Development of Dispersive Micro-Solid Phase Extraction for Determination of Neonicotinoid Insecticide Residues

Khwankaew Moyakao

A Thesis Submitted in Partial Fulfillment of Requirements for
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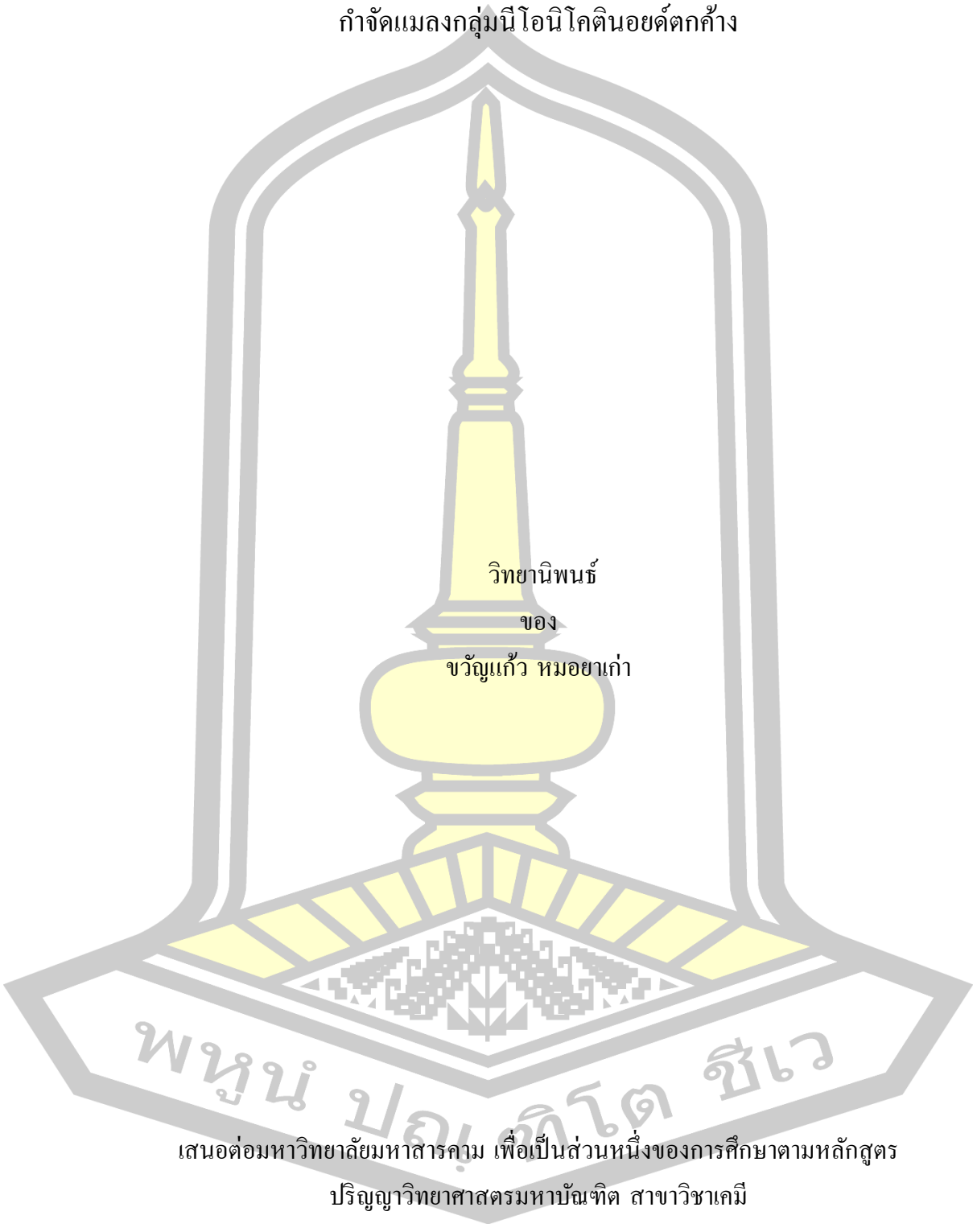

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ABSTRACT

This study presents a simple dispersive micro-solid phase extraction (d-µ-SPE) using montmorillonite (MMT) clay as an efficient adsorbent for the enrichment of neonicotinoid insecticide residues in natural surface water and fruit juice samples. High-performance liquid chromatography with UV/Visible detection was used for quantification and determination of neonicotinoid insecticide residues, including thiamethoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid. In d-µ-SPE process, the solid sorbent was dispersed into the aqueous sample solution and vortex agitation was performed to accelerate the extraction process. Finally, the solution was filtered from the solid sorbent with a membrane filter. The parameters affecting the extraction efficiency were optimized such as amount of sorbent, sample volume, salt addition, type and volume of extraction solvent, vortex time, and centrifugation time. Under optimum conditions, linear dynamic ranges were achieved between 0.5 and 1000 ng mL^{-1} with a correlation of determination (R^2) greater than 0.99. Limit of detection (LOD) ranged from 0.005 to 0.065 ng mL^{-1}, while limit of quantification (LOQ) ranged from 0.008 to 0.263 ng mL^{-1}. The enrichment factor (EF) ranged from 8 to 176. The applicability of this proposed method was successfully demonstrated for the analysis of trace target analytes in natural surface water and fruit juice samples.

Keyword : Montmorillonite, Dispersive micro-solid phase extraction, Surface water and fruit juice samples, Neonicotinoids

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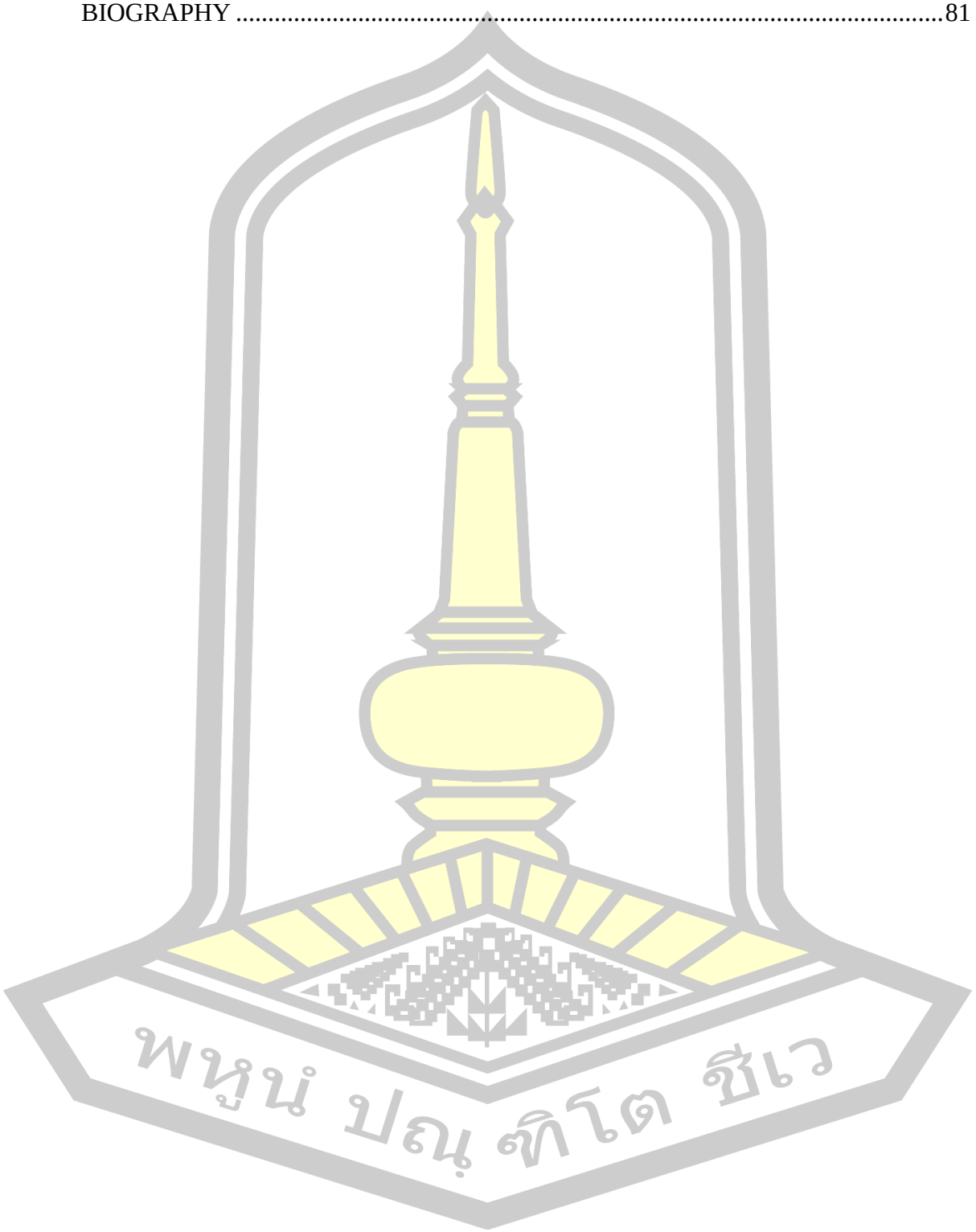

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CHAPTER 1

INTRODUCTION

1.1 Background and rational

Recently, the increasing public concerning about the health risk from pesticide residues in diet has deeply emphasized the importance on food quality and safety [1]. Neonicotinoids are a relatively new generation of pesticides introduced to the market since the launch of pyrethroids [1]. This group of insecticides includes nitro-substituted (dinotefuran, nitenpyram, thiamethoxam, imidacloprid and clothianidin) and cyano-substituted (acetamiprid and thiacloprid) compounds [2]. They are most commonly used on rice, maize, sunflowers, rapeseed, potatoes, sugar beets, vegetables, and fruits crops [3]. Neonicotinoid insecticides act as agonists at the insect nicotinic acetylcholine receptors (nAChRs), which plays an important role in synaptic transmission in the central nervous system [1]. The widespread use of neonicotinoid insecticides at various stages agricultural cultivation and during postharvest storage could give rise to serious risks for the health and safety of the consumers [4]. Consequently, restrictions in their agriculture uses and maximum residue limits (MRLs) in some food commodities have been established [5]. The MRLs for neonicotinoids in fruit, vegetable and cereals were between 0.1 and 1.0 mg kg^{-1} [6]. Thus, a sensitive and selective method for monitoring neonicotinoid residues at low concentration levels is required to secure food quality and to protect hazard for consumer.

High performance liquid chromatography (HPLC) coupled with various detection systems, including ultraviolet [7], diode array [8], fluorescence [9], and mass spectrometry [10,11], is preferred choice for neonicotinoid pesticides analysis [12], in order to obtain the concentration data at trace levels of contamination. Modern instrumental methods such as liquid chromatography coupled to mass spectrometry (LC-MS) have shown to be an excellent method for the quantitative and qualitative analysis of neonicotinoid insecticides in real samples [13], but it suffers from being a very expensive and complex instrument. However, gas chromatography

(GC) has also been reported for neonicotinoid determination [14] but requires some special condition to reduce thermal decomposition. Capillary electrophoresis (CE) has also become an attractive approach for the analysis of pesticide residues, but suffers from low sensitivity because of short optical path length of the capillary [15]. Due to their low concentrations and complex matrices in real samples, sample preparation is further step still required before instrumental analysis. Sample clean-up techniques are the most commonly employed, which comprise liquid-liquid extraction (LLE) [16], solid-phase extraction (SPE) [17], solid-phase microextraction (SPME) [18] and liquid-liquid microextraction (LLME) [19]. However, LLE suffers from the requirement of large amount of both samples and toxic organic solvents. SPE typically requires reduced amounts of organic solvents relative to LLE, but SPE sometimes suffers from analytes breakthrough when large sample volumes are analysed [6]. Two types of microextraction techniques have also been further developed, namely, solid phase microextraction (SPME) [20] and liquid phase microextraction (LPME) [21]. LPME is based on the use of very low volumes (at the level of microliter) of solvent and has its origin in the use of a drop of extraction solvent [22]. SPME integrates sampling, extraction, concentration and sample introduction into a single solvent-free step. Although, SPME and LPME eliminate and/or reduce the volume of consumed organic solvents but they are usually time-consuming processes [23]. In 2003, Anastassiades et al. reported a new approach to sample preparation named dispersive solid phase extraction (DSPE) [24]. In this technique, the solid sorbent is added directly to a sample solution without conditioning, so the clean-up procedure require only on shaking or vortex and centrifugation. DSPE cannot provide effective enough cleanup, generally for some complex matrices. More recently, a novel method called dispersive micro-solid phase extraction (d-μ-SPE) has been widely developed [24]. This technique is based on SPE methodology, but used a small amount of a solid sorbent (μg or mg range) is dispersed in the sample solution containing the target analytes to extract the target analytes. Compared to conventional DSPE, d-μ-SPE has the following advantages: simpler operation, less solvent consumption and shorter time requirement. In d-μ-SPE, nature and physicochemical properties of the solid sorbent are very important in order to achieve an accurate, sensitive and selective determination of target analytes.

Carbon nanotubes, graphene, and inorganic nanoparticles are some adsorbent materials applied for d-µ-SPE. To date, clay was investigated as sorbents which are potentially useful materials for the adsorption of environmental pollutants due to their unique polarity, pore-size distribution, and high surface areas [25]. There are three classes of clay including illite, kaonite and montmorillonite. Montmorillonite (MMT) is a class of natural clay that possesses a large surface area and high cation-exchange capacity. It has been demonstrated to serve as an effective sorbent [26]. In recent years, MMT have very successful to use as adsorbent for extraction techniques which are potentially useful materials for the adsorption of environmental pollutants [27, 28, 29]. MMT has been reported to be used as adsorbent for SPME but it is very difficult to manufacture each stand with exactly the same coating thickness [30].

The aim of this work to develop a simple d-µ-SPE for preconcentration of neonicotinoid insecticides in surface water and fruit juice samples combined with HPLC for determination of target analytes. In this research, MMT clay will be used as an efficient adsorbent in the d-µ-SPE of trace neonicotinoids. Application of such development is aimed for the determination of neonicotinoid residues in food and environmental samples.

1.2 Purposes of the research

1. To develop a simple d-µ-SPE method using MMT clay as efficient adsorbent for preconcentration of neonicotinoid insecticides.
2. To apply the proposed d-µ-SPE method for the determination of neonicotinoid insecticide residues in real samples.

1.3 Scope of research

1. Construction of calibration curves, detection limit, minimum detectable quantity and reproducibility.
2. The preconcentration method will be studied for determination of trace neonicotinoids in surface water and fruit juice samples.
3. The developed method will be applied to analysis of neonicotinoids residues in surface water and fruit juice samples.

1.4 Benefit of research

1. The optimized conditions for preconcentration method of neonicotinoids in real samples by d-µ-SPE.

2. The trace neonicotinoid contents in surface water and fruit juice samples will be obtained.

1.5 Definition of terms

1. Neonicotinoids are a relatively new class of insecticides chemically related to nicotine.
2. MMT is a kind of 2:1 type layered clay minerals and has been widely used in various branches of industry due to their high cation exchange capacity, swelling ability and high surface area.
3. Preconcentration aims to increase the concentration of the target analytes in a sample solution prior to instrumental analysis or detection. An operation (process) as the result of which microcomponents are transferred from the sample of larger mass into the sample of smaller mass, so that the concentration of the microcomponents is increased.
4. D-μ-SPE is a miniaturized extraction method based on dispersion of micro- or nanosorbents in sample solution containing of the target analytes. After extraction, the sorbent containing the target analytes is isolated by centrifugation or filtration. The target analytes can then be eluted or desorbed by an appropriate desorption solvent.
5. Detection limit or limit of detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance within a stated confidence limit.
6. The retention time is the elapsed time between the time of injection of a solute and the time of elution of the peak maximum of that soute.
7. Separation efficiency is the good signal of five neonicotinoids and the peaks obtained was not overlapped.
8. Preconcentration efficiency is the good percentage recovery of micro-solid phase extraction obtained.

