 3.** The reaction mechanism of deep eutectic solvent.

3.5.2 Optimization of the in-situ metathesis reaction of deep eutectic solvents extraction procedure

The percentage recoveries of in-situ metathesis extraction method of deep eutectic solvent were observed. The optimum conditions providing the highest percentage recoveries were selected for analysis of studied samples.

The determination of neonicotinoid insecticides was carried out by in-situ metathesis reaction of deep eutectic solvent procedure followed by HPLC-PDA. Figure 4 shown the schematic diagram of the in-situ metathesis reaction of deep eutectic solvent microextraction method.

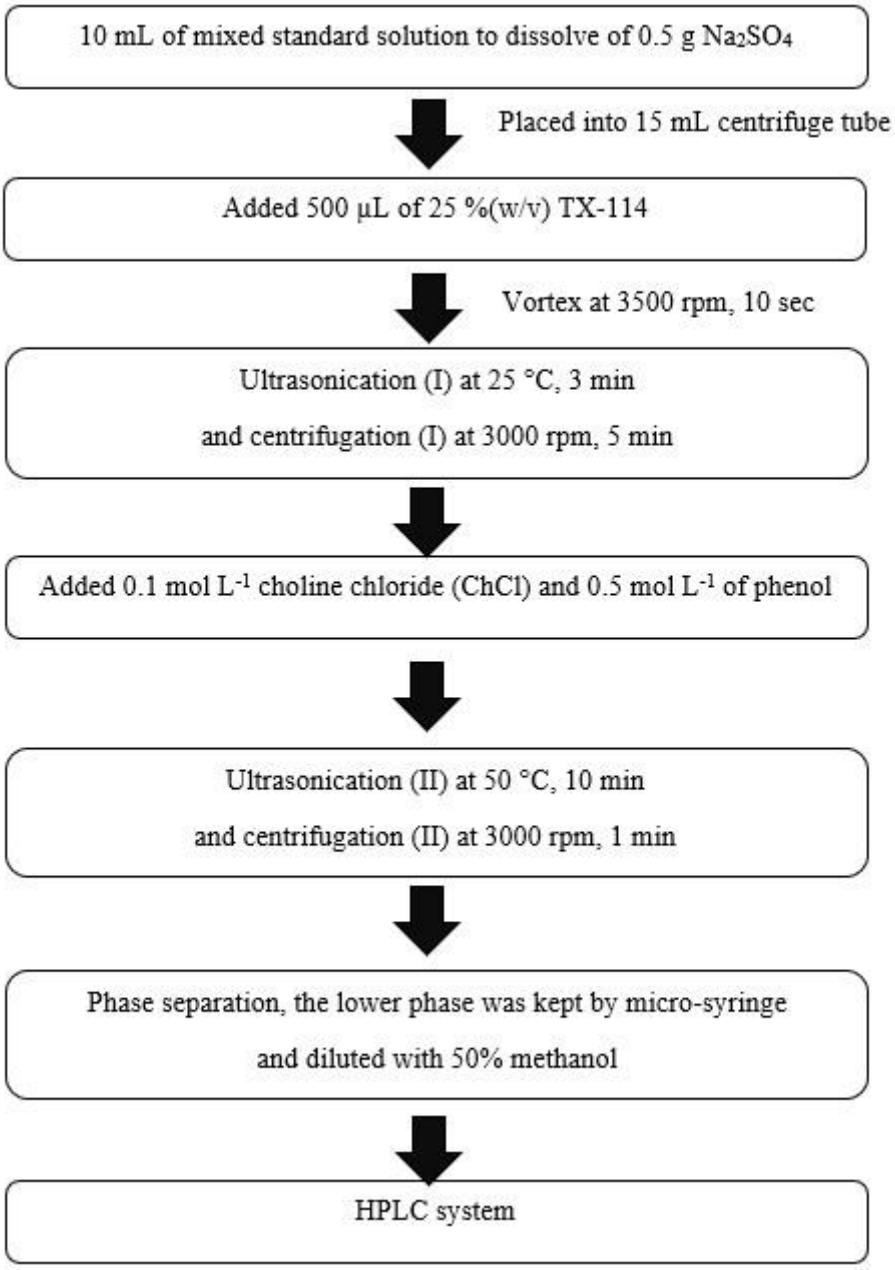



**Figure 4.** The schematic diagram of the in-situ metathesis reaction of deep eutectic solvent microextraction method.

3.5.2.1 Effect of type and concentration of salts addition

The salts addition often enhances the extraction of analytes in conventional microextraction due to the salting-out effect. To study the effect of ionic strength on the proposed extraction method, experiments were carried out by addition of different salts such as NaCl, $Na_2SO_4$, $Na_2CO_3$, and $CH_3COONa$ at 0.5 %(w/v) was investigated.

The concentration of selected salt was studied in the range of 0.25-10 %(w/v).

3.5.2.2 Effect of type and concentration of surfactants

The selection of the surfactant also greatly influenced the developed microextraction method. In this study, we investigated surfactants with different types of surfactant such as sodium dodecyl sulfate (SDS), cetyltrimethyl amonium bromide (CTAB), TX-100 and TX-114.

The concentration of surfactant was studied in the range of 0.25-2 %w/v.

3.5.2.3 Effect of vortex time

The vortex time was used for agitation during the extraction step to provide the extraction efficiency as it increases the partition of sample and the extraction solvent into aqueous solution. The vortex agitation speed was fixed at 3500 rpm and different vortex times was evaluated in the range from 0-60 sec.

3.5.2.4 Effect of ultrasonication temperature (I), (II) and ultrasonication time (I), (II)

To achieve the partition of target analytes from aqueous solution into extraction phase, ultrasonication system is important for extraction steps. The ultrasonication temperature was studied in the range of 25-60 °C at a fixed time of 5 min. The effect of ultrasonication (I) time was studied in the range of 1-20 min. Due to this extraction method divided to two parts of extraction, in ultrasonication (II) temperature was studied in the range of 25-60 °C at a fixed time of 3 min and ultrasonication (II) time was studied in the range of 1-20 min.

3.5.2.5 Effect of concentration of hydrogen bond acceptors and hydrogen bond donors

Deep eutectic solvents (DESs) are usually obtained by the complexation of a quaternary ammonium salt with a metal salt or hydrogen bond donor (HBD). The charge delocalization occurring through hydrogen bonding between for example a halide ion and the hydrogen-donor moiety is responsible for the decrease in the melting point of the mixture relative to the melting points of the individual components. The selection of a DES as an efficient extraction mean is based mainly on their electrostatic, hydrophobic, and π-π interaction capacities with the target analytes [18]. In this work, a DES has been synthesized using a hydrogen bond donor (phenol) and a hydrogen bond acceptor (choline chloride; ChCl), then used as extraction solvent.

To study the effect of concentration of hydrogen bond donor and a hydrogen bond acceptor on the proposed extraction method, experiments were carried out by addition of different concentration levels in the range of 0-2 mg $L^{-1}$.

3.5.2.6 Effect of centrifugation time (I), (II)

Centrifugation is another important step in procedure to achieve phase separation, the process of mass transfer between two phases in extraction procedure should be time-dependent. This extraction method divided to two parts of extraction, the centrifugation time(I) and centrifugation (II) time were studied in the range of 0-15 min at a fixed speed of 3000 rpm.

**3.5.3 Analytical performance of the method**

The volume of 10.00 mL of sample solution was placed in 15 mL centrifuge tube and spiked of each neonicotinoid insecticides (0.1 µg $mL^{-1}$). The in-situ metathesis reaction was investigated under the optimum conditions. The mixture of four neonicotinoid insecticide standards were prepared in deionized water. The linearity range, limits of detection (LODs), limits of quantitation (LOQs), were defined as the concentration of target analytes giving signal-to-noise ratio (S/N) of 3 and 10, respectively. The repeatability of the proposed method was done on seven different times in same day (intra-day; n = 7), the reproducibility of the proposed method was done on seven different times and three different days

(inter-day; n = 7×3 days) and calculated in term of %RSDs. The enrichment factor (EF) and extraction recoveries was evaluated.

### 3.6 Hydrophobic deep eutectic solvent based on dispersive liquid-liquid microextraction

3.6.1 Synthetic and characterization of hydrophobic deep eutectic solvents

The hydrophobic DESs were prepared using different molar ration between tetrabutylammonium bromide and decanoic acid as shown in Table 9. The mixtures were heated in water bath at 80 ˚C until transparent clear liquids were obtained. After cooling, the hydrophobic DESs were stored at room temperature. A typical structure of the prepared hydrophobic DESs is shown in Figure 5 [102].

DESs were made by combining TBABr with decanoic acid, the formation of hydrogen bonds between bond donors and ChCl was monitored using FT-IR spectroscopy.

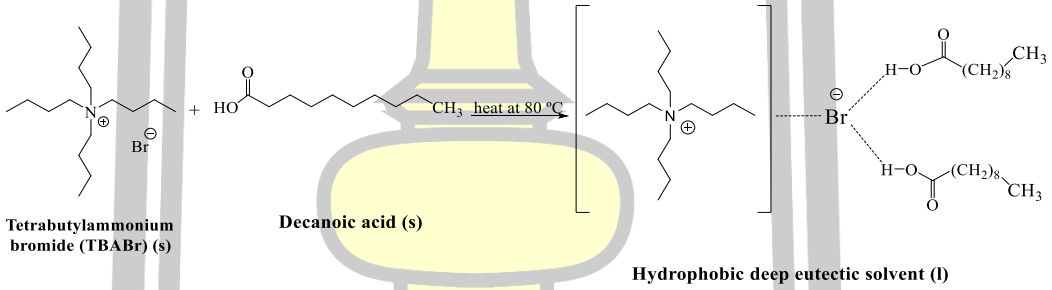



**Figure 5.** The reaction mechanism of hydrophobic DESs.

**Table 9.** Preparation of different molar ration of hydrophobic DESs used in this work.

| No. | [Hydrogen bond donor] | [Quaternary ammonium salt] | Mole ratio |
|---|---|---|---|
| 1. | *Decanoic acid* | *Tetrabutylammonium bromide (TBABr)* | 1:1 |
| 2. | *Decanoic acid* | *Tetrabutylammonium bromide (TBABr)* | 2:1 |
| 3. | *Decanoic acid* | *Tetrabutylammonium bromide (TBABr)* | 3:1 |
| 4. | *Decanoic acid* | *Tetrabutylammonium bromide (TBABr)* | 4:1 |
| 5. | *Decanoic acid* | *Tetrabutylammonium bromide (TBABr)* | 5:1 |

3.6.2 Optimization of the in-situ metathesis reaction of deep eutectic solvents extraction procedure

The determination of neonicotinoid insecticides was carried out by hydrophobic deep eutectic solvent procedure followed by HPLC-PDA. Figure 6 shown schematic diagram of the proposed microextraction method.

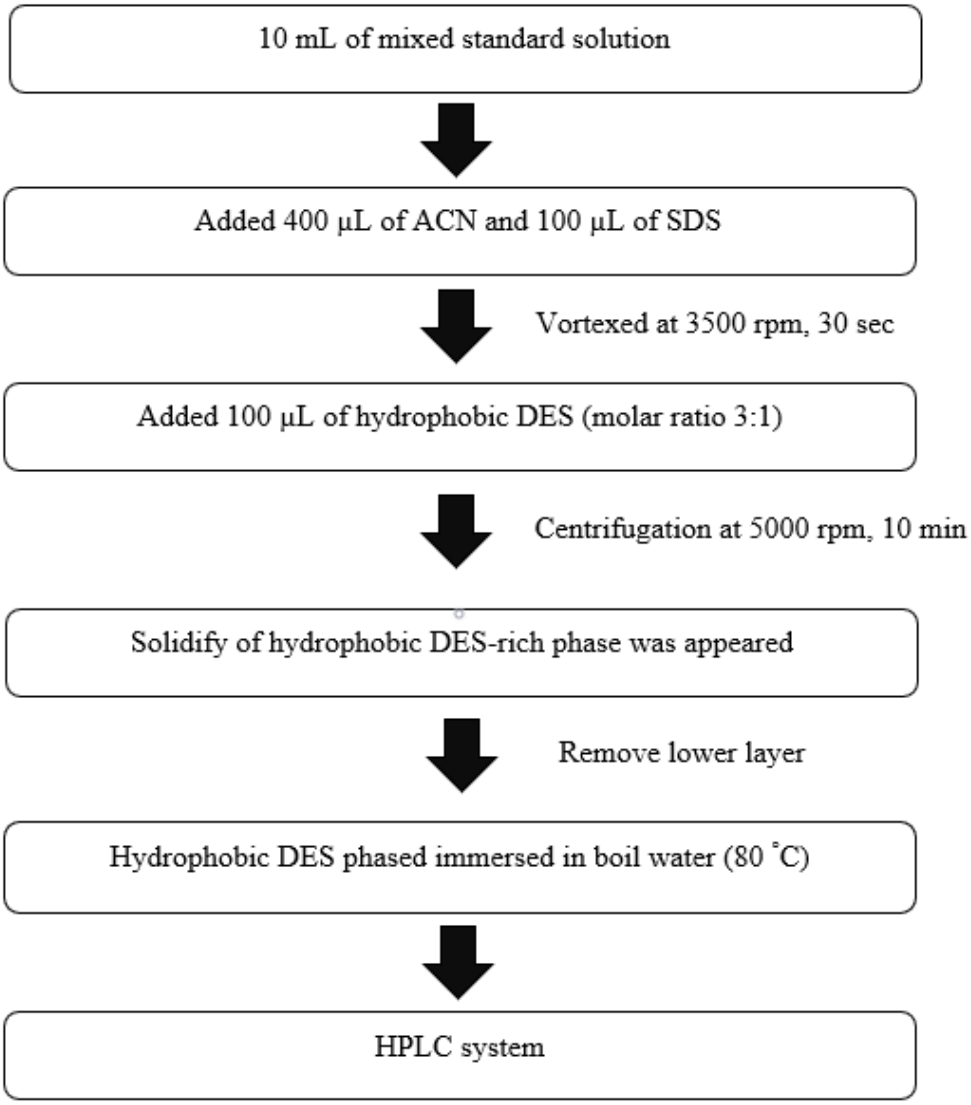



**Figure 6.** The schematic diagram of the hydrophobic deep eutectic solvent based on dispersive liquid-liquid microextraction method.

3.6.2.1 Effect of salts addition

To study the effect of ionic strength on the proposed extraction method, experiments were carried out by addition of different electrolyte salts such as NaCl, $Na_2SO_4$, $Na_2CO_3$, $CH_3COONa$ and $NH_4Cl$ at 0.3 g was investigated.

The amount of different electrolyte salts was studied at 0.05-0.5 g.

3.6.2.2 Effect of type and volume disperser solvents

To study the effect of types of disperser solvent such as acetonitrile (ACN), methanol (MeOH) and ethanol (EtOH) at 400 µL and without disperser solvent were investigated.

The volume of disperser solvent was studied in the range of 50-1,000 µL.

3.6.2.3 Effect of type and concentration of surfactant

The selection of the surfactant also greatly influenced the developed microextraction procedure. In this study, we investigated surfactants with different types of surfactant such as sodium dodecyl sulfate (SDS), cetyltrimethyl amonium bromide (CTAB), TX-100 and TX-114 to obtain a solvent with extraction efficiency of target analytes.

The effect of concentrations of surfactant were studied in the range of 3-100 mmol $L^{-1}$.

3.6.2.4 Effect of mole ratios of hydrophobic DES and it's volume.

The composition of hydrophobic DES has significant influence on its physicochemical properties, which might greatly effect of the extraction efficiency of target analytes [69]. Five different mole ratios of hydrophobic DESs prepared (Table 9) and their ability to extract the five neonicotinoid insecticides in various samples were investigated with the volume of hydrophobic DESs being kept constant at 100 µL.

The effect of five mole ratios of hydrophobic DESs were investigated.

3.6.2.5 Effect of vortex and centrifugation time

In order to increase the vortex time speeds up the distribution equilibrium of the target analytes between the selected hydrophobic DES and aqueous solution and improve the recovery.

The vortex time speed was evaluated in the range of 10-150 sec compared with that obtained from the process without vortex agitation.

To achieve the phase separation, centrifugation time is another important step. The centrifugation speed was fixed at maximum speed of instrument (5000 rpm) and fixed time of 10 min, to ensure complete phase separation in the proposed microextraction method.

**3.6.3 Analytical performance of the method**

The volume of 10.00 mL of sample solution was placed in 15 mL centrifuge tube and spiked of each neonicotinoid insecticides (0.5 µg $mL^{-1}$). The in-situ metathesis reaction was investigated under the optimum conditions. The mixture of four neonicotinoid insecticide standards were prepared in deionized water. The linearity range, limits of detection (LODs), limits of quantitation (LOQs), were defined as the concentration of target analytes giving signal-to-noise ratio (S/N) of 3 and 10, respectively. The repeatability of the proposed method was done on seven different times in same day (intra-day; n = 7), the reproducibility of the proposed method was done on seven different times and three different days (inter-day; n = 7×3 days) and calculated in term of %RSDs. The enrichment factor (EF) and extraction recoveries was evaluated.

**3.7 Data analysis**

The average result (mean) was calculated by summing the individual result and dividing by the number (n) of individual values:

$$\bar{x} = \frac{x_1 + x_2 + x_3 + \cdots}{n}$$

The standard deviation was a measure of how precise the average is, that is, how well the individual number agree with each other. It is a measure of a type of error called random error. It is calculated as follows:

$$SD = \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + \cdots}{n-1}}$$

The percentage relative standard deviations (%RSD) are calculated from the standard deviation and mean using the equation:

$$\%RSD = \frac{100 \times SD}{\bar{x}}$$

The percentage recovery (%Recovery) was calculated by concentration of sample and spiked sample using the equation:

$$\%Recovery = \frac{C_{ex} - C_0}{C_{spiked}} \times 100$$

Where $C_{ex}$ and $C_0$ are the analytes concentration in the extraction phase and the initial analyte concentration in the aqueous samples, respectively. $C_{spiked}$ is the concentration of spiked standard.

# CHAPTER 4

# Results and Discussion

## 4.1 Determination of neonicotinoid insecticide residues in surface water, soil and human urine samples using in-situ metathesis reaction of deep eutectic solvents extraction.

This chapter present the result obtained section describes a development of then extraction method and prior to high performance liquid chromatography (HPLC). The neonicotinoid insecticides such as thiamethoxam, clothianidin, imidacloprid and thiacloprid, were selected. The second section present the analytical performance of the proposed method. Finally, apply the proposed method for the analysis of neonicotinoid insecticide residues in surface water, soil and urine samples. The results were discussed.

4.1.1 Synthetic and characterization of DES

The formation of hydrogen bonding between the halide anion of choline chloride and phenol is the main force for the formation of DES. To confirm the formation of hydrogen bonding, FT-IR spectra of choline chloride and phenol were examined and the results are shown in Figure 7. In the FT-IR spectra the characteristic peaks presented at 3279 $cm^{-1}$ due to the O–H vibration of pure choline chloride and the vibration positioned at 1063 $cm^{-1}$ belongs to C–N vibration of choline chloride [88]. And FT-IR spectra the characteristic peaks presented 3345.6 $cm^{-1}$ due to the O-H vibration and C=O vibration at 1070.8 $cm^{-1}$ of phenol [103]. In the FT-IR spectrum of DESs the C=O vibrations of phenol shifted to 1092.69 $cm^{-1}$ and O–H vibrations of phenol which observed at 3334.82 $cm^{-1}$ shifted to 3360.45 $cm^{-1}$. This may be due to transfer of oxygen atom cloud electron to hydrogen bonding and consequently a decrease in force constant [104]. Thus, the shift of the O–H vibrations suggests the existence of hydrogen bonding between HBDs and choline chloride when the DESs are formed.

