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Vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction with solidification of floating organic droplet combined with HPLC for the determination of neonicotinoid pesticides

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ABSTRACT

A microextraction procedure based on vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction with solidification of floating organic droplet (VSLLME-SFO) for preconcentration of neonicotinoid pesticides, including acetamiprid, clotianidin, nitenpyram, imidacloprid, and thiamethoxam, has been developed. In VSLLME-SFO process, the addition of surfactant (as an emulsifier), could be enhance the mass-transfer from the aqueous solution into the extraction solvent. The extraction solvent could be dispersed into the aqueous by vortex process. Other experimental parameters affected the extraction efficiency, including the kind and concentration of salt, concentration and volume of HCl, kind and concentration of surfactant and its volume, kind and volume of extraction solvent, vortex time and the centrifugation extraction time, were also optimized. The optimum extraction conditions of VSLLME-SFO were 10.00 mL of sample, 0.3% (*w/v*) Na_2SO_4, 50 μL of 0.050 mol L^{-1} SDS, 1.0 mol L^{-1} HCl (400 μL), 150 μL of octanol, vortex time 1 min and centrifugation time 10 min. The sediment phase was analyzed by subjecting it to HPLC using a mobile phase of 25% acetonitrile in water, at a flow rate of 1.0 mL min^{-1}, and photodiode array detection at 254 nm. Under the optimum extraction conditions, high enrichment factors (20–100 fold) and low limit of detection (0.1–0.5 μg L^{-1}) could be obtained. This method provided high sensitivity, low toxic organic solvents used, and simplicity of the extraction processes. The proposed method was successfully applied in the analysis of neonicotinoids in fruit juice and water samples.

1. Introduction

Neonicotinoids are a new type pesticides now widely used in agriculture, instead of persistent organochlorine pesticides, due to their broad spectrum of activity and their low bioaccumulation potential [1]. Neonicotinoid pesticides act as agonists at the insect nicotinic acetylcholine receptor, which are active against many sucking and biting pest insects, including aphides, whiteflies and some *Lepidoptera* species [2]. This compound is most commonly used on rice, maize, sunflowers, rape, potatoes, sugar beets, vegetables, and fruits crops [3]. Many countries have formulated strict limit about the neonicotinoids in various matrices. The European Union (EU) legislation has established standards/regulations for the maximum residue limits (MRLs) for neonicotinoid pesticides in different agricultural products. The MRLs for neonicotinoids in fruit, vegetable and cereals were between 0.1 and 1.0 mg kg^{-1} [4]. Therefore, the evaluation and monitoring of trace levels of these pesticides in food and environmental samples is necessary and demands highly efficient, selective and sensitive analytical techniques [5].

Neonicotinoid pesticides are unsuitable for the direct analysis by gas chromatography due to their low volatility and high polarity [6]. The use of high performance liquid chromatography (HPLC) coupled with various detection systems, including ultraviolet [7,8], diode array [6,9,10], fluorescence [11], and mass spectrometry [12–14], is preferred choice for neonicotinoid pesticides analysis. Although the MS detector provides more sensitivity and selectivity than the UV for monitoring target compounds in complex samples, it is a very expensive and complex instrument [15]. Nowadays, capillary electrophoresis (CE) [5,16] has also become an attractive approach for the separation of pesticide residues, but suffers from low sensitivity because of the small sample volumes typically injected and the generally less sensitive detection systems that are employed. Although HPLC is a sensitive and selective analytical method, but neonicotinoids usually exhibit in very

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low concentrations level and the complexity of environmental matrices. Therefore, sample preparation is still required.

Good sample preparation allows not only the analyte to be preconcentrated but also the other compounds present in the sample matrix to be removed. A variety of choices have been proposed for sample pretreatment and/or preconcentration of neonicotinoids in various matrices. There are various sample preparation have been applied for the analysis of neonicotinoids in various samples including liquid–liquid extraction (LLE) [17], solid-phase extraction (SPE) [7,18], accelerated solvent extraction (ASE) [19], and QuEChERS [14]. However, big disadvantages are the large quantities of solvent utilized and the multiple operation steps needed [20]. Recently, attention is being paid to the development of miniaturized, more efficient and environmentally friendly extraction techniques that could greatly reduce the toxic organic solvent consumption [21,22].

In 2006, dispersive liquid–liquid microextraction (DLLME) [5] has been developed. Due to the large contact surface area of the two immiscible phases (organic phase and aqueous phase) in DLLME, high extraction efficiency is achieved in a relatively short time. However, the necessity of the dispersive solvent could lead to decrease of the partition coefficient of analytes into the extraction solvent [23]. In the last few years, Yiantzi et al. investigated a novel microextraction method namely vortex-assisted liquid–liquid microextraction (VALLME) [24]. In VALLME, the extraction solvent is dispersed into aqueous samples by vortex mixing, which is a mild emulsification procedure compared with ultrasound-assisted emulsification microextraction (USAEME). VALLME overcomes the main disadvantages of DLLME (using toxic dispersive solvent) and USAEME (potential analyte degradation) under some special conditions. Very recently, a vortex-assisted surfactant-enhanced emulsification liquid–liquid microextraction (VSLLME) has been introduced [25]. Surfactant could reduce the interfacial between two immiscible phases by adsorbing at the liquid–liquid interface and serve as an emulsifier to enhance the dispersion of water-immiscible solvent into aqueous phase. The low-density solvent-based vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction (LDS-VSLLME) with gas chromatography–mass spectrometry (GC–MS) was reported by Zhang et al. [26] for the fast determination of six phthalate esters in bottled water samples.

