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Analytical Methods

# Dispersive liquid–liquid microextraction combined with sweeping micellar electrokinetic chromatography for the determination of some neonicotinoid insecticides in cucumber samples

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ABSTRACT

A rapid, simple and sensitive method has been developed for the analysis of some neonicotinoid insecticides in cucumber samples by using dispersive liquid–liquid microextraction (DLLME) coupled with sweeping in micellar electrokinetic chromatography (MEKC). Under optimised conditions, the enrichment factors were achieved in the range from 4000 to 10,000. The linearity of the method was in the range from 2.7 to 200 ng $g^{-1}$ for thiacloprid, acetamiprid and imidacloprid, and in the range from 4.0 to 200 ng $g^{-1}$ for imidaclothiz in cucumber samples, with the determination coefficients ($r^2$) ranging from 0.9924 to 0.9968. The limits of detection (LODs, $S/N = 3$) ranged from 0.8 to 1.2 ng $g^{-1}$. The relative standard deviations (RSDs) at the concentration levels of 10.0 and 50.0 ng $g^{-1}$ each of the neonicotinoid insecticides in cucumber samples varied from 3.8% to 6.3%. The developed method has been successfully applied to the analysis of the neonicotinoid insecticides in cucumbers with a satisfactory result.



## 1. Introduction

Neonicotinoid insecticides are a relatively new group of active ingredients with novel modes of actions. These insecticides are active against numerous sucking and biting pests and insects, including whiteflies, aphides, beetles and some lepidoptera species as well (Meienfisch, Brandl, Kobel, Rindlisbacher, & Senn, 1999; Tomizawa & Casida, 2005). 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 (Muccio et al., 2006). They could give rise to serious risks for the health and safety of the consumers of the agricultural products due to their distribution on large areas of agricultural land. The amended European Union legislation has set the maximum residue limits (MRLs) for neonicotinoid insecticides in different agricultural products. The MRLs for fruit, vegetable and cereals were between 0.1 and 1.0 mg $kg^{-1}$ (Commission Directive 2007/11/EC). Therefore, the evaluation and monitoring of trace levels of these insecticides in vegetables is necessary and demands highly efficient, selective and sensitive analytical techniques.

Different analytical techniques, including liquid chromatography–mass spectrometry (LC–MS) (Muccio et al., 2006; Obana, Okihashi, Akutsu, Kitagawa, & Hori, 2003; Seccia, Fidente, Montesano, & Morrica, 2005), liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Radišić, Grujić, Vasiljević, & Laušević, 2009; Xiao, Li, Wang, Shen, & Ding, 2011), high-performance liquid chromatography with diode-array detection (HPLC-DAD) (Obana, Okihashi, Akutsu, Kitagawa, & Hori, 2002; Watanabe, Baba, & Eun, 2007; Wu et al., 2011), gas chromatography-mass spectrometry (GC–MS) (Rossi, Sabatini, Cenciarini, Ghini, & Girotti, 2005), and enzyme-linked immunosorbent assays (ELISAs) (Watanabe, Miyake, Baba, Eun, & Endo, 2006) have been reported for the determination of neonicotinoid insecticides in various types of samples. More recently, capillary electrophoresis (CE), because of its high separation efficiencies, short analysis time, small sample consumptions and low operation cost, has also been used for pesticide residue analysis (Zhu & Lee, 2001). However, when the most popular CE photometric detector is used, the main disadvantage of CE is its poor concentration sensitivity due to the short optical length of the capillary (Simpson, Quirino, & Terabe, 2008). This shortcoming has prevented CE from being more widely used for pesticide residues analysis. To overcome this sensitivity problem, several on-line preconcentration strategies, with the advantages of simplicity and economy, have been developed to increase the sensitivity of CE, such as field amplification (Chien & Burgi, 1992), dynamic pH junction (Britz-McKibbin, Bebault, & Chen, 2000), transient isotachophoresis (tITP) (Beckers & Boček, 2000) and sweeping (Quirino & Terabe, 1998). Sweeping is an effective on-line sample concentration technique in micellar electrokinetic chromatography (MEKC). It consists of the introduction of a large sample zone prepared in a matrix devoid of pseudostationary phase, wherein the analytes are picked-up and accumulated by the pseudostationary phase that

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penetrates the sample. This technique has been successfully applied for the on-line preconcentration of aromatic amines (Quirino, Iwai, Otsuka, & Terabe, 2000), phenoxy acid herbicides (Quirino, Terabe, Otsuka, Vincent, & Vigh, 1999), quaternary ammonium herbicides (Núñez, Kim, Moyano, Galceran, & Terabe, 2002), triazine herbicides (da Silva, de Lima, & Tavares, 2003), phenolic compounds (Huang, Lien, & Huang, 2006), carbamate pesticides (Zhang et al., 2010) and other pesticides (Breadmore, Dawod, & Quirino, 2011; El Deeb, Iriban, & Gust, 2011; See, Hauser, Ibrahim & Sanagi, 2010).

