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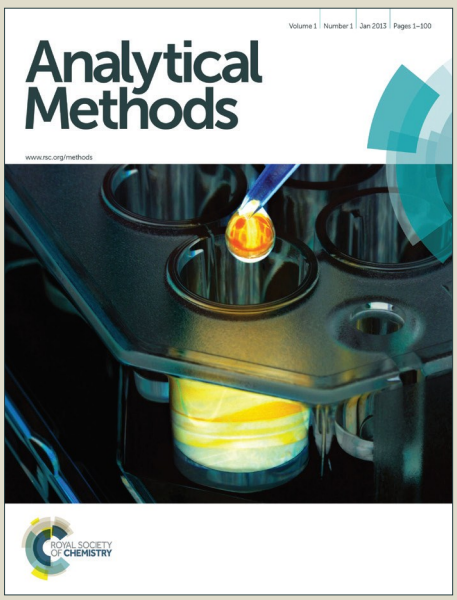

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Novel use of PVPP in a modified QuEChERS extraction for UPLC-MS/MS analysis of neonicotinoid insecticides in tea

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ABSTRACT: A rapid UPLC-ESI (+)-MS/MS method was developed and validated for simultaneous determination of eight neonicotinoid insecticides (dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid, thiacloprid and imidaclothiz) in tea based on a refined QuEChERS extraction method. In order to eliminate the matrix effect and obtain satisfactory recoveries, an inexpensive and excellent absorbent material, polyvinylpolypyrrolidone (PVPP), was used to diminish the tea polyphenols. Further, combinations of PVPP and the commonly used sorbents PSA and GCB were investigated in this study. The optimized 'quick, easy, cheap, effective, rugged and safe' protocol briefly follows. Tea sample was soaked with water and extracted with acetonitrile. Sample extracts were treated with 400 mg PVPP to remove tea polyphenols, and then cleaned up with a combination of PSA (25 mg), GCB (100 mg) and C18 (50 mg). Finally, the dried extract was dissolved with acetonitrile / water (15:85, v/v) and analyzed by UPLC-MS/MS. The recovery ratios from tea for eight neonicotinoid insecticides ranged from 60-109% at 0.01~0.5 mg kg^{-1} spiked levels. Relative standard deviations were <15.4% for all of the recovery tests. The limit of quantification were below 0.01 mg kg^{-1}. The developed method was simple, effective, and sensitive. This method should prove to be highly useful for monitoring neonicotinoid insecticides in commercial tea products.

KEYWORDS: tea leaves; matrix effect; polyvinylpolypyrrolidone; pesticide residue; 'quick, easy, cheap, effective, rugged and safe'; LC-MS/MS

Introduction

Neonicotinoids are a new class of insecticides with a distinct mode of action. They are active against numerous sucking and biting insect pests, including aphids, whiteflies, beetles, and some Lepidopteran species[1]. There are several commercialized neonicotinoids: dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid, thiacloprid and imidaclothiz, which is a new neonicotinoid insecticide produced in China and increasingly used in *Camellia sinensis* cultivation[2-5]. As highly polar compounds, neonicotinoids can be easily released from dry tea leaves into the drinkable tea infusions [2,4]. To ensure consumer health and safety, many countries and international organizations have defined temporary maximum residue levels (MRLs) for seven neonicotinoids in tea, ranging from 0.01 to 50 mg kg^{-1} [3]. In 2014, the temporary MRL for imidaclothiz (3 mg/kg) has been implemented in China [6].

Liquid chromatography, tandem mass spectrometry (LC-MS/MS) is a highly selective method when used in either ion monitoring mode or in multiple reactions monitoring mode. Despite its popularity, the technique is limited by the suppression or enhancement of analyte ionization in the electrospray ionization (ESI) source due to co-eluting compounds, known as the matrix effect[7]. [1]Although invisible in the

[1] **Abbreviations Used**

ESI, electrospray ionization; LC-MS/MS, liquid chromatography, tandem mass spectrometry; LOQ, limit of quantification; RSD, relative standard deviation; PVPP, polyvinylpolypyrrolidone; PSA, primary secondary amine; QuEChERS, quick, easy, cheap, effective, rugged and safe.

LC/MS signal, this effect very often adversely affects the accuracy and sensitivity of the method. Moreover, it has been observed that the ionization efficiency of polar compounds is more influenced by co-eluting compounds than the ionization of less polar compounds[8]. Thus, modification of the sample extraction methodology and/or improvement of chromatographic separation to remove or minimize matrix effects must be performed in order to develop a successful and robust quantitative LC-MS/MS method.