The aim of the present work was to explore and develop an efficient vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction with the solidification of floating organic droplet (VSLLME-SFO) method coupled to HPLC with photodiode array (PDA) detection for the extraction, preconcentration and analysis of neonicotinoid pesticide residues in surface water and fruit juice samples. The crucial experimental parameters affecting the VSLLME-SFO and separation conditions were also investigated and optimized. Moreover, to our knowledge, this is the first time for applicability of VSLLME-SFO for the extraction of neonicotinoid pesticides coupled with HPLC analysis.

2. Experimental

2.1. Chemicals and reagents

Common names and structures of the five neonicotinoids evaluated here are shown in Table 1. Neonicotinoid standards of highest purity were used. Acetamiprid, clotianidin, nitenpyram, imidacloprid, and thiamethoxam were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The stock standard solutions of each neonicotinoid (1000 μg mL^{-1}) were prepared by dissolving each neonicotinoid standard in methanol. Working standard solutions were prepared by diluting the stock solution with water. Deionized water obtained from RiOs™ Type I Simplicity 185 (Millipore Waters, USA) with the resistivity of 18.2 MΩ cm was used throughout the experiments. Methanol and acetonitrile (ACN) of HPLC grade were obtained from Merck (Germany). NaCl and anhydrous Na_2SO_4 were obtained from Ajax Finechem (New Zealand) and KI was perchased from Carlo Erba (France). Cetyl trimethylammonium bromide (CTAB) was purchased from Calbiochem (Germany). Sodium dodecyl sulfate (SDS), Triton X-100 and octanol were obtained from Merck (Germany).

2.2. Apparatus

Chromatographic separation was performed on an Atlantis dC18 column (4.6 mm i.d. × 150 mm, 5 μm particle diameter), using a mobile phase of 25% acetonitrile in water, at a flow rate of 1.0 mL min^{-1}. The mobile phase was filtered through a 0.45 mm membrane filter

(Millipore, USA) and degassed before use. A Waters 600 multisolvent delivery system, a Rheodyne injector with a sample loop of 20 μL, a Waters 996 photodiode array detector were recorded at 254 nm. The Millinium software was used for data acquisition. Vortex agitator (Fisher Scientific, USA) was used. A centrifuge (Centurion, England) was used for complete phase separation. Five neonicotinoid pesticides were separated within 8 min with the elution order of nitenpyram (t_R=3.25 min), thiamethoxam (t_R=4.58 min), clotianidin (t_R=5.93 min), imidacloprid (t_R=6.75 min), and acetamiprid (t_R=7.93 min).

2.3. Sample preparation

2.3.1. Surface waters

The natural surface water samples were taken from different areas located near rice fields in Mahasarakham province, northeastern of Thailand, and were filtered through a Whatman filter paper no. 42 and then passed through 0.45 μm nylon membrane filter before extraction.

2.3.2. Fruits

Commercial fruit juice samples (guava, grape, apple, orange, and passion fruit juices) available in supermarkets were also collected for analysis. Before analysis, a 30.0 mL aliquot of fruit juice was centrifuged a 5000 rpm for 10 min, and was filtered through a Whatman no. 42 filter paper. Then, the filtrate was filtered through a 0.45 μm nylon membrane filter before extraction.

2.4. VSLLME-SFO procedure

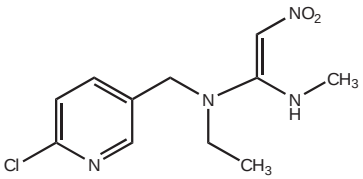
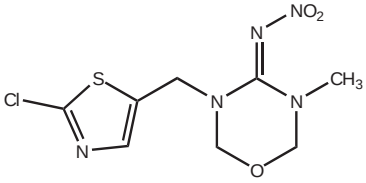
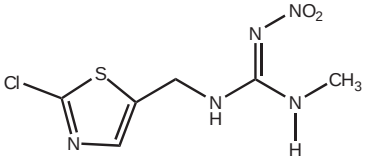
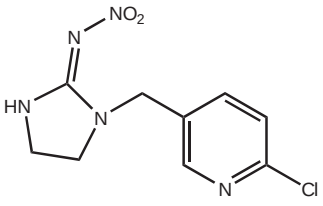
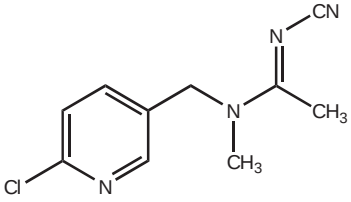
A volume of 10.00 mL of the standard solution (or sample solution) was mixed with Na_2SO_4 (0.3%, w/v) before the addition of 1.0 mol L^{-1} HCl (400 μL). After that, 50 μL of 0.050 mol L^{-1} SDS (as emulsifier) was added. The resulting solution was then vortex for 1 min. The emulsion solution was rapidly injected with octanol (150 μL) and then centrifuged at 5000 rpm for 10 min to complete the phase separation and the reconstituted solution floated on the top of the tube. The upper phase (~150 to 200 μL) was directly injected into HPLC. The concentrations of the reagents used in this work were optimized (see Section 3.1).

3. Results and discussion

3.1. Optimization of the VSLLME-SFO conditions

In the present study, VSLLME-SFO was developed for preconcentration of neonicotinoids prior to HPLC analysis. In order to obtain a high enrichment factors, various parameters to be studied

Table 1
Names and structures of five neonicotinoid evaluated.