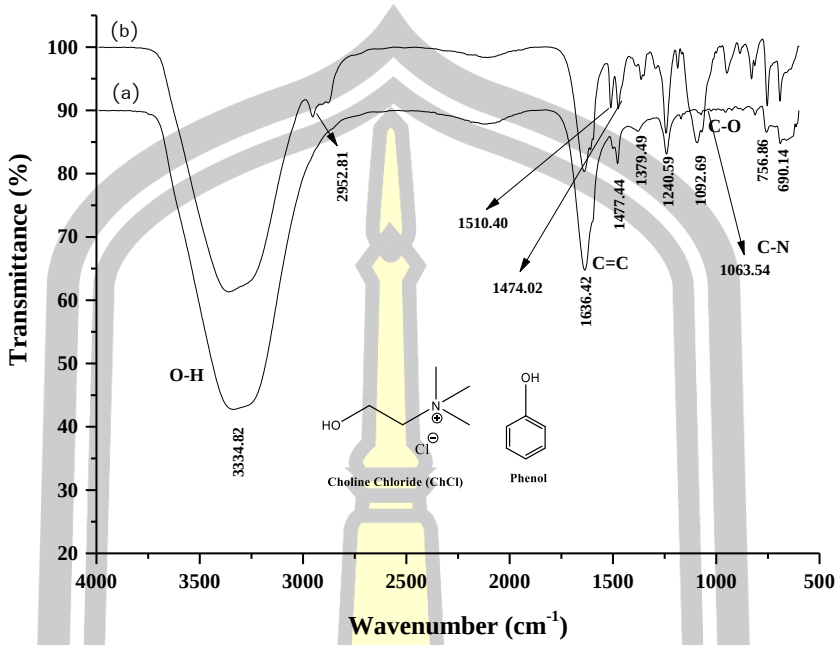



**Figure 7.** FT-IR spectra of choline chloride and phenol when DES was formed.

4.1.2 Optimization of the in-situ metathesis reaction of deep eutectic solvents extraction procedure

In order to obtain the high extraction efficiency of the proposed method, several parameters were investigated, including salt addition, concentration of surfactants, vortex time, concentration of hydrogen bond donors, concentration of hydrogen bond acceptors, temperature and time of ultrasound and centrifugation time. To identify of optimum extraction conditions, the peak area of the analytes was applied to evaluate extraction efficiency. In this experiment, various parameters were studied by a one parameter at a time while the other remaining factors were kept constant. The optimization was carried out on the aqueous solution (10.00 mL) containing 0.10 μg $mL^{-1}$ of each analyte. All the experiments were performed triplicated and the mean of the results were used to optimization.

4.1.2.1 Effect of salt addition

Salts addition can potentially decrease the solubility of the analytes in the aqueous solution and enhance their partitioning into the adsorbent or organic phases in conventional microextraction due to the salting-out effect. On the other hand, as the ionic strength of the medium increases, the viscosity and density of the aqueous solution are also enhanced, leading to a reduction of the mass transfer efficiency process [105]. To study the effect of ionic strength on the proposed extraction method, the experiments were carried out by addition of different salts such as NaCl, $Na_2SO_4$, $Na_2CO_3$, $CH_3COONa$ and the amount of each salt being kept constant at 5 %(w/v). The results were compared with that obtained from the procedure without salt addition. It was found that, the addition of $Na_2SO_4$ provided higher extraction efficiency in term of peak area. Thus, $Na_2SO_4$ was selected for further studies, the results are shown in Figure 8. On the other hand, with increase of salt concentration and ionic strength, salting in effect occurred. Whereby, polar molecules may participate in electrostatic interactions with the salt ions in solution; therefore, the mass transfer is decreased [106]. With salt addition, at first, the predominant process is the interaction of salt with water (salting out effect). With increasing salt concentration, salt molecules start interacting with analytes (salting in effect) [107]. To investigate the effect of salinity on extraction performance, experiments were performed by adding different amount of $Na_2SO_4$ in the range of 0.25-10 %(w/v). The result was shown in Figure 9. It was found that increase of $Na_2SO_4$ amount from 0.25 to 5 %(w/v) led to increase of extraction efficiency, while further increase of salt amount of 7 and 10 %(w/v) of $Na_2SO_4$, no phase separation was occurred. Therefore, 5 %(w/v) of $Na_2SO_4$ was chosen.

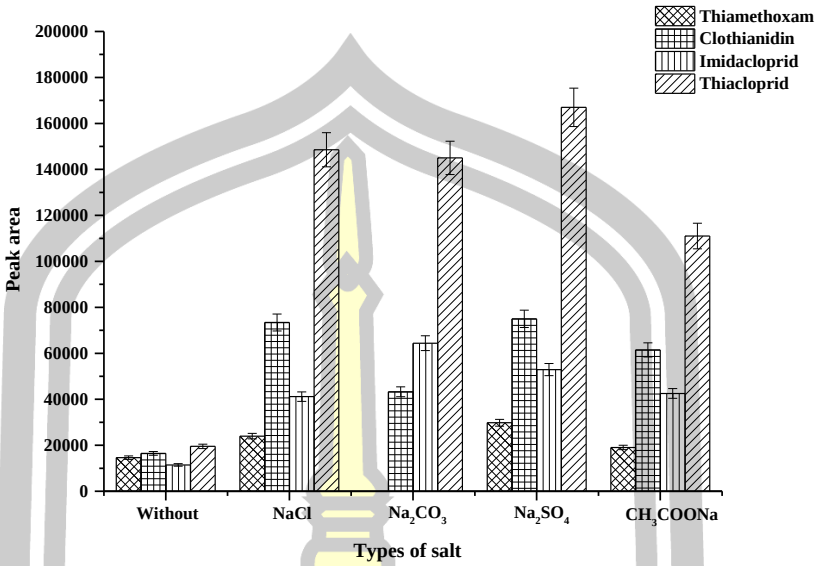



**Figure 8.** Effect of types of salt. Conditions: 5 %(w/v) of each salt; 25 %(w/v) of each surfactant; vortex time 30 sec; ultrasonication (I) temperature at 30 °C for 3 min; 100 µL of 1.00 mol $L^{-1}$ ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 60 °C for 20 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

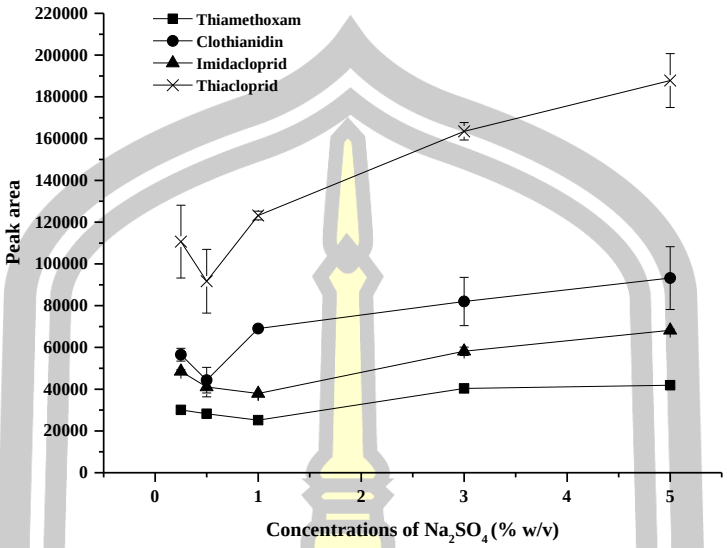



**Figure 9.** Effect of the amounts of salt. Conditions: 5 %(w/v) of $Na_2SO_4$; 25 %(w/v) of each surfactant; vortex time 30 sec; ultrasonication (I) temperature at 30 °C for 3 min; 100 µL of 1.00 mol $L^{-1}$ ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 60 °C for 20 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.1.2.2 Effect of type and concentration of surfactants

The selection of the surfactant also greatly influenced the extraction efficiency of the proposed microextraction method. The structure of surfactants affects its physical and chemical properties, which may affect the extraction efficiency of key factors [108]. The effect of surfactant on cloud point extraction is considered to be very important because there is a narrow range for easy phase separation within maximum extraction efficiency and analytical signal detection. The solubilization/partition of non-polar organic molecules in the hydrophobic micellar core is an inherent property of all surfactant formulations. The efficiency of these procedures relies on the magnitude of analyte solubilization into the micelle (non-polar core and polar micelle-water interface). Non-ionic surfactants have always remained good vector for extraction of analyte from complex matrices [109]. Different types of surfactant were studied, including sodium dodecyl sulfate (SDS),

cetyltrimethyl ammonium bromide (CTAB), TX-100 and TX-114. When SDS and CTAB were added, it was found that phase separation was not occurred. When TX-100 and TX-114 were added, it was found that phase separation was occurred. The chromatograms were shown in Figure 10 and Figure 11. When TX-114 was added, clear chromatogram and high extraction efficiency in term of peak area were obtained. Moreover, the result obtained with and without TX-114 were compared. It was found that, the addition of TX-114 provided higher extraction efficiency than without TX-114 addition (Figure 12). Therefore, TX-114 was used in the subsequent experiments. The effect of concentration of TX-114 was investigated in the range of 0.25-2.0 %(w/v). The result was shown in Figure 13. It was found that the concentration of 1.0 %(w/v) of TX-114 provided higher extraction efficiency, beyond this point, the extraction efficiency was sharply decreased. Because of the large volume of surfactant-rich phase was occurred. Therefore, the concentration of 1.0 %(w/v) of TX-114 was selected for further studied.

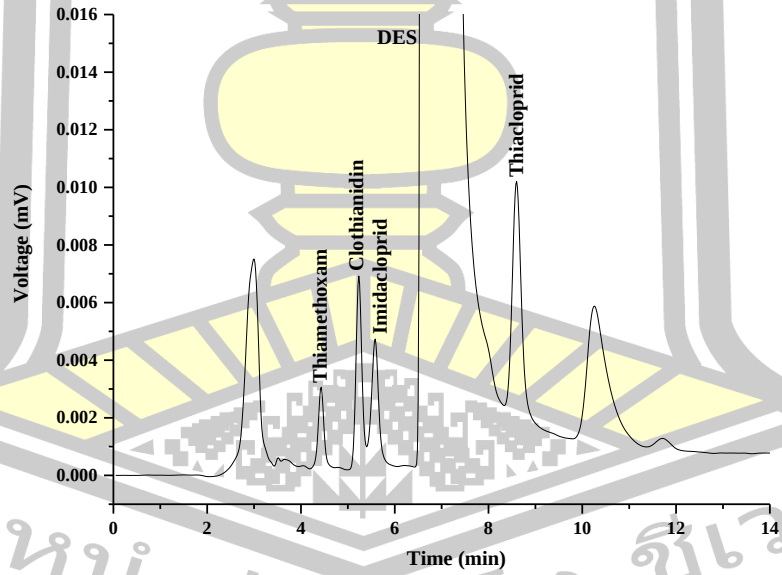



**Figure 10.** The chromatogram of the proposed method using TX-100.

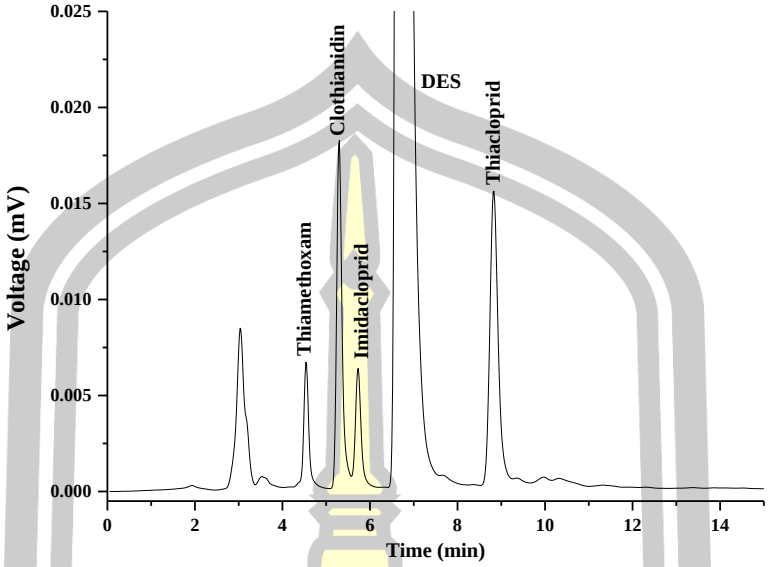



**Figure 11.** The chromatogram of the proposed method using TX-114.

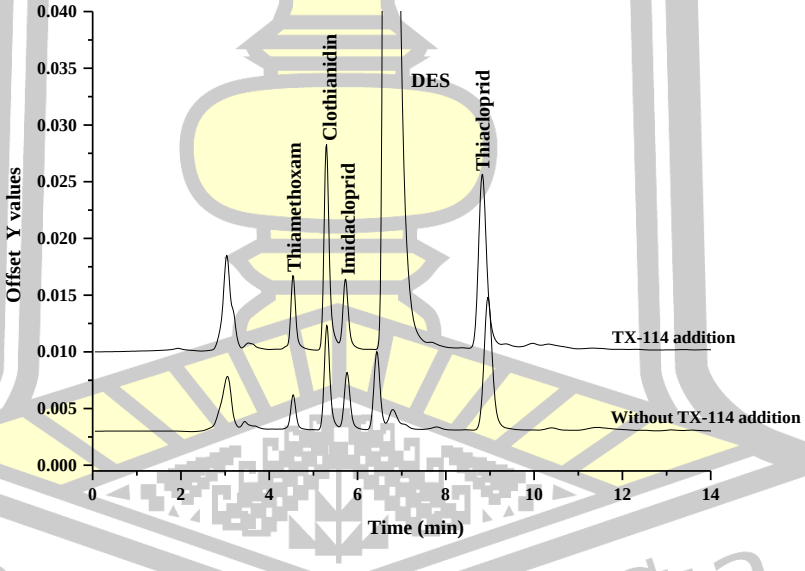



**Figure 12.** The overlaid chromatograms of without TX-114 addition and with TX-114.

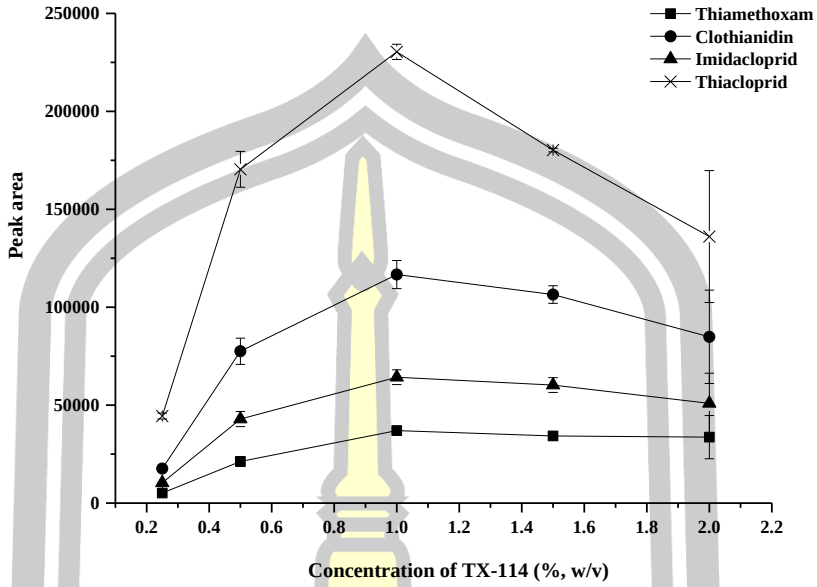



**Figure 13.** Effect of concentration of Triton X-114. Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 30 sec; ultrasonication (I) temperature at 30 °C for 3 min; 100 µL of 1.00 mol $L^{-1}$ ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 60 °C for 20 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.1.2.3 Effect of vortex time

The efficiency of phase formation and separation is an important parameter that affects the microextraction process. Enough vortex time ensures that the extraction solvent disperses entirely into the sample solution resulting in higher extraction efficiency [110]. It is known that generally the dispersion of the extraction solvent into the aqueous sample can depend on the rotational speed and vortex time [56]. In this work, the effect of vortex time was investigated in the range of 0-60 sec, keeping the vortex agitator rotational speed at 3500 rpm. The results that show in Figure 14, the maximum peak areas were obtained at vortex time of 10 sec. It was found that the peak areas of the analytes slightly increased since 15-60 sec. Therefore, 10 sec was selected as the optimum vortex time for further experiments.

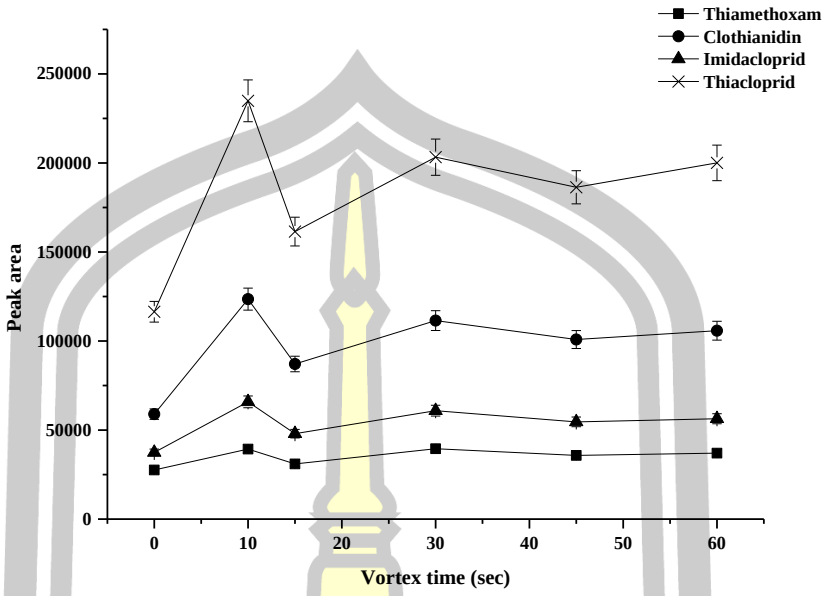



**Figure 14.** Effect of vortex time (sec). Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 30 °C for 3 min; 100 µL of 1.00 mol $L^{-1}$ ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 60 °C for 20 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.1.2.4 Effect of ultrasound time (I), (II) and temperature (I), (II)

Whether ultrasound facilitates mass transfer between two immiscible phases are arguable if one considers the ability of this form of energy to facilitate emulsification. Probably for this reason, analytical chemists have been reluctant to test ultrasound as a means for improving liquid-liquid extraction (LLE). In fact, ultrasound application most often produces stable emulsions that result in long phase separation times; therefore, Ultrasound favors mass transfer between phases provided the partitioning equilibrium involved facilitates the transfer. Efficient, fast LLE entails avoiding or minimizing the former effect and maximizing the latter. These are the two major factors to be optimized in ultrasound assisted liquid-liquid extraction (USALLE) [111]. The ultrasound extraction time plays an important role in emulsification and mass transfer. The ultrasound extraction time was defined as the time interval between the addition of the extraction solvent and the end

of the sonication stage [112], which directly influences the levels of dispersion. Therefore, the ultrasound assisted processing time was varied from 1-20 min at 25-60 °C to evaluate its effect. It was found that a fast achievement of the stability was obtained within 3 min of ultrasonication (I) time at 25°C and 10 min of ultrasonication (II) time at 50 °C. Beyond this point, the extraction efficiency in term of peak area was decreased. Therefore, 3 min of ultrasonication (I) time at 25°C (Figure 15 and Figure 16) and 10 min of ultrasonication (II) time at 50 °C (Figure 17 and Figure 18) were used for the proposed method. Sufficient treatment time accelerates the formation of a fine dispersive mixture and results in higher recoveries. However, the extension of ultrasonic treatment time can also result in the loss of volatile analytes and extractants due to heat generation [113].