To date, single- and multi-residue analytical methods for the neonicotinoid pesticides in food have been reported using conventional HPLC and the more sensitive and accurate LC-MS[1,3,9-16]. The neonicotinoids are prime candidates for this analysis, in part due to their low volatility. Several LC-MS/MS-based methods using solid phase extraction (SPE) cleanup are available for some neonicotinoid insecticides from tea samples[17-19]. While the time-consuming and costly SPE clean-up may improve the method sensitivity, it may also increase variation and limit the scope of the target analytes. However, a QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction approach has been developed with a pretreatment method for analysis of multiple pesticides in food [2,15-16, 20-26]. PSA, GCB and C18 are commonly used absorbents in the QuEChERS method for multi-residue analysis in food matrices [2,15-16,18,27-28]. PSA absorbs polar compounds (sugars or fatty acids), GCB absorbs pigments and sterols, and C18 absorbs nonpolar compounds. However, these materials are relatively expensive. The goal of this investigation is to decrease the dosages of expensive materials or to develop more effective and economical sorbents

used in pre-extraction. Recently, an inexpensive and excellent absorbent, Polyvinylpolypyrrolidone (PVPP), has been verified by us to eliminate polyphenols in tea matrix, which is rich in polyphenols[3]. To us, PVPP had the potential to serve as an inexpensive pretreatment material that would diminish the tea matrix effect. To our knowledge, the development of a modified QuEChERS using PVPP in the extraction for analysis of pesticide residues with LC-MS/MS in tea has not been published.

The aim of the present study was to develop a simple, selective and reliable method - based on the QuEChERS extraction approach - for the determination of eight neonicotinoids using UPLC-MS/MS. In this study, PVPP and other several sorbents, which are typically used for QuEChERS sample pretreatment, were evaluated for decreasing the matrix effect and providing high recoveries of neonicotinoid residues. The goal was to find a pretreatment that balanced low matrix effect from the different kinds of tea matrix with high recoveries of each neonicotinoid residue. This research represents the first developmental trial of the modified QuEChERS method to co-recover eight neonicotinoid insecticides from tea.

Materials and method

Chemicals and reagents

Certified neonicotinoid insecticide standards dinotefuran, 98.6%; nitenpyram, 98.6%; thiamethoxam, 98.5%; clothianidin, 99%; imidacloprid, 98.0%; acetamiprid, 98.1%; and thiacloprid, 98% were obtained from Dr. Ehrenstorfer (Augsburg, Germany) while imidaclothiz, (at 100 μg mL^{-1} in acetonitrile, ACN) was purchased

from Agro-environmental Protection Institute, Ministry of Agriculture (Tianjin, China). Stock standard solutions for seven insecticides (expect imidaclothiz) were prepared in ACN at 500 μg mL^{-1}. Working standard solutions were prepared by diluting the stock solution with ACN:water (15:85). Matrix-matched calibration standards were prepared by adding to blank tea sample extracts in appropriate volumes to generate standard working solutions at six different levels (0.001, 0.005, 0.01, 0.05, 0.1, 0.2 μg mL^{-1}). Both solutions were stored at 4 ºC and protected from light. ACN was HPLC-grade (Tedia Company, OH, USA). HPLC-grade water was produced with a Milli-Q water purification system (Millipore, Bedford, MA). Polyvinylpolypyrrolidone (PVPP) was purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Graphitized carbon black (GCB, Supelclean ENVI-Carb, 120/400 mesh) were obtained from Supelco Company (Bellefonte, PA, USA). Primary secondary amine (PSA, 230~400 Mesh) and (C18, 230~400 Mesh, 60Å; SiliCycle, Canada) absorbents were obtained from Shanghai ANPEL Scientific Instrument Co., Ltd. Anhydrous Na_2SO_4 , $MgSO_4$ (dried at 550 ºC for 5 h and stored in desiccators) , $C_{14}H_4O_6KNa.4H_2O$, $FeSO_4.7H_2O$，$Na_2HPO_4.12H_2O$ and KH_2PO_4 were of analytical grades. 1.0 g $FeSO_4.7H_2O$ and 5.0 g $C_{14}H_4O_6KNa.4H_2O$ was dissolved in distilled water and made up to mark in 1000 mL measuring flask to generate the ferrous tartrate solution; 23.9 g $Na_2HPO_4.12$ H_2O was dissolved in distilled water and made up to mark in 1000 mL measuring flask to form the 1/15 mol L^{-1} $Na_2HPO_4.12H_2O$ solution; 9.0

8 g KH_2PO_4 was dissolved in distilled in water and made up to mark in 1000 mL measuring flask to form 1/15 mol L^{-1} Na_2HPO_4 solution; 85 mL of 1/15 mol L^{-1} $Na_2HPO_4.12H_2O$ solution and 1/15 mol L^{-1} of KH_2PO_4 was mixed to form the phosphate buffer (pH 7.5).