Pesticide	Structure	Abbreviation	Molecular weight (g/mol)	Formula
Nitenpyram		NTR	270.72	$C_{11}H_{15}ClN_4O_2$
Thiamethoxam		TMX	291.71	$C_8H_{10}ClN_5O_3S$
Clothianidin		CTD	249.68	$C_6H_8ClN_5O_2S$
Imidacloprid		ICL	255.66	$C_9H_{10}ClN_5O_2$
Acetamiprid		ACT	222.67	$C_{10}H_{11}ClN_4$

include the kind and concentration of salt, concentration and volume of HCl, kind and concentration of surfactant and its volume, kind and volume of extraction solvent, vortex time and the centrifugation time. The optimization was carried out on the aqueous solution containing 3.0 μg mL^{-1} of each analytes. The EFs, which was defined as the ratio between the analyte concentration in extraction solvent after the extraction process and the concentration of analyte in the sample before the extraction process, was used to evaluate the extraction efficiency [25]. All experiments were operated at least in triplicate.

3.1.1. *Effect of salt addition*

Salt addition to the aquatic solutions may have several different effects on extraction (salting-out, salting-in or no effect) [26]. In general, salt is often added into the sample solution to decrease the solubility of the analytes in the aqueous phase and improve the extraction efficiency of the relatively more polar analytes. In this study, addition of different electrolyte salts (NaCl, Na_2SO_4 and KI) at 0.1% (*w*/*v*) was investigated and the results were compared with that obtained from the process without salt addition. From the results (Fig. 1(a)), it is clearly seen that the addition of Na_2SO_4 provided higher extraction efficiency in term of peak area of neonicotinoids. Therefore, the concentration of Na_2SO_4 on the extration efficiency of the target analyte were also studied within the the ranged of 0%–0.7% (*w*/*v*). The results in Fig. 1(b) showed that an improvement of extraction efficiency for all neonicotinoids when 0.3% (*w*/*v*) Na_2SO_4 was added.

3.1.2. *Kind, concentration of surfactant and its volume*

In the VSLLME-SFO method, surfactant is one of great important parameter because it serves as an emulsifier. Under the vortex mixing, the surfactant could accelerate the emulsification of organic solvent into the aqueous samples [25]. Three types of surfactant were studied including the cationic (CTAB), the anionic (SDS) and the non-ionic (Triton X-100). Their critical micellar concentrations (CMCs) are 7, 0.91 and 0.24 mmol L^{-1}[26]. The concentration of each surfactant was fixed at 150 μL of surfactant 0.01 mol L^{-1} and the other conditions were controlled as follows: 10.00 mL of sample, 0.3% (*w*/*v*) Na_2SO_4, 1000 μL of 1.0 mol L^{-1} HCl, 300 μL of octanol, vortex time 1 min and centrifugation time 5 min. The relevant data are shown in Fig.2(a). It was found that, high extraction efficiency (in term of peak area) can be obtained when SDS was used.

Surfactant concentration is another parameter which is important for effective extraction. Different concentrations of SDS were investigated in the range of 0.005–0.10 mol L^{-1}. The results are shown in Fig. 2(b). The peak areas of all the analytes slightly increased with an increase in SDS concentration up to 0.050 mol L^{-1}, then slightly decreased. Therefore, 0.050 mol L^{-1} of SDS was selected for further studies.

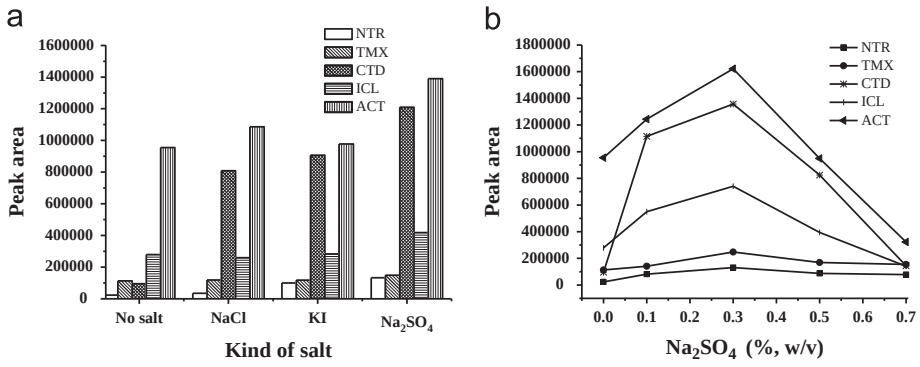


Fig. 1. Effect of salt addition; kind (a) and concentration (b) of salt.

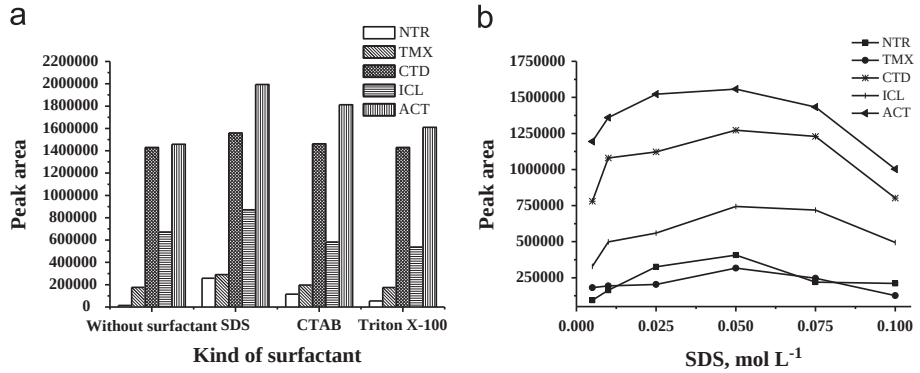


Fig. 2. Kind (a) and concentration (b) of surfactant.