CHAPTER 2

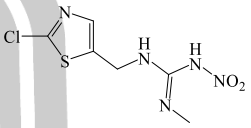
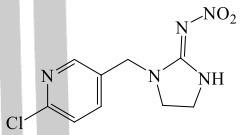
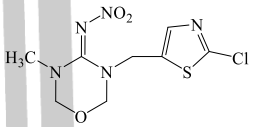
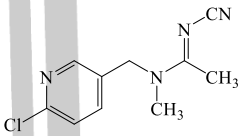
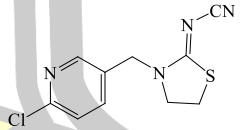
LITERATURE REVIEW

2.1 Neonicotinoid insecticides

Neonicotinoid (new nicotine-like substance) is a type of pesticide that is often used recently. It is named after a similar poisonous substance called nicotine, which is found in cigarettes. Neonicotinoid was developed around 1900 after the organophosphorous pesticides. The neonicotinoids: 1) are systemic pesticides 2) are persistent in the environment 3) have neurotoxity and there are concerns over its effect towards insects including honeybees, ecosystems, and people. The insecticidal property of neonicotinoids is due to overstimulation of nicotinic acetylcholine receptors (nAChRs) in the insect nervous system at the neuromuscular junction, resulting in paralysis and death [31]. Neonicotinoids can also be classified into *N*-nitroguanidines (imidacloprid, thiamethoxam, clothianidin and dinotefuran), nitromethylenes (nitenpyram), and *N*-cyano-amidines (acetamiprid and thiacloprid) [32]. Neonicotinoid is also known as systemic pesticides as it permeates into the plant because of its water solubility. A new type (phenylpyrazole) of systemic pesticides called fipronil is also being used frequently. It is used for eradicating fleas in pets, household insecticides, and pesticides. This also has neurotoxicity, and is gathering attention as one of the causes for honeybee losses. Furthermore, it is reported that neonicotinoids can be more persistent in the environment depending on the conditions, and it can stay in the soil for extended periods (over 1 year) [33].

The physicochemical properties of neonicotinoids are shown in Table 1. Molecular weights range from 160 to 292. Neonicotinoids have greater water solubility than other insecticides. Water solubility of neonicotinoids can also be altered by commercial formulations of the insecticides [34].

Table 1. Properties of the studied neonicotinoid insecticides from other chemical classes [35].

Neonicotinoid Insecticide	**Water Solubility (mg L^{-1}) @ 20 °C**	**Log Kow**	**Structure**
N-nitro-guanidines			
Clothianidin	340	0.91	
Imidacloprid	610	0.57	
Thiamethoxam	4100	-0.13	
N-cyano-amidines			
Acetamiprid	2950	0.80	
Thiacloprid	184	1.26	

2.2 D-μ-SPE

D-μ-SPE is a miniaturized extraction method based on dispersion of micro- or nanosorbents in sample solution and isolation of solid sorbent by centrifugation, filtration or using an external magnetic field. D-μ-SPE is based on the SPE methodology, but a small amount of solid sorbent (μg or mg range) is dispersed in a sample solution containing the target analytes without conditioning. Dispersion phenomenon enables the sorbent to interact rapidly and uniformly with all the target analytes which lead to enhance the precision of method and reduce the extraction time [23]. D-μ-SPE technique has been successfully applied to the separation and the preconcentration of pesticides in different types of solid sorbent. The following paragraphs and Table 2 summarize the reports related the d-μ-SPE technique for preconcentration of pesticides.

Jiménez-Soto *et al*. (2012) [36] reported dispersive micro solid phase extraction (DMSPE) using single-walled carbon nanohorns (SWNHs) as sorbent material for the extraction of triazines from waters followed by GC-MS. DMSPE method, a 10 mL of the standard solution (or sample solution) was mixed with 1 mL of the SWNHs, stirred at 1600 rpm for 2 min and 0.2 mL of methanol as elution solvent. The organic extraction was directly injected into GC-MS for identification and quantification of the analytes. The linearity was in the range from 0.05-200 μg L^{-1}. The LODs were in the range between 0.015 and 0.1 μg L^{-1}. The proposed method was applied to the identification and quantification of eleven triazines in the different water samples. The recoveries were between 63 and 100%.

Galán-Cano *et al*. (2013) [37] used of methylimidazolium-hexafluorophosphate functionalized silica is evaluated under d-μ-SPE approach for the extraction of organophosphate pesticides (OPs) from water samples follow by UPLC-DAD. D-μ-SPE conditions were: 8 mL of the sample or standard, 1.6 g of NaCl, 100 mg of SiO_2-MIM-PF_6 as sorbent, vortex for 1 min and centrifuged for 5 min at 5000 rpm. After centrifugation, the sample is removed. The sorbent was mixed with 500 μL of acetonitrile as elution solvent and filtered through a 0.45 μm nylon filter. The LODs were in the range between 0.3 to 0.6 μg L^{-1}. The enrichment factors (EFs) were range from 74-100. The recoveries were between 86 and 99%.

Li *et al*. (2015) [38] reported dynamic microwave assisted extraction coupled with d-μ-SPE using MIL-101 as sorbent for determination of triazine and phenylurea herbicides in soybean samples by HPLC-DAD. D-μ-SPE conditions were: 5 mg of MIL-101, ultrasonic bath for 30 s, shook for 5 min, and centrifuged for 5 min at 8390 g. The supernatant was removed and the residue was eluted by 2 mL of methanol under ultrasonication. After centrifugation, the obtained eluate was dried under nitrogen stream and the resulting residue was dissolved in 250 mL of methanol. The resulting solution was filtered with 0.22 μm PTFE filter. At the optimal conditions, LODs was in the range of 1.56-2.00 μg kg^{-1}. The recoveries were obtained in the range of 91.1-106.7%.

Chen *et al*. (2015) [39] reported a novel DMSPE clean-up method based on a PCX sorbent is established for the simultaneous determination of melamine and cyromazine residues in milk and milk powder by ultra high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS). Milk powder samples were first extracted with 1% formic acid in acetonitrile/water (1:1, v/v), and milk samples were cleaned up directly without any pre-extraction. Then, melamine and cyromazine in the extracts or milk were adsorbed to the PCX powder. Subsequently, the analytes in PCX sorbent were eluted with ammonium hydroxide/acetonitrile (2.5:97.5, v/v) through a simple unit device equipped with 1 mL syringe and 0.22 μm nylon syringe filter. Under the optimum conditions, the LODs were in the range of 0.05-0.06 μg kg^{-1} (milk) and 0.60 μg kg^{-1} (milk powder). The recoveries were between 78.1 and 107.1%.

Table 2. Literatures on d-μ-SPE for determination of pesticides.

Author (year)	**Analyte/ Sample**	**D-μ-SPE conditions**	**Detection**	**LODs**
Jiménez-Soto *et al.* (2012)	Triazines/ water	Sample volume: 10 mL Sorbent: carbon nanohorns (SWNHs) Elution solvent: 0.2 mL of methanol Agitation: stirred at 1600 rpm for 2 min	GC-MS	0.015-0.1 (μg L^{-1})
Galán-Cano *et al.* (2013)	organophosphate pesticides/ water	Sample volume: 8 mL Salt addition: 1.6 g of NaCl Sorbent: 100 mg of [SiO_2-MIM-PF_6] Elution solvent: 500 μL of acetonitrile Agitation: vortex for 1 min and centrifuged for 5 min at 5000 rpm	UPLC-DAD	0.3-0.6 (μg L^{-1})
Li *et al.* (2015)	Triazine and phenylurea herbicides/ soybean	Sample: 1 g Sorbent: 5 mg of MIL-101 Elution solvent: 2 mL of methanol Agitation: ultrasonic bath for 30 s, centrifuged for 5 min at 8390 g	HPLC-DAD	1.56-2 (μg kg^{-1})

Table 2. Literatures on d-μ-SPE for determination of pesticides (cont.).

Author (year)	**Analyte/ Sample**	**D-μ-SPE conditions**	**Detection**	**LODs**
Chen *et al*. (2015)	Melamine and cyromazine residues/ milk and milk powder	Sample: 2 mL (milk), 1.0 g (milk powder) Sorbent: 25 mg of PCX sorbent Elution solvent: ammonium hydroxide/ acetonitrile (2.5:97.5 v/v) Agitation: vortex for 30 s	UHPLC-HRMS	0.05-0.6 (μg kg^{-1})

2.3 Sample preparation and chromatographic determination of neonicotinoids

There are a number of reports for determination of neonicotinoids in samples by various sample preparation and preconcentration techniques in combination with instruments such as LC-MS, GC and HPLC. The following paragraphs and Table 3 summarize the reports related the chromatographic analysis of neonicotinoids.

Seccia *et al*. (2005) [40] reported SPE prior to LC-MS for determination of four neonicotinoid insecticides (acetamiprid, imidacloprid, thiacloprid and thiamethoxam) in drinking water by LC-electrospray ionization-mass spectrometry (LC-ESI-MS). The separation was performed using a LichroCart 125-4 Lichrosphere 100 (5 μm) column. The mobile phase was water and methanol, both acidified with 0.01% acetic acid under a gradient elution. The column temperature was 40 °C, the flow rate was 1.0 mL min^{-1}. Under the optimum condition, the linearity was in the range from 0 to 1 mg L^{-1}. The LODs for each of the four insecticides was 0.03 μg L^{-1}. The recoveries were between 95 and 104%.

Radišić *et al*. (2009) [16] developed matrix solid-phase dispersion (MSPD) for the determination of acephate, monocrotophos, carbendazim, acetamiprid, dimethoate, simazine, carbofuran, atrazine, diuron, DNOC (4,6-dinitro-*o*-cresol), malathion and tebufenozide in fruit juices by $LC\text{-}MS^2$. The separation was achieved using a reversed-phase Zorbax Eclipse® XDB-C18 column (4.6 mm × 75 mm and 3.5 μm particle size). Before the separation column, pre-column was installed (4.6 mm × 12.5 mm i.d. and 5 μm particle size). The mobile phase was water, methanol and acetic

acid under a gradient elution. The flow rate was 0.5 mL min^{-1}. Extracts were obtained by MSPD using diatomaceous earth as dispersant and dichloromethane as eluent. Under the optimum condition, the recoveries were in the range of 71-118%. The relative standard deviations, were in general between 5% and 15%. The LODs were in the range of 0.01-0.94 ng L^{-1}.

Dujaković *et al*. (2010) [41] presented SPE followed by LC-MS for the selected insecticides, fungicides and herbicides belong to seven chemical classes (organophosphates, neonicotinoids, carbamates, diacylhydrazines, benzimidazoles, triazines and phenylureas). The separation was achieved using reversed-phase Zorbax Eclipse® XDB-C18 column (75 mm × 4.6 mm i.d. and 3.5 µm particle size). The mobile phase was water, methanol and acetic acid under a gradient elution. The flow rate was 0.5 mL min^{-1}. SPE condition were: HLB cartridge (200 mg/6 mL) is preconditioned with 5 mL of methanol-dichloromethane mixture (1:1) followed by 10 mL of deionized water; 250 mL of the water sample, with the pH-value adjusted to 6, is applied to preconditioned HLB cartridge at a flow rate of 1 mL min^{-1}; the cartridge is dried under vacuum for 10 min; the cartridge is eluted with 10 mL of methanol-dichloromethane mixture (1:1); extract is evaporated to dryness and reconstituted with 1 mL of methanol; the final extract is filtered through 0.45µm PVDF filter into the auto sampler vial. Under the optimum condition, the recoveries were in the range of 72-129%. The LODs were in the range of 0.4-5.5 ng L^{-1}. The LOQs were in the range of 1.1-18.2 ng L^{-1}.

Xie *et al*. (2011) [13] reported multi-residue LC-MS/MS method for detection, confirmation and quantification of six neonicotinoid pesticides (dinotefuran, thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid) in agricultural samples. The separation was achieved using a ZORBAX Eclipse XDB-C_8 column (150 mm × 4.6 mm i.d., 5 µm) with a column oven temperature of 30 °C. The mobile phase was 0.1% formic acid and acetonitrile under a gradient elution. The flow rate was set at 0.4 mL min^{-1}. SPE was performed with the activated carbon and Oasis HLB SPE cartridges. In activated carbon cartridge, the cartridge was conditioned with 5 mL of acetonitrile, flow through under the action of gravity, 5 mL of acetonitrile as elution solvent and dryness on a water bath at 50 °C under a flow of nitrogen. The dried extract was reconstituted in 10 mL water, vortex mixed for 60 s. In the Oasis

HLB SPE cartridge, the cartridge was conditioned with 5 mL of methanol, 5 mL of ultra-pure water and flow through under the action of gravity. The cartridge was vacuum-dried for 3 min, methanol as elution solvent and dryness by vacuum rotary evaporation on a water bath at 50 °C. The residue was reconstituted in 2 mL of mobile phase and filtered through a 0.22 µm PTFE syringe filter. Under the optimum condition, the linearity was range from 4 to 100 µg mL^{-1}, the recoveries were in the range of 82.1-108.5%. The LOQs were in the range of 0.1 mg kg^{-1} for chestnut, shallot, ginger and 0.02 mg kg^{-1} for tea sample.

Cunha and Fernandes (2011) [42] developed QuEChERS method combined with DLLME procedure for multiclass determination of pesticides in maize, emerged by GC-MS. Chromatographic separation was performed using a DB-5MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) with helium carrier gas. The oven temperature was programmed from 80 °C to 280 °C and the total analytical time was 26 min. QuEChERS method was as follow: 2.5 g of thoroughly homogenized sample was put into a 50 mL polypropylene centrifugation tube, then 50 µL of TPP solution, 10 mL of deionized water and 10 mL of acetonitrile were added and the tube was sealed. The tube was vortexed and put it on a wrist action shaker for 30 min before adding 4 g of anhydrous $MgSO_4$ and 1 g of NaCl. The tube was sealed and shaken vigorously by hand for 1 min. The floating phase was further centrifuged at 5000 rpm for 4 min. Then a DLLME procedure was performed: transfer 1 mL of the acetonitrile extract to a 4 mL vial tube and add 50 µL of isotopically labeled internal standards (ISTD) solution, add 100 µL of carbon tetrachloride, transfer rapidly the mixture to a 25 mL screw cap plastic tube with conical bottom containing 4 mL of deionized water, seal the tube and shake gently by hand for 30 s, After centrifugation for 1 min (5000 rpm), transfer 100 µL of the settled volume into a vial and inject 1 µL of the extract in to the GC-MS system. The recoveries were between 58 to 117%. The LODs were in the range between 9 and 52 µg kg^{-1}.

Wang *et al*. (2012) [14] developed dispersive solid-phase extraction (DSPE) and DLLME for determination of seven neonicotinoid insecticide residues (nitenpyram, dinotefuran, clothianidin, thiamethoxam, acetamiprid, imidacloprid and thiacloprid) in grains by HPLC. The separation was performed using C_{18} column (Agilent TC-C_{18}, 4.6 × 250 mm i.d. × 5 µm). The temperature of the column was kept

at 25 °C. The mobile phase was acetonitrile and 0.3% (v/v) of formic acid in water system (20:80, v/v). The flow rate was set at 1.0 mL min^{-1}. The detection was performed using diode array detector (DAD) set at 260 nm. For DSPE, 10 mL centrifuge tube containing 125 mg PSA, 125 mg C_{18} and 25 mg graphitized carbon black was used as the extraction device, vortex for 2 min and centrifuged at 4000 rpm for 5 min. DLLME was used in this clean-up procedure, a 50 mL centrifuge tube with conical bottom was used as the extraction device containing 10 mL water, 0.8 g of NaCl and 2.0 mL of $CHCl_3$ and CH_2Cl_2 (1:1, v/v) (as extraction solvent), vortex for 30 s and centrifugation at 4000 rpm for 5 min. The $CHCl_3$ and CH_2Cl_2 phase was sediment at the bottom and then evaporated, dryness by a gentle nitrogen stream with a water bath at 50 °C. Finally, the residue was reconstituted in 1 mL acetonitrile:water (20:80; v/v) and filtered with a 0.45 µm organic filter. Under the optimum condition, the linearity was in the range of 0.02 to 4.5 µg mL^{-1}. The recoveries were between 76 to 123%. The LODs were in the range between 0.002 to 0.005 mg kg^{-1}.

