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Application of Box–Behnken design to optimize multi-sorbent solid phase extraction for trace neonicotinoids in water containing high level of matrix substances

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ABSTRACT

Extensive use of neonicotinoid insecticides has raised great concerns about their ecological risk. A reliable method to measure trace neonicotinoids in complicated aquatic environment is a premise for assessing their aquatic risk. To effectively remove matrix interfering substances from field water samples before instrumental analysis with HPLC/MS/MS, a multi-sorbent solid phase extraction method was developed using Box-Behnken design. The optimized method employed 200 mg HLB/GCB (w/w, 8/2) as the sorbents and 6 mL of 20% acetone in acetonitrile as the elution solution. The method was applied for measuring neonicotinoids in water at a wide range of concentrations (0.03–100 μg/L) containing various amounts of matrix components. The recoveries of acetamiprid, imidacloprid, thiacloprid and thiamethoxam from the spiked samples ranged from 76.3% to 107% while clothianidin and dinotefuran had relatively lower recoveries. The recoveries of neonicotinoids in water with various amounts of matrix interfering substances were comparable and the matrix removal rates were approximately 50%. The method was sensitive with method detection limits in the range of 1.8–6.8 ng/L for all target neonicotinoids. Finally, the developed method was validated by measurement of trace neonicotinoids in natural water.

1. Introduction

Since the first neonicotinoid, imidacloprid, was commercialized in the early 1990s, the demand for neonicotinoid insecticides has rapidly increased all over the world. Nowadays the seven patented neonicotinoids have gained approximately one fourth of the total insecticide market [1]. With systemic activity, neonicotinoids have been extensively used as granules or seed-coatings to be buried into the soil during crop planting and dominated approximately 80% of the market share for seed treatment [1]. The intensive use of neonicotinoids has shown to endanger the pollinator species [2–5]. Besides, neonicotinoids tend to move into surface water and pose a risk to aquatic organisms as a consequence of their high solubility in water and persistence in soil (Table S1 in the Supplementary Data, "S" represents figures and tables in the Supplementary Data thereafter) [6].

Neonicotinoids have been frequently detected in freshwater environment at sub-μg/L to μg/L levels and reached hazardous levels in some areas [7,8]. As the most extensively used neonicotinoids in the world, imidacloprid was found in 89% of surface water samples which were collected from agricultural regions in California, the U.S., with the highest concentration of 3.29 μg/L [9]. Anderson et al. [7] found that the concentrations of thiamethoxam and acetamiprid in playa lakes in Texas, the U.S. were as high as 225 and 44.1 μg/L, respectively, which exceeded the benchmarks for protecting aquatic organisms in most countries [6].

Most of studies so far on the occurrence and risk of neonicotinoids in freshwater ecosystems were performed in the developed countries. On the other hand, neonicotinoids have also been intensively applied in the developing countries. Jin et al. [10] reported that acetamiprid was one of the three most widely used insecticides in China, but little information was available on environmental occurrence of neonicotinoids in this country. Lacking an effective method to measure trace neonicotinoids in complicated aquatic environment with high level of matrix interfering substances is one of the reasons.

Solid phase extraction (SPE) was the most commonly used sample preparation method for analyzing neonicotinoids in water and hydrophilic-lipophilic balance (HLB) [11–13] and C18 [14,15] have been previously used as SPE sorbents. Although these single-sorbent methods

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worked well for concentrating neonicotinoids from water which contained relatively low level of organic matters, they failed to effectively remove matrix components in dirty water, such as pigments. Unfortunately, severe matrix effects may interfere instrumental analysis of trace neonicotinoids [16,17]. Compared with the developed countries, freshwater systems, e. g. rivers and lakes, in the countries which are undergoing rapid development, such as China, were more severely deteriorated [18,19]. For example, most streams in Guangzhou, China were highly polluted and most water quality parameters, like dissolved oxygen, chemical oxygen demand, biochemical oxygen demand, total nitrogen and total phosphorus, frequently exceeded the threshold values for the worst Grade V water quality standards of 2, 40, 10, 2 and 0.4 mg/L, respectively [20]. The presence of abundant interfering materials may obstruct the measurements of trace neonicotinoids in these types of water samples [16].

The main objective of the current study was to develop a method for analyzing trace neonicotinoids in water with high level of matrix components using high performance liquid chromatography/ mass spectrometry/ mass spectrometry (HPLC/MS/MS) detection after multi-sorbent SPE. A response surface methodology (RSM) was employed to optimize the SPE conditions to gain high recovery of target analytes while minimizing matrix effects. As a collection of statistical techniques, RSM describes interactive effects among variables and obtains the optimal value for each variable [21]. Box-Behnken design (BBD) is one of RSM experimental designs and is based on three-level factorial designs with its experimental points being located on a hypersphere equidistant from the central point [22,23]. After optimization with a three-level-three-factor BBD, the developed SPE method was validated by assessing neonicotinoids in both laboratory-spiked and field-collected water samples.