The influent of 0.050 mol L^{-1} of SDS volume was studied in the range of 30–1000 μL. The other conditions were kept constant as follows: 10.00 mL of sample, 0.3% (*w*/*v*) Na_2SO_4, 1000 μL of 1.0 mol L^{-1} HCl, 300 μL of octanol, vortex time 1 min and centrifugation time 5 min. It was observed that, the highest peak area was obtained for all neonicotinoids using an SDS volume of 50 μL (data not shown). At higher volumes than 50 μL, the peak areas of all the neonicotinoids slightly decreased. Therefore, 50 μL of 0.050 mol L^{-1} SDS was used for further experiments.

3.1.3. *Effect of HCl*

Hydrochloric acid (HCl) is required as essential parameter to optimize for obtaining two isotropic phases (L–L) formed from anionic surfactant solutions [27]. The concentration of HCl increased with decreasing pH of solution. Therefore, 1.0 mol L^{-1} HCl was selected throughout this work. A 1.0 mol L^{-1} HCl volume was varied between 0 and 1000 μL, and the other conditions were kept constant as follow: 10.00 mL of sample, 0.3% (*w*/*v*) Na_2SO_4, 50 μL of 0.050 mol L^{-1} SDS, 300 μL of octanol, vortex time 1 min and centrifugation time 5 min. Fig. 3 shows that, the peak area of all neonicotinoids slightly increased with increasing the volume to 400 μL of HCl, and slightly decreased afterward. Therefore, HCl 400 μL was chosen for further experiments.

3.1.4. *Effect of kind and volume of extraction solvent*

It is known that the extraction solvent used in VSLLME-SFO should meet the following requirements: good extraction efficiency for the target analytes to ensure high enrichment, immiscible with water, a lighter density than water and good chromatographic behavior [26]. Based on these considerations, toluene, n-hexane, 1-dodecanol and octanol were selected as potential extraction solvents for the study. The results are shown in Fig. 4. It was found

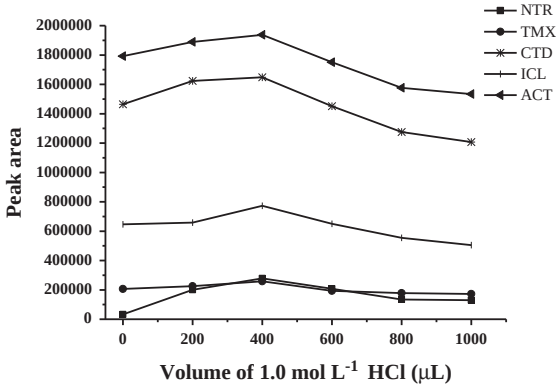


Fig. 3. Effect of volume of hydrochloric acid.

that octanol provided the highest extraction performance for all the analytes, except for nitenpyram and imidacloprid where the extraction did not depend on the type of extraction solvent used. Therefore, octanol was selected as the extraction solvent for further experiments.

The effect of extraction solvent volume was studied between 50 and 1000 μL (data not shown). It was found that, the extraction solvent volume is 50 μL, the solution can not complete phase separation. Moreover, the extraction solvent volume more than 150 μL decreased the peak area of neonicotinoids. Therefore, octanol 150 μL was selected as extraction solvent.

3.1.5. *Effect of vortex time*

Duration of the vortexing is one of the main parameters in VSLLME-SFO. It affects both the emulsification and mass transfer

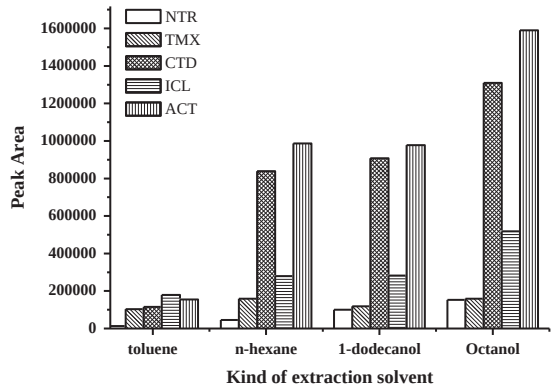


Fig. 4. Kind of extraction solvent.

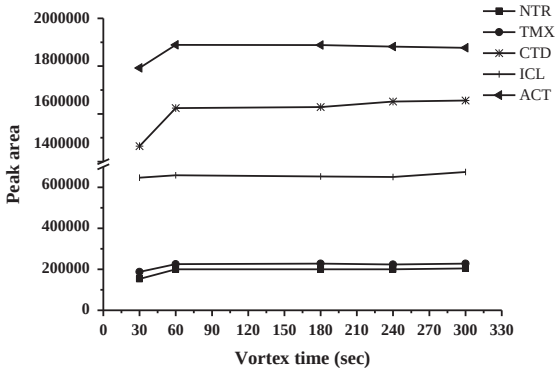


Fig. 5. Effect of vortex time.