Zhang *et al*. (2012) [4] developed DLLME coupled with sweeping in micellar electrokinetic chromatography (MEKC) for determination of neonicotinoid insecticides (thiacloprid, acetamiprid, imidaclothiz and imidacloprid) in cucumber samples. Chromatographic separation was performed using an uncoated fused-silica capillary of 50 cm (40 cm × 75 µm i.d.). DLLME condition was as follow: 0.8 mL of acetonitrile (as dispersive solvent) were mixed with 100.0 µL of $CHCl_3$ (as extraction solvent), vortex for 1 min and centrifuged for 5 min at 3500 rpm. The sedimented phase (about 90 µL) was completely transferred to another 1.0 mL conical bottom vial, evaporated to dryness under a mild nitrogen stream, and finally reconstituted with 20.0 µL 150 mmol L^{-1} H_3BO_3 (pH 4.7). Under optimum conditions, the EFs were in the range from 4000 to 10,000. The linearity was range from 2.7 to 200 ng g^{-1} for thiacloprid, acetamiprid and imidacloprid, and 4.0 to 200 ng g^{-1} for imidaclothiz. The LODs were in the range from 0.8 to 1.2 ng g^{-1}.

Kapoor *et al.* (2013) [43] reported QuEChERS method for determination of imidacloprid residues in fruits, fruit juices, baby foods, vegetables, and cereals by HPLC-PDA. The separation was performed using a reversed-phase, C-18 ODS analytical column (75 × 4.6 mm inner diameter, 3.5 µm particle size), with a precolumn of the same phase, mobile phase of acetonitrile and water (20:80, v/v) was

set at flow rate 1.0 mL min^{-1} and the injection volume was 50 µL. The detection was performed using PDA at set 270 nm. For QuEChERS, samples (10 g) of each commodity were mixed with 10 mL acetonitrile and 4 g of anhydrous $MgSO_4$ in a centrifuge tube, shaken for 10 min at 50 rpm and centrifuged for 10 min at 10,000 rpm. Supernatant was collected and evaporated to dryness under a slow stream of nitrogen at 40 °C. Dried extracts were reconstituted with 1 mL of acetonitrile. A further 1 mL of extract was cleaned with the mixture of 50 mg PSA, 150 mg anhydrous $MgSO_4$, and 10 mg activated charcoal. The extract was shaken for 10 min at 50 rpm. Under the optimum condition, the LODs were in the range between 0.004-0.01 mg kg^{-1}.

Vichapong *et al*. (2013) [12] developed vortex-assisted surfactant-enhanced-emulsification liquid-liquid microextraction with solidification of floating organic droplet (VSLLME-SFO) for preconcentration of neonicotinoid pesticides (acetamiprid, clotianidin, nitenpyram, imidacloprid, and thiamethoxam) by HPLC. The separation was performed using an Atlantis dC18 column (4.6 mm i.d. ×150 mm, 5 µm particle diameter) and mobile phase of 25% (v/v) acetonitrile in water flow rate was set at 1.0 mL min^{-1}. The detection was performed using PDA at 254 nm. For VSLLME-SFO, a 10.00 mL of the standard solution (or sample solution) was mixed with Na_2SO_4 (0.3%, w/v) and added of 1.0 mol L^{-1} HCl (400 µL), 0.050 mol L^{-1} SDS (50 µL) as emulsifier, vortex for 1 min, octanol (150 µL) as extraction solvent. The extraction was performed by centrifuged at 5000 rpm for 10 min. The upper phase comprising the organic extractant (~150 to 200 µL) was directly injected into HPLC. Under optimum conditions, the linearity was in the range from 0.0005-5 mg mL^{-1}. The EFs were ranged from 20-100. The LODs were in the range from 0.1-0.5 µg L^{-1}.

Giroud *et al*. (2013) [44] developed QuEChERS method using acetonitrile-based extraction for determination pyrethroids and neonicotinoids in beebread by UHPLC-MS/MS. The separation was achieved using a Kinetex Phenyl-Hexyl (100 × 2.1 mm, 2.6 µm) column. The mobile phase was mixture of acetic acid/ ammonium acetate and MeOH under a gradient elution. The flow rate was set at 0.4 mL min^{-1}, the oven temperature was 60 °C and the injection volume was 2 µL. QuEChERS condition were: 2 g of beebread was weighed in a 50 mL polypropylene centrifuge tube, 5 mL of pure water, 5 mL of heptane and 10 mL of acetonitrile with

TEA 2%, added 200 µL of internal standards at 100 µg L^{-1} and the mixture was vortex for 15 s. A packet of acetate buffer was added and the tube was immediately manually shaken for 10 s to prevent the coagulation of $MgSO_4$ and swirled on a vortex mixer for 20 s. The mixture was then centrifuged at 5000 g for 2 min and 8 mL of the supernatant (acetonitrile phase) was transferred to a 15 mL tube and incubated for 15 h at -18 °C. Afterwards, a 6 mL volume of the extract was transferred to a 15 mL centrifuge tube containing 150 mg of PSA and 900 mg of $MgSO_4$ then swirled on a vortex mixer for 10 s and centrifuged at 5000 rpm for 2 min. The solvent was evaporated to dryness under a gentle stream of N_2 at 40 °C. The dry residue was dissolved in 400 µL MeOH. Lastly, 40 µL were added to 160 µL of pure water. Under optimum conditions, the recoveries varied from 53% to 119%. LODs were in the range from 0.05 to 5.7 ng g^{-1}.

Costa *et al*. (2014) [45] compared QuEChERS (original, acetate and citrate) sample preparation methods for the analysis of pesticide residues in canned and fresh peach by GC-MS. Chromatographic separation was performed using an Rtx®-5MS capillary column (30 m × 0.25 mm × 0.25 µm) and helium carrier gas at a flow rate of 1.5 mL min^{-1}. The injection temperature was set at 280 °C and the interface temperature maintained at 300 and 280 °C. The oven temperature was programmed from 70 to 300 °C. Original QuEChERS condition were: 10 g of sample, 10 mL acetonitrile, 4 g $MgSO_4$ and 1 g NaCl, vortexed for 1 min, centrifuged for 5 min at 5000 rpm. The upper phase comprising acetonitrile was transferred to a 15 mL polypropylene tube and then cleaned up using dispersive solid-phase extraction (d-SPE) with 25 mg PSA and 150 mg $MgSO_4$. The tube was vortexed for 1 min and centrifuged for 6 min at 1000 rpm. Acetate QuEChERS condition were: 15 g of sample, 15 mL 1% CH_3COOH in acetonitrile, manually homogenised for 1 min, 6 g $MgSO_4$ and 1.5 g CH_3COONa, centrifuged for 1 min at 5000 rpm. The upper phase comprising acetonitrile was cleaned up with 50 mg PSA and 150 mg $MgSO_4$ and then mixed for 1 min and centrifuged for 5 min at 5000 rpm. Citrate QuEChERS condition were: 10 g of sample, 10 mL acetonitrile, shaken for 1 min, 1 g $C_6H_5Na_3O_7 \cdot 2H_2O$ and 0.5 g $C_6H_6Na_2O_7 \cdot 1.5H_2O$, 1 g NaCl and 4 g $MgSO_4$ were added and the tube was shaken for 1 min and centrifuged for 2 min at 4000 rpm. The upper phase comprising acetonitrile was cleaned up with 25 mg PSA and 150 mg $MgSO_4$. After that, the

mixture was manually homogenised for 20 s and centrifuged for 2 min at 4000 rpm. Under optimum conditions, LODs were in the range from 1 to 10 µg kg^{-1}. The recoveries varied from 68 to 124%.

López-Fernández *et al.* (2015) [46] developed SPE for preconcentration and determination of neonicotinoid insecticide residues in dietary bee pollen by HPLC-MS/MS. The separation was performed using a Gecko 2000 column heater linked to a PC running Xcallibur version 5.0. A Hypersil GOLD™ column (100 mm × 4.6-mm inner diameter; 5 µm) with a Hypersil GOLD™ drop-in guard cartridge (10 mm × 4.6-mm inner diameter, 5 µm). The mobile phase was acetonitrile and water under gradient elution from 10 to 95% acetonitrile at a flow rate of 1.0 mL min^{-1} and total run time of 30 min. For SPE, Supelclean™ Envi-Carb II/PSA cartridges was employed as the extraction vessel, acetonitrile with 1% acetic acid as the extraction solvent and primary-secondary amine (PSA) as dispersive solvent. Under optimum conditions, the LODs were in the range between 0.2 and 2.2 µg kg^{-1}. The recoveries were between 81% and 99%.

Vichapong *et al.* (2015) [47] presented the in-coupled syringe assisted octanol-water partition microextraction for preconcentration and determination of neonicotinoid insecticide residues (imidacloprid, acetamiprid, clothianidin, thiacloprid, thiamethoxam, dinotefuran, and nitenpyram) in honey by HPLC. The separation was performed using an Atlantis dC18 (4.6 x 150 mm, 50 µm) column with isocratic elution using 25% (v/v) acetonitrile in water. The injection volume was 20 µL. The detection was obtained using photodiode array detector (PDA) set at 254 nm. The total analytical time was 18 min. In-coupled syringe assisted octanol-water partition microextraction condition were: 10.00 mL of aqueous sample, 10% (w/v) Na_2SO_4, 1-octanol (100 µL) as an extraction solvent, shooting 4 times and extraction time 2 min. Under the optimum condition, the linearity were in the range of 0.1-3000 ng mL^{-1}. LODs were in the range from 0.25-0.50 ng mL^{-1}. The recoveries were between 96.93 and 107.70%.

Rodríguez-Cabo *et al.* (2016) [5] used LC-MS for the determination of five neonicotinoid insecticides (thiamethoxam, imidacloprid, acetamiprid, clothianidin and IMI-d_4) in red and white wines. Chromatographic separation was achieved on a Zorbax Eclipse SDB C_{18} column (100 mm × 2 mm, 3.5 µm) connected to a C_{18} guard

cartridge (4 mm × 2 mm). The temperature of the column was kept at 40 °C. The mobile phase was acetonitrile and water under gradient elution from 2 to 100% acetonitrile. For SPE, 200 mg OASIS HLB cartridges was used as an extraction vessel. A mixture of acetonitrile and ethanol: water (12:88) as the extraction solvent and 2 mL of acetonitrile as the dispersive solvent were used. Under optimum conditions, the linearity was obtained in the range of 1-500 ng mL^{-1}. The recoveries were between 77 and 119%.

Vichapong *et al*. (2016) [48] developed ionic liquid-based cold-induced aggregation microextraction for determination of neonicotinoid insecticide residues (clothianidin, imidacloprid, dinotefuran and thiacloprid) in honey by HPLC with photodiode array detection. The separation was performed using a LiChrospher® 100 RP-18 endcapped (4.6×150 mm, 5.0 µm) column. The mobile phase was 25% (*v/v*) acetonitrile in water. Ionic liquid-based cold-induced aggregation microextraction procedure were 200 µL room temperature ionic liquids $[C_4MIM][PF_6]$ containing 0.05 mol L^{-1} SDS, 0.75 g sodium carbonate, vortex agitation speed of 1800 rpm for 30 s and centrifugation at 3500 rpm for 10 min. Under optimum conditions, the high enrichment factor of 200 was obtained. The linearity was in the range of 0.25-500 mg L^{-1}. The recoveries were between 86 and 100%. LODs were in the range of 0.25-0.50 µg L^{-1}.

Shi *et al*. (2017) [49] used graphene (CH_3NH-G) as SPE sorbent to cleanup the acetonitrile extract of sunflower seeds for the determination of neonicotinoid insecticides by UPLC-MS/MS. Chromatographic separation was performed on an ACQUITY UPLC® BEH C_{18} column (2.1×100 mm i.d., 1.7 µm) preceded by a BEH C_{18}VanGuard™ pre-column (2.1×5 mm i.d., 1.7 µm). The mobile phase was mixture of 0.1% formic acid and acetonitrile under a gradient elution 20-90% acetonitrile. The flow rate was 0.4 mL min^{-1}. The temperature of the column was kept at 30 °C and 15 °C. The injection volume was 10 µL. For extraction, 2.0 g of sample was accurately weighed into a 50 mL plastic centrifuge tube. 5 mL of water, 20 mL of acetonitrile and 3.0 g of sodium chloride were added into the centrifuge tube, and vortex for 3 min and centrifuged for 5 min at 4000 rpm. The supernatant was used in the following cleanup. Sample cleanup was performed with SPE cartridge packed with 20 mg (dry weight) of CH_3NH-G. Before the sample cleanup procedure, the SPE

cartridge was conditioned with 3 mL of methanol, 3 mL of acetonitrile, 3 mL of acetone and 9 mL of water. Then 1 mL of the above clarified supernatant sample solution was loaded onto the SPE cartridge and left to flow through the cartridge under the action of gravity. Finally, the analytes were eluted out using 1 mL of acetonitrile. Under optimum conditions, the linearity was obtained in the ranges of 0.025-100 ng g^{-1} for thiamethoxam and imidacloprid, 0.001-20 ng g^{-1} for acetamiprid and 0.05-100 ng g^{-1} for thiacloprid, respectively. LODs were between 0.05 and 5.7 ng kg^{-1}. The recoveries were between 74.3-119.1%.

Arnnok *et al.* (2017) [50] reported dispersive solid phase extraction (DSPE) using polyaniline (PANI)-modified zeolite NaY as a sorbent for multi-class pesticides in food and environmental samples by HPLC-PDA. The separation was performed using a Symmetry Shield RP18 (4.6×150 mm, 5 μm) analytical column. The mobile phase was acetonitrile and water under gradient elution from 20-80% of acetonitrile. The flow rate was 1.0 mL min^{-1}. For DSPE, 150 mg of PANI-coated zeolite NaY as a sorbent and 3-mL polypropylene syringe column as the extraction vessel were used. The mixture of 0.01 mol L^{-1} sodium hydroxide in 90% acetonitrile was used as elution solvent. Under optimum conditions, the LODs were in the range of 0.001-0.1 mg L^{-1}. The linearity was obtained in the range of 0-25 mg L^{-1}. The recoveries were between 64 and 128% with RSDs less than 12%.

Table 3. Literatures on chromatographic determination of neonicotinoid insecticides using various preconcentration techniques.

Author (year)	**Analyte/ Sample**	**Chromatographic conditions/ Preconcentration technique**
Seccia *et al*. (2005)	Acetamiprid, imidacloprid, thiacloprid, and thiamethoxam /Drinking water	LC-ESI-MS; Column: LichroCart 125-4 Lichrosphere 100 (5 μm) Column temperature: 40 °C Mobile phase: water and methanol/0.01% acetic acid Flow rate: 1.0 mL min^{-1} SPE; Extraction device: LiChrolut EN cartridges (200 mg) Extraction solvent: 3 mL Ethyl acetate/ methanol 50:50 (v/v) Disperser solvent: acetic acid 0.01%, of water (60%) and methanol (40%)
Radišić *et al*. (2009)	Acephate, monocrotophos, carbendazim, acetamiprid, dimethoate, simazine, carbofuran, atrazine, diuron, DNOC,malathion and tebufenozide/ Fruit juices	LC-MS; Column: reversed-phase Zorbax Eclipse® XDB-C18 column (4.6 mm × 75 mm and 3.5 μm particle size) connected to pre-column (4.6 mm × 12.5 mm i.d. and 5 μm particle size) Mobile phase: water, methanol and acetic acid Flow rate: 0.5 mL min^{-1} MSPD; Extraction device: 6 ml SPE tube Sorbent: diatomaceous earth Extraction solvent: 10 ml of dichloromethane

Table 3. Literatures on chromatographic determination of neonicotinoid insecticides using various preconcentration techniques (cont.).