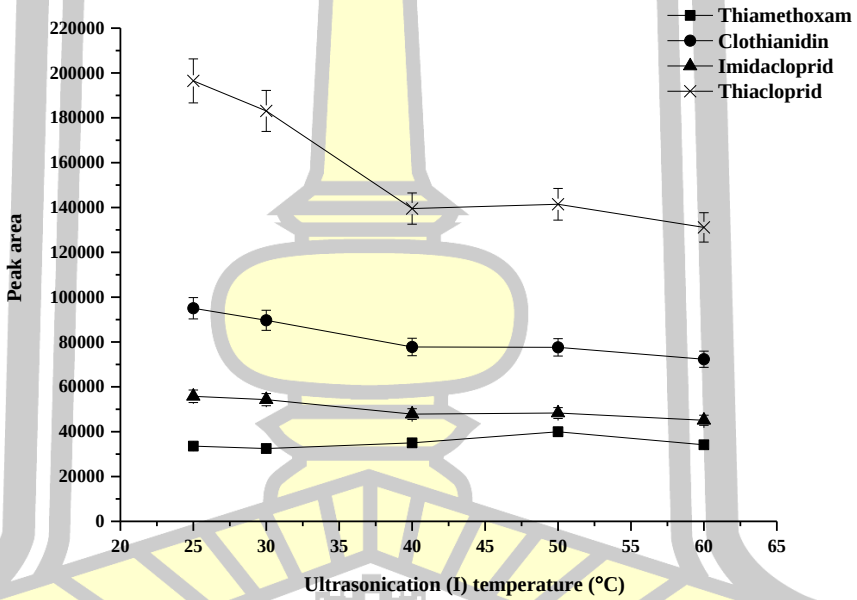



**Figure 15.** Effect of ultrasonication (I) temperature. Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 25 °C for 3 min; 100 µL of 1.00 mol $L^{-1}$ ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 60 °C for 20 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

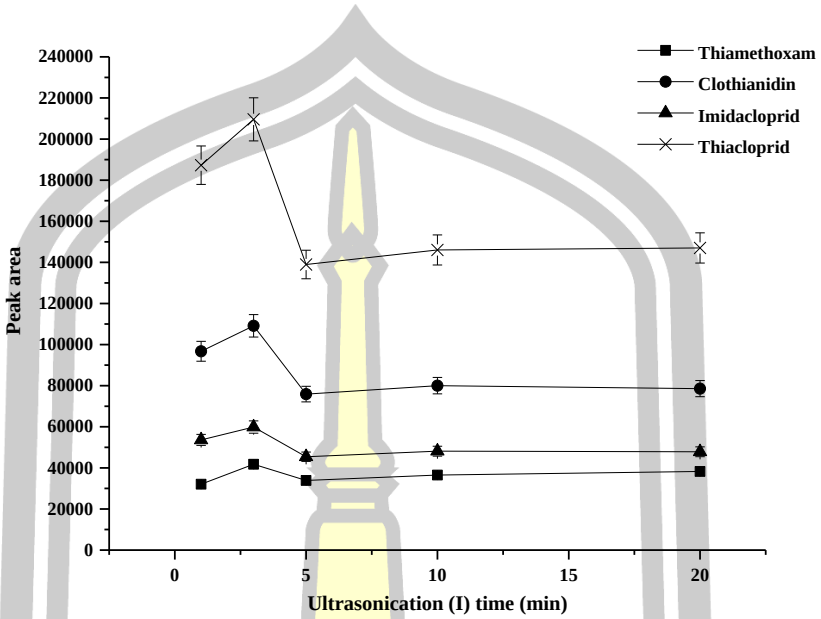



**Figure 16.** Effect of ultrasonication (I) time. Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 25 °C for 1 min; 100 µL of 1.00 mol $L^{-1}$ ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 60 °C for 20 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

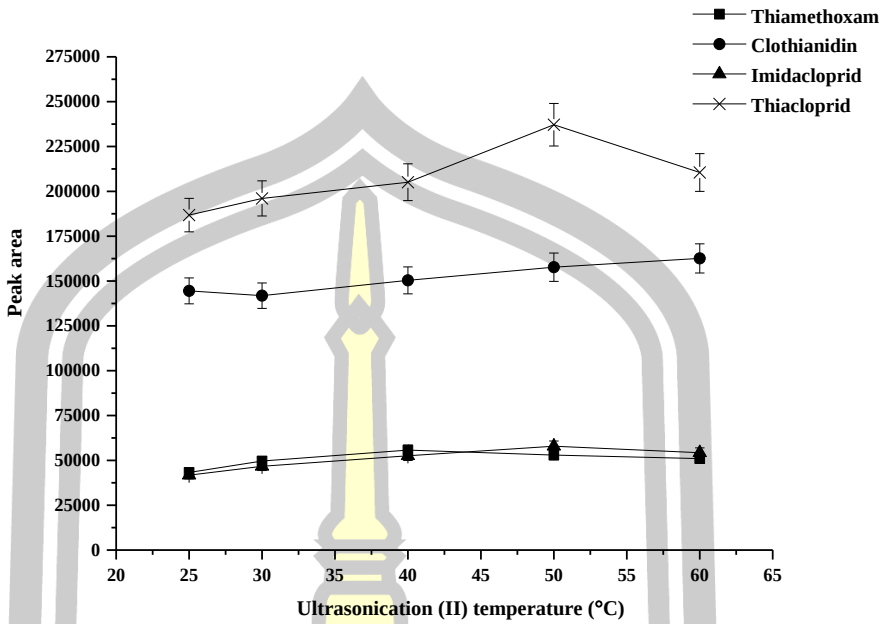



**Figure 17.** Effect of ultrasonication (II) temperature. Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 25 °C for 3 min; 100 µL of 1.00 mol $L^{-1}$ ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 50 °C for 20 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

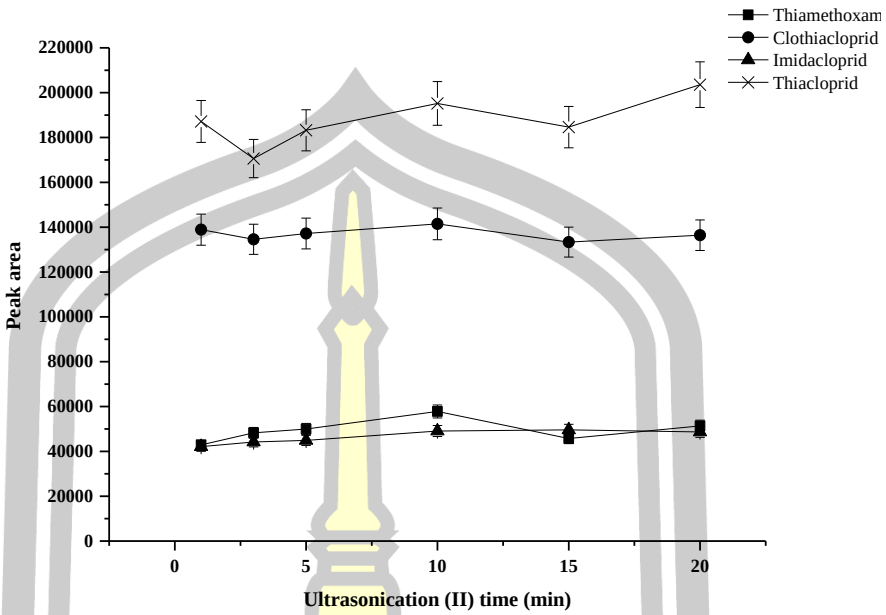



**Figure 18.** Effect of ultrasonication (II) temperature. Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 25 °C for 3 min; 100 µL of 1.00 mol $L^{-1}$ ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 50 °C for 10 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.1.2.5 Effect of concentration of hydrogen bond acceptors and hydrogen bond donors

Deep eutectic solvents (DESs) are usually obtained by the complexation of a quaternary ammonium salt with a metal salt or hydrogen bond donor (HBD). The charge delocalization occurring through hydrogen bonding between for example a halide ion and the hydrogen-donor moiety is responsible for the decrease in the melting point of the mixture relative to the melting points of the individual components. The selection of a DES as an efficient extraction mean is based mainly on their electrostatic, hydrophobic, and π-π interaction capacities with the target analytes [18]. Nowadays, researchers well recognized how slight changes in the molecular structures of the HBD portion of DESs or on their molar ratio led to appreciable differences not only on their extraction capacities but on their overall physic-chemical properties [27]. In this work, a DES has been synthesized using

a hydrogen bond acceptor (ChCl) and a hydrogen bond donor (phenol), then used as extraction solvent. The proposed reaction mechanism was shown in Figure 4. To study the effect of in-situ forming, concentration of hydrogen bond acceptor (ChCl) on the extraction efficiency of neonicotinoids, different concentration of ChCl (0.00, 0.10, 0.30, 0.50, 0.70 and 1.00 mol $L^{-1}$) were tested when the concentration of phenol was fixed at 0.30 mol $L^{-1}$. As illustrated in Figure 19, the extraction efficiency increased with the increasing of ChCl from 0.00 mol $L^{-1}$ up to 0.10 mol $L^{-1}$ and then slightly decreased. However, high concentration of ChCl caused decrease of extraction efficiency probable due to the dilution effect. Then keeping the concentration of ChCl at 0.10 mol $L^{-1}$, the effect of concentration of hydrogen bond donor (phenol) was investigated (0.00, 0.10, 0.30, 0.50, 0.70 and 1.00 mol $L^{-1}$). The result that shown in Figure 20. It was found that the high response was obtained at 0.50 mol $L^{-1}$. As a result, the following optimizations were performed using 0.10 mol $L^{-1}$ ChCl and 0.50 mol $L^{-1}$ phenol, corresponding to 1:5 mole ratio of ChCl to phenol.

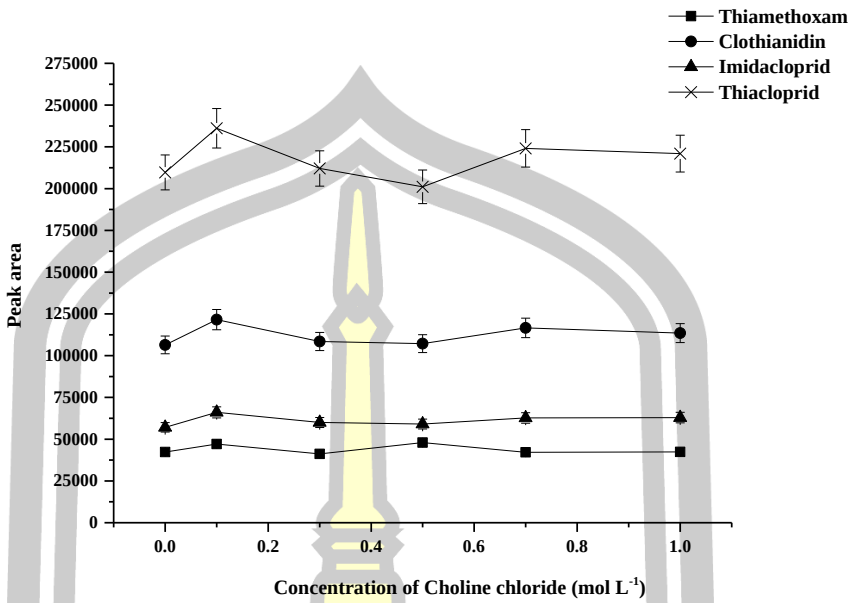



**Figure 19.** Effect of concentration of hydrogen bond acceptors (Choline chloride; ChCl). Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 25 °C for 3 min; 1.00 mol $L^{-1}$ of ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 50 °C for 10 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

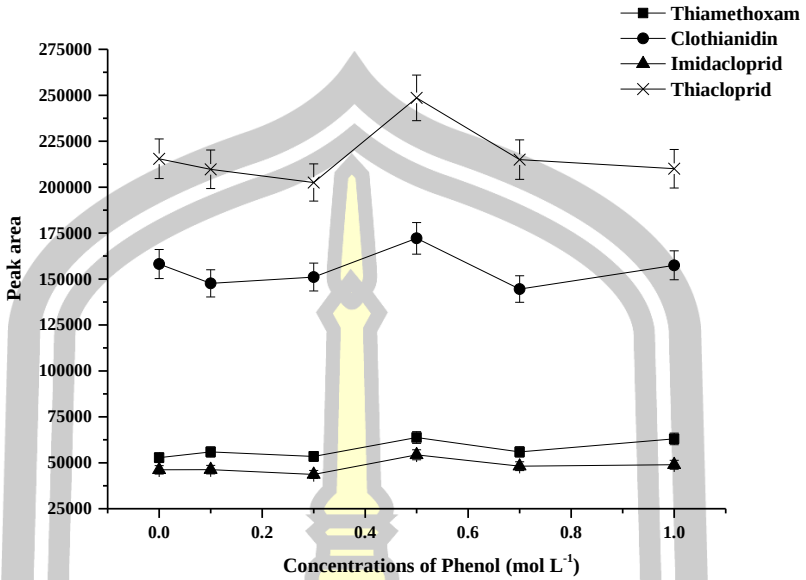



**Figure 20.** Effect of concentration of hydrogen bond donors (phenol). Conditions: 5 %(w/v) g of $Na_2SO_4$; 1.00 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 25 °C for 3 min; 1.00 mol $L^{-1}$ of ChCl; 100 µL of 1.00 mol $L^{-1}$ phenol; ultrasonication (II) temperature at 50 °C for 10 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.1.2.6 Effect of centrifugation time (I), (II)

In this method, centrifugation was used to separate the DES phase from the aqueous phases after the extraction procedure. This step is critical, as it has an obvious impact on the quality of the recovered DES and can directly affect the recovery amounts [112]. In order to achieve the best extraction efficiency, centrifugation time in the range of 0-15 min at 3500 rpm was evaluated for centrifugation (I) time and centrifugation (II) time. As shown in Figure 21 and Figure 22, the highest peak area at 5 min for centrifugation (I) time and 1 min for centrifugation (II) time, respectively. Therefore, 5 min of centrifugation (I) time and 1 min of centrifugation (II) time was used for the proposed method.

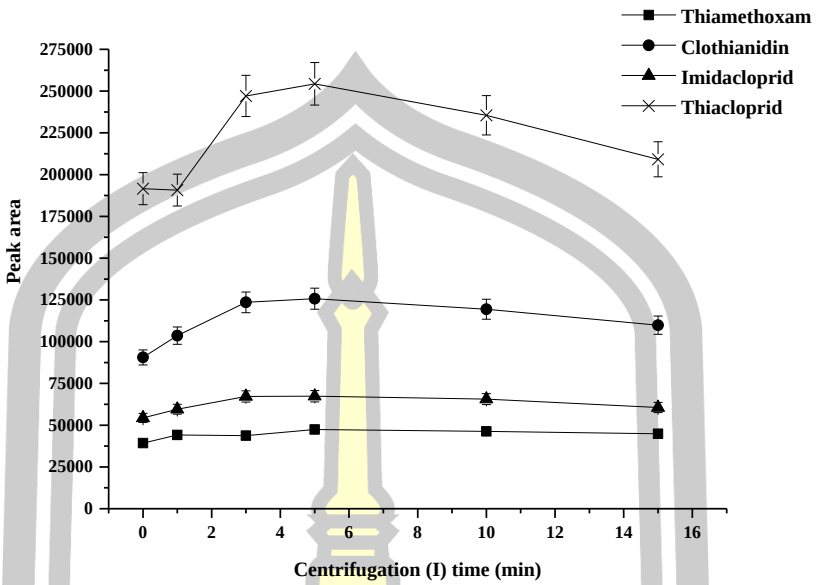



**Figure 21.** Effect of centrifugation (I) time. Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 25 °C for 3 min; 1.00 mol $L^{-1}$ of ChCl; 0.50 mol $L^{-1}$ of phenol; ultrasonication (II) temperature at 50 °C for 10 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

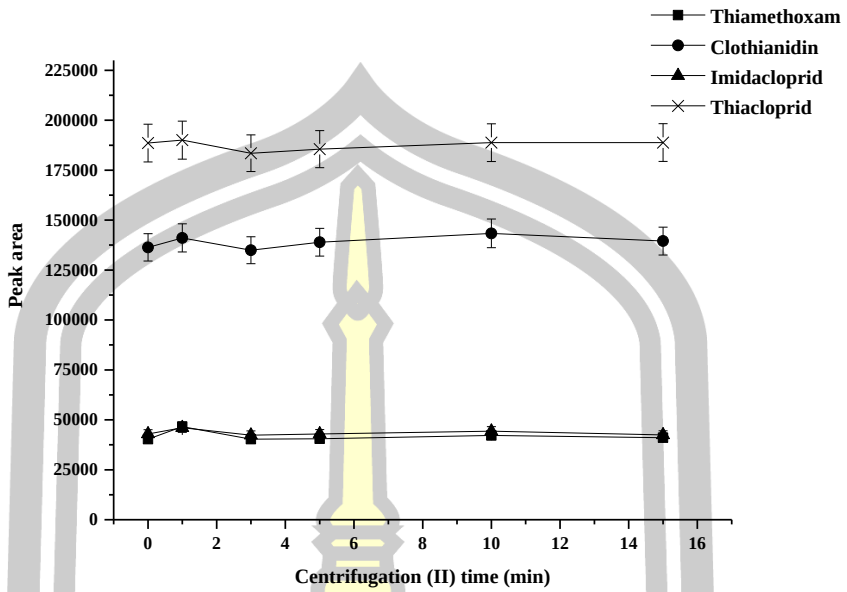



**Figure 22.** Effect of centrifugation (II) time. Conditions: 5 %(w/v) of $Na_2SO_4$; 1.0 %(w/v) of TX-114; vortex time 10 sec; ultrasonication (I) temperature at 25 °C for 3 min; 1.00 mol $L^{-1}$ of ChCl; 0.50 mol $L^{-1}$ of phenol; ultrasonication (II) temperature at 50 °C for 10 min; centrifugation (I) time at 3000 rpm for 5 min; centrifugation (II) time at 3000 rpm for 1 min (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

**Table 10.** The optimum conditions of in-situ metathesis reaction of deep eutectic solvent for preconcentration of neonicotinoid insecticides.

| Parameters | Optimum conditions |
|---|---|
| Type of salts | $Na_2SO_4$ |
| Amount of salts | 0.5 g |
| Type of surfactants | TX-114 |
| Volume of surfactants | 500 µL |
| Vortex time | 10 sec |
| Ultrasonication (I) temperature | 25 °C |
| Ultrasonication (I) time | 3 min |
| Centrifugation (I) time | 5 min |
| Concentrations of hydrogen bond donor | 1 mol $L^{-1}$ of Choline chloride |
| Volumes of hydrogen bond donor | 100 µL |
| Concentrations of hydrogen bond acceptor | 0.5 mol $L^{-1}$ of Phenol |
| Volumes of hydrogen bond acceptor | 500 µL |
| Ultrasonication (II) temperature | 50 °C |
| Ultrasonication (II) time | 10 min |
| Centrifugation (II) time | 1 min |