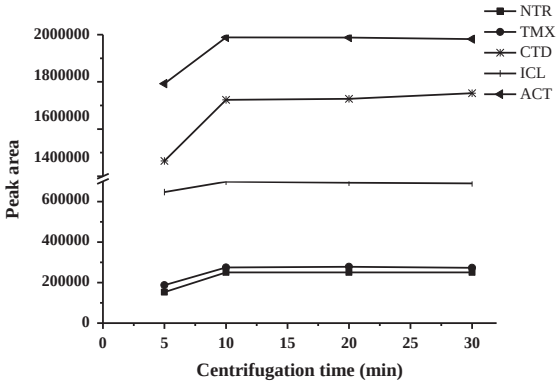


Fig. 6. Effect of centrifugation time.

processes, and thus affects the extraction efficiency of the proposed method. For the present study, the effect of the vortex time was studied in range of 30–300 s (Fig. 5). It can be observed that the extraction efficiency increased with increasing the vortex time from 30 s to 60 s and then keep constant. Due to the contact surface between the extractions solvent and aqueous sample was greatly enhanced by the addition of surfactant and the vortex agitation, therefore the mass transfer increase. As a result, the equilibrium vortex time was 60 s.

3.1.6. *Effect of centrifugation time*

Although centrifugation time has less influence on the extraction efficiency in VSLLME-SFO, optimum centrifugation time is still required because the process of mass transfer between two phases in extraction procedure should be time-dependent, the influence of centrifugation time on the peak area was examined. A series of centrifugation extraction times at 5000 rpm to complete phase separation and short time analysis was studied within the range of 5–30 min (Fig. 6) while other experimental conditions were kept constant (10.00 mL of sample, 0.3% (*w/v*) Na_2SO_4, 50 μL of 0.05 mol L^{-1} SDS, 400 μL of 1.0 mol L^{-1} HCl, 150 μL of octanol and vortex time 1 min). The extraction performance of neonicotinoids slightly increased with time up to 10 min and was kept constant, therefore 10 min was chosen to ensure the extraction performance of the proposed method.

3.2. *Analytical performance*

Under the above-selected conditions, the analytical performance of VSLLME-SFO combined with HPLC for analysis of neonicotinoids was evaluated. A series of experiments was designed for obtaining linear range, precision, limit of detection (LOD) and enrichment factor. Linear equations in the range of 0.0005–5 μg mL^{-1} with high correlation coefficients ($r^2 > 0.96$) were obtained. The characteristic calibration data obtained are listed in Table 2. The relative standard deviation (RSD) was determined using five solutions of the 3.0 μg mL^{-1} of each neonicotinoids. The RSD values of the retention times and peak areas range from 0.75%–1.45% and 1.54%–3.45%, respectively. The sensitivity was evaluated in term of LOD as concentration giving the signal-to-noise ratio of 3 (S/N=3) and ranged between 0.0001 and 0.0005 μg mL^{-1}. The LOQ (S/N=10) ranged between 0.002 and 0.003 μg mL^{-1}. The enhancement factors, calculated by comparing the slopes of the calibration graphs with and without preconcentration, were in the range of 20–100. The chromatograms obtained for the separation of neonicotinoids by direct HPLC injection (Fig. 7 (a)) and VSLLME-SFO combined with HPLC (Fig. 7(b)) were compared. Using the VSLLME-SFO procedure, the chromatographic signals were enhanced.

Table 2
Analytical performance of the method.

Pesticide	HPLC					Vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction with solidification of floating organic droplet (VSLLME–SFO)					
	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	%RSD (n=5)		Linear range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	%RSD (n=5)		EF
				t_R	Peak area				t_R	Peak area	
Nitenpyram	0.03–5	0.03	0.10	1.54	2.54	0.001–5	0.0005	0.002	1.45	3.45	60
Thiamethoxam	0.01–5	0.01	0.03	0.83	1.76	0.001–5	0.0005	0.003	0.98	1.54	20
Clothianidin	0.01–5	0.01	0.03	0.70	2.84	0.0005–5	0.0001	0.002	0.75	2.47	100
Imidacloprid	0.03–5	0.03	0.01	1.25	2.63	0.001–5	0.0005	0.002	1.32	2.73	60
Acetamiprid	0.01–5	0.01	0.03	1.10	3.45	0.0005–5	0.0001	0.003	1.22	3.28	100