2. Materials and methods

2.1. Chemicals and reagents

Neat compounds of acetamiprid, imidacloprid, thiacloprid and thiamethoxam were purchased from Shanghai Pesticide Research Institute (Shanghai, China) and dinotefuran and clothianidin were bought from Dr. Ehrenstrofer Gmbh (Augsburg, Germany). Deuterated surrogate standards (acetamiprid-d_3 and clothianidin-d_3) and internal standards (imidacloprid-d_4 and thiamethoxam-d_3) were gained from CDN Isotopes (Quebec, Canada). All the compounds had a purity of greater than 98% as indicated by the manufacturers. Physicochemical properties of target neonicotinoids are presented in Table S1.

The HLB and graphic carbon black (GCB) sorbents were purchased from Agela Technologies Company (Tianjin, China), and HPLC grade methanol and acetonitrile were obtained from Oceanpak Alexative Chemical, Limited (Gothenburg, Sweden). Analytical grade acetone was bought from Tianjin Chemical Reagent Factory (Tianjin, China) and redistilled before use. The HPLC/MS grade acetonitrile was used as the mobile phase in HPLC/MS/MS analysis and was purchased from Merck Corporation (Darmstadt, Germany).

2.2. Method development

To analyze neonicotinoids in water, both instrumental analysis (HPLC/MS/MS) and extraction conditions (multi-sorbent SPE) were optimized.

2.2.1. Instrumental analysis (HPLC/MS/MS)

The target compounds were analyzed on a Shimadzu DGU-30A LC coupled to an AB SCIEX TRIPLE QUAD™ 5500 tandem MS system [13]. The separation of analytes was achieved using an Agilent Zorbax Eclipse Plus C18 column (100×2.1 mm i.d., 1.8 μm), and the column temperature was at 40 °C. Isocratic elution was performed with a mixture of water containing 0.1% formic acid (A) and acetonitrile (B) as the mobile phase and the flow rate was 0.3 mL/min. A gradient with the A: B ratio was used as following: 0 min, 63/37; 3 min, 30/70; 5 min, 30/70; 5.1 min, 63/37; 8 min, 63/37. The MS system was operated with an electrospray ionization (ESI) in positive mode and the multiple-reaction-monitoring (MRM) transitions were used in data collecting. The MS parameters were as follows: source temperature: 550 °C; curtain gas (CUR): 40 psi; collision gas (CAD): 7 psi; ionspray voltage (IS): 5500 V; ion source gas1 (GS1): 55 psi; ion source gas 2 (GS2): 55 psi; entrance potential (EP): 10 V; collision cell exit potential (CXP): 16 V. The injection volume was 2 μL and the total run time was 8.1 min. Quantification of target insecticides was achieved using an internal standard calibration method and the calibration curve for individual neonicotinoids was linear over a range of 1–500 μg/L. To check the sensitivity of the developed HPLC/MS/MS method for analyzing neonicotinoids, instrumental detection limits (IDLs) were calculated to gain a signal-to-noise (S/N) ratio of 3.

2.2.2. Optimization of extraction conditions (BBD)

Neonicotinoids were extracted from the water samples using home-packed SPE cartridges with a mixture of two sorbents and the total mass of sorbents was 200 mg. Our previous study [13] found SPE with HLB only worked well for most neonicotinoids, but failed to recover dinotefuran from water, calling for the use of multiple SPE sorbents. Li et al. [24] reported that GCB sorbent in combination with other sorbents can reduce matrix enhancement effects during instrumental analysis. Considering the complex water environment in China and the capacity of GCB sorbent to remove large molecular interferences, e. g. pigments [24,25], GCB was selected as another sorbent, in addition to the commonly used HLB sorbent. Before loading the samples, the SPE cartridges were conditioned with 3 mL of methanol and 10 mL of water, sequentially. Water samples were then passed through the cartridges at a flow rate of 3–5 mL/min and the target analytes were finally eluted out of the cartridges with the chosen eluents.

To achieve good recovery for the analytes but minimize the co-extracted matrix components, three-level-three-factor BBD was used to optimize SPE conditions for neonicotinoids in water using the software Design Expert 8.0.6 (Stat-Ease, Inc., MI, USA). As the critical variables, the type of sorbent (X_1), and the type (X_2) and volume (X_3) of eluent would significantly affect extraction efficiency, so they were optimized to achieve the highest recovery (Y). A total of 17 experiments were conducted following the BBD experimental design and the original data were processed using Microsoft Excel 2010 (Microsoft Inc., Washington DC, USA). The designated coded values for the variables, −1, 0 and 1, were used to represent low, middle and high levels, respectively, and the coded and actual levels of the independent variables are listed in Table 1.