**4.1.3 Analytical performance of the in-situ metathesis reaction of deep eutectic solvents extraction**

Under the optimum conditions, the analytical performance of the proposed method coupled with HPLC-PDA were evaluated for extracting some neonicotinoid insecticides by testing linearity, limits of detection (LODs), limits of quantitation (LOQs), repeatability, extraction recovery and enrichment factor (EF). The analytical performances of the proposed method are summarized in Table 11. The linearity range of 0.0001-1 µg $mL^{-1}$ were obtained with the correlation coefficient (R2) more than 0.997. LODs and LOQs were defined as the concentration of target analytes giving S/N = 3 and 10, respectively. LODs of the studied analytes were from 0.0003-0.001 µg $mL^{-1}$, while LOQs ranges between 0.001-0.003 µg $mL^{-1}$. To test the repeatability of the proposed method, precision in term of intra-day (n = 7) and inter-day (n = 7×3 days) were expressed as %RSDs of retention time (tR) and peak area of studied compounds by replicate injection of the mixed standard solution of 0.1 µg $mL^{-1}$. Good precision with RSDs less than 10.0% were obtained. The enrichment factors (EFs), defined as the concentration ratio of LODs passed extraction procedure ($C_{ext.}$) and LODs of non-extraction procedure ($C_{non}$-$C_{ext.}$) ranged from 50-250 folds. Figure 23. show the chromatogram of standard neonicotinoids obtained by without perconcentration (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide) and Figure 24. show the chromatogram of standard neonicotinoids obtained by in-situ metathesis reaction of deep eutectic solvent procedure (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

**Table 11.** Analytical performances of the proposed method for four neonicotinoid insecticides.

| Analyte | Linear range ($\mu g\ mL^{-1}$) | Linear equation | $R^2$ | LOD ($\mu g\ mL^{-1}$) | LOQ ($\mu g\ mL^{-1}$) | RSD (%) | | EF |
|---|---|---|---|---|---|---|---|---|
| | | | | | | $t_R$ | Peak area | |
| Thiamethoxam | 0.0001-1 | y = 470820x + 8155.7 | 0.9985 | 0.0005 | 0.0017 | 0.05 | 5.30 | 250 |
| Clothianidin | 0.0001-1 | y = 1,498,433.80x – 373.3 | 0.9999 | 0.0003 | 0.0010 | 0.06 | 4.27 | 150 |
| Imidacloprid | 0.0001-1 | y = 482381x + 9657.8 | 0.9972 | 0.0005 | 0.0017 | 0.06 | 8.03 | 125 |
| Thiacloprid | 0.0001-1 | y = 2,273,602.02x – 2434.6 | 0.9995 | 0.001 | 0.0030 | 0.12 | 5.15 | 50 |

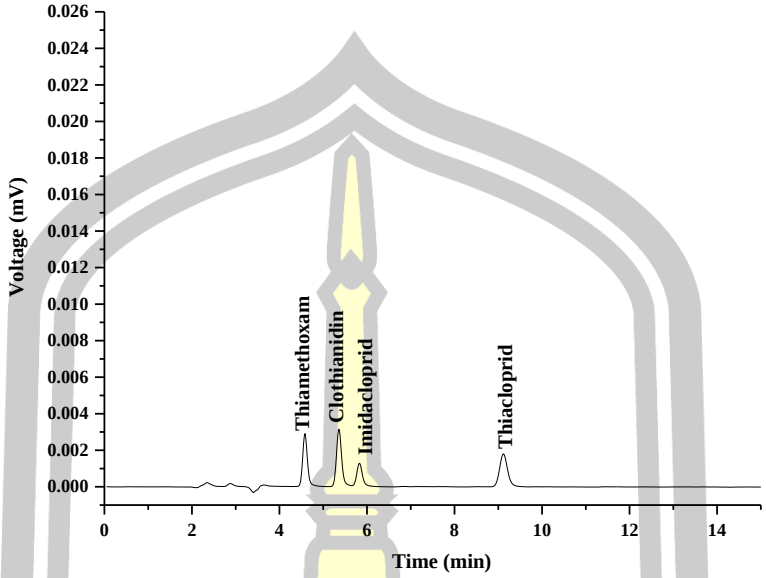



**Figure 23.** Chromatograms of standard neonicotinoid insecticides obtained by without preconcentration (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

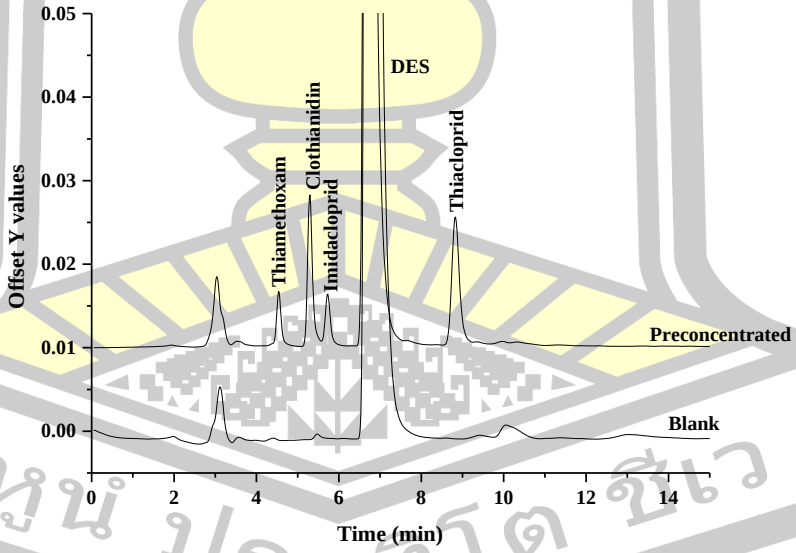



**Figure 24.** The overlaid chromatograms of standard neonicotinoid insecticides obtained by blank and in-situ metathesis reaction of deep eutectic solvent procedure (sample volume 10 mL, 0.1 µg $mL^{-1}$ of each neonicotinoid insecticide).

### 4.1.4 Analysis of real sample samples

The applicability of the proposed method was evaluated for the determination of neonicotinoid insecticides in surface water, soil and urine samples. The recoveries of the proposed method were studied by spiking surface water samples with neonicotinoid insecticides at three different concentration of 0.10, 0.50, 1.0 µg $mL^{-1}$, spiking soil samples with neonicotinoid insecticides at three different concentration of 0.05, 0.10, 0.50 µg $mL^{-1}$ and spiking human urine samples with neonicotinoid insecticides at three different concentration of 0.25, 0.50, 1.0 µg $mL^{-1}$. As shown in Table 12, Table 13 and Table 14, the recoveries samples were in the range of 80.00-115.11% for surface water and urine samples and 50.73-95.16% for soil samples, with the relative standard deviations (RSDs) of less than 10.0%, at the estimated spiking different concentration levels. The overlaid chromatograms of blank and spiked surface water samples are shown in Figure 25-27), soil samples (Figure 28-30) and urine samples (Figure 31-37). The results show that no neonicotinoids insecticide residues were detected in the studied samples. The developed method was effective, sensitive and reliable analytical method for screening of neonicotinoid insecticide residues in various samples.

**Table 12.** Recoveries of the studied neonicotinoids spiked in surface water samples obtained by the proposed method (n=3).

| Samples | Spiked ($\mu g\ mL^{-1}$) | Recovery ± RSD (%) | | | |
|---|---|---|---|---|---|
| | | TMX | CTD | ICP | TCP |
| Surface water I | 0.00 | ND | ND | ND | ND |
| | 0.10 | 82.99 ± 0.90 | 90.98 ± 3.60 | 91.37 ± 6.94 | 93.58 ± 6.02 |
| | 0.50 | 89.45 ± 1.27 | 80.00 ± 2.89 | 92.51 ± 0.06 | 109.82 ± 4.18 |
| | 1.00 | 88.97 ± 8.87 | 80.23 ± 1.17 | 92.86 ± 4.48 | 115.09 ± 6.92 |
| Surface water II | 0.00 | ND | ND | ND | ND |
| | 0.10 | 83.39 ± 4.54 | 80.16 ± 0.11 | 91.06 ± 3.67 | 103.96 ± 2.14 |
| | 0.50 | 81.02 ± 2.24 | 80.50 ± 0.74 | 92.51 ± 6.50 | 108.62 ± 3.57 |
| | 1.00 | 81.75 ± 7.23 | 82.78 ± 1.30 | 86.41 ± 4.34 | 103.97 ± 0.52 |
| Surface water III | 0.00 | ND | ND | ND | ND |
| | 0.10 | 115.11 ± 2.54 | 80.68 ± 4.45 | 80.27 ± 3.75 | 114.53 ± 2.85 |
| | 0.50 | 95.07 ± 0.56 | 81.05 ± 0.94 | 87.78 ± 2.89 | 109.45 ± 2.50 |
| | 1.00 | 106.01 ± 0.34 | 80.06 ± 1.23 | 99.08 ± 2.90 | 107.79 ± 3.31 |

*ND: not detected, TMX: thiamethoxam, CTD: clothianidin, ICP: imidacloprid, TCP: thiacloprid

**Table 13.** Recoveries of the studied neonicotinoids spiked in soil samples obtained by the proposed method (n=3).

| Samples | Spiked ($\mu g\ mL^{-1}$) | Recovery ± RSD (%) | | | |
|---|---|---|---|---|---|
| | | TMX | CTD | ICP | TCP |
| Soil I | 0.00 | ND | ND | ND | ND |
| | 0.05 | 74.21 ± 5.40 | 67.67 ± 1.19 | 52.35 ± 2.77 | 64.84 ± 0.50 |
| | 0.10 | 95.16 ± 2.58 | 64.65 ± 4.50 | 50.73 ± 6.79 | 64.25 ± 5.64 |
| | 0.50 | 60.19 ± 7.41 | 62.82 ± 5.59 | 67.64 ± 6.27 | 61.95 ± 6.04 |
| Soil II | 0.00 | ND | ND | ND | ND |
| | 0.05 | 84.47 ± 0.69 | 73.99 ± 1.25 | 80.93 ± 0.95 | 72.64 ± 1.03 |
| | 0.10 | 80.28 ± 2.37 | 65.24 ± 7.17 | 72.99 ± 3.45 | 64.19 ± 7.26 |
| | 0.50 | 88.62 ± 6.92 | 61.23 ± 7.18 | 67.60 ± 2.59 | 61.57 ± 7.43 |
| Soil III | 0.00 | ND | ND | ND | ND |
| | 0.05 | 78.72 ± 7.24 | 69.82 ± 0.15 | 67.42 ± 8.64 | 63.13 ± 1.58 |
| | 0.10 | 80.78 ± 4.70 | 68.15 ± 2.82 | 78.38 ± 2.13 | 64.91 ± 3.22 |
| | 0.50 | 86.47 ± 2.02 | 68.41 ± 2.07 | 73.19 ± 1.56 | 66.52 ± 2.76 |

*ND: not detected, TMX: thiamethoxam, CTD: clothianidin, ICP: imidacloprid, TCP: thiacloprid

**Table 14.** Recoveries of the studied neonicotinoids spiked human urine samples obtained by the proposed method (n=3).

| Samples | Spiked | Recovery ± RSD (%) | | | |
|---|---|---|---|---|---|
| | (μg $mL^{-1}$) | TMX | CTD | ICP | TCP |
| Urine I | 0.00 | ND | ND | ND | ND |
| | 0.25 | 109.31 ± 4.90 | 80.25 ± 0.22 | 90.00 ± 1.96 | 83.48 ± 0.34 |
| | 0.50 | 110.48 ± 1.19 | 80.08 ± 1.70 | 94.34 ± 2.15 | 108.99 ± 2.84 |
| | 1.00 | 83.89 ± 4.60 | 80.22 ± 1.17 | 85.61 ± 5.09 | 102.92 ± 8.36 |
| Urine II | 0.00 | ND | ND | ND | ND |
| | 0.25 | 80.57 ± 2.24 | 80.02 ± 3.02 | 82.81 ± 2.72 | 103.52 ± 0.30 |
| | 0.50 | 82.19 ± 5.25 | 80.07 ± 4.35 | 89.74 ± 4.83 | 106.10 ± 0.84 |
| | 1.00 | 88.29 ± 1.19 | 80.39 ± 2.62 | 90.08 ± 1.27 | 104.25 ± 0.96 |
| Urine III | 0.00 | ND | ND | ND | ND |
| | 0.25 | 80.55 ± 0.25 | 80.42 ± 5.34 | 88.04 ± 0.72 | 105.82 ± 2.15 |
| | 0.50 | 90.84 ± 4.13 | 81.02 ± 3.25 | 96.75 ± 8.03 | 109.56 ± 5.41 |
| | 1.00 | 81.38 ± 3.27 | 80.02 ± 3.54 | 89.40 ± 6.29 | 95.91 ± 5.047 |
| Urine IV | 0.00 | ND | ND | ND | ND |
| | 0.25 | 85.65 ± 3.87 | 80.85 ± 3.87 | 85.05 ± 4.35 | 102.93 ± 5.10 |
| | 0.50 | 87.08 ± 2.39 | 80.15 ± 0.29 | 87.11 ± 1.35 | 102.27 ± 1.80 |
| | 1.00 | 84.40 ± 1.62 | 80.02 ± 1.94 | 85.92 ± 0.84 | 99.32 ± 1.98 |
| Urine V | 0.00 | ND | ND | ND | ND |
| | 0.25 | 81.65 ± 7.82 | 90.60 ± 2.51 | 95.89 ± 3.23 | 103.03 ± 4.42 |
| | 0.50 | 80.24 ± 2.89 | 80.98 ± 3.91 | 93.04 ± 6.34 | 108.19 ± 4.76 |
| | 1.00 | 86.29 ± 9.07 | 80.98 ± 3.85 | 91.30 ± 0.47 | 99.12 ± 6.58 |
| Urine VI | 0.00 | ND | ND | ND | ND |
| | 0.25 | 87.30 ± 2.65 | 92.57 ± 4.29 | 90.89 ± 6.53 | 111.34 ± 0.58 |
| | 0.50 | 92.18 ± 6.88 | 80.17 ± 0.25 | 97.76 ± 5.74 | 113.28 ± 4.02 |
| | 1.00 | 87.85 ± 0.16 | 80.67 ± 2.24 | 92.84 ± 1.76 | 109.07 ± 1.62 |
| Urine VII | 0.00 | ND | ND | ND | ND |
| | 0.25 | 90.99 ± 6.23 | 81.70 ± 2.09 | 97.83 ± 4.45 | 112.32 ± 4.45 |
| | 0.50 | 82.71 ± 1.07 | 80.32 ± 0.65 | 89.20 ± 5.05 | 98.78 ± 1.70 |
| | 1.00 | 90.38 ± 2.74 | 80.17 ± 0.28 | 100.98 ± 2.73 | 108.82 ± 3.47 |

*ND: not detected, TMX: thiamethoxam, CTD: clothianidin, ICP: imidacloprid, TCP: thiacloprid