Author (year)	**Analyte/ Sample**	**Chromatographic conditions/ Preconcentration technique**
Dujaković *et al*. (2010)	Organophosphates, neonicotinoids, carbamates, diacylhydrazines, benzimidazoles, triazines, and phenylureas/ Surface and ground waters	LC-MS; Column: reversed-phase Zorbax Eclipse® XDB-C18 column (75 mm × 4.6 mm i.d. and 3.5 μm particle size) Mobile phase: water, methanol and acetic acid Flow rate: 0.5 mL min^{-1} SPE; Extraction device: HLB cartridge (200 mg/6 mL) Extraction solvent: 5 mL of methanol-dichloromethane mixture (1:1) Sample volumes: 250 mL pH-value: 6 Disperser solvent: 1 mL of methanol
Xie *et al*. (2011)	Dinotefuran, thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid / Gricultural samples	LC-MS/MS; Column: ZORBAX Eclipse XDB-C_8 column (150 mm × 4.6 mm i.d., 5 μm) Column temperature: 30 °C Mobile phase: 0.1% formic acid and acetonitrile Flow rate: 0.4 mL min^{-1} SPE; Extraction device: HLB SPE cartridges Extraction solvent: acetonitrile Disperser solvent: ethyl acetate

Table 3. Literatures on chromatographic determination of neonicotinoid insecticides using various preconcentration techniques (cont.).

Author (year)	**Analyte/ Sample**	**Chromatographic conditions/ Preconcentration technique**
Cunha and Fernandes (2011)	Pesticides/Maize	GC-MS; Column: DB-5MS column (30 m × 0.25 mm I.D. × 0.25 µm film thickness) Injector temperature: 280 °C Oven programme: 80-300 °C Carrier gas: Helium Run time: 26 min QuEChERS; Extraction device: 50 mL polypropylene centrifugation tube Extraction: 50 µL of TPP solution, 10 mL of deionized water and 10 mL of acetonitrile DLLME; Extraction: carbon tetrachloride Disperser solvent: QuEChERS extract
Wang *et al*. (2012)	Nitenpyram, dinotefuran, clothianidin, thiamethoxam, acetamiprid, imidacloprid and thiacloprid /Grains	HPLC-DAD; Column: Agilent-C18 column (4.6 x 250 mm i.d., 5.0 µm) Column temperature: 25 °C Mobile phase: acetonitrile: 0.3% (v/v) of formic acid in water system (20:80; v/v) Flow rate: 1.0 mL min^{-1} DSPE; Extraction device: 10 mL centrifuge tube Extraction: 125 mg PSA, 125 mg C_{18} and 25 mg graphitized carbon black

Table 3. Literatures on chromatographic determination of neonicotinoid insecticides using various preconcentration techniques (cont.).

Author (year)	**Analyte/ Sample**	**Chromatographic conditions/ Preconcentration technique**
		DLLME; Extraction solvent: $CHCl_3$ and CH_2Cl_2 (1:1, v/v)
Zhang *et al*. (2012)	Thiacloprid, acetamiprid, imidaclothiz and imidacloprid / Cucumber samples	MEKC; Column: uncoated fused-silica capillary of 50 cm (40 cm ×75 μm i.d) Detector: DAD DLLME; Extraction device: 20 ml centrifuge tube Extraction solvent: $CHCl_3$ Disperser solvent: acetonitrile
Kapoor *et al*. (2013)	Imidacloprid / fruits, fruit juices, baby foods, vegetables, and cereals	HPLC-PDA; Column: reversed-phased, C-18 ODS analytical column (75 × 4.6 mm inner diameter, 3.5 μm particle size), with a precolumn Mobile phase: acetonitrile and water (20:80, v/v) Flow rate: 1.0 mL min^{-1} Injection volume: 50 μL Detection: PDA at set 270 nm. QuEChERS; Extraction: 10 ml acetonitrile and 4 g of anhydrous $MgSO_4$ Disperser: 50 mg PSA, 150 mg anhydrous $MgSO_4$, and 10 mg activated charcoal

Table 3. Literatures on chromatographic determination of neonicotinoid insecticides using various preconcentration techniques (cont.).

Author (year)	**Analyte/ Sample**	**Chromatographic conditions/ Preconcentration technique**
Vichapong *et al*. (2013)	Acetamiprid, clothianidin, nitenpyram, imidacloprid, and thiamethoxam / Surface water samples and fruit juice samples	HPLC-PDA; Column: Atlantis dC18 column (4.6 mm i.d.×150 mm, 5 µm particle diameter) Mobile phase: 25% (v/v) acetonitrile in water Flow rate: 1.0 mL min^{-1} VSLLME-SFO; Emulsifier solvent: 0.050 mol L^{-1} Sodium dodecyl sulfate (SDS) Extraction solvent: 150 µl of octanol
Giroud *et al*. (2013)	Pyrethroids and neonicotinoids/ Beebread	UHPLC-MS/MS; Column: Kinetex Phenyl-Hexyl (100 × 2.1 mm, 2.6 µm) Mobile phase: acetic acid/ ammonium acetate and MeOH Oven temperature: 60 °C Flow rate: 0.4 mL min^{-1} QuEChERS; Extraction device: 50 mL polypropylene centrifuge tube Extraction solvent: acetonitrile Disperser solvent: MeOH
Costa *et al*. (2014)	Trichlorphon, dimethoate, atrazine-d5, fenitrothion, malathion, fenthion,	GC-MS; Column: Rtx®-5MS capillary column (30 m × 0.25 mm × 0.25 µm) Injector temperature: 280 °C Oven programme: 70-300 °C Interface temperature: 280 and 300 °C

Table 3. Literatures on chromatographic determination of neonicotinoid insecticides using various preconcentration techniques (cont.).

Author (year)	**Analyte/ Sample**	**Chromatographic conditions/ Preconcentration technique**
	thiamethoxam, ciproconazole, tebuconazole, difenoconazole, and azoxystrobin/ Canned and fresh peach	Carrier gas: Helium Flow rate: 1.5 mL min^{-1} QuEChERS; Extraction solvent: acetonitrile, CH_3COOH and citrate Disperser solvent: 25 mg PSA and 150 mg $MgSO_4$
López-Fernández *et al*. (2015)	Neonicotinoid insecticide residues /dietary bee	HPLC-MS/MS; Column: Symmetry Shield RP18 (4.6×150 mm, 5 µm) Mobile phase: acetonitrile and water under gradient elution from 20-80% acetonitrile Flow rate was 1.0 mL min^{-1} SPE; Extraction device: Supelclean™ Envi-Carb II/PSA cartridges Extraction solvent: acetonitrile with 1% acetic acid Disperser solvent: PSA
Vichapong *et al*. (2015)	Imidacloprid, acetamiprid, clothianidin, thiacloprid, thiamethoxam, dinotefuran, and nitenpyram/ Honey	HPLC-PDA; Column: Atlantis dC18 (4.6 x 150 mm, 5.0 µm) Mobile phase: 25% (v/v) acetonitrile in water Detector: PDA In-coupled syringe assisted octanol-water partition microextraction; Extraction solvent: 1-octanol (100 µL)

Table 3. Literatures on chromatographic determination of neonicotinoid insecticides using various preconcentration techniques (cont.).

Author (year)	**Analyte/ Sample**	**Chromatographic conditions/ Preconcentration technique**
Rodríguez-Cabo *et al*. (2016)	Thiamethoxam, imidacloprid, acetamiprid, clothianidin and IMI-d_4/ Red and white wines	LC-MS/MS; Column: Zorbax Eclipse SDB C_{18} column (100 mm × 2 mm, 3.5 µm) connected to a C_{18} guard cartridge (4 mm × 2 mm) Column temperature: 40 °C Mobile phase: acetonitrile and water SPE; Extraction device: SPE cartridges Extraction solvent: acetonitrile and ethanol: water (12:88) Disperser solvent: 2 mL of acetonitrile
Vichapong *et al*. (2016)	Clothianidin, imidacloprid, dinotefuran and thiacloprid / Honey	HPLC-PDA; Column: LiChrospher® 100 RP-18 endcapped (4.6×150 mm, 5.0 µm) Mobile phase: 25% (*v/v*) acetonitrile in water Flow rate: 1 mL min^{-1} ionic liquid-based cold-induced aggregation microextraction; Emulsifier solvent: SDS Extraction solvent: $[C_4MIM][PF_6]$
Shi *et al*. (2017)	Neonicotinoid insecticides/ Sunflower seeds	UPLC-MS/MS; Column: ACQUITY UPLC® BEH C_{18} column (2.1×100 mm i.d., 1.7 µm) preceded by a BEH C_{18}VanGuard™ pre-column (2.1×5 mm i.d., 1.7 µm) Column temperature: 30 and 15 °C

Table 3. Literatures on chromatographic determination of neonicotinoid insecticides using various preconcentration techniques (cont.).

Author (year)	**Analyte/ Sample**	**Chromatographic conditions/ Preconcentration technique**
		Mobile phase: 0.1% formic acid and acetonitrile SPE; Extraction device: SPE cartridge Sorbent: graphene (CH_3NH-G) Elution solvent: 1 mL of acetonitrile
Arnnok *et al.* (2017)	Twenty pesticides/ food and environmental samples	HPLC-PDA; Column: Symmetry Shield RP18 (4.6×150 mm, 5 μm) Mobile phase: acetonitrile and water under gradient elution from 20-80% acetonitrile Flow rate was 1.0 mL min^{-1}. DSPE; Extraction device: 3-mL polyropylene syringe column Sorbent: PANI-coated zeolite NaY Elution solvent: 0.01 mol L^{-1} sodium hydroxide in 90% acetonitrile

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 4.

Table 4. Chemical and reagents used in this work.

No	Chemical	Formula	Company	Country
1	Thiametoxam	$C_8H_{10}ClN_5O_3S$	Dr. Ehren-storfer	Germany
2	Clothianidin	$C_6H_8ClN_5O_2S$	Dr. Ehren-storfer	Germany
3	Imidacloprid	$C_9H_{10}ClN_5O_2$	Dr. Ehren-storfer	Germany
4	Acetamiprid	$C_{10}H_{11}ClN_4$	Dr. Ehren-storfer	Germany
5	Thiacloprid	$C_{10}H_9ClN_4S$	Sigma-Aldrich	Germany
6	Acetonitrile	CH_3CN	Merck	Germany
7	Methanol	CH_3OH	Merck	Germany
8	Sodium sulphate	Na_2SO_4	Ajax Finechem	New Zealand
9	Sodium chloride	NaCl	Ajax Finechem	New Zealand
10	Sodium acetate	CH_3COONa	CarloErba	France
11	Sodium carbonate	Na_2CO_3	Ajax Finechem	New Zealand
12	Sodium nitrite	$NaNO_2$	Ajax Finechem	New Zealand
13	Montmorillonite (cloisite 10A)	-	Rheologie additive	Germany
14	Deionized water	-	Millipore Water	USA

3.2 Instrumentation

The HPLC system consists of a Waters 1525 Binary HPLC pump (USA), and a Waters 2489 UV/Visible detector operated at 254 nm. Table 1 show the chromatographic condition used for separation of neonicotinoid.

Table 5. The chromatographic conditions used for separation of neonicotinoids.

Parameter	**Conditions**
Detector	UV/Visible detector (λ_{max} 254 nm)
Column	A LiChrospher® 100 RP-18 endcapped (4.6x150 mm, 5.0 µm) (Merck, Germany)
Mobile phase	Isocratic elution with acetonitrile: water (25:75, v/v)
Flow rate	1.0 mL min^{-1}
Injection volume	20 µL

3.3 Surface water and fruit juice samples

The natural surface water samples were taken from the different areas located near rice fields in Maha Sarakham province, Northeast Thailand.

Fruit samples including longan, watermelon and grape were randomly purchased from local markets and supermarkets in Maha Sarakham province in Northeast Thailand.

3.4 Experimental

3.4.1 Preparation of standard neonicotinoid insecticides.

Stock standard solution (1000 µg mL^{-1}) of all neonicotinoids were prepared by dissolving 0.01 g of each standard and adjusted to the mark (10.00 mL) with methanol. The stock standard solutions were stored in dark bottles, kept at 4 °C and used for not longer than six months. Working solutions were prepared by appropriated dilution of the stock solution with water.

3.4.2 Linearity, detection limits and repeatability

The mixture of neonicotinoids including thiamethoxam, imidacloprid, clothianidin, acetamiprid and thiacloprid, were prepared in methanol and working solution were diluted in water before injected into HPLC with the optimum

conditions. The relationship between the concentration and peak area were plotted. The linearity range was evaluated by the calibration curve (y=ax+b) and the R^2 value.

The sensitivity of the method was evaluated by limit of detection (LOD), using a signal to noise (S/N) ratio of 3:1 and limit of quantitation (LOQ) using a signal to noise (S/N) ratio of 10:1.

The repeatability was evaluated in terms of percentage relative standard deviations.

3.5 Sample preconcentration

The precentage recoveries of d-µ-SPE method was observed. The condition giving the highest percentage recoveries were selected to analysis of samples.

3.5.1 D-µ-SPE procedure

The determination of neonicotinoid insecticides was carried out by the d-µ-SPE procedure using MMT Cloisite 10A clay sorbent followed by HPLC-UV/Visible. Figure 1 shows schematic diagram of the proposed extraction method.

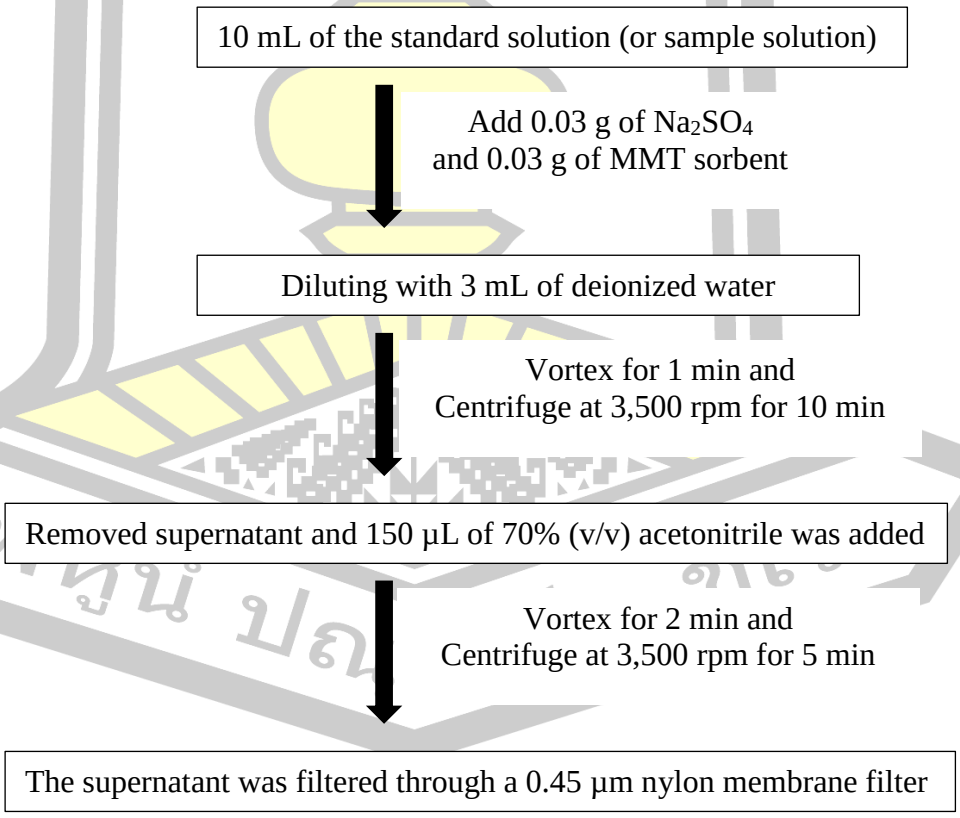


Figure 1. Schematic diagram of the D-µ-SPE

The experimental parameters affected the extraction efficiency were studied.

3.5.1.1 Effect of amount of MMT sorbent

MMT has previously been demonstrated by some researcher to be effective as a sorbent for the removal of pollutants from wastewaters. MMT provides a high specific surface area due to its non-smooth, porous structure, resulting in higher loading capacity and thermal stability [26]. In this study, MMT was employed as the sorbent for d-µ-SPE procedure. It was studied in the range of 5-100 mg.