In the BBD experimental design, the relationship between the natural and coded values was conforming to the following equation as suggested by Bezerra et al. [21].

$$X_i = (x_i - x_0)/\Delta x_i \quad (1)$$

Where X_i is a coded value of an independent variable, x_i is the natural value of an independent variable, x_0 is the natural value of an independent variable in the center point, and $\triangle x_i$ is the step change value of an independent variable.

Table 1
Experimental design levels of chosen variables.

Variables	Factor levels		
Coded level	Low (−1)	Middle (0)	High (+1)
Sorbents ratio	HLB: GCB (8:2)[a]	HLB: GCB (1:1)	HLB: GCB (2:8)
Eluent Ratio	ACE: ACN (8:2)[b]	ACE: ACN (1:1)	ACE: ACN (2:8)
Eluent volume (mL)	2	6	10

[a] HLB and GCB stand for hydrophilic-lipophilic balance and graphic carbon black, respectively.
[b] ACE and ACN represent acetone and acetonitrile, respectively.

Then, the response variable was fitted to a non-linear quadratic model (Eq. (2)) [21].

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \quad (2)$$

Where Y is the process response (recovery), β_0 is the intercept, β_i is the slope calculated from the observed experimental values for Y, β_{ii} is the quadratic effect of the independent variable X_i, and β_{ij} is the interaction effect between the two independent variables X_i and X_j.

At last, the optimum conditions of sorbent type as well as the composition and volume of the elution solvents were determined through the BBD analysis and the accurate optimal values were calculated.

2.3. Method validation

To validate extraction performance of the optimized SPE method, the accuracy (recovery) and precision (relative standard deviation, RSD) were evaluated for the target neonicotinoids in water at four concentrations of 0.03, 1, 20 and 100 μg/L. In the meantime, to evaluate the efficiency of the method to remove the matrix interfering substances, the recoveries of neonicotinoids in water containing various levels of matrices (0.5, 2.0 and 10 g matrix/L) were also validated. The performance of the newly developed multiple-sorbent SPE method was also compared with the SPE method with HLB only.

In addition to using IDLs to assess the instrumental sensitivity, the method detection limits (MDL) of the target neonicotinoids were calculated to assess the sensitivity of the optimized SPE-HPLC/MS/MS method. The MDL is defined as the minimum concentration of an analyte in water which can be quantified and reported with 99% confidence that the chemical concentration is greater than zero [25]. The MDLs were computed by analyzing neonicotinoids in water samples which were spiked with 30 ng/L of dinotefuran and 10 ng/L of other neonicotinoids. The analysis was conducted in seven replicates and the calculation was following Eq. (3) [25].

$$\mathrm{MDL} = t_{(n-1,\,1-\alpha=0.99)} \times \mathrm{SD} \quad (3)$$

Where SD is the standard deviation of replicate measurements and $t_{(n-1,\,1-\alpha=0.99)}$ represents the Student's t-value used at a confidence level of 99% with a degree of freedom of $n-1$. In the current study, as we set seven replicates, a t-value of 3.14 is used.

2.4. Method application

Three water samples from an urban stream where the water was evaluated as grade V were analyzed using the newly developed method. Samples were collected in 4-L pre-washed glass jars and transported to the laboratory immediately. Water quality parameters (conductivity, pH, dissolved oxygen and temperature) were measured for each sample. Before chemical analyses (within 48 h of collection), samples were refrigerated at 4 °C and filtered through a 0.7 μm pore size glass fiber filter (GF/F) membranes.

3. Results and discussion

3.1. Method development

Target neonicotinoids were analyzed by HPLC/MS/MS after extraction from water using SPE. To achieve good performance for analyzing neonicotinoids, both instrumental analysis and extraction conditions were optimized.

3.1.1. Optimization of HPLC/MS/MS analysis

To gain the preferable separation and sensitive detection of the target neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid and thiamethoxam) within a reasonable timeframe, gradient elution program in HPLC was optimized. All the compounds were well separated within 8 min using the conditions represented in the section of instrumental analysis (HPLC/MS/MS).