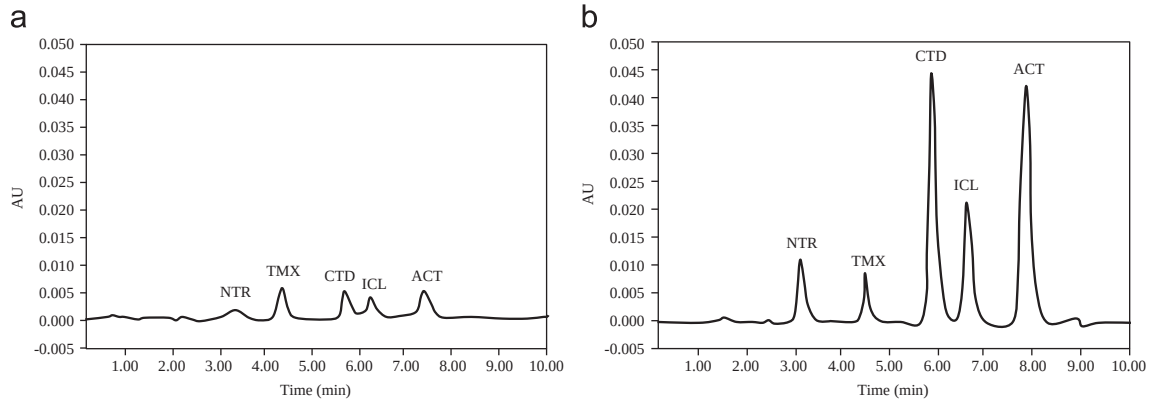


Fig. 7. Chromatograms of standard neonicotinoids obtained by (a) direct injection without preconcentration and (b) using VSLLME-SFO procedure: concentration of all standards was 3.0 μg mL^{-1}. Conditions: HPLC, Atlantis dC18 column (4.6 × 150 mm), isocratic elution of 25% (*v*/*v*) of ACN with the flow rate of 1.0 mL min^{-1} and detection at 254 nm. VSLLME-SFO conditions: Na_2SO_4 (0.3%, *w*/*v*), 1.0 mol L^{-1} HCl (400 μL), 50 μL of 0.05 mmol L^{-1} SDS, octanol (150 μL), vortex time 1 min and centrifugation time (5000 rpm) 10 min.

Table 3
Analysis of neonicotinoid pesticides in real samples.

Sample	Amount found ± SD, μg mL^{-1} (n=3)				
	Nitenpyram	Thiamethoxam	Clothianidin	Imidacloprid	Acetamiprid
Water sample					
Water I	–	–	–	–	–
Water II	–	–	–	–	–
Water III	–	–	–	–	–
Fruit juice sample					
Guava	0.92 ± 0.02	2.92 ± 0.11	–	–	–
Grape	–	0.67 ± 0.06	–	0.06 ± 0.01	0.01 ± 0.00
Apple	0.32 ± 0.03	–	–	–	–
Orange	–	0.79 ± 0.03	–	–	–
Passion fruit juice	–	1.13 ± 0.09	0.04 ± 0.00	0.59 ± 0.03	0.10 ± 0.02

-; Not detected

Table 4
Recoveries of the studied neonicotinoids in spiked samples.

Sample	Spike (μg mL^{-1})	Nitenpyram		Thiamethoxam		Clothianidin		Imidacloprid		Acetamiprid	
		RR (%)	RSD (%)	RR (%)	RSD (%)	RR (%)	RSD (%)	RR (%)	RSD (%)	RR (%)	RSD (%)
Water sample											
Water I	0.5	88	1.8	92	2.1	95	2.5	85	1.3	91	1.6
	1.0	93	2.3	98	1.5	102	2.1	95	2.8	97	1.7
Water II	0.5	98	1.5	89	1.3	93	1.8	99	1.4	101	2.8
	1.0	95	2.4	88	1.7	95	1.6	93	1.7	105	2.1
Water III	0.5	98	2.8	105	1.8	98	1.5	91	2.8	102	1.5
	1.0	100	1.7	89	1.8	91	1.2	97	2.6	94	2.2
Fruit juice sample											
Guava	0.5	97	1.8	98	2.1	89	1.6	94	2.3	92	1.8
	1.0	95	3.2	96	2.5	99	1.6	89	1.8	90	1.5
Grape	0.5	97	1.5	89	2.2	100	1.5	102	1.7	98	3.2
	1.0	95	2.8	99	1.8	101	1.7	89	1.8	95	1.8
Apple	0.5	98	2.3	96	1.4	87	0.8	89	0.8	98	1.4
	1.0	93	1.1	95	1.7	97	1.8	96	1.8	89	2.6
Orange	0.5	95	1.5	98	2.5	89	2.2	98	1.7	97	2.5
	1.0	91	2.8	93	1.3	99	1.7	88	2.8	95	2.3
Passion fruit juice	0.5	91	1.7	99	2.2	100	1.7	99	3.2	105	1.6
	1.0	99	0.8	98	2.7	89	1.8	96	1.6	103	1.6

RR relative recovery (on average, n=3)

3.3. *Application to real samples*

To evaluate the applicability and accuracy of the developed VSLLME-SFO method in real sample determination, surface water samples and commercial fruit juice samples (guava, grape, apple, orange, and passion fruit juices) were analyzed. Each sample was pre-treated as described in Section 2.3, and then extracted using the VSLLME-SFO procedure (see Section 2.4) before analysis by