3.5.1.2 Effect of sample volume

The effect of sample volume on the extraction of neonicotinoids were studied in the range of 1-15 mL.

3.5.1.3 Effect of type and amount of the salt addition

The salt addition can decrease the solubility of the target analytes and either reduce the solubility of organic solvent in water or reinforce partitioning of the target analytes into organic phase [51]. Therefore, different kinds of salts including NaCl, $NaNO_2$ Na_2SO_4, CH_3COONa, and Na_2CO_3 were investigated with the amount of each salt being kept constant at 0.1 g.

The concentration of selected salt was studied in the range of 0.01-0.1 g.

3.5.1.4 Effect of type and volume of the desorption solvent

The desorption solvent (acetonitrile) was tested in the range of 20-100% (v/v). The volume of desorption solvent was varied in the range of 100-250 µL.

3.5.1.5 Effect of vortex time

The vortex was used for agitation during the extraction step to enhance the extraction efficiency as it provided vigorous stirring of sample and the sorbent. The effect of vortex time was evaluated in the range from 30-240 s.

3.5.1.6 Effect of centrifugation time

Centrifugation time is another important step in d-µ-SPE for achieving phase separation. Therefore, the effect of centrifugation time was studied in the range of 5-20 min at 3500 rpm.

3.5.1.7 Reusability of adsorbent

The reusability of the MMT sorbent for extraction of neonicotinoid insecticides was investigated 4 cycles. In order to ensure elimination of residues on

the MMT before extraction using the proposed method, the adsorbent was washed with 5 mL of methanol.

3.5.2 Preparation of samples

Three natural surface water samples were taken from different areas located near rice fields in Maha Sarakham province (Northeastern of Thailand) province and were filtered through a Whatman No. 42 filter paper. Then, the filtrate was filtered through a 0.45 µm nylon membrane filter.

Fruit samples (watermelon, grape, and longan) were purchased from different markets in Maha Sarakham province (Northeastern of Thailand). Before analysis, a 30.0 mL aliquot of fruit juice was centrifuged at 3500 rpm for 15 min and was filtered through Whatman No. 42 filter paper. Then, the solution was filtered through a 0.45 mm nylon membrane filter before extraction by the proposed method.

3.6 Analysis of neonicotinoids in natural surface water and fruit juice samples

Neonicotinoid in natural surface water and fruit juice samples were analyzed by using the optimum conditions.

3.7 Data analysis

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

$$\overline{X} = \frac{X_1 + X_2 + X_3 \ldots}{n}$$

2. 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:

$$SD = \sqrt{\frac{(X_1 - \overline{X})^2 + (X_2 - \overline{X})^2 + (X_3 - \overline{X})^2 + \ldots}{n-1}}$$

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

$$\%RSD = \frac{100xSD}{\overline{X}}$$

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

$$\%\text{Recovery} = \frac{(C_{ex} - C_0)}{C_{spiked}} \text{x}100$$

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

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Determination of neonicotinoid insecticide residues in surface water and fruit juice samples using dispersive micro-solid phase extraction (d-µ-SPE)

This chapter present the results obtained section describes a development of the d-µ-SPE prior to analysis by HPLC. The studied neonicotinoids include thiametoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid, were studied. The second section presents the analytical performance of the proposed method. Finally, apply the proposed method for the analysis of neonicotinoid insecticide residues in surface water and fruit juice samples. The results were discussed.

4.1.1 Optimization of the d-µ-SPE procedure

In order to obtain the high extraction efficiency of the proposed d-µ-SPE method, several parameters were investigated, including amount of sorbent, sample volume, salt addition, type and volume of extraction solvent, vortex time and the centrifugation extraction time. To identify the optimal extraction conditions, the peak area of the analytes was applied to evaluate extraction efficiency under various conditions. A one-at-a time procedure was followed to understand the individual influence of each parameter. The optimization was carried out on an aqueous solution containing 500 ng mL^{-1} of each analyte. All the experiments were performed in triplicate and the mean of the results were used for optimization.

4.1.1.1 Effect of amount of MMT sorbent

It is a primarily consideration to study an appropriate amount of sorbent on the extraction efficiency of the proposed extraction method. In the present work, the sorbent was directly added into the sample, and dispersed with the aid of vortex agitation [31]. MMT was used as a sorbent in this work, the adsorption of the pesticides in the interlayers of MMT, when occurring by substitution of water molecules hydrating the exchangeable cation, is favoured when the exchangeable cation has a small ionic potential. This is because the substitution of coordinated water molecules by pesticide molecules is facilitated and the opening of the silicate

layers is easier [52]. Different amount of MMT Cloisite 10A clay sorbent in the range of 5-100 mg were investigated, while keeping the other parameters constant. As can be seen in Figure 2, the extraction efficiency increased as the amount of MMT sorbent up to 30 mg. This may be due to the number of active sites and high surface area of MMT that increase the extraction efficiency. Efficiency then decreased for values higher than 30 mg. The reason may be that excessively strong adsorption leads to difficulty during the desorption process. With higher amounts of MMT, the extraction efficiency did not present a marked enhancement. Therefore, 30 mg of MMT sorbent was sufficient for effective extraction and was used for further experiments.

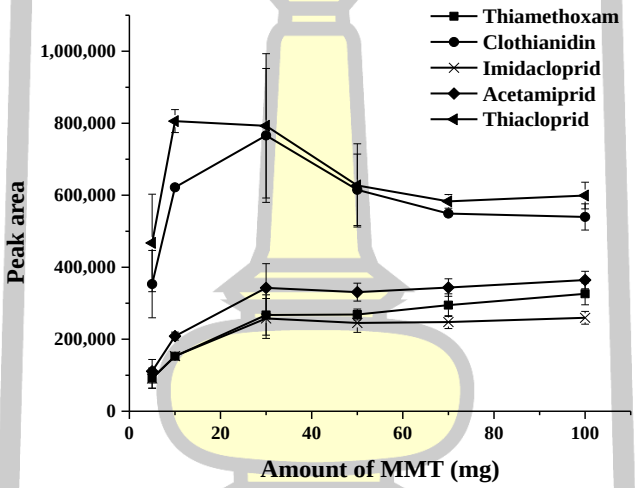


Figure 2. Effect of sorbent amount. Conditions: 100% (v/v) of acetonitrile (300 µL) as the desorption solvent; vortex time 30 s and centrifugation time at 3500 rpm, 10 min (10 mL, 500 ng mL-1 of each neonicotinoids).

4.1.1.2 Effect of sample volume

Sample volume is one important parameter which influences the extraction efficiency. An increase in the ratio of volume of aqueous phase to desorbing phase lead to a significant increase in the extraction efficiency. On the other hand, an increase in the sample volume may result in a decrease in extraction efficiency in a given time. The effect of sample volume on the extraction efficiency were studied in the range of 1-15 mL. The results shown in Figure 3, the highest peak areas were obtained using the sample volume of 13 mL. Therefore, 13 mL of sample volume was selected for further study.

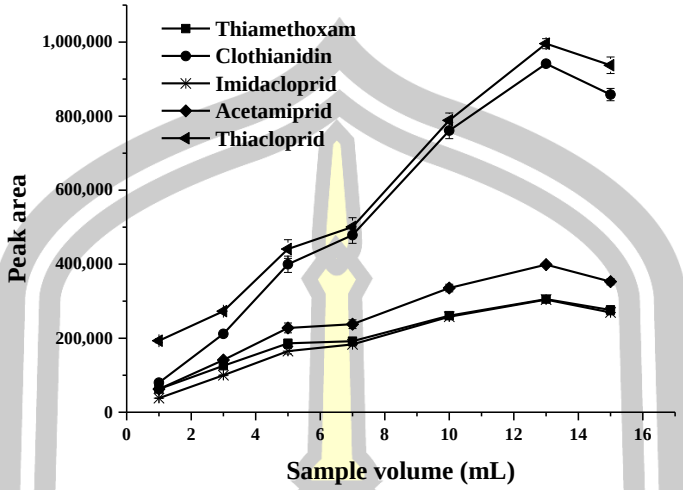


Figure 3. Effect of the sample volume. Conditions: 30 mg of MMT sorbent; 100% (v/v) of acetonitrile (300 µL) as the desorption solvent; vortex time 30 s and centrifugation time at 3500 rpm, 10 min (500 ng mL^{-1} of each neonicotinoids).

4.1.1.3. Salt addition

Salt addition plays various roles in the microextraction processes, depending on the analyte and sorbent natures in terms of hydrophobicity and hydrophilicity and their interaction [53]. It can reduce the solubility of the target analytes and either reduce the solubility of organic solvent in water or reinforce partitioning of the target analytes into organic phase [51]. Therefore, several salts ($NaNO_2$, NaCl, Na_2SO_4, CH_3COONa, Na_2CO_3) were examined with the amount of each salt being kept constant at 0.1 g and the results were compared with that obtained from the process without salt addition (Figure 4 and Figure 5). It was found that Na_2SO_4 provided higher extraction efficiency in terms of peak area of neonicotinoids and better chromatogram than other salts. Therefore, Na_2SO_4 was selected for the further studied. On the other hand, with increase of salt concentration and ionic strength, salting in effect can be a dominant phenomenon [54]. Whereby, polar molecules may take part in electrostatic interactions with the salt ions in solution; thus, the mass transfer is diminished [55]. To probe the effect of salinity on extraction performance, experiments were accomplished by adding different amounts of Na_2SO_4 in the range of 0.01-0.1 g. As shown in Figure 6, the peak area increased to a maximum when

amount of Na_2SO_4 increased to 0.03 g, then decreased with further increasing of Na_2SO_4 amount. Therefore, Na_2SO_4 0.03 g was used in this study.

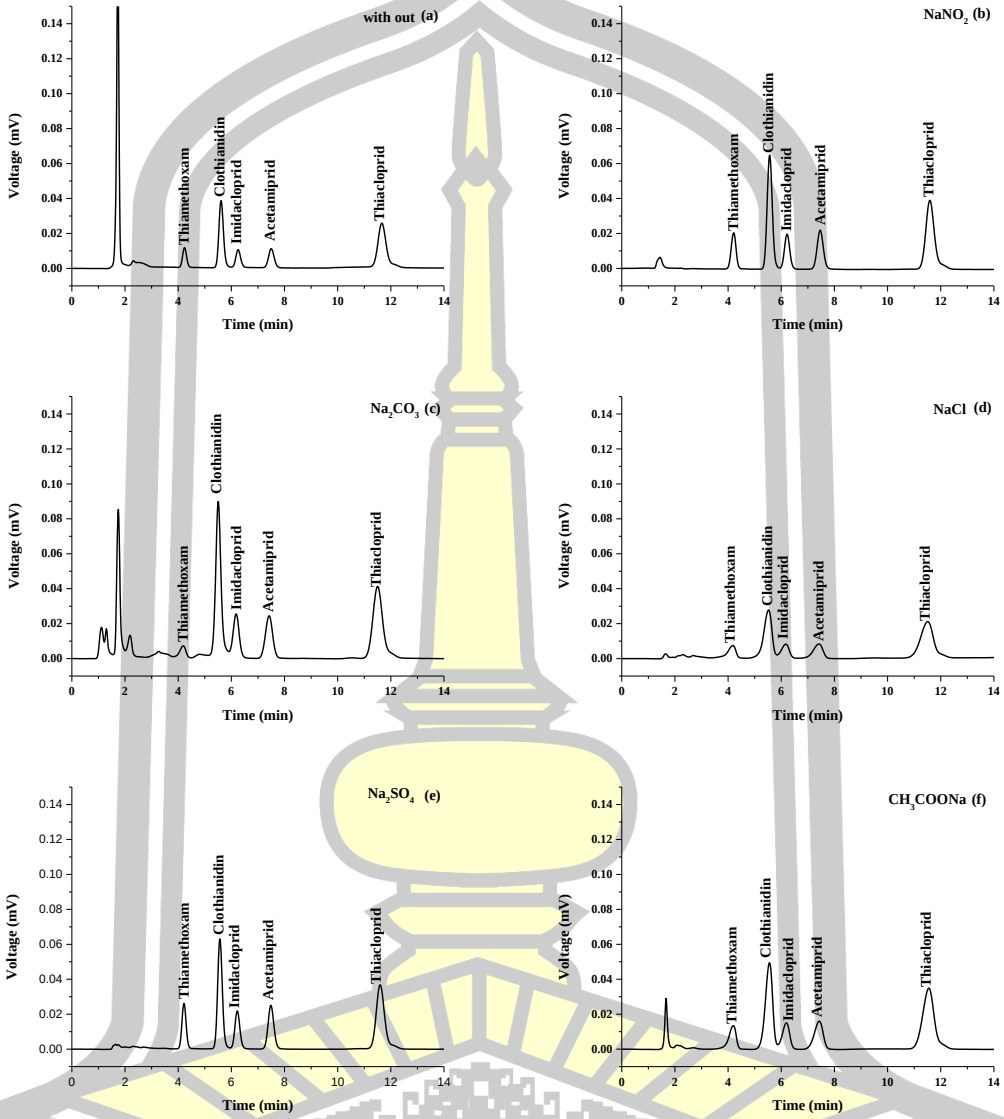


Figure 4. Chromatograms of salt addition obtained by (a) without salt, (b) $NaNO_2$, (c) Na_2CO_3, (d) NaCl, (e) Na_2SO_4, and (f) CH_3COONa: concentration of all standards was 500 ng mL^{-1}.

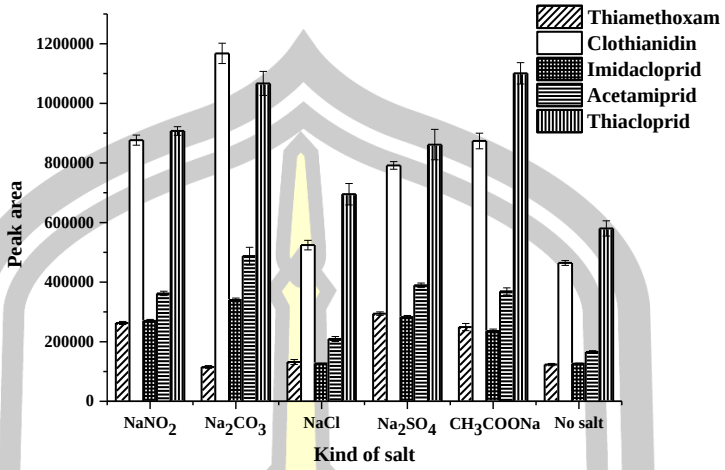


Figure 5. Effect of the salt addition. Conditions: 30 mg of MMT sorbent; 100% (v/v) of acetonitrile (300 µL) as the desorption solvent; vortex time 30 s and centrifugation time at 3500 rpm, 10 min (13 mL, 500 ng mL^{-1} of each neonicotinoids).

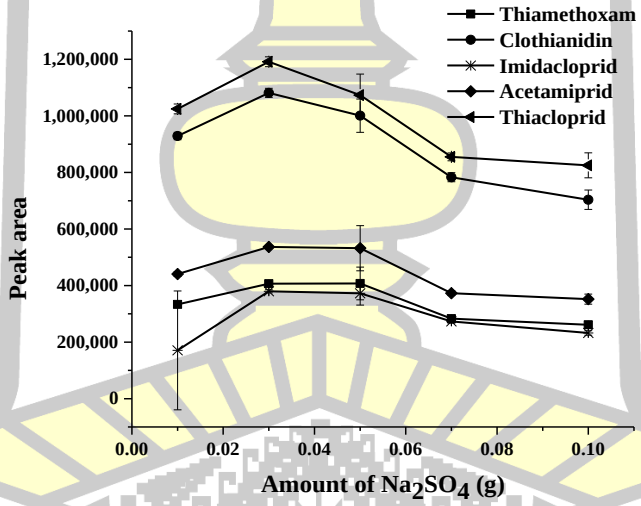


Figure 6. Effect of Na_2SO_4 concentration. Conditions: 30 mg of MMT sorbent; 100% (v/v) of acetonitrile (300 µL) as the desorption solvent; vortex time 30 s and centrifugation time at 3500 rpm, 10 min (13 mL, 500 ng mL^{-1} of each neonicotinoids).