The MS system was operated with an ESI in positive mode and MRM transitions were used in data collecting. To achieve the maximum sensitivity of MS system, the ESI parameters were optimized. Standard solution of the analytes was directly injected into MS detector and the protonated molecular ions $[M+H]^+$ were recorded in full scan mass spectra. The qualification of the target analytes was confirmed by the co-monitoring of two MRM transitions from the parent ion to the most abundant daughter ions, and quantification was performed with the ion pair which had relatively higher intensity in the transitions. In addition, declustering potential and collision energy were also optimized to gain high sensitivity of MS system. The optimized MS parameters and retention time for individual neonicotinoids are listed in Table S2. The linear range of the calibration curve was 1–500 μg/L, with IDLs being from 0.26 to 0.89 μg/L for the target neonicotinoids.

3.1.2. Model fitting and Box-Behnken statistical analysis

The equations of response surface quadratic models for individual neonicotinoids during Box-Behnken analysis are shown in Table 2. As the example of thiacloprid, a second-order polynomial regression in coded form was used to fit the experimental data (Eq. (4)) and the optimum values of the factors could be estimated by solving this equation.

$$Y = 68.0 + 0.05X_1 + 1.21X_2 - 1.17X_3 + 0.34X_1X_2 + 0.83X_1X_3 - 0.77X_2X_3 - 1.80X_1^2 - 6.96X_2^2 - 5.11X_3^2 \quad (4)$$

The analysis of variance is essential to the model and the fitness of the model was verified by regression model analysis and statistical analysis of variance (ANOVA). The ANOVA analysis was used to check the significance of the variables and the results are shown in Table S3. The regression model was significant, as suggested by a F_{model} value of 5.33 and a p_{model} value of 0.019. Relative to the pure errors, the lack of fit in this model was insignificant with a p-value being 0.31. Furthermore, the goodness of the model was checked by the coefficient of determination (R^2) and adjusted coefficient of determination (AdjR^2). As shown in Table S3, R^2 and AdjR^2 were 0.87 and 0.71,

Table 2
Significant factors and the equations of response surface quadratic models of individual neonicotinoids during Box-Behnken analysis.

Compound	Significant factor	Equation
Acetamiprid	X_1X_2, X_2^2 and X_3^2	$Y = 64.7 - 1.05X_1 - 1.84X_2 - 1.59X_3 - 3.42X_1X_2 + 0.10X_1X_3 - 0.82X_2X_3 - 2.26X_1^2 - 3.92X_2^2 - 4.33X_3^2$
Clothianidin	X_1 and X_2	$Y = 36.0 - 18.5X_1 + 10.7X_2 - 0.03X_3$
Dinotefuran	X_2, X_1X_3 and X_2^2	$Y = 25.1 + 1.03X_1 + 2.09X_2 + 0.04X_3 - 1.64X_1X_2 - 6.72X_1X_3 + 0.53X_2X_3 + 0.13X_1^2 - 4.94X_2^2 - 0.03X_3^2$
Imidacloprid	NONE	$Y = 62.9 - 3.50X_1 - 0.35X_2 - 2.40X_3 - 4.11X_1X_2 - 2.00X_1X_3 + 0.30X_2X_3 - 1.78X_1^2 - 4.89X_2^2 - 4.33X_3^2$
Thiacloprid	X_2^2 and X_3^2	$Y = 68.0 + 0.05X_1 + 1.21X_2 - 1.17X_3 - 0.34X_1X_2 + 0.83X_1X_3 - 0.77X_2X_3 - 1.80X_1^2 - 6.96X_2^2 - 5.11X_3^2$
Thiamethoxam	X_3	$Y = 62.9 + 0.35X_1 + 0.92X_2 - 2.93X_3 - 1.32X_1X_2 - 2.06X_1X_3 + 0.70X_2X_3 - 0.05X_1^2 - 2.62X_2^2 - 3.03X_3^2$

X_1, X_2, and X_3 stand for sorbent type, eluent type and eluent volume, respectively.
Y represents the response (recovery).