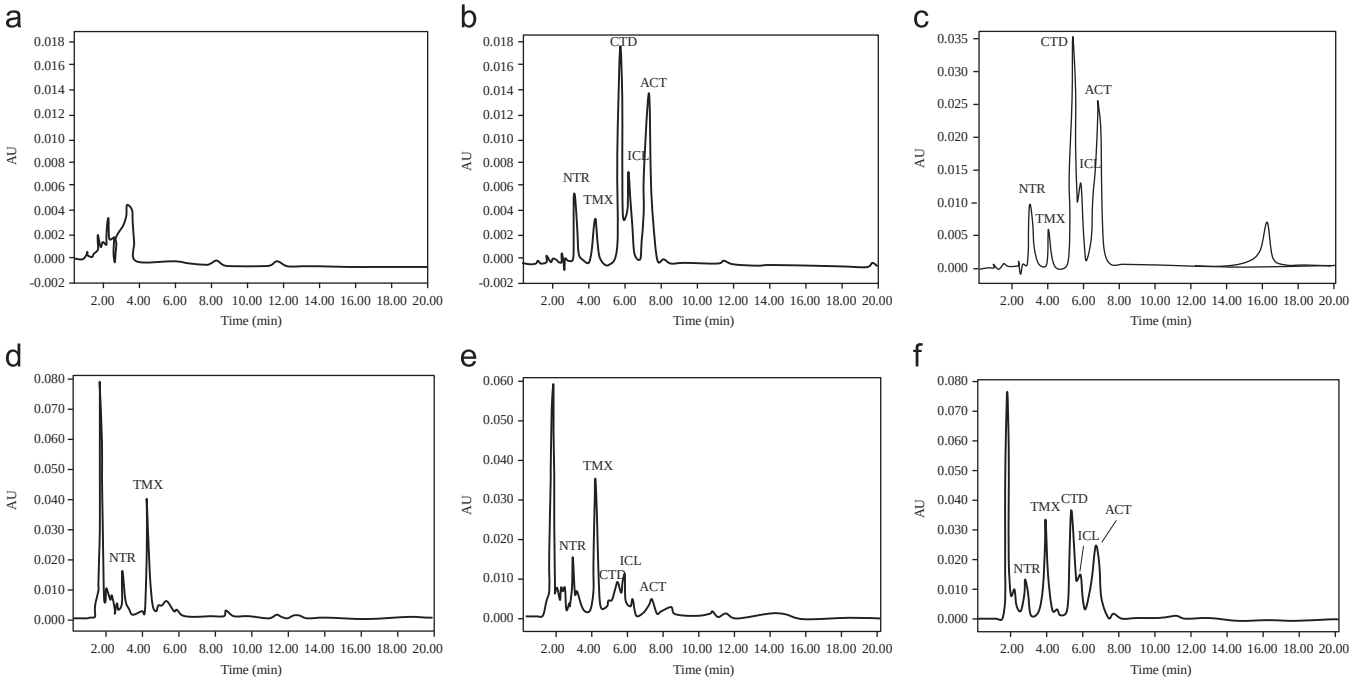


Fig. 8. Typical chromatograms of the studied samples: (a) water, (b) water spiked with 0.5 μg mL^{-1} of each neonicotinoids, (c) water spiked with 1.0 μg mL^{-1} of each neonicotinoids, (d) guava, (e) guava spiked with 0.5 μg mL^{-1} of each neonicotinoids, and (f) guava spiked with 1.0 μg mL^{-1} of each neonicotinoids.

Table 5
The performance of the proposed VSLLME-SFO with other sample preconcentrations and determination of neonicotinoids.

Method	Analytes	Sample	Analytical technique	LOD	Recovery (%)	Enrichment factor	Ref.
DSPE –DLLME	Nitenpyram, Dinotefuran, Clothianidin, Thiamethoxam, Acetamiprid, Imidacloprid, Thiacloprid	Grain	HPLC–DAD	0.002–0.005 mg kg^{-1}	76–123	–	[6]
SPE	Acetamiprid, Imidacloprid, Thiacloprid, Thiamethoxam	Drinking water	LC–ESI–MS	0.01 μg L^{-1}	95–104	–	[28]
Subcritical water extraction	Dinotefuran, Nitenpyram, Thiamethoxam, Imidacloprid, Clothianidin, Acetamiprid, and Thiacloprid	Eels	UPLC–MS/MS	0.12–0.30 μg kg^{-1}	84.6–102.0	–	[29]
SPE	Acetamiprid, Imidacloprid, Thiacloprid, Thiamethoxam	Bovine milk	HPLC–DAD	0.01–0.04 mg kg^{-1}	85.1–99.7	–	[9]
–	Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid and Thiamethoxam	Beeswax	LC–ESI–MS	0.4–2.3 μg kg^{-1}	85–105	–	[14]
DLLME	Thiacloprid, Acetamiprid, Imidaclothiz and Imidacloprid	Cucumber	MEKC	0.8–1.2 ng g^{-1}	79.7–98	–	[5]
VSLLME-SFO	Acetamiprid, Clotianidin, Nitenpyram, Imidacloprid, Thiamathoxam	Water and fruit juice	HPLC–DAD	0.1–0.5 μg L^{-1}	85–105	20–100	This study

HPLC. The results are summarized in Table 3. It was observed that no residue of the studied neonicotinoids was observed in the water samples. All neonicotinoid pesticides commonly found in the studied fruit juice samples. However, the amounts of neonicotinoid pesticides found in the fruit samples were lower than the maximum residue limits (MRLs) established by EU (acetamiprid, 1.0 mg kg^{-1}, 0.8 mg kg^{-1} and 0.5 mg kg^{-1} in orange, apple and grape, respectively; imidacloprid, 1.0 mg kg^{-1}, 0.5 mg kg^{-1} and 1.0 mg kg^{-1} in orange, apple and grape, respectively; thiamethoxam and clothianidin, 0.5 mg kg^{-1}, 0.5 mg kg^{-1} and 0.9 mg kg^{-1} in orange, apple and grape, respectively).

The recovery experiments were carried out to investigate the method accuracy and precision. The samples were spiked with standard neonicotinoids at two concentration levels of 0.50 and 1.00 μg mL^{-1} before analysis by the whole analytical process as proposed. As listed in Table 4, the recoveries were observed in the range of 85%–105% and 87%–105% for water samples and fruit juice samples, respectively. Fig. 8 shows the typical chromatograms of the studied samples (a) water, (b) water spiked with 0.5 μg mL^{-1} of each neonicotinoids, (c) water spiked with 1.0 μg mL^{-1} of each neonicotinoids, (d) guava, (e) guava spiked with 0.5 μg mL^{-1} of each neonicotinoids, and (f) guava spiked with 1.0 μg mL^{-1} of each neonicotinoids.