4.1.1.4. Effect of type and volume of the desorption solvent

To select a proper desorption solvent for d-µ-SPE method, two points should be considered, one is that the solvent preferably be miscible with water and another is that it should have good chromatographic behavior. The appropriate desorption

solvent tested for desorption of neonicotinoid insecticides were 20-100% (v/v) of acetonitrile (500 µL). As can be seen in Figure 7, the highest extraction recovery for neonicotinoids was obtained with 70% (v/v) of acetonitrile as the desorption solvent. Therefore, 70% (v/v) acetonitrile was chosen as an desorption solvent for next experiments.

A series of experiments was investigated to detect the optimum volume of the desorption solvent as 70% (v/v) acetonitrile. The different volumes of desorption solvent ranging from 100 to 250 µL were investigated. As can be seen from Figure 8, the highest extraction efficiency in term of peak areas of the target analytes was obtained with 150 µL of desorption solvent and remained almost decreased afterward. Therefore, 150 µL of 70% (v/v) acetonitrile was selected for further optimization of the experiments.

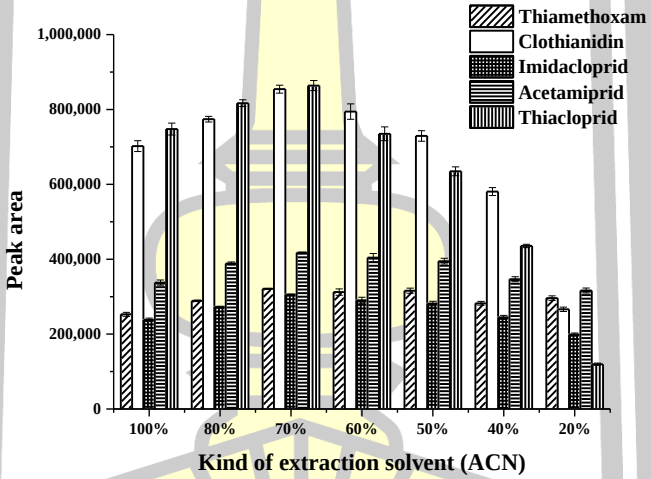


Figure 7. Effect of the different desorption solvent. Conditions: 30 mg of MMT sorbent; 0.03 g of Na_2SO_4; vortex time 30 s and centrifugation time at 3500 rpm, 10 min (13 mL, 500 ng mL^{-1} of each neonicotinoids).

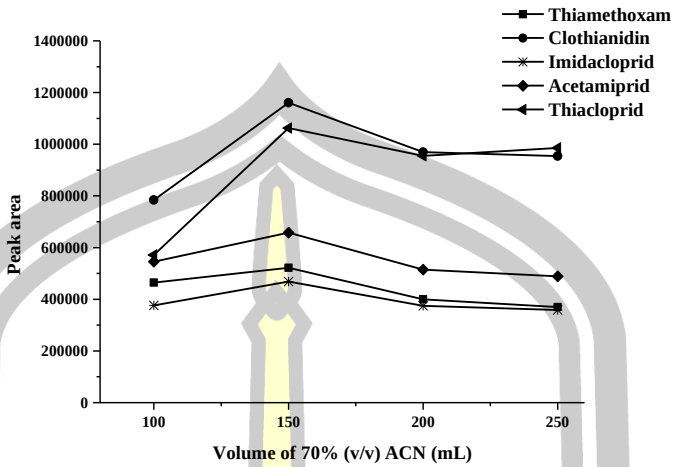


Figure 8. Effect of the volume of 70% (v/v) ACN. Conditions: 30 mg of MMT sorbent; 0.03 g of Na_2SO_4; 70% (v/v) of acetonitrile as the desorption solvent; vortex time 30 s and centrifugation time at 3500 rpm, 10 min (13 mL, 500 ng mL^{-1} of each neonicotinoids).

4.1.1.5. Effect of vortex time

The vortex was selected for agitation during the extraction step to enhance the extraction efficiency as it provided vigorous stirring of sample and the sorbent [56]. The effect of vortex time was investigated in the range of 30-240 s. As show in Figure 9, it was found that a fast accomplishment of the equilibrium was recieved within 60 s of loading time and 120 s of eluting time. Beyond this point, the extraction efficiency in term of peak area was decreased. Therefore, 60 s of loading time and 120 s of eluting time was used for d-μ-SPE process.

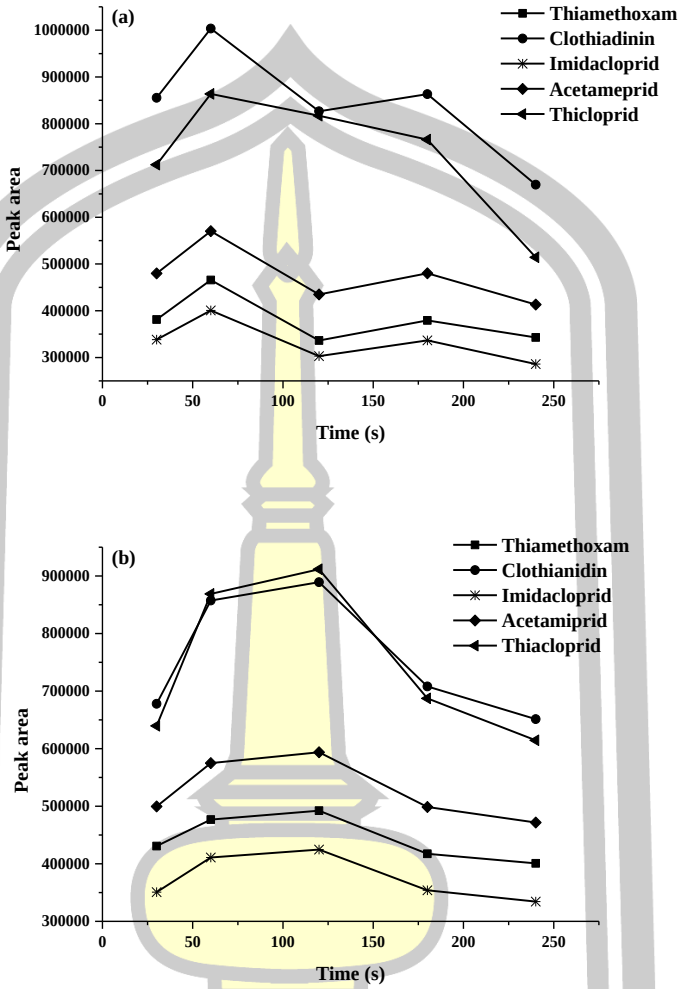


Figure 9. Effect of vortex time (a) loading time and (b) eluting time. Conditions: 30 mg of MMT sorbent; 0.03 g of Na_2SO_4; 70% (v/v) of acetonitrile (150 µL) as the desorption solvent and centrifugation time at 3500 rpm, 10 min (13 mL, 500 ng mL^{-1} of each neonicotinoids).

4.1.1.6 Effect of centrifugation time

Other parameters that may affect extraction efficiency of the developed method are centrifugation time, optimum centrifugation time is still required because the process of mass transfer between two phases in extraction procedure should be time-dependent. The effect of centrifugation time was investigated in the range of 5-15 min for loading time and 5-20 min for eluting time while centrifugation time at 3500 rpm was kept. As show in Figure 10, the extraction performance of neonicotinoids slightly increased with time up to 10 min and 5 min for loding time

and eluting time, respectively. Therefore, 10 min of loading time and 5 min of eluting time was used for d-μ-SPE process.

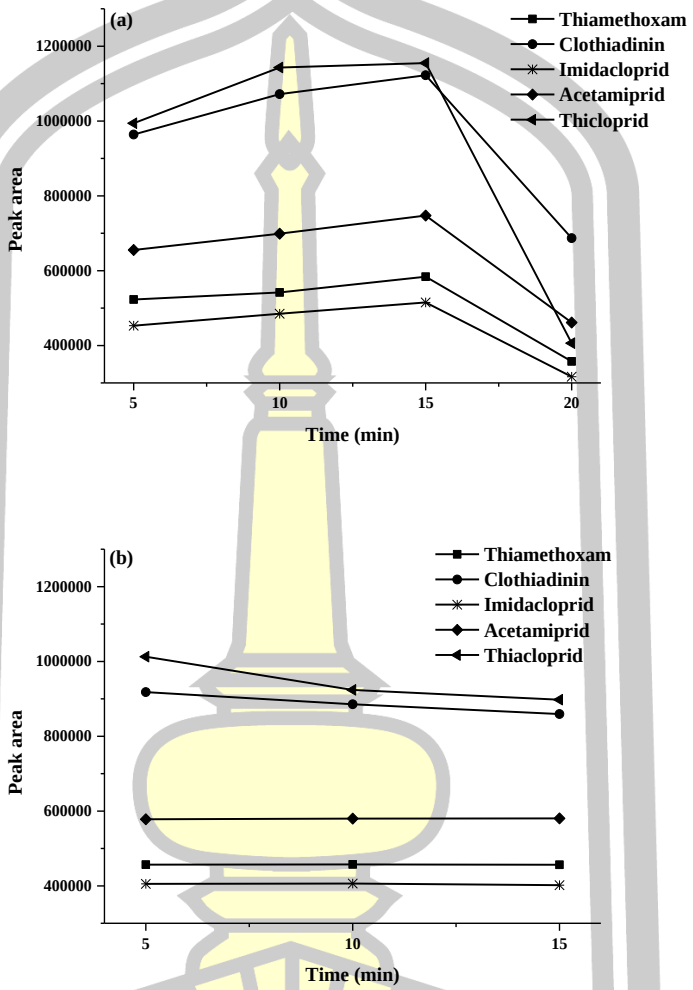


Figure 10. Effect of centrifugation time (a) loading time and (b) eluting time. Conditions: 30 mg of MMT sorbent; 0.03 g of Na_2SO_4; 70% (v/v) of acetonitrile (150 μL) as the desorption solvent; vortextime 60 s (loading time) and 120 s (eluting time) (13 mL, 500 ng mL^{-1} of each neonicotinoids).

The optimum conditions of the d-μ-SPE for analysis of neonicotinoid insecticides are summarized in Table 6.

Table 6. The optimum conditions of d-µ-SPE for analysis of neonicotinoid insecticides.

Extraction process	Extraction condition
Sorbent amount	30 mg
Sample volume	13 mL
Salt addition	Na_2SO_4 0.03 g
Desorption solvent	150 µL of 70% (v/v) acetonitrile
Vortex time	60 s of loading time
	120 s of eluting time
Centrifugation time	3500 rpm 10 min of loading time
	3500 rpm 5 min of eluting time

4.1.1.7 Reusability of adsorbent

The reusability of the MMT sorbent for extraction of neonicotinoid insecticides was studied. In order to ensure elimination of residues on the MMT before extraction using the proposed method, the adsorbent was washed with 5 mL of methanol. It was found that the extraction efficiency in terms of peak area decreased after 4 cycles (data not show). This indicates that MMT possesses excellent reusability as an efficient adsorbent.

4.1.2 Analytical performance of the developed d-µ-SPE procedure

To study the analytical performance of the proposed method, the analytical parameters included linear ranges, correlation coefficients (R^2), precision, limit of detection (LOD), limit of quantitation (LOQ) and enrichment factors (EFs) were investigated under the selected condition. The experimental results are summarized in Table 7. All analytes exhibited good linearity in the ranges of 0.5-1000 ng mL^{-1} with a correlation of determination (R^2) greater than 0.99. LOD and LOQ were evaluated by the analytes concentration giving the signal to noise ratio (S/N) of 3 and 10, respectively. LODs of the studied analytes were from 0.005-0.065 ng mL^{-1}, while LOQs ranges between 0.008-0.263 ng mL^{-1}. To test the reproducibility of the proposed method, precision in terms of intra-day and inter-days were studied by replicate injection of the standard mixture of 50 ng mL^{-1} each in a day (n = 3) and several days (n = 3×3). Good precisions were obtained with relative standard

deviations (RSDs) less than 0.46% for retention time and 7.17% for peak area. The enrichment factor (EF), defined as the concentration ratio of the analytes in the settled phase (Cset) and in the aqueous sample (Co), ranged from 8-176 folds. Chromatograms of the studied neonicotinoid insecticides obtained from direct HPLC and preconcentrated by the proposed d-μ-SPE are shown in Figure 11.

Table 7. Analytical performance of the proposed method.

Insecticide	Linear equation	Linearity (ng mL^{-1})	Coefficient of determination (R^2)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Intra-day (n=3) RSD		Inter-days (n=3 days x 3) RSD		EF
						t_R	Peak area	t_R	Peak area	
Thiamethoxam	y = 1894030x + 256 (y = 256063x + 6414)[a]	0.5-1000	0.9951	0.009 (0.021)	0.031	0.46	2.75	0.21	4.58	73
Clothianidin	y = 3990993x - 3842 (y = 232549x -655)	0.5-1000	0.9972	0.013 (0.006)	0.042	0.30	2.97	0.16	5.71	176
Imidecloprid	y = 1385642x - 6607 (y = 154139x + 4041)	0.5-1000	0.9940	0.006 (0.028)	0.010	0.30	2.58	0.16	5.10	8
Acetamiprid	y = 945451x - 4414 (y = 116891x + 16181)	0.5-1000	0.9963	0.005 (0.135)	0.008	0.28	3.24	0.44	4.99	8
Thiacloprid	y = 2249620x + 43266 (y = 137214 + 9689)	0.5-1000	0.9980	0.065 (0.067)	0.263	0.23	3.26	0.14	7.17	16

LOD: limit of detection, LOQ: limit of quantitation, RSD: relative standard deviation, EF: enrichment factor

[a]Values reported in parentheses are obtained from the standard neonicotinoids without preconcentration (direct injection).

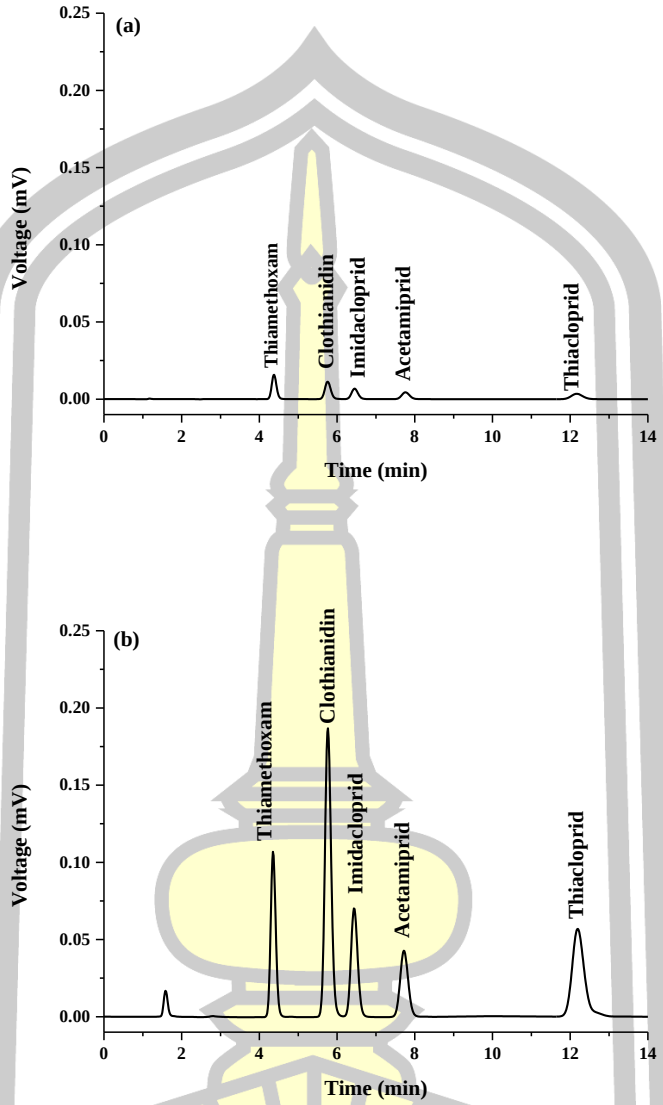


Figure 11. Chromatograms of standard neonicotinoids obtained (a) without preconcentration, and (b) with preconcentration using the proposed method (concentration of all standards was 500 ng mL^{-1}).