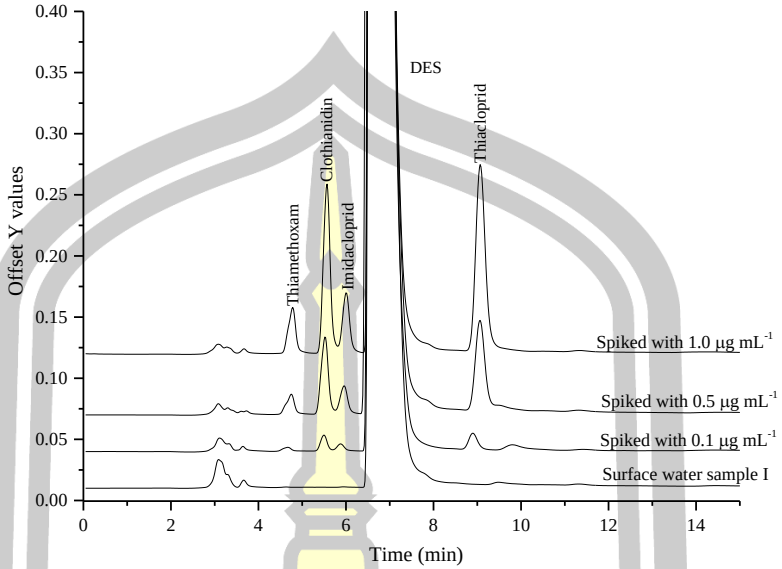



**Figure 25.** The overlaid chromatograms of blank and spiked surface water sample I.

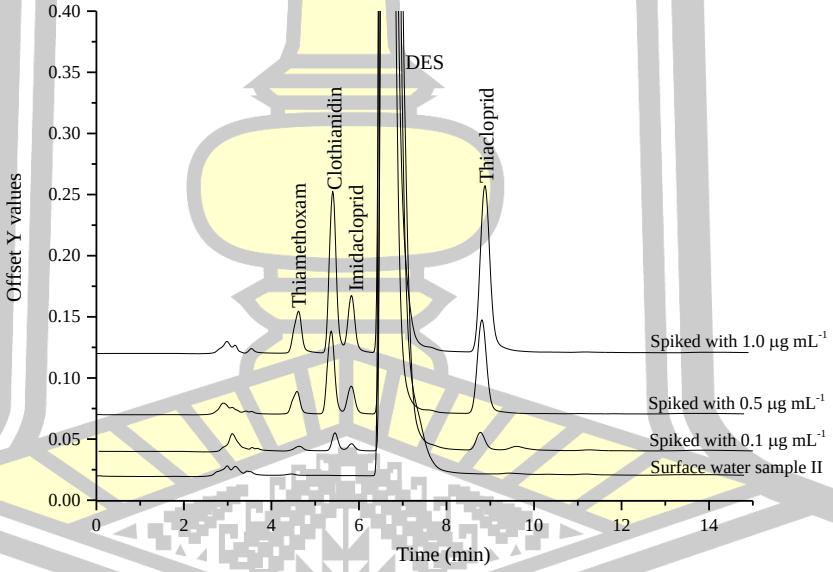



**Figure 26.** The overlaid chromatograms of blank and spiked surface water sample II.

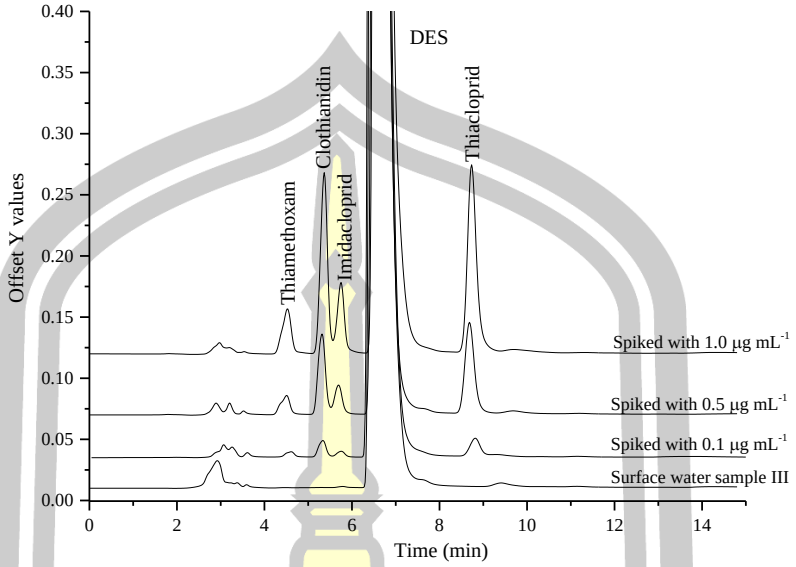



**Figure 27.** The overlaid chromatograms of blank and spiked surface water sample III.

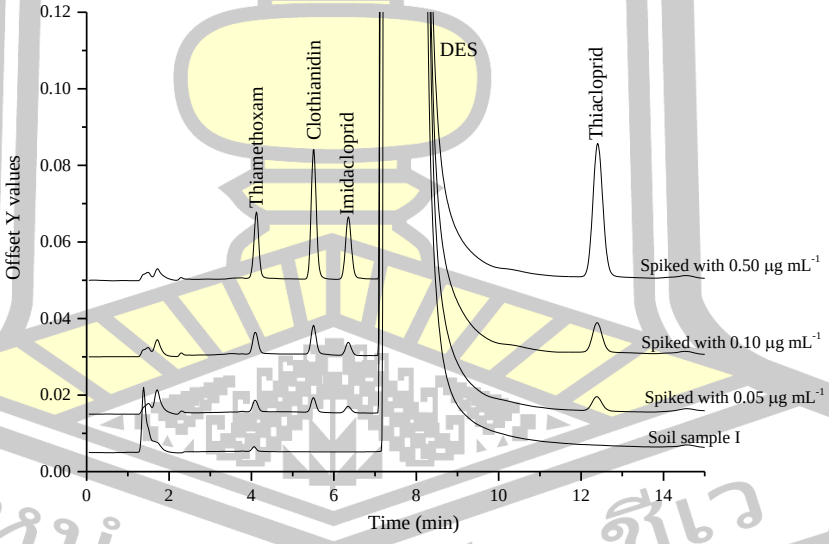



**Figure 28.** The overlaid chromatograms of blank and spiked soil sample I.

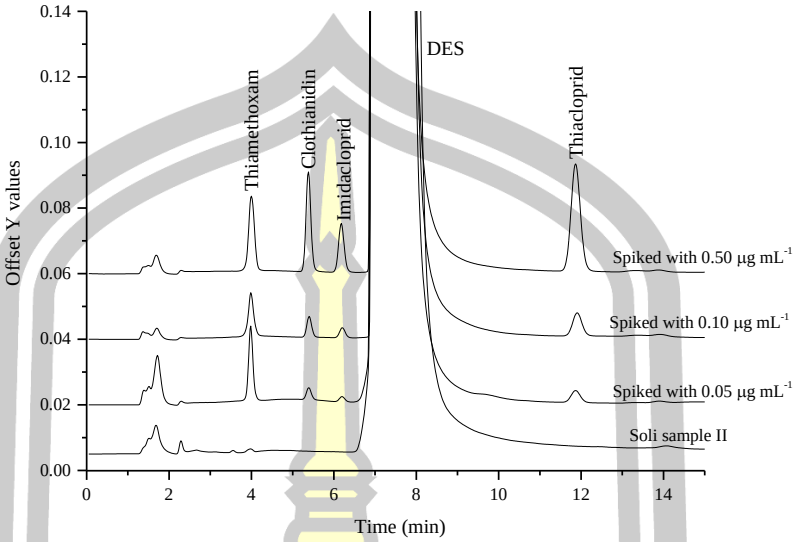



**Figure 29.** The overlaid chromatograms of blank and spiked soil sample II.

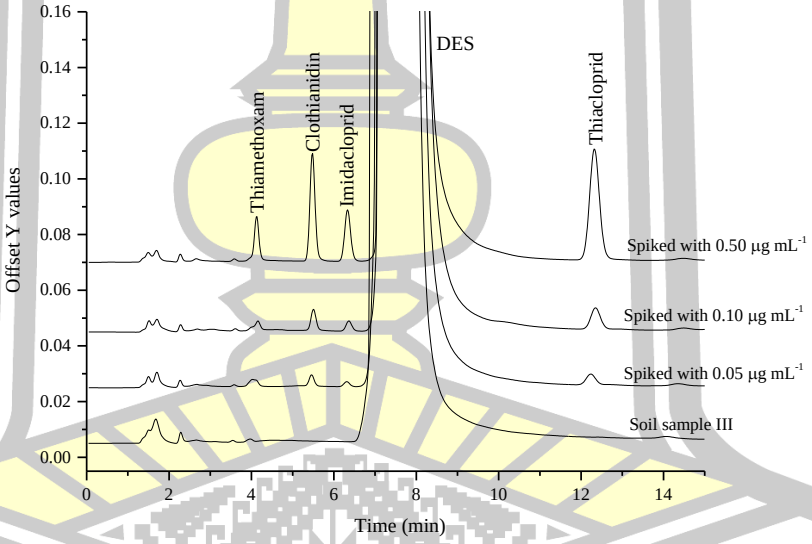



**Figure 30.** The overlaid chromatograms of blank and spiked soil sample III.

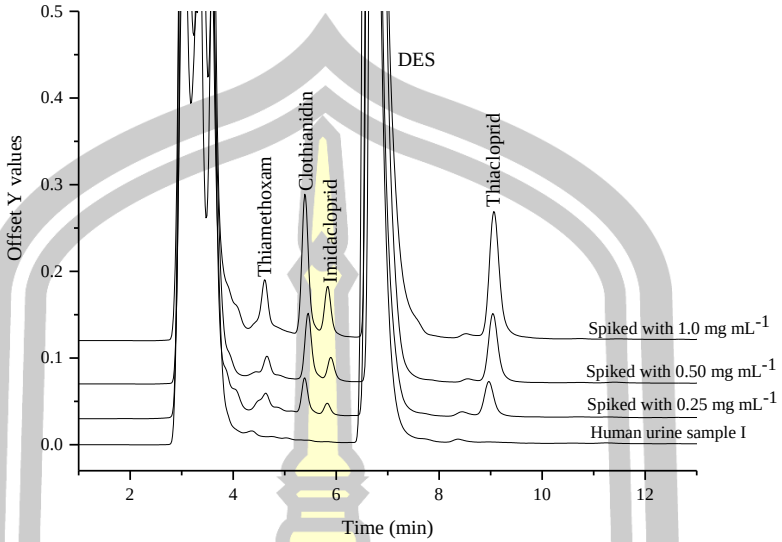



**Figure 31.** The overlaid chromatograms of blank and spiked human urine sample I.

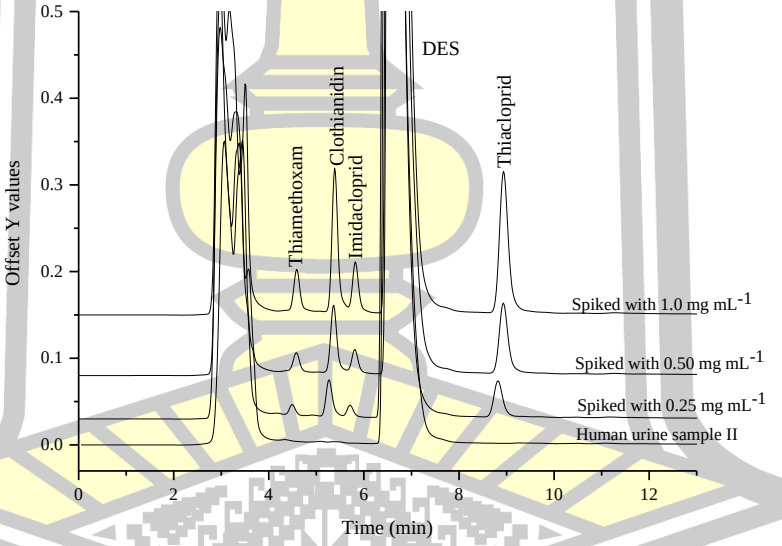



**Figure 32.** The overlaid chromatograms of blank and spiked human urine sample II.

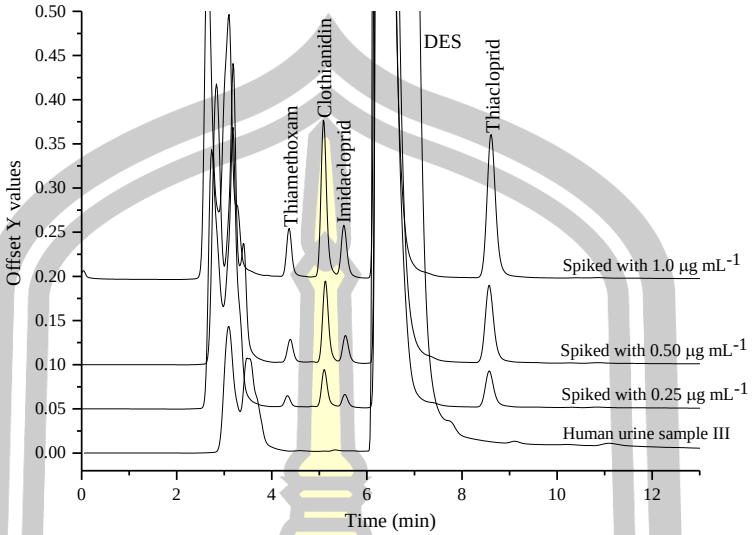



**Figure 33.** The overlaid chromatograms of blank and spiked human urine sample III.

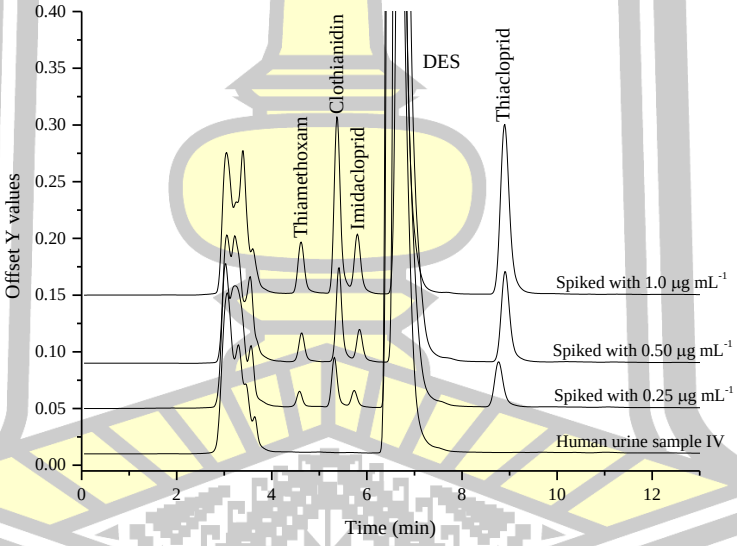



**Figure 34.** The overlaid chromatograms of blank and spiked human urine sample IV.

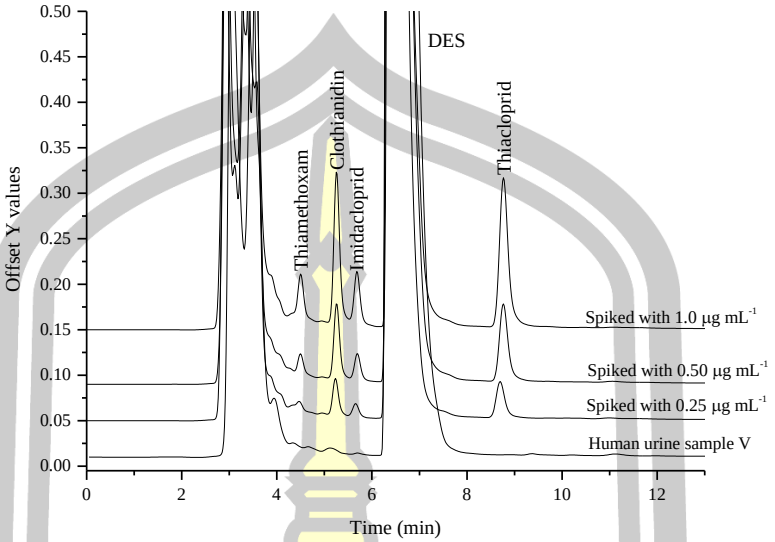



**Figure 35.** The overlaid chromatograms of blank and spiked human urine sample V.

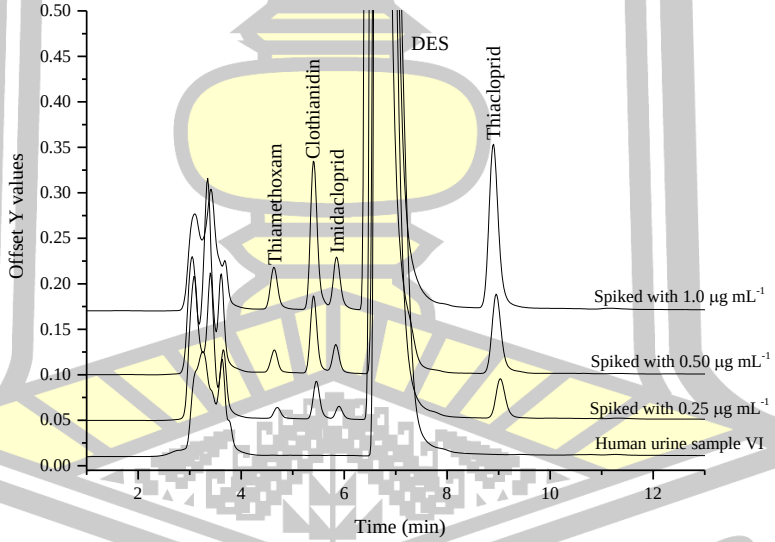



**Figure 36.** The overlaid chromatograms of blank and spiked human urine sample VI.

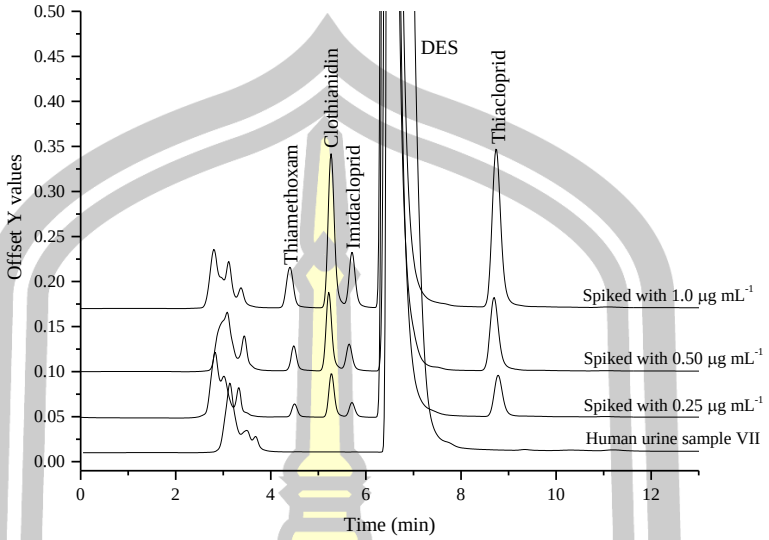



**Figure 37.** The overlaid chromatograms of blank and spiked human urine sample VII.

**4.1.5 Comparison of the proposed method with other sample preparation methods**

The proposed method as prepared to other sample preparation method for analysis of neonicotinoid insecticide residues. As summarized in Table 15, the proposed method coupled to HPLC is better than other sample preparation method in term of high analytical performance, short extraction time and used of environmentally friendly solvent. The sensitivity of the proposed method in term of LODs are almost comparable to that obtained from other microextraction method. The presented method achieves low LODs, which are below the MRLs of neonicotinoid insecticide residues in various samples.

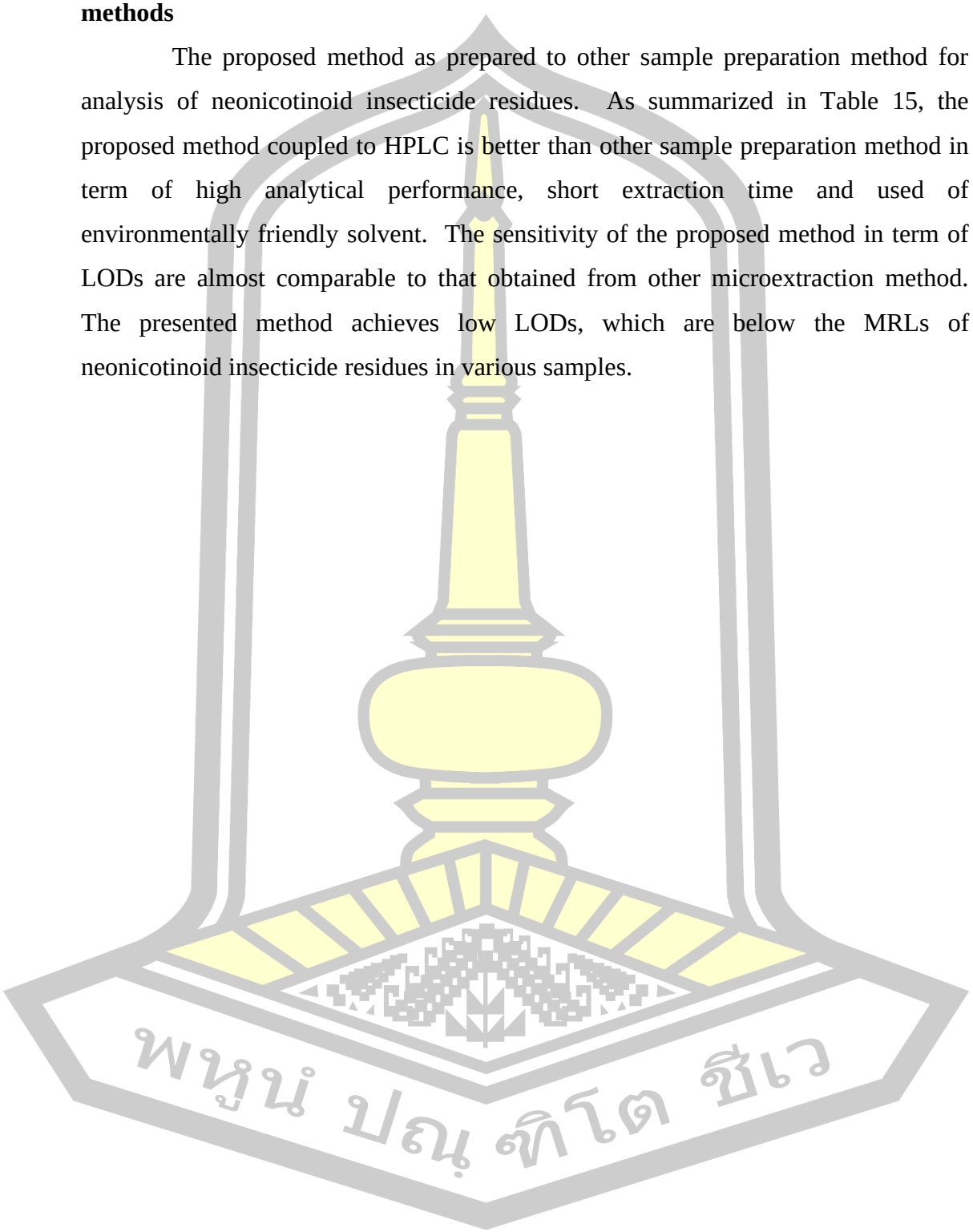


**Table 15.** Comparison of the present extraction method with other extraction method for the determination of neonicotinoid insecticides.

| Extraction method | Technique | Concentration range | Recovery (%) | LOD | EF | Ref. |
|---|---|---|---|---|---|---|
| dSPE and DLLME | HPLC-DAD | 0.030-4.5 µg $mL^{-1}$ | 76-123 | 0.002-0.05 mg $kg^{-1}$ | - | [13] |
| DLLME | HPLC DAD | LOQ-100.0 µg $kg^{-1}$ | 66.6-105.9 | | | [95] |
| QuEChERS | with | for MMC* | 72.2-85.2 | 1.5-2.5 µg $kg^{-1}$ | - | |
| | LC-MS/MS | 1.5-100 µg $kg^{-1}$ for SC** | | | | |
| SPE | LC-MS/MS | 0.0125-5 ng $mL^{-1}$ | 72.8-106.5 | - | - | [114] |
| VSLLME-SFO | HPLC | 0.0005-5 µg $mL^{-1}$ | 85-105 | 0.01-0.0005 µg $mL^{-1}$ | 20-100 | [115] |
| DLLME and | LC-MS/MS | LOQ-100.0 µg $L^{-1}$ | 69.2-113.4 | 0.5-1.5 µg $L^{-1}$ | 67.8-95.0 | [116] |
| QuEChERS | | | | | | |
| QuEChERS | LC-MS/MS | LOQ-100.0 µg $L^{-1}$ | 71.8-94.9 | 1.0-2.5 µg $L^{-1}$ | 51.3-96.2 | [117] |
| Methylamine-modified | | | | | | [118] |
| graphene- based SPE | UPLC-MS/MS | 0.001-100 ng $g^{-1}$ | 74.3-119.1 | 0.05-5.7 ng $kg^{-1}$ | - | |
| In-situ metathesis of | | | | | | This |
| deep eutectic solvent | HPLC-UV | 0.0001-1 µg $mL^{-1}$ | 80-115 | 0.0003-0.001 µg $mL^{-1}$ | 50-250 | work |

MMC*; Matrix match calibration curve, SC**; Standard curve

**4.2 Determination of neonicotinoid insecticide residues in surface water, soil and egg yolk samples using hydrophobic deep eutectic solvents based on dispersive liquid-liquid microextraction.**

This chapter present the result obtained section describes a development of then extraction method and then prior to high performance liquid chromatography (HPLC). The neonicotinoid insecticides such as thiamethoxam, clothianidin, acetamiprid and thiacloprid, were selected. The second section present the analytical performance of the proposed method. Finally, apply the proposed method for the analysis of neonicotinoid insecticide residues in surface water, soil and egg yolk samples. The results were discussed.