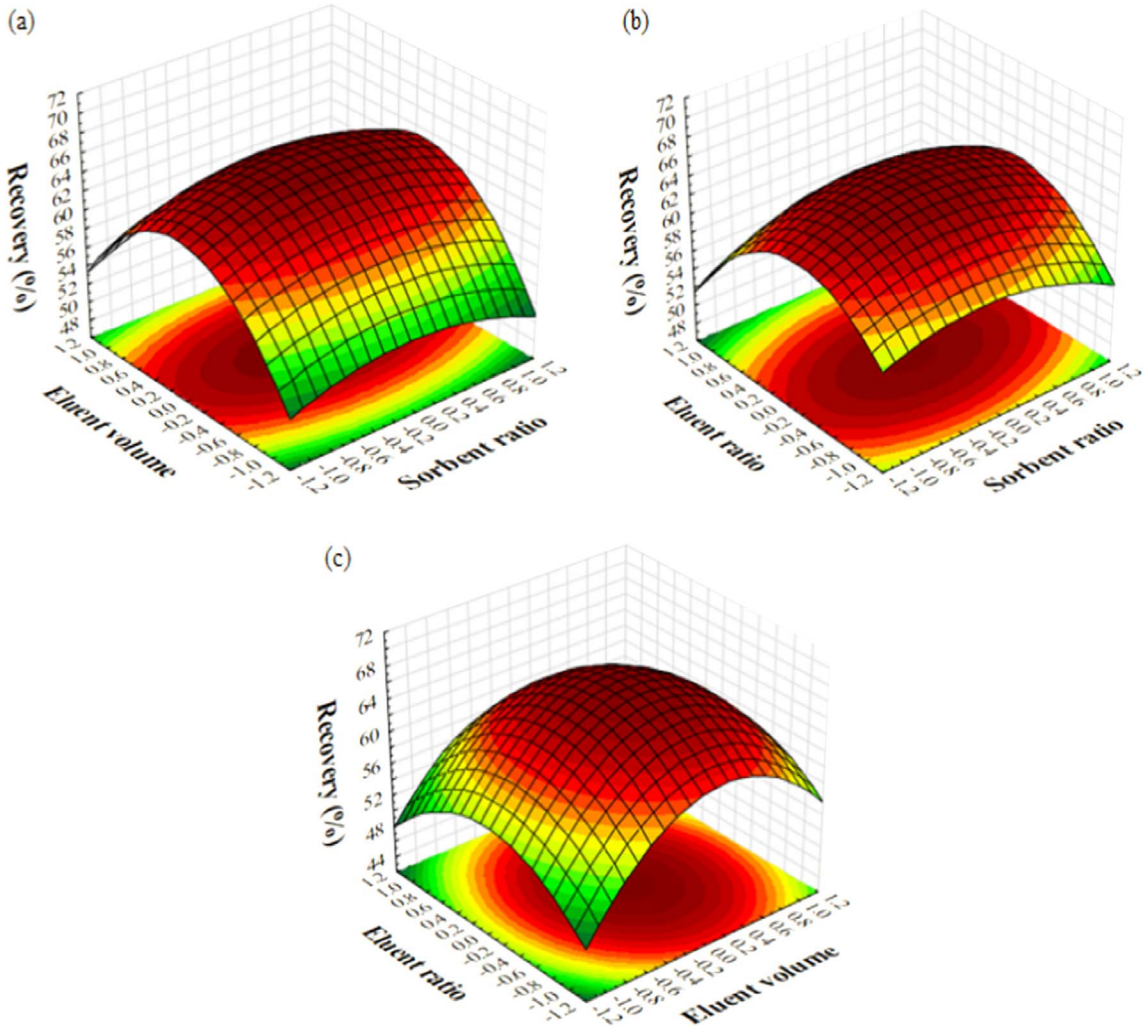

Fig. 1. Response surface plots for effects of the ratios of sorbents and eluent and eluent volume on extracting thiacloprid from water samples. (a) effects of sorbent ratio and eluent volume on recovering thiacloprid from water when the composition of the eluents (acetonitrile and acetone) was fixed at 1:1; (b) effects of the ratios of sorbent and eluent on recovering thiacloprid from water when eluent volume was fixed at zero level; (c) effects of the ratio and volume of the eluent on recovering thiacloprid from water when the sorbent ratio was held at zero level.

respectively, demonstrating that 87% of the variability could be described by the regression model. Overall, statistical analysis suggested that the experimental values fit well with the models with a good accuracy and reliability.

3.1.3. Selection of optimal conditions with response surface graphs

In order to better understand the effect of the variables on the recovery of target compounds and the interaction between variables, three-dimensional response surface (3D-RS) graphs were plotted using a statistical software STATISTICA 8.0 (StatSoft Inc., Tulsa, OK, USA). In these graphs, a factor was set at a constant level when the other two factors were changed within the experimental ranges. The undersurface of the 3D-RS plots was the contour plot, correspondingly. The advantage of the 3D-RS plots was that it can examine the effects of the experimental factors on the responses more straightforward [26]. Since 3D-RS plots were similar for all the seven neonicotinoids, only the 3D-RS plots for thiacloprid are shown in Fig. 1 and the figures for the other neonicotinoids are provided in the Supplementary Data (Fig. S1–S5).