4.1.3 Application to real samples

The proposed d-μ-SPE method was utilized for the simultaneous determination of neonicotinoid insecticides in natural surface water and fruit juice samples from local markets and supermarket in Maha Sarakham province. The results are summarized in Table 8. It was found that no residue of the studied neonicotinoids was observed in the natural surface water and longan samples. For watermelon and

grape studied, all the studied neonicotinoid insecticides were detected in the range of 0.005-0.27 ng mL^{-1}. However, the amounts of neonicotinoid pesticides found in the fruit samples were lower than the maximum residue limits (MRLs) established by EU (acetamiprid, 0.5 mg kg^{-1} in grape; imidacloprid, in grape; clothianidin, 0.9 mg kg^{-1} in grape).

In order to validate the accuracy of the established method, the fruit juice samples were spiked with neonicotinoid insecticides at concentration levels of 250 and 500 ng mL^{-1}. As indicated in Table 9, the recoveries of the studied neonicotinoid insecticides in fruit and natural surface water samples were obtained in the range of 12-138% and 62-151%, respectively. The chromatograms of fruit and natural surface water samples are shown in Figure 12 to Figure 20.

Table 8. Analysis of neonicotinoid insecticides in real samples.

Sample		**Amount found ± SD, mg L^{-1} (n=2)**				
		Thiametoxam	**Clothianidin**	**Imidacloprid**	**Acetamiprid**	**Thiacloprid**
Watermelon	Local Market	0.03±0.007	0.05±0.003	-	-	0.007±0.01
	Super Market	0.11±0.08	0.01±0.007	0.07±0.1	0.27±0.1	0.005±0.01
Grape	Local Market	0.04±0.01	0.04±0.002	-	0.06±0.03	0.01±0.01
	Super Market	0.26±0.02	0.03±0.003	-	-	-
Longan	Local Market	-	-	-	-	-
	Super Market	-	-	-	-	-
Water sample	Water I	-	-	-	-	-
	Water II	-	-	-	-	-
	Water III	-	-	-	-	-

- ; not detection

Table 9. The recoveries of neonicotinoid insecticides in spiked using d-µ-SPE.

Sample	Thiametoxam				Clothianidin				Imidacloprid				Acetamiprid				Thiacloprid			
	RR (%)		RSD (%)		RR (%)		RSD (%)		RR (%)		RSD (%)		RR (%)		RSD (%)		RR (%)		RSD (%)	
	250	500	250	500	250	500	250	500	250	500	250	500	250	500	250	500	250	500	250	500
Watermelon																				
Local market	78	72	7.0	1.1	65	58	5.7	0.1	49	43	2.8	1.6	77	69	6.4	1.1	68	66	3.0	0.9
Supermarket	88	80	4.4	1.8	68	63	4.6	0.7	51	49	6.2	1.8	84	74	0.5	4.2	79	67	0.2	1.1
Grape																				
Local market	138	102	6.2	7.0	62	53	1.1	5.5	53	44	3.6	6.8	30	40	0.5	4.6	59	56	0.3	5.5
Supermarket	92	65	2.2	1.1	55	59	2.5	0.5	41	40	7.5	3.3	53	67	7.0	1.2	68	70	1.2	1.1
Logan																				
Local market	78	66	6.4	2.0	48	55	2.3	3.1	59	55	0.7	5.4	79	69	4.3	2.2	82	74	4.9	3.0
Supermarket	59	79	0.9	5.8	57	64	0.6	5.4	13	32	5.1	4.8	44	12	0.5	5.6	69	77	1.1	4.3
Water samples																				
Water l	128	110	2.8	1.5	115	99	2.7	1.0	87	76	3.4	0.2	133	113	2.5	0.3	151	123	2.8	1.4
Water ll	113	93	3.8	7.4	101	80	4.4	7.0	77	62	3.9	6.3	97	126	8.7	9.2	94	119	7.5	8.7
Water lll	125	105	8.1	0.4	101	97	8.5	0.1	82	68	7.8	0.6	126	109	9.2	0.1	119	126	8.7	0.1

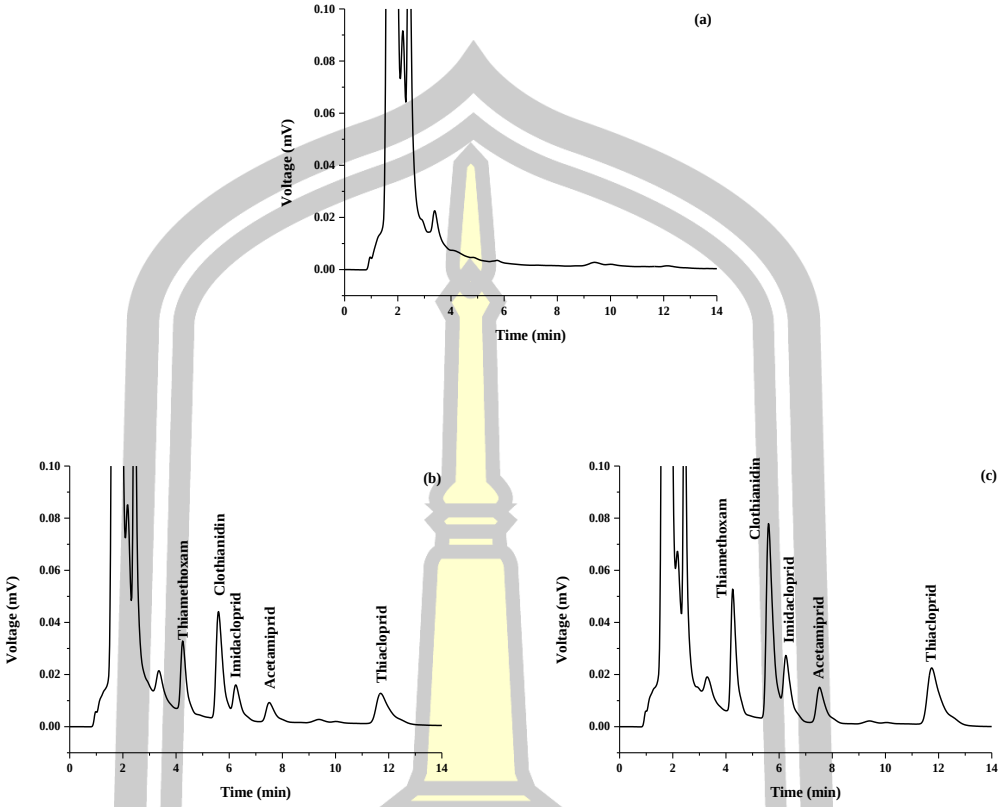


Figure 12. Chromatograms of (a) longan sample (local market), (b) longan sample spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) longan sample spiked with 500 ng mL^{-1} of each neonicotinoid.

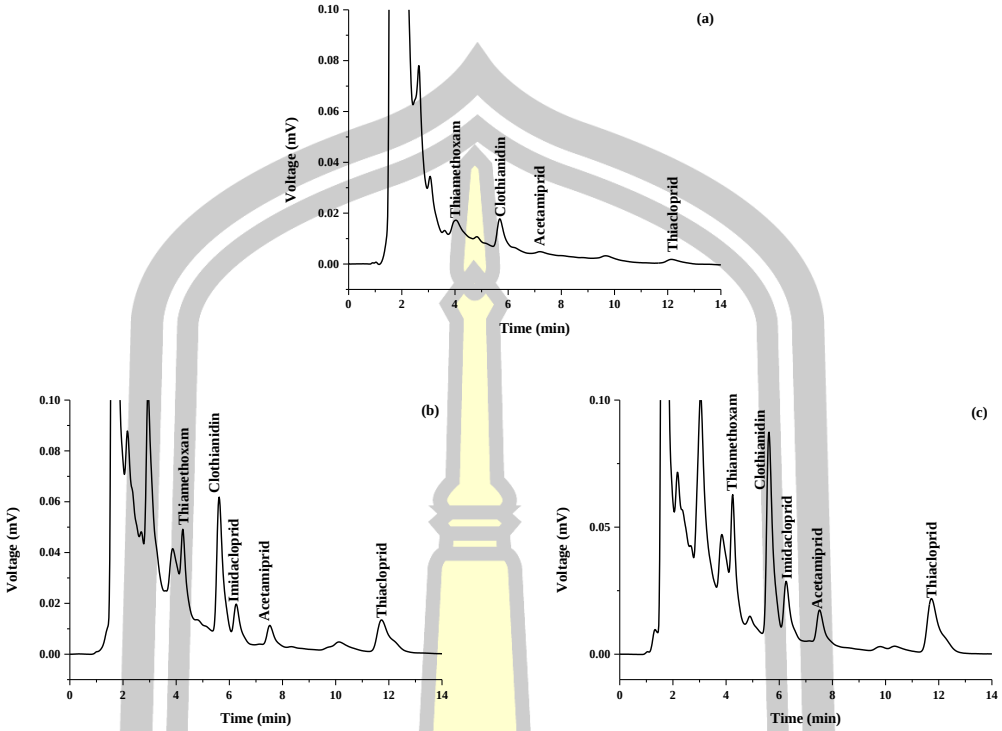


Figure 13. Chromatograms of (a) grape sample (local market), (b) grape sample spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) grape sample spiked with 500 ng mL^{-1} of each neonicotinoid.

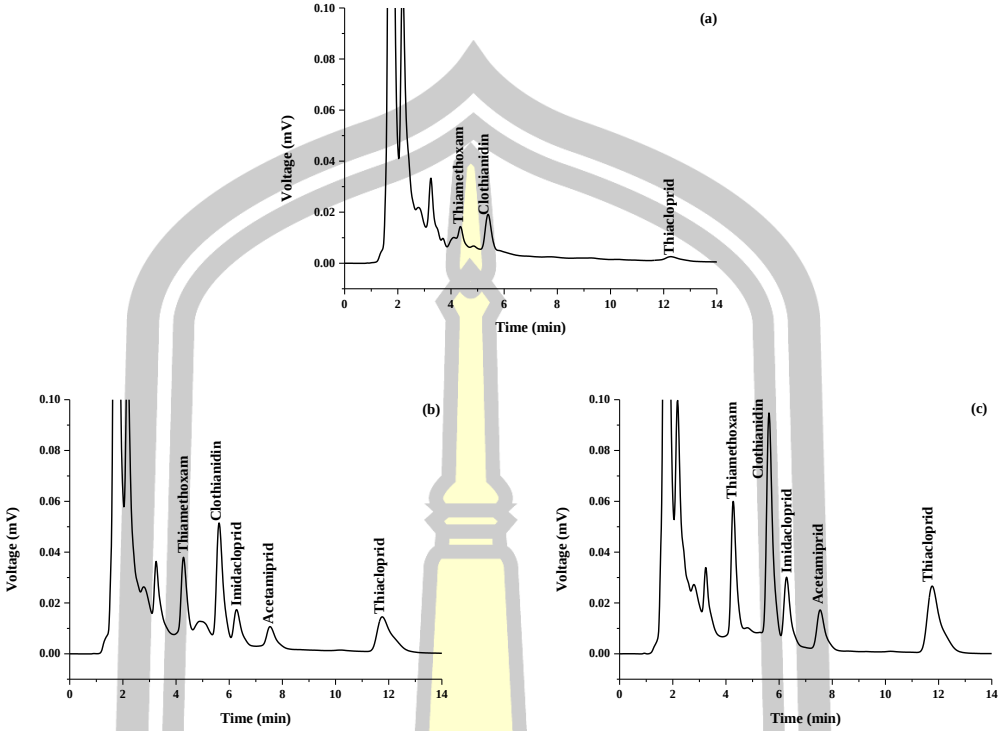


Figure 14. Chromatograms of (a) watermelon sample (local market), (b) watermelon sample spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) watermelon sample spiked with 500 ng mL^{-1} of each neonicotinoid.

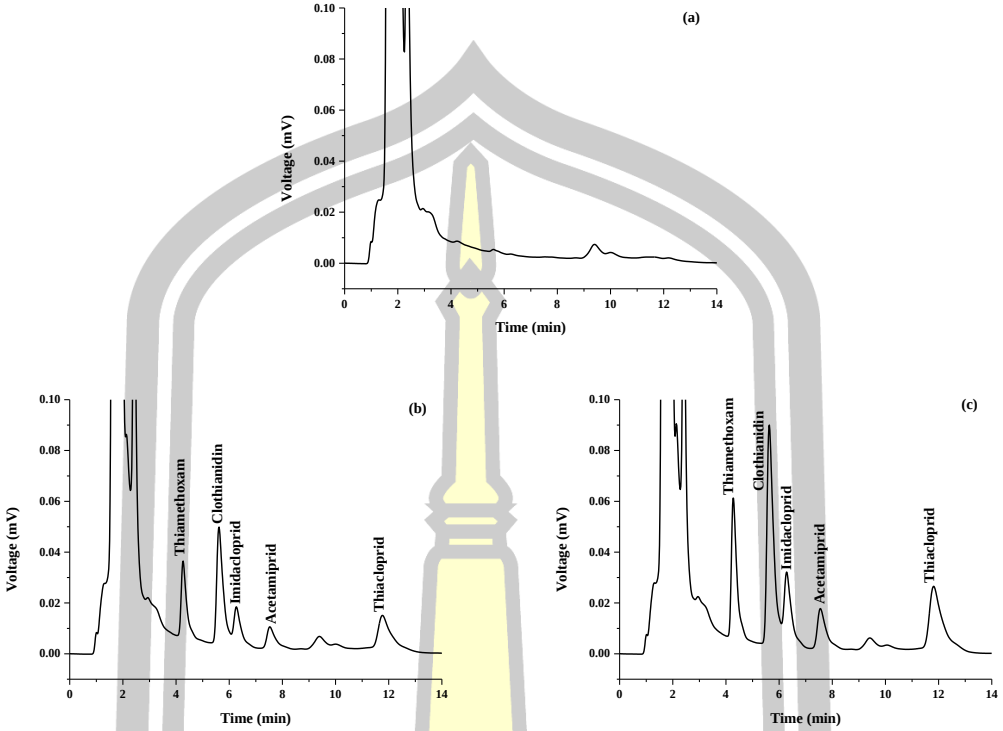


Figure 15. Chromatograms of (a) logan sample (super market), (b) logan sample spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) logan sample spiked with 500 ng mL^{-1} of each neonicotinoid.

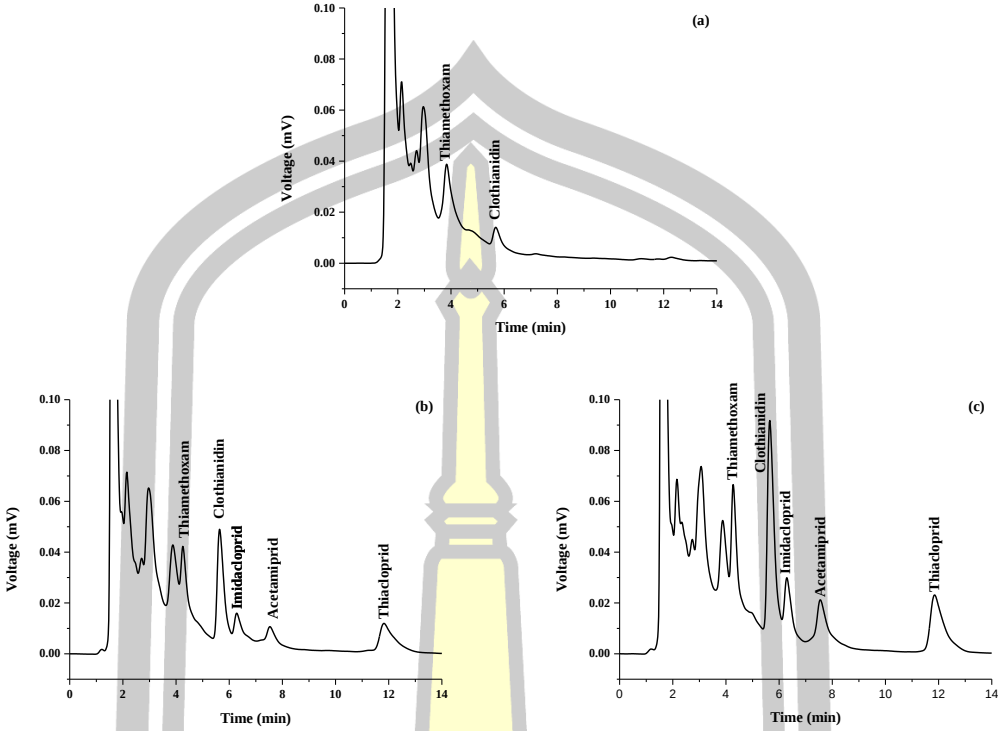


Figure 16. Chromatograms of (a) grape sample (super market), (b) grape sample spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) grape sample spiked with 500 ng mL^{-1} of each neonicotinoid.