Green, black and oolong tea samples that tested negative for pesticide residues were used to create blank and spiked samples for recovery assays and to generate matrix-matched standards for calibration in the experiments. Samples for the monitoring study were tea samples collected from local markets in Hefei.

Sample preparation

Tea samples were ground with a pulverizer (A11, IKA, Germany) and sized by 50 mesh sieve. A 1.0 g aliquot of sieved sample was weighed into a 50 mL centrifuge tube and soaked with water (2.0 mL) for 30 min before ACN (20 mL) was added. The mixture was homogenizeed for 1 min then allowed to rest for 10 min. A 5 mL aliquot of the supernatant was obtained by filtration (through Whatman No.1 paper) into a 35 mL centrifuge tube.

To this extract, 2 g of anhydrous sodium sulfate and 400 mg of PVPP were added. The sample was shaken by vortex for 2 min and then centrifuged at 8000 rpm for 5 min. An aliquot (2.0 mL) of the extract, equivalent to 0.1 g of sample, was transferred into a 5 mL centrifuge tube to which 25 mg of PSA, 100 mg of GCB, 50 mg of C18 and 150 mg of anhydrous $MgSO_4$ had been added. The mixture was shaken by vortex for 2 min before a 1 mL aliquot of the supernatant was evaporated to nearly dryness with a nitrogen evaporator (N-EVAP, Organomation, USA) at 40 °C.

The residue was dissolved in 0.5 mL ACN:water (15:85, v/v) before being passed through a 0.22 μm pore size filter membrane (Millipore, Billerica, MA). This test solution, equivalent to 0.1 g of sample, was ready for injection into LC-MS/MS.

Investigation the abilities of PVPP and PSA to diminish polyphenols: Different amount of PVPP or PSA and 2 g of anhydrous sodium was added to (25, 50, 75, 100，200, 300, 400, 500 mg) 5 mL aliquot tea extract (prepared according to the method 2.2, sect.1) separately. The sample was shaken by vortex for 2 min and then centrifuged at 8000 rpm for 5 min. 1 mL aliquot of the supernatant was added to 25 ml measuring flask, then 4 ml water and 5 ml ferrous tartrate solution was added and mixed well. After this, the flask was made up to the mark with phosphate buffer (pH 7.5). The absorbance of the mixture at 540 nm was measured against the reagent as blank. The weight of the polyphones in tea extract was calculated according to the method of ferrous tartrate method (GB/T8313-2002) [29], as follows:

$$\text{The weight of polyphones} = A \times 1.957 \times 5 \times 10^3 \text{ (mg)} \qquad (1)$$

LC-MS/MS analysis.

The extracts were analyzed on an Agilent Series 1290 ultra performance liquid chromatography system (UPLC), consisting of a quaternary pump with a vacuum degasser, a thermostatted column compartment, and an autosampler. The mass analyzer was a triple quadrupole Mass Spectrometer (QQQ; Agilent Technologies, Palo Alto, CA, USA) operating in positive ion mode. A Waters HSS T3 column (particle size: 1.8 μm, Length: 100 mm and internal diameter: 2.1 mm) was used at a flow rate of 0.2 mL min^{-1}. The column compartment temperature was set at 40 ºC.

The injection volume was 10 μL. The mobile phase consisted of 5 mM ammonium formate in water (phase A) and 100% ACN (phase B). During elution, the gradient of ACN increased linearly from 15 to 38% over 10 min, and then decreased back to 15% by min 12. Mass spectra were acquired using electrospray ionization (ESI) in the positive ionization mode over the range of m/z 50 to 500. The settings were: a drying gas flow of 6 L min^{-1} with a drying gas temperature of 325 ºC, nebulizer pressure of 45 psi, sheath gas temp of 350 ºC, sheath gas flow of 11.0 L min^{-1}, and capillary voltage of 3364 V.

Analysis of the insecticides was performed in multiple reactions monitoring (MRM) mode. For each insecticide, at least one precursor ion and two fragment/product ions were monitored. The most abundant product ion was selected for quantification and the second most intense ion for qualification. The quantification (MR1) and qualification ion transitions (MR2) of the respective insecticides and the optimum collision energies [(collision energy 1 and collision energy 2 cell acceleration voltage were programmed **(Table 1)**] were acquired and processed using MassHunter software.