The response surface plot in Fig. 1a showed the effects of sorbent ratio and eluent volume on recovering thiacloprid from water when the composition of the eluents (acetonitrile and acetone) was fixed at 1:1. Both the ratio of sorbents and eluent volume displayed a quadratic impact on the response. No matter what sorbent ratios were, the recovery of thiacloprid increased as soon as eluent volume increased, yet the recovery started to drop later when the volume of the eluents exceeded a certain value. On the contrary, the influence of sorbent ratio on the recovery was not significant when eluent volume was fixed at a certain value. Fig. 1b plotted the relationship among recovery, sorbent ratio and eluent ratio at a fixed eluent volume of 6 mL. Similar as Fig. 1a, eluent ratio had greater influence on the recovery of thiacloprid than sorbent ratio. Fig. 1c was the response surface plot describing the effects of the composition and volume of the eluents on the recovery of thiacloprid at a fixed sorbent ratio of HLB: GCB (*w/w*, 1/1). Both composition and volume of the eluents had a quadratic influence on the recovery with an initial increase of the recovery with increasing eluent composition and volume, but then a decrease when the variables reached a certain value.

The optimal conditions were selected for individual neonicotinoids and the recoveries were predicted from the equations in Table 2. For all insecticides, the predicted recoveries were exceeding 60%, except for dinotefuran (48%) (Table S4). The relatively lower recovery of dinotefuran than other analytes was likely because of its higher solubility in water [7]. As shown in Table S1, water solubility of dinotefuran (39,830 mg/L) was one to two orders of magnitude higher than those of other neonicotinoids, thus the chemical was more preferred to staying in water. As a result, a SPE method merely using HLB sorbent failed to extract dinotefuran from water [13]. Rather, GCB had a good

affinity for dinotefuran and increasing the fraction of GCB in the mixture sorbents enhanced recovering dinotefuran from water (Fig. S3). On the other hand, when the mass of GCB sorbent increased, the recovery of clothianidin quickly dropped (Fig. S2). The influence of HLB: GCB ratio on the recovery of the remaining four neonicotinoids was not as significant as dinotefuran and clothianidin. Overall, to recover dinotefuran from water at the same time to reduce the matrix interfering substances without significant sacrifice the recovery of clothianidin, the optimal sorbent composition of HLB/GCB (*w/w*, 8/2) was chosen.

The individual optimal conditions of clothianidin, dinotefuran and thiamthoxam which used a sorbent composition of HLB/GCB (*w/w*, 8/2) were further evaluated as the candidate conditions for extracting all neonicotinoids from water simultaneously (Table S4). The predicted recoveries of other neonicotinoids were relatively low (< 50%) under the optimal conditions for dinotefuran. Instead, all neonicotinoids had good predicted recoveries (> 60%) with an exception of dinotefuran when the optimal conditions of thiamthoxam were used. Accordingly, the optimal conditions of thiamthoxam were used as the SPE method for all neonicotinoids. That is, all neonicotinoids were extracted from water samples using SPE cartridges packed with a mixture sorbents of HLB/GCB (*w/w*, 8/2), and a mixture of acetonitrile and acetone (*v/v*, 8/2) was applied as the elution solution with a volume of 6 mL.

3.2. Method validation

In order to validate the accuracy of model prediction and check the performance of the newly developed method, the target neonicotinoids were measured in spiked water samples in triplicate under the optimal conditions. The recovery of neonicotinoids were all exceeded 80%, except dinotefuran (37.8 ± 1.3%) and clothianidin (60.3 ± 1.6%). The measurements had a good fit with the model prediction but with higher recovery values. The impacts of chemical concentrations and matrix components on extraction efficiency as well as the sensitivity of the developed method (MDLs) were also evaluated.

3.2.1. Effects of neonicotinoid concentrations on recovery

To validate the applicability of the optimized method to measure neonicotinoids in water at different concentrations, water samples (500 mL each) spiked with neonicotinoids at 0.03, 1, 20 and 100 μg/L were analyzed. As shown in Fig. 2, good recoveries were achieved for acetamiprid (81.1 ± 10.0% – 105 ± 24%), imidacloprid (76.3 ± 6.4% – 107 ± 20%), thiacloprid (76.4 ± 4.8% – 101 ± 19%) and thiamethoxam (88.5 ± 2.2% – 107 ± 7.2%) at water concentrations across four orders of magnitudes with RSDs from 1.73% to 21.4%. This showed the suitability of the method for analyzing neonicotinoids in water with a wide range of contamination levels. The recoveries were comparable with a previous study which reported the recoveries of imidacloprid, thiacloprid and thiamethoxam in water samples ranging from 87% to 97% [27].