Prior to the instrumental determination of the residues, extraction and preconcentration of the sample is often required. For the preconcentration and cleanup of the neonicotinoid insecticides, liquid–liquid extraction (LLE) (Watanabe et al., 2006) and solid-phase extraction (SPE) (Obana et al., 2002, 2003; Seccia et al., 2005; Muccio et al., 2006; Watanabe et al., 2007) are the most commonly used techniques. However, LLE suffers from the disadvantage of requiring large amount of both samples and toxic organic solvents. SPE techniques typically require reduced amounts of organic solvents relative to LLE, but SPE sometimes suffers from analytes breakthrough when large sample volumes are analysed. Moreover, both techniques are tedious, time-consuming and expensive. To overcome these shortcomings in LLE and SPE, in recent years, extensive efforts have been made to the development of new sample preparation techniques that can save time, labour and solvent consumption and, therefore, can improve the analytical performance of the procedure. Dispersive liquid–liquid microextraction (DLLME), which was first reported by Rezaee and co-workers in 2006 (Rezaee et al., 2006), can overcome some of the above-mentioned limitations with the advantages of simplicity of operation, rapidity, low cost and high enrichment factor (Fattahi, Assadi, Hosseini, & Jahromi, 2007; Nagaraju & Huang, 2007; Wu, Wang, Liu, Wu, & Wang, 2009). DLLME is based on the formation of the fine droplets of an extractant in an aqueous sample solution when a water-immiscible extraction solvent (extractant) dissolved in a water-miscible organic dispersive solvent is rapidly injected into the aqueous sample solution. The analytes in the sample solution are extracted into the fine droplets, which are further separated by centrifugation, and the enriched analytes in the sedimented phase are determined by either chromatographic or spectrometric methods. However, until now, there are very few literature reports about the applications of DLLME in combination with capillary electrophoresis for the analysis of organic pollutants in real samples. Therefore, the exploration of the potential applications of the DLLME technique in combination with CE for the analysis of more complex matrix samples, such as fruits and vegetables, is very desirable.

Previously, we have reported a new strategy to apply DLLME procedure with sweeping MEKC (DLLME-sweeping-MEKC) for the analysis of some carbamate pesticides in apples (Zhang et al., 2010). In continuation to our previous endeavours, herein, we explore the DLLME-sweeping-MEKC method for the determination of some neonicotinoid insecticides in cucumber samples. Thiacloprid, acetamiprid, imidaclothiz and imidacloprid, which are most widely used in the local area, were selected as the analytes. As a result, the sensitivity of the analysis was much improved and satisfactory analytical results were achieved.

## 2. Experimental

### 2.1. Reagents, chemicals and materials

Thiacloprid, acetamiprid, imidaclothiz and imidacloprid (all >99%) were purchased from Agricultural Environmental Protection Institution (Tianjin, China). Sodium dodecyl sulphate (SDS) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Boric acid ($H_3BO_3$), hydrochloric acid (HCl), sodium hydroxide (NaOH, 98%), acetonitrile, acetone, ethanol and methanol (HPLC-grade) were from Sinopharm Chemical Reagent Co., (Beijing, China). Dichloromethane ($CH_2Cl_2$), chloroform ($CHCl_3$), carbon tetrachloride ($CCl_4$), 1,2-dichloroethane ($C_2H_4Cl_2$), 1,2-dichlorbenzene ($C_6H_4Cl_2$) and chlorobenzene ($C_6H_5Cl$) were purchased from Beijing Chemical Reagent Co. (Beijing, China). All the solvents were filtered through a 0.45 μm MicroScience membrane filter from Tianjin Automatic Science Instrument Co., (Tianjin, China). The pH of $H_3BO_3$ solutions was adjusted with 1.0 mol $l^{-1}$ HCl. The background solution (BGS) was newly prepared everyday and sonicated for 5 min prior to use. Cucumber samples were purchased from local supermarket (Baoding, China).

The mixture stock standard solution containing 10.0 μg $ml^{-1}$ each of the neonicotinoids was prepared in methanol and stored in glass-stoppered bottles at 4 °C. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with 150 mmol $l^{-1}$ $H_3BO_3$ (pH 4.7) after dryness under a stream of nitrogen.

### 2.2. Apparatus

All CE experiments were performed on a Beckman P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA, USA) equipped with an auto sampler and a diode array detector (DAD). An uncoated fused-silica capillary (Yongnian Ruifeng Optical Fibre Factory, Hebei, China) of 50 cm (effective length, 40 cm) ×75 μm i.d was used throughout the experiments. All of the operations were computer-controlled using Beckman P/ACE MDQ 32 karat software.

### 2.3. Sample preparation

After homogenisation with a laboratory homogenizer, a 20.0 g portion of the homogenised cucumber sample was accurately weighed, put into a 20-ml centrifuge tube and diluted to 20.0 ml with double-distilled water. Then, the sample was centrifuged at 3500 rpm for 10 min. A 5.0 ml aliquot of the above supernatant was then transferred to a 10.0 ml screw cap glass tube with conic bottom. Then, 0.8 ml of acetonitrile (as dispersive solvent) containing 100.0 μl of $CHCl_3$ (as extraction solvent) was rapidly added into the tube. After vortexing for 1 min, a cloudy solution that consisted of very fine droplets of $CHCl_3$ dispersed into the aqueous sample was formed, and the analytes were extracted into the fine droplets. After centrifugation at 3500 rpm for 5 min, the $CHCl_3$ phase was sedimented at the bottom of the centrifuge tube. The sedimented phase (about 90 μl) was completely transferred to another 1.0 ml conical bottom vial using a 100.0 μl microsyringe, 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) for CE analysis.

### 2.4. General electrophoresis procedure

New capillaries was conditioned prior to use with 0.1 mol $l^{-1}$ NaOH (10 min), water (10 min), methanol (10 min), and water (5 min). To ensure repeatability, the capillary was flushed between consecutive analyses with 0.1 mol $l^{-1}$ NaOH at 20 psi for 3 min, then with double-distilled water for 3 min, and finally with the BGS for 5 min.

For sweeping, sample was prepared in 150 mmol $l^{-1}$ $H_3BO_3$ (pH 4.7). The BGS was 50 mmol $l^{-1}$ $H_3BO_3$ (pH 2.0) containing 80 mmol $l^{-1}$ SDS and 25% methanol. The sample was introduced into the capillary by hydrodynamic injection at 0.5 psi for 90 s. Electrophoresis was performed at a constant voltage of −20.0 kV