The performance of the proposed VSLLME-SFO method was compared with other extraction methods, as summarized in Table 5. The results show that linearity, LODs, and recovery obtained from the proposed method are comparable. High preconcentration factors were obtained. Moreover, less consumption

of toxic organic solvents and samples are required in the proposed VSLLME-SFO method.

4. Conclusion

In the present study, a VSLLME-SFO method was proposed for preconcentration of neonicotinoid pesticides prior to analysis by HPLC. The method provided good precision, an efficient recovery, high preconcentration factor without using organic dispersive solvent and showed reliability with well suited analytical detection range for application in fruit juice samples. VSLLME-SFO provides high efficacy for extraction with the obtained high enrichment factor. The proposed method has potential to be used as an alternative green extraction method for the determination of neonicotinoids in various sample matrices with good recovery in the range of 85%–105%.

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References

[1] E. Watanabe, K. Baba, H. Eun, S. Miyake, Food Chem. 102 (2007) 745–750.
[2] K. Matsuda, S.D. Buckingham, D. Kleier, Trends Pharmacol. Sci. 22 (11) (2001) 573–580.
[3] R.C. Biever, J.R. Hoberg, B. Jacobson, Environ. Toxicol. Chem. 22 (01) (2009) 164–174.
[4] EU pesticides database, ⟨http://ec.europa.eu/sanco_pesticides/public/index.cfm⟩.
[5] S. Zhang, X. Yang, X. Yin, C. Wang, Z. Wang, Food Chem. 133 (2012) 544–550.
[6] P. Wang, X. Yang, J. Wang, J. Cui, A.J. Dong, H.T. Zhao, L.W. Zhang, Z.Y. Wang, R. B. Xu, W.J. Li, Y.C. Zhang, H. Zhang, J. Jing, Food Chem. 134 (2012) 1691–1698.
[7] Q. Zhou, Y. Ding, J. Xiao, Anal. Bioanal. Chem. 385 (2006) 1520–1525.
[8] S.B. Singh, G.D. Foster, S.U. Khan, J. Agric. Food Chem. 52 (2004) 105–109.
[9] S. Seccia, P. Fidente, D. Montesano, P. Morrica, J. Chromatogr. A 1214 (2008) 115–120.
[10] Q. Wu, Z. Li, C. Wang, C. Wu, W. Wang, Z. Wang, Food Anal. Methods 4 (2011) 559–566.
[11] M.D.G. García, M.M. Galera, R.S. Valverde, A. Galanti, S. Girotti, J. Chromatogr. A 1147 (2007) 17–23.
[12] A.D. Muccio, P. Fidente, D.A. Barbini, R. Dommarco, S. Seccia, P. Morrica, J. Chromatogr. A 1108 (2006) 1–6.
[13] P. Jovanov, V. Guzsvány, M. Franko, S. Lazić, M. Sakač, B. Šarić, V. Banjac, Talanta 111 (2013) 125–133.
[14] K.P. Yáñez, J.L. Bernal, M.J. Nozal, M.T. Martín, J. Bernal, J. Chromatogr. A 1285 (2013) 110–117.
[15] Y. Santaladchaiyakit, S. Srijaranai, Anal. Methods 4 (2012) 3864–3873.
[16] A.S. Carretero, C. Cruces-Blanco, S.P. Durán, A.F. Gutiérrez, J. Chromatogr. A 1003 (2003) 189–195.
[17] C. Jansson, T. Pihlstrom, B.G. Österdahl, K.E. Markides, J. Chromatogr. A 1023 (1) (2004) 93–104.
[18] D. Štajnbaher, L. Zupančič-Kralj, J. Chromatogr. A 1015 (1) (2003) 185–198.
[19] C.L. Chitescu, E. Oosterink, J. de Jong, A.A. Maria (Linda) Stolker, Talanta 88 (2012) 653–662.
[20] J. Vichapong, R. Burakham, S. Srijaranai, K. Grudpan, Talanta 84 (2011) 1253–1258.
[21] Q.H. Wu, X. Zhou, Y.M. Li, X.H. Zang, C. Wang, Z. Wang, Anal. Bioanal. Chem. 393 (2009) 1755–1761.
[22] Q. Wu, Q. Chang, C. Wu, H. Rao, X. Zeng, C. Wang, Z. Wang, J. Chromatogr. A 1217 (2010) 1773–1778.
[23] J. Regueiro, M. Llompart, C. Garcia-Jares, J.C. Garcia-Monteagudo, R. Cela, J. Chromatogr. A 1190 (2008) 27–38.
[24] E. Yiantzi, E. Psillakis, K. Tyrovola, N. Kalogerakis, Talanta 80 (2010) 2057–2062.
[25] Z.H. Yang, Y.L. Lu, Y. Liu, T. Wu, Z.Q. Zhou, D.H. Liu, J. Chromatogr. A 1218 (2011) 7071–7077.
[26] Y. Zhang, H.K. Lee, J. Chromatogr. A 1274 (2013) 28–35.
[27] A. Santalad, S. Srijaranai, R. Burakham, T. Sakai, R.L. Deming, Microchem. J. 90 (2008) 50–55.
[28] S. Seccia, P. Fidente, D.A. Barbini, P. Morrica, Anal. Chim. Acta 553 (2005) 21–26.
[29] Z. Xiao, Y. Yang, Y. Li, X. Fan, S. Ding, Anal. Chim. Acta 777 (2013) 32–40.