4.2.1 Synthetic and characterization of hydrophobic DESs

In addition, in order to elucidate the interactions between the two components resulting in the formation of hydrophobic DESs, FT-IR spectra were recorded. A comparison of FT-IR spectra of pure components [119,120] and the DESs formed is shown in Figure 38. The synthesis of hydrophobic deep eutectic solvents is accomplished by the formation of hydrogen bonds between HBA and HBD. The location of the bonds depends on the structure of the reactants. An inspection of FT-IR spectra of all the investigated hydrophobic deep eutectic solvents also reveals a O-H band of decanoic acid at 3435.12 $cm^{-1}$, C=O band at 1716.73 $cm^{-1}$, C-O band at 1037.23, methylene group ($-CH_2$) band at 2929.80 $cm^{-1}$and methyl group ($-CH_3$) band at 2857.40 $cm^{-1}$. FT-IR spectra of tetrabutyl ammonium bromide (TBABr) reveals a methylene group ($-CH_2$) and methyl group ($-CH_3$) band at wavenumbers: 2854.37 $cm^{-1}$, 2923.23 $cm^{-1}$, and 2955.80 $cm^{-1}$, respectively. But the observed of N-H group due to quaternary amine not shown in band of FT-IR spectra. In addition, the spectra of DESs formed from TBABr and decanoic acid reveal a characteristic shift of the bands corresponding to stretching vibrations of the carbonyl group towards higher wavenumber: from 1711.18 $cm^{-1}$ to 1716.73 $cm^{-1}$, which indicates the formation of new hydrogen bonds in the vicinity of the COOH group. Thus, the shift of the O–H vibrations suggests the existence of hydrogen bonding between TBABr and decanoic acid when the hydrophobic DES is formed.

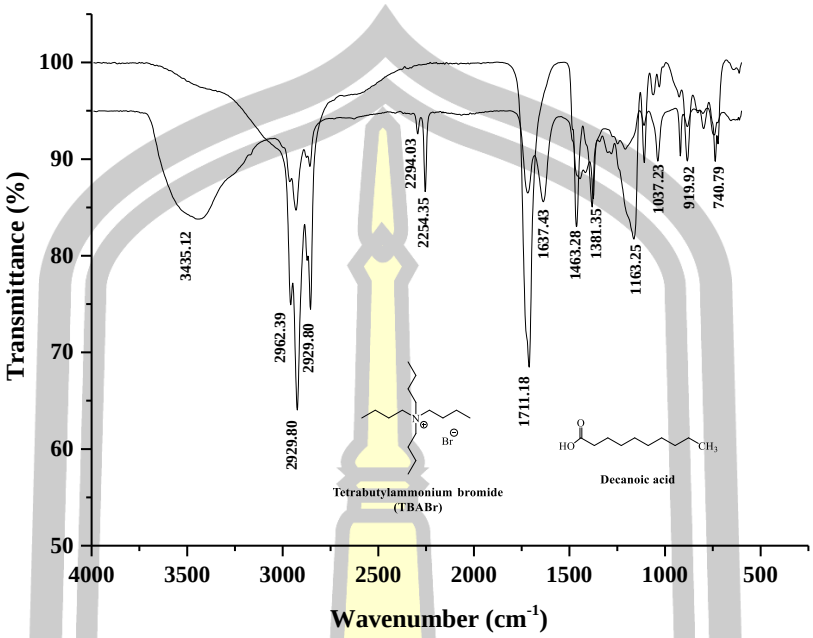



**Figure 38.** FT-IR spectra of TBABr and decanoic acid when hydrophobic DES was formed.

4.2.2 Optimization of dispersive liquid-liquid microextraction using hydrophobic deep eutectic solvents procedure

In order to obtain the high extraction efficiency of the proposed method, several parameters were investigated, including type and volume of disperser solvents, molar ratio and volume of hydrophobic DES, salts addition and extraction time were optimized. To identify of optimal extraction conditions, the peak area of the analytes was applied to evaluate extraction efficiency under the various conditions. In this experiment, various parameters were studied by a one parameter at a time while the other remaining factors were kept constant. The optimization was carried out on the aqueous solution (10.00 mL) containing 0.50 μg $mL^{-1}$ of each analyte. All experiments were performed in triplicate and average values were used for optimization.

4.2.2.1 Effect of salts addition

The addition of salt to aqueous solutions mainly cases decrease in the solubility of organic solvents in water, the addition of salt has been widely used to improve the extraction recovery of analytes [121]. To study the effect of ionic strength on the proposed method, different electrolyte salts such as NaCl, $Na_2SO_4$, $Na_2CO_3$, $CH_3COONa$ and $NH_4Cl$ at 0.3 g were investigated. The results were compared with that obtained from the procedure without salt addition. The experiment results shown in Figure 39, indicated that the extraction efficiency decreased with adding the different electrolyte salts in term of peak area of the studied neonicotinoid insecticides. Thus, no salt was added in subsequent experimental.

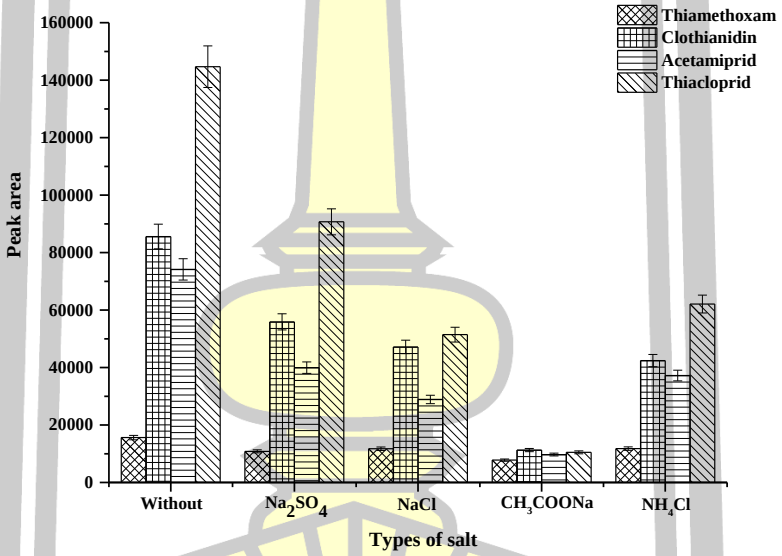



**Figure 39.** Effect of types of salt. Conditions: 400 µL of ACN; 100 µL of 0.1 mol $L^{-1}$ SDS; vortex time 60 sec; 100 µL of hydrophobic DES (mole ratio 3:1); centrifugation time at 5000 rpm for 10 min (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.2.2.2 Effect of type and volume of disperser solvents

In the proposed method, experiment for choosing the disperser solvents were performed using 400 µL of acetonitrile (ACN), methanol (MeOH) and ethanol (EtOH) and the results were compared with that obtained from the procedure without no disperser solvent. The results that shown in Figure 40. Among of these ACN was

found to provide the highest extraction efficiency due to cooperate effect of good compatibility of ACN with aqueous solutions and the low distributive ratio of analytes in mixture of ACN and water [122]. Thus, ACN was chosen as the disperser solvent in the further experiments.

The volume of ACN (50, 100, 200, 400, 600, 800 and 1,000 µL) in the presence of constant volume of 50 µL SDS and 100 µL hydrophobic DES were tested. The results that shown in Figure 41. It was found that extraction efficiency increased up to 400 µL of the disperser solvent and decreased after which was thought to be due to higher solubility of target analytes in the aqueous phase [121] in the presence of high volume of ACN and at 1,000 µL of ACN, no phase separation. As a result, 400 µL of ACN was selected as the optimum disperser solvent.

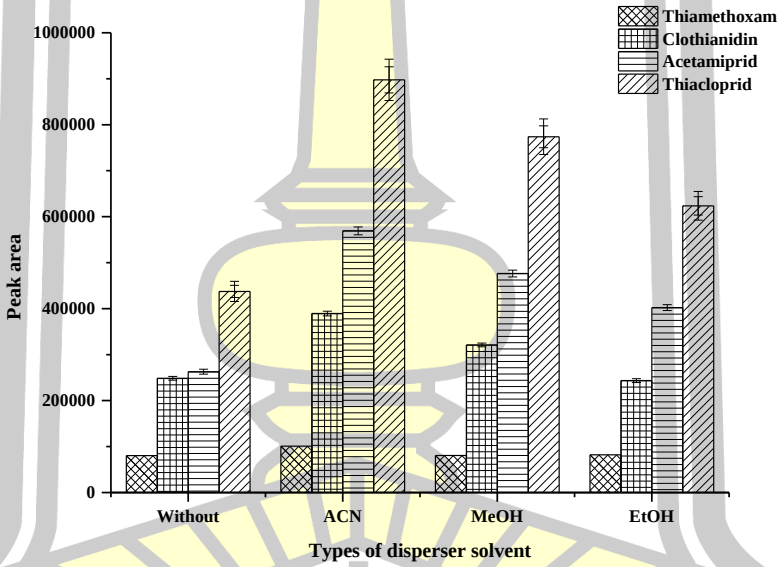



**Figure 40.** Effect of types of disperser solvent. Conditions: 400 µL of ACN; 100 µL of 0.1 mol $L^{-1}$ SDS; vortex time 60 sec; 100 µL of hydrophobic DES (mole ratio 3:1); centrifugation time at 5000 rpm for 10 min (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

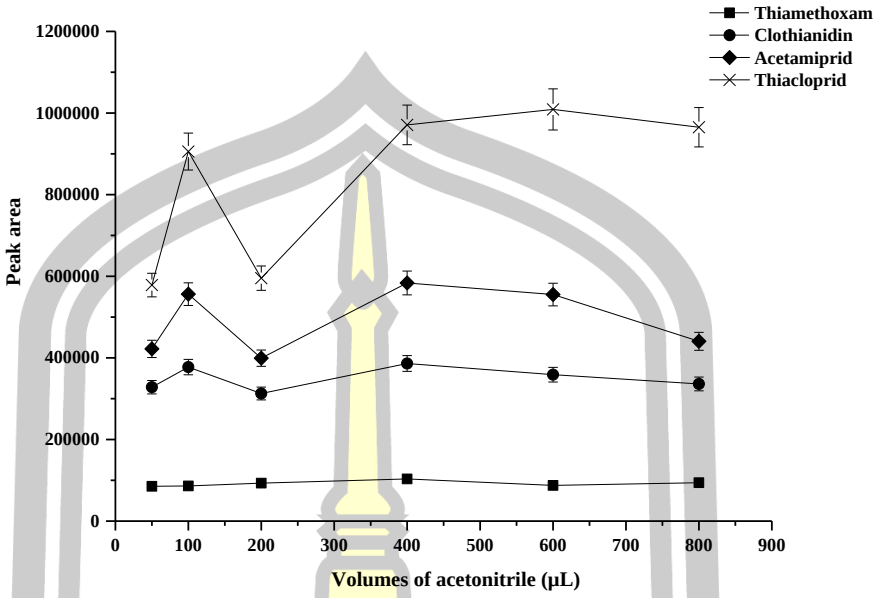



**Figure 41.** Effect of the volume of acetonitrile (disperser solvent). Conditions: 400 µL of ACN; 100 µL of 0.1 mol $L^{-1}$ SDS; vortex time 60 sec; 100 µL of hydrophobic DES (mole ratio 3:1); centrifugation time at 5000 rpm for 10 min (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.2.2.3 Effect of type and concentration of surfactant

The selection of the surfactant also greatly influenced the developed microextraction procedure. The structure of surfactants affects its physical and chemical properties, which may affect the extraction efficiency of key factors [108]. In this method, different types of surfactant including sodium dodecyl sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB), Triton X-100 (TX-100) and Triton X-114 (TX-114) at 100 µL were investigated and the results were compared with that obtained from the procedure without surfactant. The results that shown in Figure 42. It was found that the addition of SDS provided higher extraction efficiency in term of peak area of studied neonicotinoid insecticides. A surfactant aggregate orientates its hydrocarbon tails towards the center to create a non-polar core. Isolated hydrophobic substances, which is presented in the aqueous solution, is favorably partitioned in the hydrophobic core of micelles [123]. Thus, SDS was selected as the surfactant in the proposed method.

The effect of concentration of SDS on the extraction efficiency were studied in the range of 3-100 mmol $L^{-1}$. The results that shown in Figure 43. When increasing the concentration of SDS from 3-10 mmol $L^{-1}$, the extraction efficiency increased and constant at 30 mmol $L^{-1}$. And the concentration of SDS from 50-100 mmol $L^{-1}$, more turbid solution and no phase separation. Therefore, the concentration of 10 mmol $L^{-1}$ SDS was used.

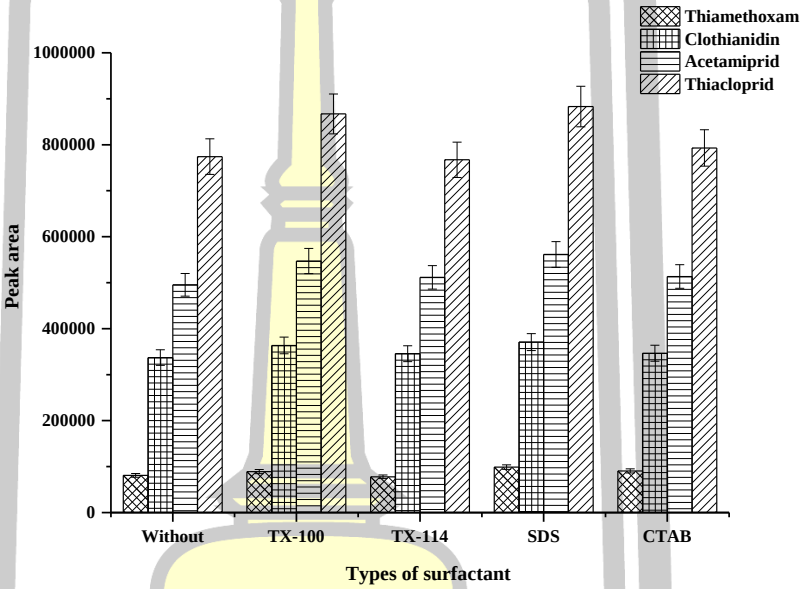



**Figure 42.** Effect of types of surfactant. Conditions: 400 µL of ACN; 100 µL of 0.1 mol $L^{-1}$ SDS; vortex time 60 sec; 100 µL of hydrophobic DES (mole ratio 3:1); centrifugation time at 5000 rpm for 10 min (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

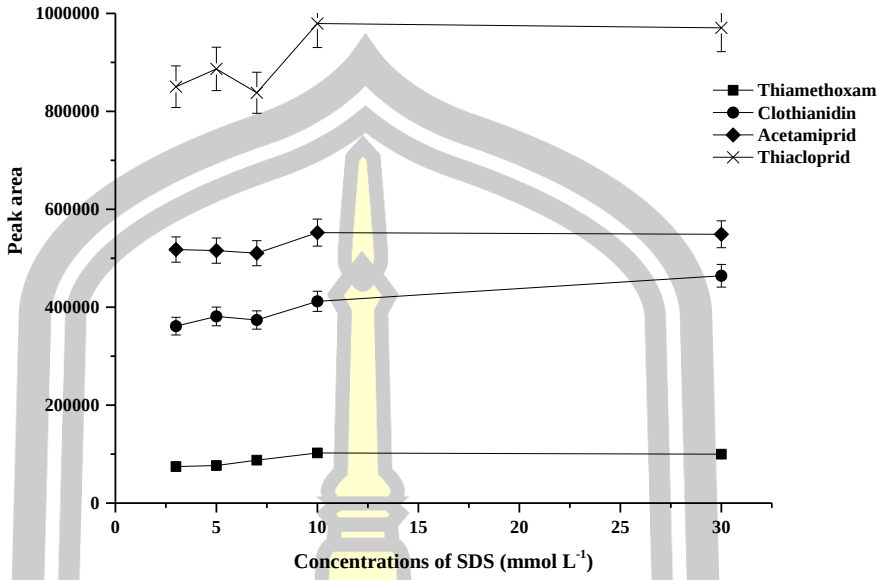



**Figure 43.** Effect of concentrations of SDS. Conditions: 400 µL of ACN; 100 µL of 0.1 mol $L^{-1}$ SDS; vortex time 60 sec; 100 µL of hydrophobic DES (mole ratio 3:1); centrifugation time at 5000 rpm for 10 min (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.2.2.4 Effect of vortex and centrifugation time

In order to increase the vortex time speeds up the distribution equilibrium of the target analytes between hydrophobic DES (mole ratio 3:1) and aqueous solution and improve the recovery. The results as shown in Figure 44. The vortex time speed was evaluated in the range of 10-150 sec compared with that obtained from the process without vortex agitation. The extraction efficiencies were improved with extraction time over the range 10-60 sec, with a maximum at 30 sec. And the vortex time more than 60 sec, no phase separation was occurred. Therefore, the vortex time of 30 sec was selected.