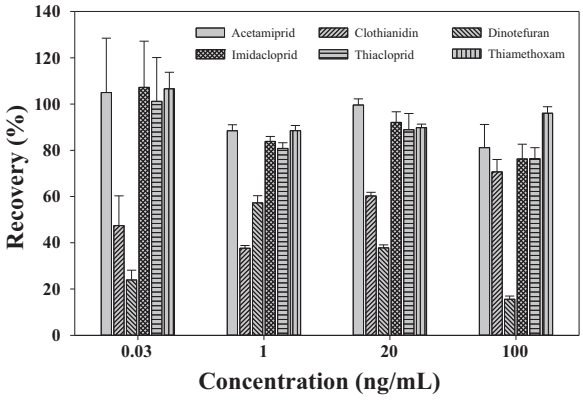

Fig. 2. Recovery of individual neonicotinoids in water at different concentrations (0.03, 1, 20 and 100 μg/L) using the optimized method. The error bars stand for standard deviations of three replicates for each sample.

On the other hand, extraction efficiency of clothianidin and dinotefuran was significantly affected by their concentrations in water (Fig. 2). The recovery of clothianidin was less than 50% when water concentrations were lower than 1 μg/L, but achieved acceptable levels of 60.3 ± 1.6% and 70.7 ± 5.4% at relatively high concentrations of 20 and 100 μg/L, respectively. Conversely, the recovery of dinotefuran decreased from 57.2 ± 3.1% to 15.6 ± 1.4% when water concentrations increased from 1 to 100 μg/L. The different concentration-dependent trends of clothianidin and dinotefuran may be explained by their differing affinity for GCB and HLB sorbents. Compared with other neonicotinoids, GCB had a stronger sorption capacity to clothianidin [28], resulting in a greater difficulty to elute clothianidin out of the SPE cartridges containing GCB. This effect of strong retention by GCB for clothianidin was more prominent when water concentrations were low. With the increase of water concentrations, more active sites of GCB sorbent were filled and higher portion of clothianidin could be eluted out of the SPE cartridge, and greater recovery could be achieved (Fig. 2). This hypothesis was supported by the observation of higher clothianidin recovery (75.4 ± 1.0% – 122 ± 1.7%) by SPE cartridges packing with HLB only [13].

On the other hand, Zhang et al. [13] found sorption capacity of HLB-only SPE cartridges for dinotefuran was extremely low and could not recover this compound from 500 mL of water samples at any spiking levels. Alternatively, almost half of dinotefuran could be recovered with the current multi-sorbent SPE method when chemical concentrations were ≤20 μg/L (Fig. 2). The results suggested that the retention of dinotefuran was only achieved by GCB while mixture sorbents of HLB and GCB were used. The interaction of GCB with contaminants was mainly on the surface of the adsorbent and was possibly saturated when chemical concentrations were high, particularly in the case of low amount of GCB being used (40 mg in the current study). Saturation of the active sites on GCB sorbent would lead to a breakthrough of dinotefuran from the SPE cartridges during the loading step. Herein, the recovery of dinotefuran decreased when the concentration of dinotefuran in water increased.

3.2.2. Effects of matrix components on extracting neonicotinoids from water

To accurately quantify trace neonicotinoids in water containing high level of interfering substances, minimizing the co-extracts was essential besides of good recovery of target analytes. Therefore, the recovery and standard deviations were evaluated for 20 μg/L of individual neonicotinoids in water with various amounts of matrix substances using the optimized method (Fig. 3). As shown in Fig. 3, increasing co-extracted matrix components had trivial impact on their recovery for most target neonicotinoids, however, the recovery of clothianidin increased with increasing matrix components in water. This served as another piece of evidence for the observed concentration-dependent recovery for clothianidin in water samples (low recovery at low concentration). Matrix components could also fill the active sites on GCB surface and reduced the strong affinity of GCB for clothianidin. As a consequence, higher recovery of clothianidin was noted in water with greater amount of matrix substances (60.3 ± 1.6% and 83.0 ± 9.8% for water samples containing 0 and 10 g/L matrix, respectively).

To validate the matrix removal rate of the newly developed method, in addition to estimating recovery of the neonicotinoids, the amounts of matrix components in the original extracts and the effluents after SPE were compared. The contents of matrix components were 104, 270 and 356 μg in the original water samples spiked with 0.5, 2 and 10 g matrix /L, respectively. After passing the SPE cartridges, matrix removal rates of the three water samples were all approximate 50%, which indicated that the newly method has superiority in removing high level of matrix components.

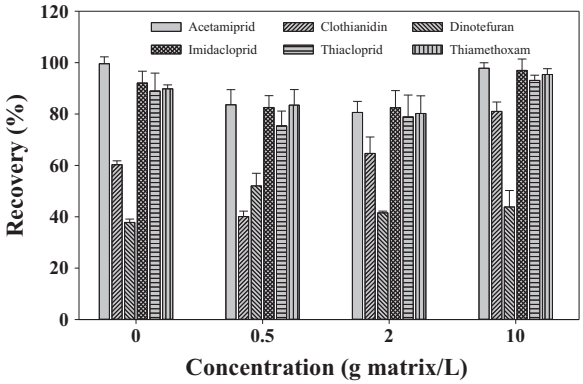

Fig. 3. Recovery of individual neonicotinoids in water with various amounts of matrix interfering substances (0.5, 2 and 10 g matrix /L) using the optimized method. The error bars stand for standard deviations of three replicates for each sample.