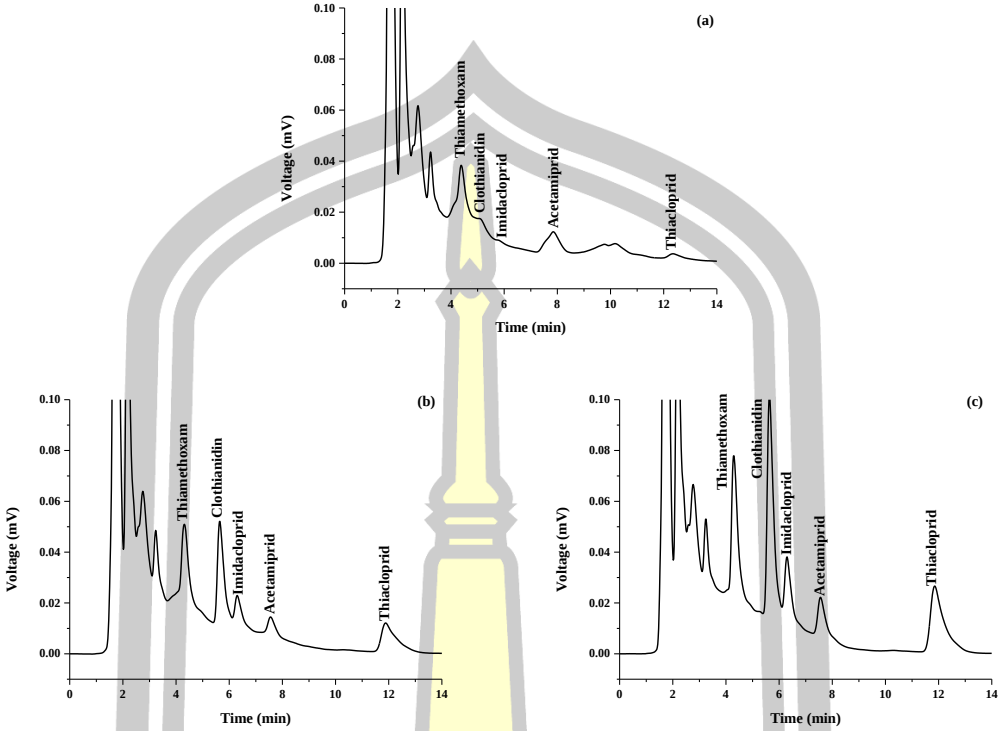


Figure 17. Chromatograms of (a) watermelon sample (super market), (b) watermelon sample spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) watermelon sample spiked with 500 ng mL^{-1} of each neonicotinoid.

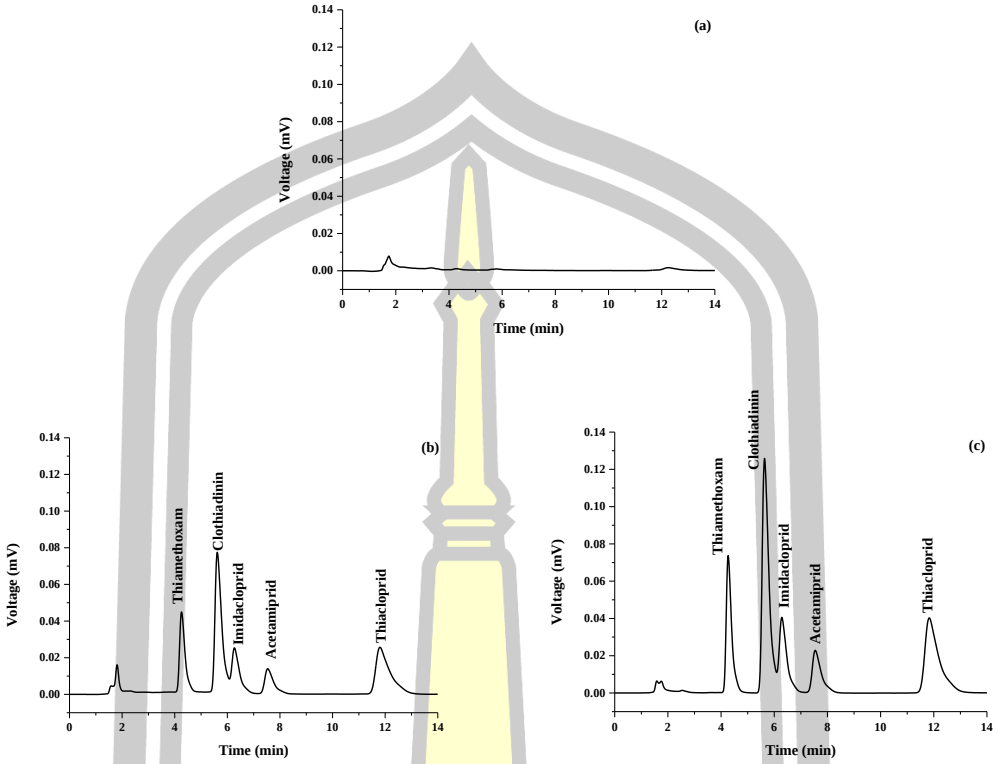


Figure 18. Chromatograms of (a) surface water sample I, (b) surface water sample I spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) surface water sample I spiked with 500 ng mL^{-1} of each neonicotinoid.

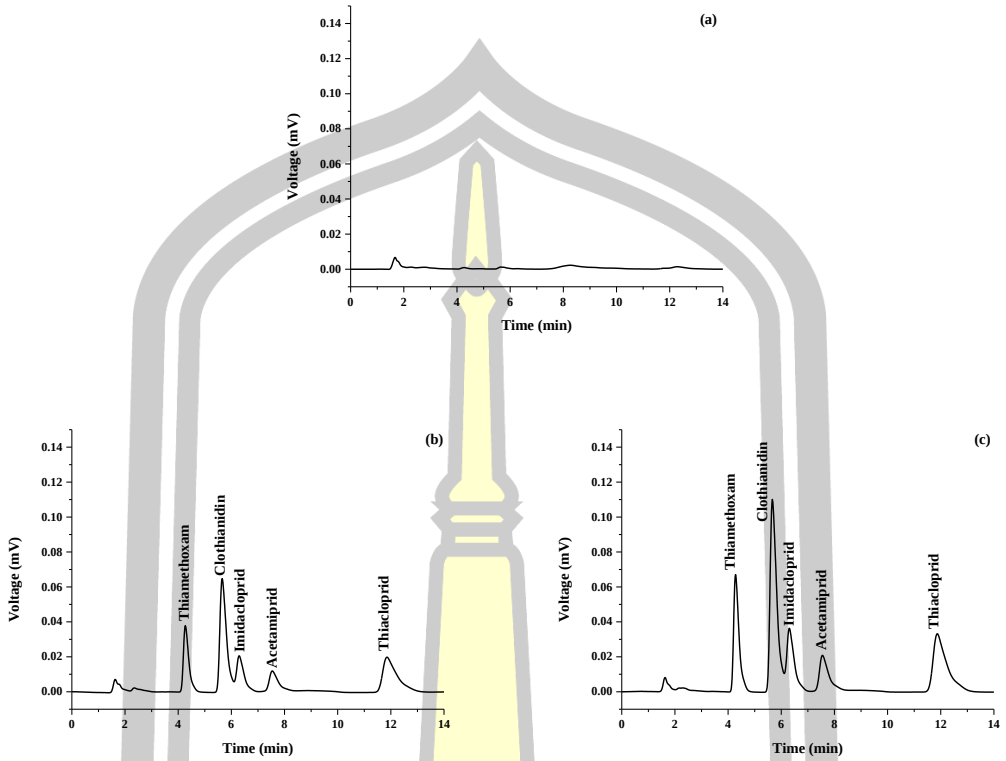


Figure 19. Chromatograms of (a) surface water sample II, (b) surface water sample II spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) surface water sample II spiked with 500 ng mL^{-1} of each neonicotinoid.

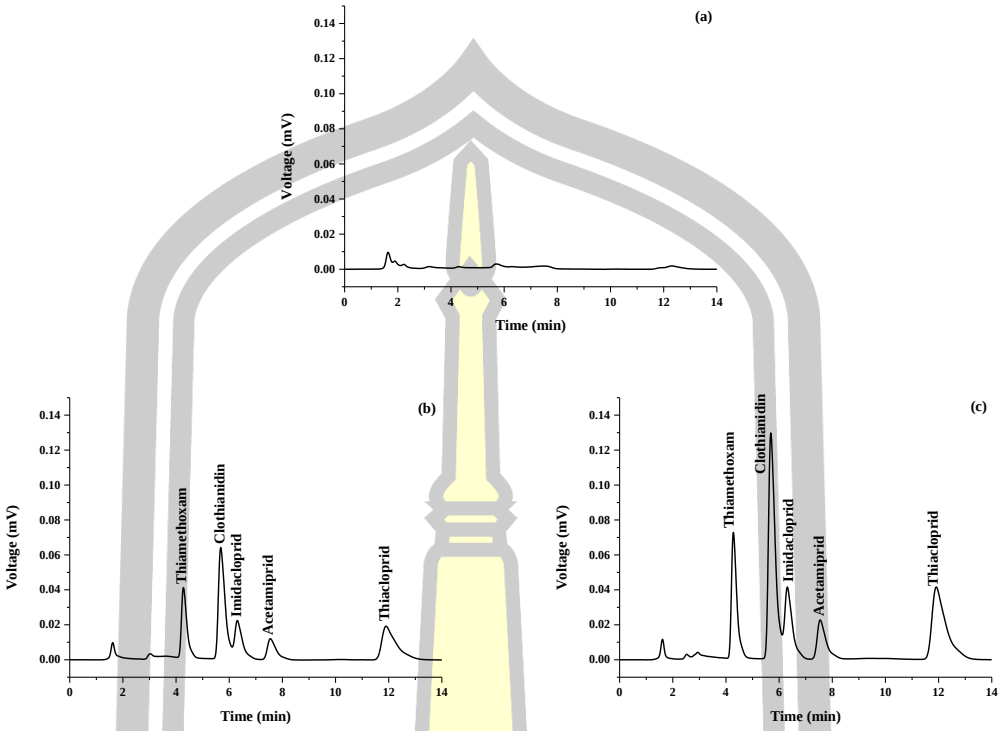


Figure 20. Chromatograms of (a) surface water sample III, (b) surface water sample III spiked with 250 ng mL^{-1} of each neonicotinoid, and (c) surface water sample III spiked with 500 ng mL^{-1} of each neonicotinoid.

4.2 Comparison of the proposed d-μ-SPE method with other sample preparation methods

The proposed d-μ-SPE was prepared to other sample preparation method for analysis of neonicotinoid insecticide residues. As summarized in Table 9, the proposed d-μ-SPE coupled to HPLC is superior to the others in term of high analytical performance, short analysis time and environmetally friendly since it required just a low cost of sorbent. The sensitivity of the proposed method in term of LOD is 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 agricultural product.

Table 10. Comparison of the proposed d-µ-SPE with other sample preparation methods for the determination of neonicotinoids.

Method	Analytes	Sample	Analytical technique	Linearity	LOD	Recovery (%)	Ref.
DSPE-DLLME	Nitenpyram, Dinotefuran, Clothianidin, Thiamethoxam, Acetamiprid, Imidacloprid, Thiacloprid	Grain	HPLC-DAD	0.02-4.5 µg mL^{-1}	0.002-0.005 mg kg^{-1}	76-123	[14]
VSLLME-SFO	Acetamiprid, clotianidin, nitenpyram, imidacloprid, thiamathoxam	Water and fruit juice	HPLC-DAD	0.0005-5 µg mL^{-1}	0.1-0.5 µg L^{-1}	85-105	[12]
SPE	Acetamiprid, imidacloprid, thiacloprid, thiamethoxam	Drinking water	LC-ESI-MS	0-1 mg L^{-1}	0.01 µg L^{-1}	95-104	[37]
DLLME	Acetamiprid, clothianidin, thiamethoxam, imidacloprid, dinotefuran, thiacloprid, nitenpyram	Honey	LC-MS/MS	1.5-100.0 µg kg^{-1}	0.5-1.0 µg kg^{-1}	74.3-113.9	[38]
SPE-DLLME	Thiamethoxam, clothianidin, imidacloprid, acetamiprid, thiacloprid	Honey	LC-APCI-IT-MS/MS	0.1-7500 ng g^{-1}	0.2-1.0 ng g^{-1}	90-104	[39]
DLLME	Thiacloprid, acetamiprid, imidaclothiz,imidacloprid	Cucumber	MEKC	2.7-200 ng g^{-1}	0.8-1.2 ng g^{-1}	79.7-98	[40]
D-µ-SPE	Imidacloprid, acetamiprid, clothianidin, thiacloprid, thiamethoxam	Fruit juice and surface water	HPLC-UV/Visible	0.5-1000 ng mL^{-1}	0.005-0.065 ng mL^{-1}	30-138	This study

CHAPTER 5

CONCLUSION

In this research, a simple dispersive micro-solid phase extraction (d-µ-SPE) method was proposed for preconcentration of neonicotinoid insecticides prior to analysis by HPLC. Monmorillonite was chosen as a solid sorbent for extraction of the target analytes. For d-µ-SPE, the optimal extraction conditions were sample 13 mL, monmorillonite 0.03 g, Na_2SO_4, 0.03 g, and 150 µL of 70% (v/v) acetonitrile as extraction solvent. The extraction was then analyzed by using HPLC with UV/Visible detection. A LiChrospher® 100 RP-18 endcapped (4.6x150 mm, 5.0 µm) with isocratic elution with 25% acetonitrile in water at a flow rate of 1.0 mL min^{-1} was used for separation of the studied neonicotinoid insecticides. Separation of five neonicotinoids was achieved within 14 min.

Under the optimum condition, high extraction efficiency (8-176), LODs (0.005-0.065 ng mL^{-1}) lower than the regulatory limit for insecticides residues, and good repeatability with a very small amount of sorbent and organic solvent consumption were obtained. The optimum conditions were applied to analysis of five neonicotinoid insecticides in natural surface water and fruit juice samples (logan, grape, and watermelon). The results were not found neonicotinoid insecticides in the natural surface water and logan samples. For watermelon and grape studied, all the studied neonicotinoid insecticides were detected in the range of 0.005-0.27 ng mL^{-1}. The proposed method has been successfully appiled to the preconcentration and determination of neonicotinoid insecticide residues in real samples.

The method represented here has acceptable relative recoveries, good repeatability, and a wide linear range. When compared to other extraction methods for neonicotinoids analysis, this method reduces the exposure to toxic solvents used in the conventional extraction procedures, is environmentally friendly since it requires just a low cost of sorbent and has a much faster extraction time with high extraction efficiency. The method showed reliability with an appropriate analytical detection range for application in natural surface water and fruit juice samples.

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