To achieve the phase separation, centrifugation time is another important step. The centrifugation speed was fixed at maximum speed of instrument (5000 rpm) and fixed time of 10 min, to ensure complete phase separation in the proposed microextraction method.

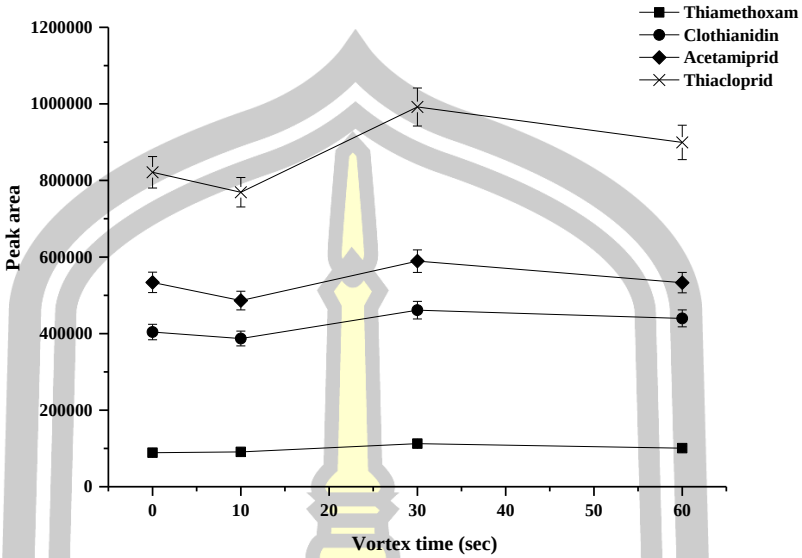



**Figure 44.** Effect of vortex time. Conditions: 400 µL of ACN; 100 µL of 10 mmol $L^{-1}$ SDS; vortex time 60 sec; 100 µL of hydrophobic DES (mole ratio 3:1); centrifugation time at 5000 rpm for 10 min (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

4.2.2.5 Selection of hydrophobic DES and it's volume.

The composition of hydrophobic DES has significant influence on its physicochemical properties, which might greatly effect of the extraction efficiency of target analytes [69]. Five different mole ratios of hydrophobic DES were prepared (Table 9) and their ability to extract the five neonicotinoid insecticides in various samples were investigated. The extraction efficiencies of three different molar ratio of hydrophobic DES as shown in Figure 45. The result of ability of extraction using DES (mole ratio 4:1) provided higher extraction efficiency in term of peak area but its property has non-stable (solidified at room temperature). Thus, in this method hydrophobic DES (mole ratio 3:1) was used in the further experimental.

The volume of hydrophobic DES (mole ratio 3:1) has influence on the extraction efficiency. Different volumes of hydrophobic DES (mole ratio 3:1) (50, 100, 300, 500, 700, 1,000 µL) were investigated, the results that shown in Figure 46. It was found that an enhancement of extraction efficiency for all neonicotinoid insecticides in term of peak area when 100 µL hydrophobic DES (mole ratio 3:1) was added.

At the volume of hydrophobic DES (mole ratio 3:1) higher 100 µL (300-1,000 µL), peak of clothianidin and imidacloprid was overlap and the large volumes of hydrophobic DES (mole ratio 3:1) did not increase efficiencies because dilution effect. Therefore, the volume of 100 µL was selected as extraction solvent.

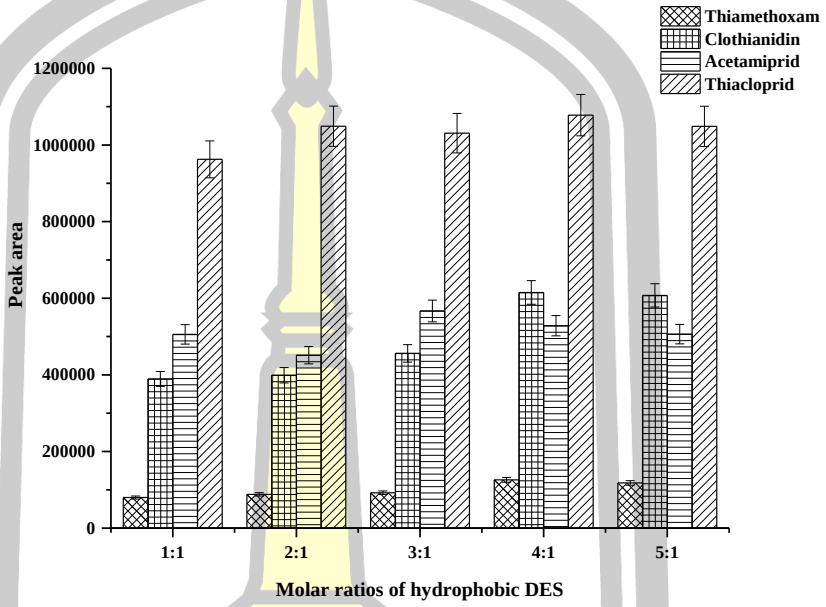



**Figure 45.** Effect of mole ratios of hydrophobic DES. Conditions: 400 µL of ACN; 10 mmol $L^{-1}$ of SDS; vortex time 30 sec; 100 µL of hydrophobic DES (molar ratio 3:1); centrifugation time at 5000 rpm for 10 min (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

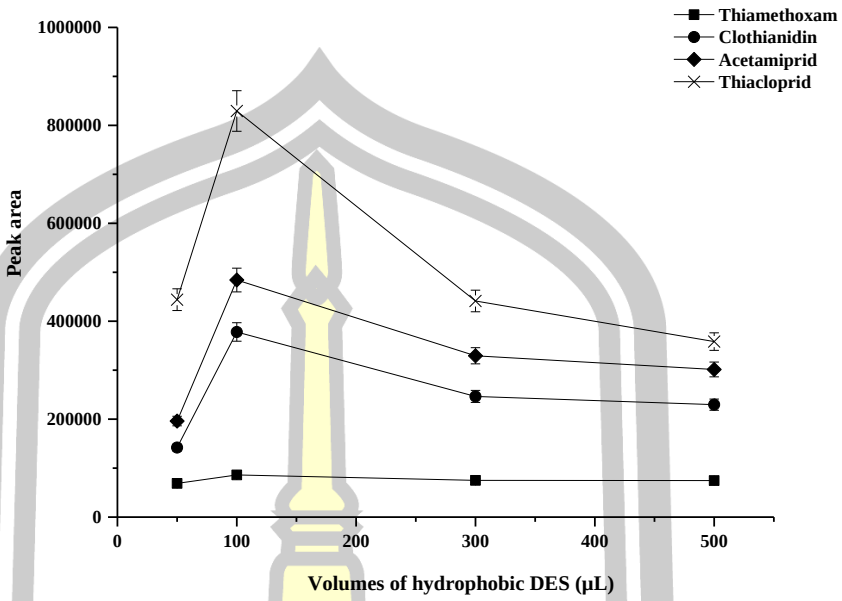



**Figure 46.** Effect of the volume of hydrophobic DES. Conditions: 400 µL of ACN; 10 mmol $L^{-1}$ of SDS; vortex time 30 sec; 100 µL of hydrophobic DES (mole ratio 3:1); centrifugation time at 5000 rpm for 10 min (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

**Table 16.** The optimum conditions of dispersive liquid-liquid microextraction using hydrophobic deep eutectic solvent for preconcentration of neonicotinoid insecticides.

| Parameters | Optimum conditions |
|---|---|
| Type of salts | Without salt addition |
| Types of disperser solvent | Acetonitrile |
| Volume of disperser solvent | 400 µL |
| Concentration of surfactant | 10 mmol $L^{-1}$ of SDS |
| Vortex time | 30 sec |
| Mole ratio of hydrophobic DES | 3:1 (Decanoic acid:TBABr) |
| Volume of hydrophobic DES | 100 µL |
| Centrifugation | 5000 rpm; 5 min |

### 4.2.3 Analytical performance of the proposed method

The developed hydrophobic deep eutectic solvent based on dispersive liquid-liquid microextraction method coupled with HPLC-PDA for determination of neonicotinoid insecticide residues in various samples was evaluated for linear range, limits of detection (LODs), limits of quantitation (LOQs), repeatability, extraction recovery and enrichment factor (EF) of each target analyte. The analytical performances of the proposed method were performed by enriching 10 mL of working standard solution, and the results as summarized in Table 17. The proposed method gives a good linearity in the range of 0.001-0.1 μg $mL^{-1}$ with the correlation coefficient ($R^2$) greater than 0.99. The sensitivity was characterized by limits of detection as the concentration providing a signal-to-noise ratio of 3 and limits of quantitation as the concentration providing a signal-to-noise ratio of 10, LODs were in the range of 0.001-0.003 μg $mL^{-1}$ and LOQs were in the range of 0.009-0.003 μg $mL^{-1}$, respectively. Four neonicotinoid insecticide solutions of 0.01 and 0.10 μg $mL^{-1}$ were employed to determine the precision as the relative standard deviation. The relative standard deviations of the retention times and peak areas were less than 10.0%. The enrichment factor (EF), defined as the concentration ratio of the analytes in the settled phase ($C_{set}$) and in aqueous samples ($C_0$), ranged from 10-30 folds. The overlaid chromatograms of the studied neonicotinoid insecticides obtained from direct HPLC and preconcentrated by the proposed microextraction method are shown in Figure 47.

**Table 17.** Analytical performance of the proposed method for four different neonicotinoid insecticides.

| Analyte | Linear range ($\mu g\ mL^{-1}$) | Linear equation | $R^2$ | LOD ($\mu g\ mL^{-1}$) | LOQ ($\mu g\ mL^{-1}$) | RSD (%) | | EF |
|---|---|---|---|---|---|---|---|---|
| | | | | | | $t_R$ | Peak area | |
| Thiamethoxam | 0.003-1 | y = 203253x + 366.71 | 0.9996 | 0.001 | 0.003 | 0.10 | 1.37 | 10 |
| Clothianidin | 0.003-1 | y = 1,041,035.06 x + 691.91 | 0.9991 | 0.001 | 0.003 | 0.19 | 1.67 | 10 |
| Acetamiprid | 0.009-1 | y = 875385x – 2215.8 | 0.9989 | 0.003 | 0.009 | 0.18 | 1.07 | 30 |
| Thiacloprid | 0.009-1 | y = 2,038,651.97 x – 5337 | 0.9994 | 0.003 | 0.009 | 0.32 | 1.63 | 30 |

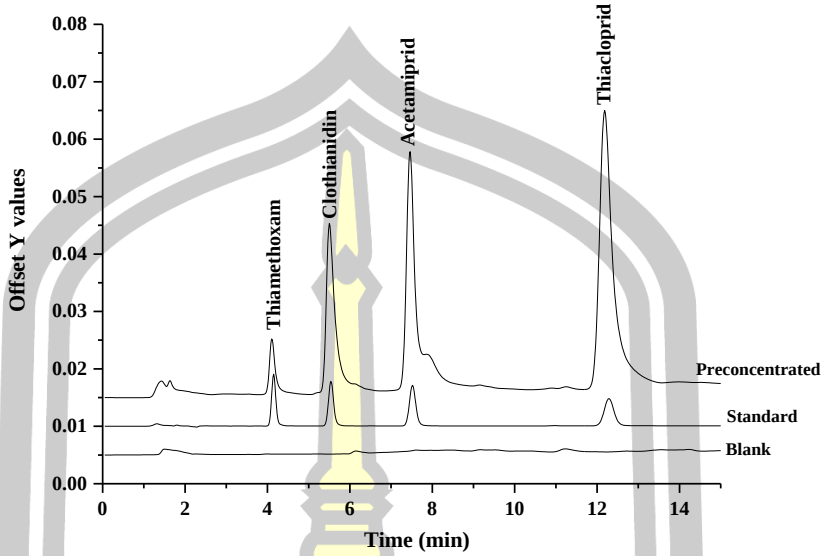



**Figure 47.** The overlaid chromatograms of the studied neonicotinoid insecticides obtained from blank, direct HPLC and preconcentrated by the proposed microextraction method (sample volume 10 mL, 0.5 µg $mL^{-1}$ of each neonicotinoid insecticide).

### 4.2.4 Real samples analysis

For evaluating of the applicability and recovery of the proposed method, three types of sample including surface water, soil and egg yolk samples were examined. These samples were spiked with the neonicotinoid insecticides at the different concentration of 0.01, 0.05 and 0.1 µg $mL^{-1}$, before extraction and analysis. The overlaid chromatograms of blank and spiked surface water (Figure 48-50), soil (Figure 51-53) and egg yolk samples are shown in Figure 54-56. The recoveries of the studied neonicotinoid insecticides were obtained in the range of 30.04-114.33%, 46.37-113.72% and 35.02-106.04% for surface water, soil and egg yolk samples, respectively. The results were shown in Table 18. The relative standard deviations (RSDs) were less than 10.0%, at the estimated spiking different concentration levels. The results show that no neonicotinoids insecticide residues were detected in the studied samples because LODs of this method is lower than the maximum residue limit (MRL) established by EU.

**Table 18.** Recoveries of the studied neonicotinoids spiked in surface water, soil and egg yolk samples obtained by the proposed method (n=3).

| Samples | Spiked ($\mu g\ mL^{-1}$) | Recovery ± RSD (%) | | | |
|---|---|---|---|---|---|
| | | TMX | CTD | ATP | TCP |
| Surface water I | 0 | ND | ND | ND | ND |
| | 0.01 | 94.34 ± 2.16 | 77.59 ± 5.42 | 81.16 ± 1.73 | 104.45 ± 2.41 |
| | 0.05 | 97.19 ± 1.49 | 74.04 ± 8.74 | 89.42 ± 2.43 | 90.88 ± 7.39 |
| | 0.1 | 98.93 ± 7.64 | 86.65 ± 8.22 | 108.85 ± 3.06 | 101.81 ± 1.54 |
| Surface water II | 0 | ND | ND | ND | ND |
| | 0.01 | 76.70 ± 2.32 | 47.80 ± 7.47 | 108.21 ± 2.64 | 101.23 ± 7.73 |
| | 0.05 | 90.35 ± 2.12 | 73.86 ± 6.53 | 81.53 ± 7.64 | 92.05 ± 3.87 |
| | 0.1 | 85.43 ± 9.28 | 70.98 ± 9.28 | 84.96 ± 3.65 | 91.45 ± 2.83 |
| Surface water III | 0 | ND | ND | ND | ND |
| | 0.01 | 62.05 ± 0.08 | 30.04 ± 0.13 | 91.79 ± 0.02 | 114.56 ± 0.02 |
| | 0.05 | 114.33 ± 4.46 | 80.22 ± 1.35 | 104.83 ± 5.07 | 111.56 ± 4.73 |
| | 0.1 | 92.81 ± 5.04 | 90.89 ± 2.41 | 98.06 ± 4.07 | 90.05 ± 4.88 |
| Soil I | 0 | ND | ND | ND | ND |
| | 0.01 | 105.32 ± 3.24 | 46.37 ± 0.33 | 99.74 ± 3.31 | 108.35 ± 5.29 |
| | 0.05 | 85.97 ± 4.54 | 58.66 ± 8.99 | 90.78 ± 0.18 | 94.60 ± 1.79 |
| | 0.1 | 95.99 ± 3.95 | 62.21 ± 8.37 | 94.87 ± 5.29 | 90.40 ± 2.95 |
| Soil II | 0 | ND | ND | ND | ND |
| | 0.01 | 58.27 ± 5.38 | 65.88 ± 6.99 | 109.94 ± 2.11 | 86.46 ± 6.67 |
| | 0.05 | 86.16 ± 8.41 | 73.52 ± 2.14 | 107.93 ± 6.70 | 97.56 ± 3.29 |
| | 0.1 | 111.78 ± 1.24 | 81.64 ± 4.87 | 105.01 ± 1.97 | 93.65 ± 4.44 |
| Soil III | 0 | ND | ND | ND | ND |
| | 0.01 | 94.72 ± 9.07 | 63.18 ± 0.77 | 102.78 ± 0.87 | 81.61 ± 1.28 |
| | 0.05 | 90.33 ± 1.36 | 80.89 ± 4.89 | 97.58 ± 1.43 | 113.72 ± 0.04 |
| | 0.1 | 95.64 ± 7.16 | 80.10 ± 2.86 | 109.20 ± 1.89 | 96.63 ± 3.73 |
| Chicken egg | 0 | ND | ND | ND | ND |
| | 0.01 | 66.24 ± 5.69 | 47.00 ± 3.09 | 67.69 ± 6.53 | 90.67 ± 0.49 |
| | 0.05 | 71.94 ± 0.52 | 47.27 ± 4.31 | 85.16 ± 3.34 | 81.41 ± 0.56 |
| | 0.1 | 70.44 ± 4.69 | 36.88 ± 3.54 | 78.05 ± 9.81 | 100.41 ± 3.17 |
| Duck egg | 0 | ND | ND | ND | ND |
| | 0.01 | 65.42 ± 2.29 | 46.48 ± 6.32 | 106.04 ± 0.16 | 88.91 ± 5.09 |
| | 0.05 | 76.69 ± 0.24 | 48.65 ± 1.42 | 105.37 ± 0.99 | 84.55 ± 5.33 |
| | 0.1 | 84.28 ± 4.28 | 46.59 ± 6.72 | 90.01 ± 3.49 | 80.81 ± 1.91 |
| Quail egg | 0 | ND | ND | ND | ND |
| | 0.01 | 84.96 ± 0.50 | 35.02 ± 0.71 | 67.11 ± 0.38 | 63.69 ± 2.29 |
| | 0.05 | 81.46 ± 3.90 | 42.76 ± 0.02 | 88.47 ± 2.33 | 82.48 ± 2.12 |
| | 0.1 | 85.96 ± 0.91 | 53.40 ± 0.56 | 90.61 ± 2.72 | 85.02 ± 3.31 |

*ND: not detected, TMX: thiamethoxam, CTD: clothianidin, ATP: acetamiprid, TCP: thiacloprid