Table 3
Concentrations of neonicotinoids in field water samples collected from urban streams in Guangzhou, China and the method detection limits (MDLs) of individual neonicotinoids at the optimal conditions.

Insecticide	MDL[a] (ng/L)	Concentrations (ng/L)		
		S1	S2	S3
Acetamiprid	1.8	20.6	22.8	23.4
Clothianidin	4.5	ND[b]	ND	ND
Dinotefuran	6.8	ND	ND	ND
Imidacloprid	3.6	32.8	193	127
Thiacloprid	1.8	ND	ND	ND
Thiamthoxam	2.1	< MDL[c]	< MDL	< MDL

[a] MDL: Method detection limit.
[b] ND: Not detected.
[c] < MDL: Less than the MDL.

3.2.3. Method detection limits

In addition to accuracy (recovery) and precision (RSD), the sensitivity of the new method was also evaluated by determining the MDLs, and they ranged from 1.8 to 6.8 ng/L for target neonicotinoids (Table 3). The use of HPLC/MS/MS techniques which had relatively low IDLs for neonicotinoid insecticides helped increasing method sensitivity for analyzing neonicotinoids from different matrices [5,29]. Average recoveries of the target neonicotinoids at 10 ng/L (except for dinotefuran at 30 ng/L) ranged from 91.6% to 116% with RSDs of 5.26–11.5%, with an exception of clothianidin and dinotefuran, which had recoveries of 33.7% and 47.0%, respectively. The recovery of neonicotinoids at concentrations near the MDLs was similar to those observed for the respective insecticides at other dosing levels (Figs. 2 and 3). With relatively lower recovery, the detection sensitivity of clothianidin and dinotefuran (MDL of 4.5 and 6.8 ng/L, respectively) were also slightly lower than other four compounds (MDL of 1.8–3.6 ng/L). The MDLs for neonicotinoids in the current study were in the same range as those reported by Hladik and Calhoun (3.6–6.2 ng/L) [30] and lower than those by de Perre et al. (2.6–12.3 ng/L) [31], indicating that the newly developed method had a good sensitivity.

3.2.4. Application the methods in natural water samples

Eventually, three field samples were collected from urban streams in Guangzhou, China and neonicotinoids in the water samples were analyzed under the optimized conditions (Table 3). Acetamiprid and imidacloprid were detected in all the samples and their concentrations ranged from 20.6–23.4 ng/L and 32.8–193 ng/L, respectively. In addition, thiamthoxam was detectable in the water samples, but its concentrations were less than the MDL. As the sampling sites were not in an agricultural region, the source of the neonicotinoid insecticides may be originated from upper stream where vegetable planting areas located.

While the concentrations of imidacloprid in the current study were similar to those detected in Georgia, the U.S., [30] higher concentrations of acetamiprid were detected in Guangzhou, China and it was corresponded to the high demand of acetamiprid in China. Zhang [32] reported that the production of acetamiprid in the first quarter of 2012 was 905 tonnes and it has become one of the most commonly used pesticides in China [10].

4. Conclusions

In summary, the current study established a sensitive analytical method based on multi-sorbent SPE coupled with HPLC/MS/MS and the method was capable to determine neonicotinoids in natural water at environmentally relevant concentrations. Under the optimal conditions of SPE procedure, HLB/GCB mixture (*w/w*, 8/2) as the sorbents and acetonitrile/ acetone mixture (*v/v*, 8/2) as the elution solvents, the highest recoveries of the majority of neonicotinoids were achieved. The method had also been validated with a series of experiments. Good recoveries were gained at the different concentrations of acetamiprid, imidacloprid, thiacloprid and thiamethoxam ranged from 0.03 to 100 μg/L and it indicated that the newly developed method was suitable for various concentrations of neonicoinoids in polluted water. Moreover, the recoveries of neonicotinoids in water with various amounts of matrix interfering substances were also comparable and the matrix removal rates were approximately 50%. Furthermore, low MDLs (< 7 ng/L) indicated that this method had a good sensitivity. Finally, the analytical method was successfully applied for extracting neonicotinoids from field waters. The advantage of the newly developed method is that it can lessen the contamination burden to the HPLC/MS/MS system, reduce matrix effects during analysis and prolong the lifetime of the instrument.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2017.04.031.

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