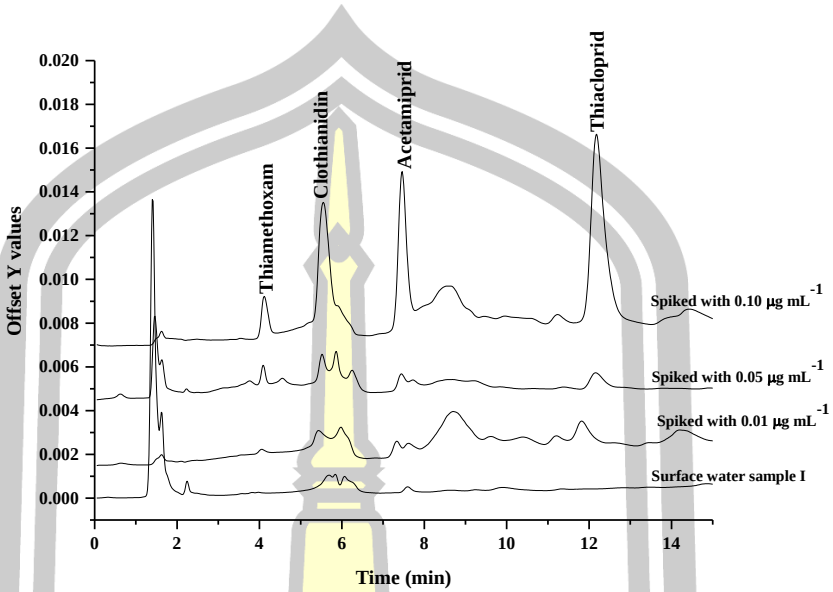



**Figure 48.** The overlaid chromatograms of blank and spiked surface water sample I.

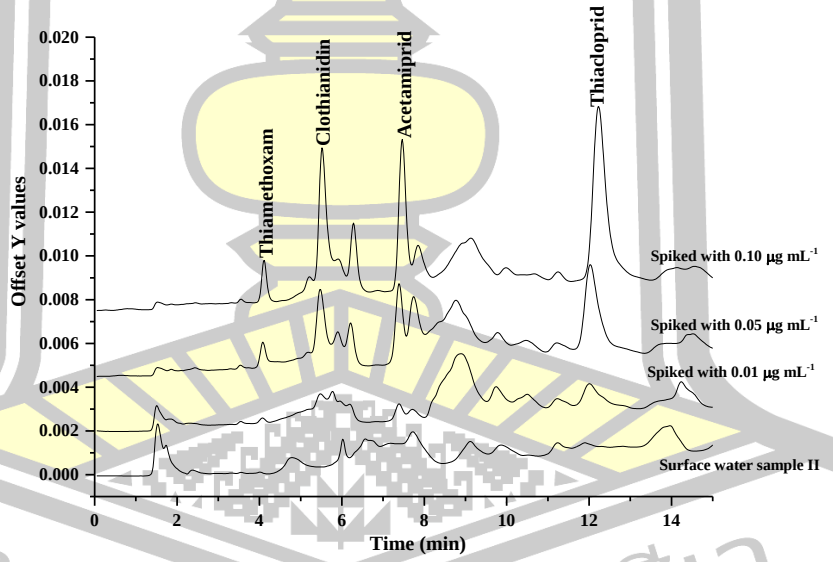



**Figure 49.** The overlaid chromatograms of blank and spiked surface water sample II.

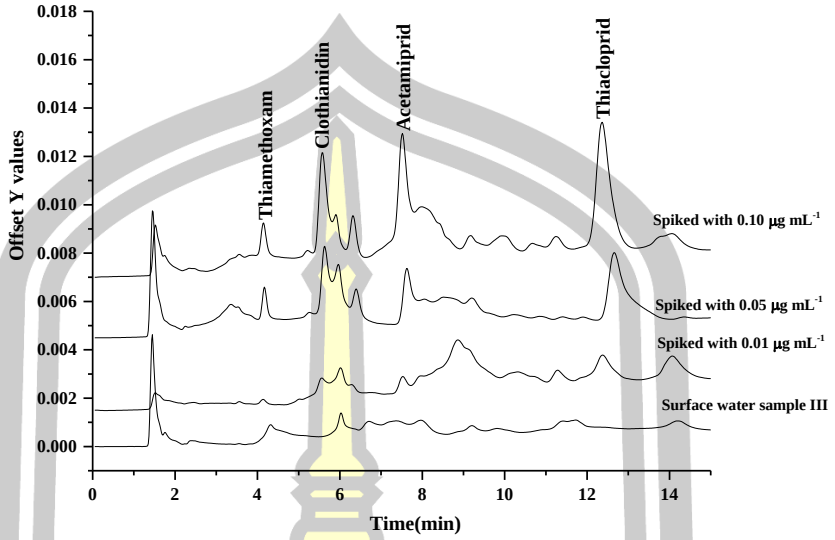



**Figure 50.** The overlaid chromatograms of blank and spiked surface water sample III.

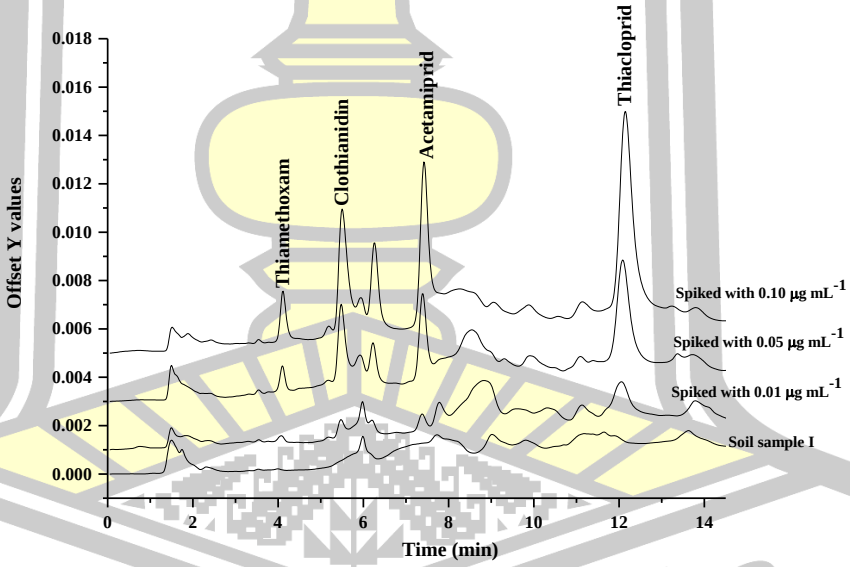



**Figure 51.** The overlaid chromatograms of blank and spiked soil sample I.

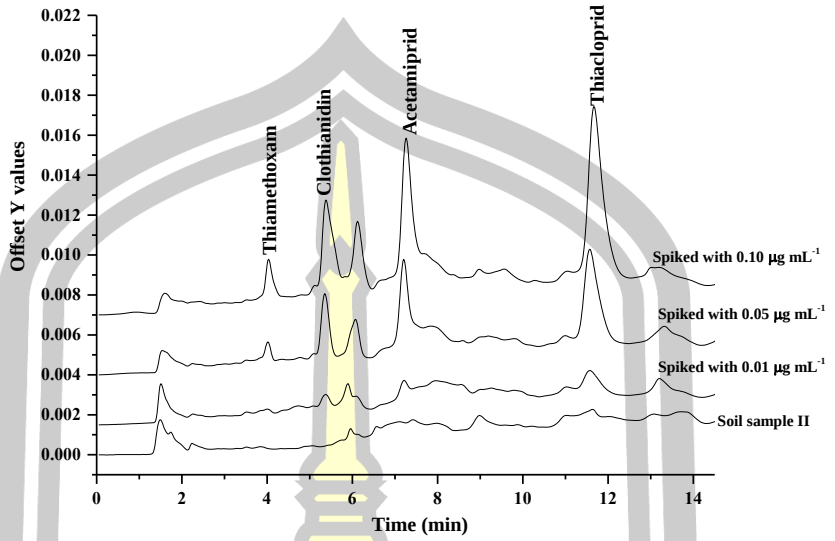



**Figure 52.** The overlaid chromatograms of blank and spiked soil sample II.

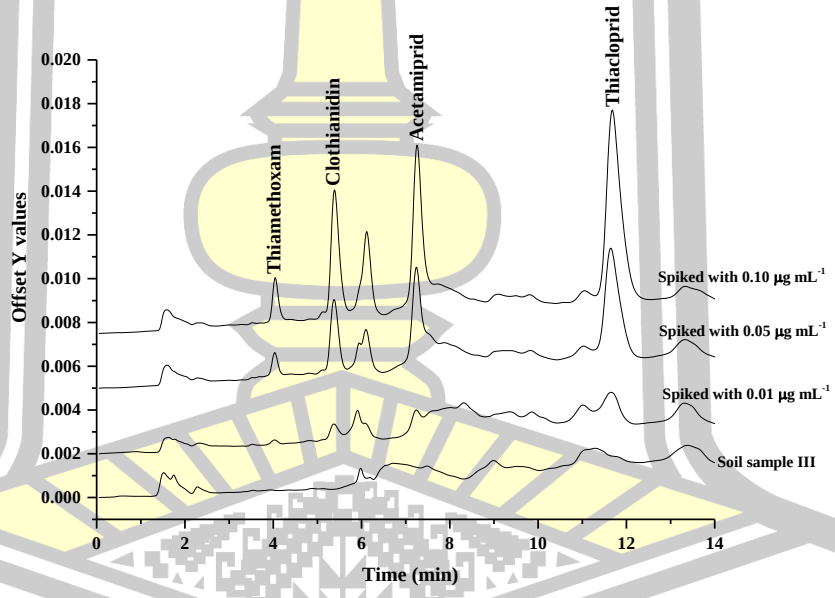



**Figure 53.** The overlaid chromatograms of blank and spiked soil sample III.

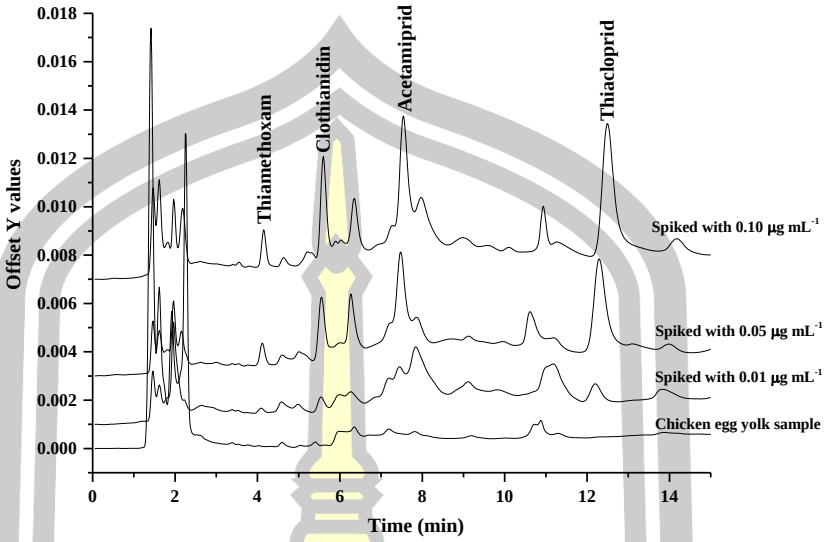



**Figure 54.** The overlaid chromatograms of blank and spiked chicken egg yolk sample.

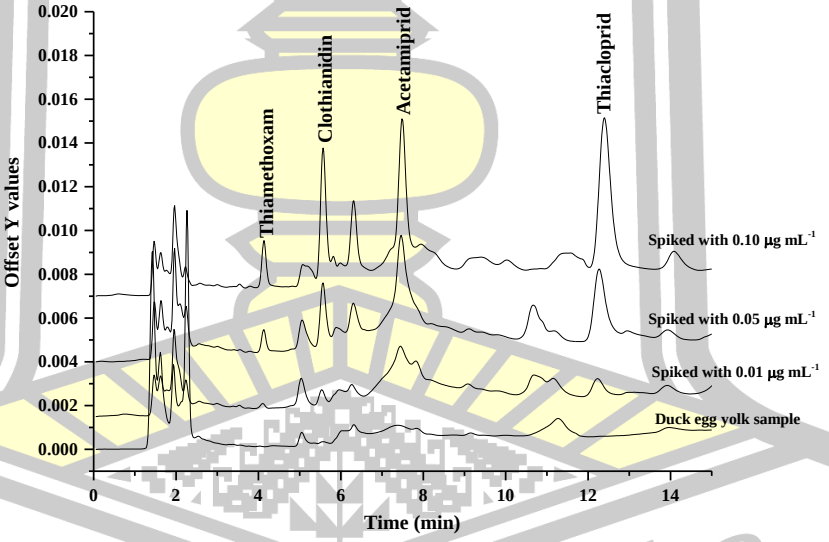



**Figure 55.** The overlaid chromatograms of blank and spiked duck egg yolk sample.

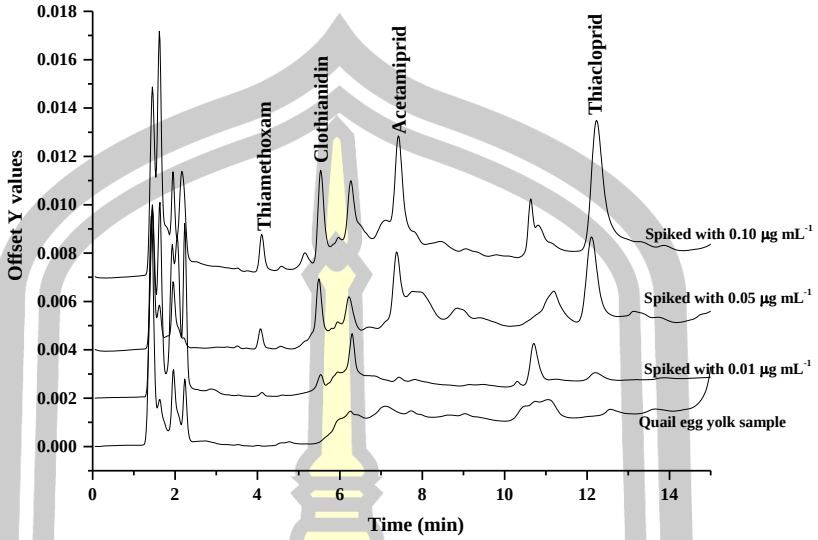



**Figure 56.** The overlaid chromatograms of blank and spiked quail egg yolk sample.

**4.2.5 Comparison of the proposed hydrophobic deep eutectic solvent based on dispersive liquid-liquid microextraction with other sample preparation methods**

Comparisons between the current DLLME-hydrophobic DES method and previous sample pretreatment methods for the preconcentration and determination of neonicotinoid insecticide residues are shown in Table 19. The results showed that the parameters of this method such as linearity, LODs, LOQs and recovery were the same as or greater than those of most of the reported methods. Moreover, the advantages of the DLLME-hydrophobic DES method can be summarized as follows: (i) the extraction solvent (hydrophobic DES) are a new generation of green solvents, which is necessary from environmentally friendly and economical attitudes. (ii) simple preparation procedures, the components (HBD, neutral compounds such as carboxylic acid, alcohol, sugars and salts) can be easily mixed to obtain target eutectic mixture. (iii) the proposed method is miniaturized, making it possible to reduce dramatically the amounts of samples, reagents and solvents consumed and wastes generated. The present method achieves low LODs which are below the MRLs established by EU for neonicotinoid insecticide residues in environmental and animal products.

**Table 19.** Comparisons of the proposed method with other methods for the quantitation of neonicotinoid insecticides.

| Method | Linear range | Extraction solvents | Solvent usage | Extraction time | LOD | Ref. |
|---|---|---|---|---|---|---|
| QuEChERS-LC-MS/MS | 1.56-400 µg $L^{-1}$ | 1% acetic acid in acetonitrile | 10 mL | 50 min | 1.0-3.0 µg $kg^{-1}$ | [45] |
| PSE-LC-MS/MS | - | Methanol | 5 mL | 20 min | 0.8-1.5 µg $kg^{-1}$ | [92] |
| LLE-LC-ESI-MS | 2.0-1000 µg $kg^{-1}$ | n-hexane/isopropanol (8:2 %v/v) | 15 mL | 18 min | 0.6-2.3 µg $kg^{-1}$ | [93] |
| DLLME-HPLC-DAD | 1.5-100 µg $L^{-1}$ | acetonitrile/ dichloromethane | 2.5 mL | 17 min | 1.5-2.5 µg $L^{-1}$ | [95] |
| DLLME-LC-MS/MS | 1.5-100.0 µg $kg^{-1}$ | acetonitrile/ dichloromethane | 2.5 mL | 20 min | 0.5-1.0 µg $kg^{-1}$ | [124] |
| Capillary-ESI-MS | 4.0-1000 µg $L^{-1}$ | n-hexane/isopropanol (8:2 %v/v) | 15 mL | 25 min | 2.6-4.7 µg $L^{-1}$ | [125] |
| Hydrophobic DES-DLLME-HPLC-UV | 0.001-1 µg $mL^{-1}$ | Hydrophobic DES | 600 µL | 10.3 min | 0.003-1 µg $mL^{-1}$ | This work |

# CHAPTER 5
# CONCLUSION

Two liquid phase microextraction methods including in-situ metathesis reaction of deep eutectic solvent and DLLME using hydrophobic DESs under study were simple, fast and inexpensive for sample preconcentration of neonicotinoid insecticide residues in various samples. In the first part, in-situ metathesis reaction of deep eutectic solvents was developed for preconcentration and determination of neonicotinoid insecticides prior to HPLC analysis. By employing in-situ reaction, the formation of hydrogen bonding between the halide anion of choline chloride and functional groups of hydrogen bond donor agent is responsible for the decrease in the freezing point of DESs in relation to the melting point of the individual components. For in-situ metathesis of DES extraction, the optimal conditions were 10 mL of sample, 0.5 g $Na_2SO_4$, 500 µL of TX-114, vortex 10 sec, ultrasound (I) temperature at 25 °C for 3 min, ultrasound (II) temperature at 50 °C for 10 min, 100 µL of ChCl, 500 µL of Phenol, centrifugation (I) time at 5 min and centrifugation (II) time at 1 min. The extraction was then analyzed by Chromolith® Highresolution RP-18 endcapped column (100 mm × 4.6 mm) (Merck, Germany) was used as an analytical column carried out at room temperature. The injection volume was 20 µL. For data processing, Empowers 3 software was used. Chromatographic analysis using isocratic elution with 26 %v/v acetonitrile in water as the mobile phase at a flow rate of 0.5 mL $min^{-1}$ was used for separation of the studied neonicotinoid insecticide residues. Separation of four neonicotinoid insecticide residues was achieved less than 10 min. Under the optimum conditions, low limits of detection (LODs) were 0.0003-0.001 µg $L^{-1}$ for all target analytes which below the acceptable maximum residue limits (MRLs) for neonicotinoid insecticides. High enrichment factor (50-250 folds) proved that the proposed microextraction method is simple and efficient in the analysis of neonicotinoids in surface water, soil and human urine samples. The proposed method has been successfully applied to the preconcentration and determination of neonicotinoid insecticide residues in real samples.

In the second part, an efficient hydrophobic DES was synthesized from decanoic acid and terabutylammonium bromide (TBABr). Based on the use of

hydrophobic DES, a green, fast, and inexpensive technique (DLLME) was proposed for the preconcentration, extraction and separation of neonicotinoid insecticide residues coupled with HPLC-PDA. For hydrophobic DES based on DLLME, the optimal conditions were 10 mL of sample, 400 µL of acetonitrile, 100 µL of SDS, vortex 30 sec, 100 µL of hydrophobic DES (molar ratio 3:1) and centrifugation at 5000 rpm for 10 min. The extraction was then analyzed by Purosphere® STAR RP-18 endcapped column at room temperature. The injection volume was 20 µL. For data processing, Empowers 3 software was used. Chromatographic analysis using isocratic elution with 25 %v/v acetonitrile in water as the mobile phase at a flow rate of 1.0 mL $min^{-1}$ was used for separation of the studied neonicotinoid insecticide residues. Separation of four neonicotinoid insecticide residues was achieved less than 13 min. Under the optimum conditions, low limits of detection (LODs) were 0.001-0.003 µg $mL^{-1}$ for all target analytes which below the acceptable maximum residue limits (MRLs) established by EU for neonicotinoid insecticide residues. The proposed method is simple and efficient in the analysis of neonicotinoids in surface water, soil and egg yolk samples. Although the application of DESs in environmental analysis will grow in the near future, there is still a need for further investigations using DESs as extractive agents. For example, new hydrophobic DESs with different polarities should be synthesized to improve their application to various samples.

Both methods represented here has acceptable relative recoveries, good repeatability and a wide linear range. When compare to the other microextraction methods, this method utilizing very low volumes of organic solvent, is environmentally friendly method, provided a high extraction efficiency and showed reliability with as appropriate analytical detection range for application in surface water, soil, human urine and egg yolk samples.

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