Matrix effect.

The matrix effect (ME), used to describe the analyte ionization efficiency, was expressed as the signal from the insecticide in matrix compared to the signal in solvent (% ME), calculated as follows:

$$ME\ (\%) = [(\text{area of post-extraction spiked}/\text{area of standard})-1] \times 100 \quad (2)$$

To supplement the analysis, matrix effects were also assessed by comparing the slopes of six-point, matrix-matched calibration (MMC) curves with the slopes of calibration curves in the solvent, calculated as follows:

$$ME_s\ (\%) = [(\text{slope in matrix/slope in solvent})-1)] \times 100 \quad (3)$$

A mean suppression or enhancement effect (SSE) of less than 20% was considered a soft matrix effect. Matrix effects in that range are low enough to be treated as negligible. An SSE in the range of >20% but <50% was considered a medium matrix effect. Strong matrix effects were in the range of enhancement/suppression >50% [30].

Method performance

The analytical method optimized for tea was validated using spiked blank tea samples. Several tea samples were analyzed in advance to obtain a sample that was free of analyte at the particular retention time (tR) of the analyte. Validation parameters assessed were linearity, recovery and limit of quantification (LOQ).

Linearity was evaluated using MMC curves generated by spiking blank samples of green, oolong and black tea at six concentration levels (0.001~0.2 μg mL^{-1}). Peak area was used as analyte response. Calibration curves were constructed by plotting the peak areas (y) versus the concentration of analytes (x) and the determination coefficients (R^2) for each insecticide. Calculations were performed on the average peak areas (n=3).

The sensitivity and precision of the method were evaluated by use of spiked blank tea samples. LOQs were established at the value more than 10 times the background

noise of the spiked blank sample at the retention time of each pesticide. Recoveries and relative standard deviations (RSD) were determined for six replicates at three concentration levels (0.01, 0.05 and 0.5 mg kg^{-1}). The recovery rate was quantified by addition of known levels of external standards to blank sieved sample. Spiked sample was allowed to stand for 0.5 h before extraction.

Result and Discussion

Optimization of LC-MS/MS conditions.

The ESI source was tuned for each insecticide by introducing the analyte (0.5 µg mL^{-1}) into the mass spectrometer through direct infusion via a syringe pump at a flow rate of 10 µl min^{-1}. In the tuning mode, the molecular ion $[M+H]^+$ for the first quadrupole, Q1, and for scanning at Q3 were optimized. Two characteristic fragment ions were selected for the Q3 for each analyte. The quantification (MR1) and qualification ion transitions (MR2) of the respective insecticides and the collision energies were optimized for the pair ions in the MRM mode for all the tested analytes. Apart from the selection of two fragment ions, the relative ion intensity (peak area secondary ion/peak area primary ion*100) [31] of the two transitions was additionally assessed to an identification criteria. The relative ion intensities of the standards were compared with that of matrix samples. Optimized MS conditions are summarized in **Table 1**.

Although MS/MS can discriminate neonicotinoid analytes without chromatography, the LC elution was optimized to improve separation of the tested

compounds. In reports of neonicotinoid insecticide analytical methods, good separation of these neonicotinoids was achieved when the mobile phase was modified by formic acid [15-17,19, 33] or, in a few QuEChERS-based analytical method, ammonium formate [22,32]. Different mobile phases were compared in our test (**Fig. 1**). In the initial stages of method development, the mobile phase (A) was water containing 0.1% or 0.3% (v/v) formic acid. The ionization of most of the neonicotinoids in tea matrix was either not obviously changed or was decreased when formic acid was added to phase A. However, in phase A containing formic acid, the signal of the nitenpyram fragment ion (m/z, 237, data not shown) was 0.07 times as high and that of fragment ion (m/z, 224) was 1.2 times as high as those using purified water. Interestingly, when 5 mM ammonium formate was added to phase A, the signals of all neonicotinoids in MRM were strongly increased, from 2.2 times for nitenpyram to 13.7 times for imidaclothiz. In addition, the peak uniformity was also improved. When formic acid and 5 mM ammonium formate were both added to Phase A, the signals of all eight neonicotinoid insecticides were suppressed slightly. Hence, a mobile phase based on water with 5 mM ammonium formate was selected. As shown in Fig. 2, all the insecticides were eluted with good separation and MS sensitivity in a gradient run of 12 min.

Extraction solvent selection and evaluation of cleanup

In multi-residue determination methods, the most critical step is the optimization of the extraction and clean-up procedure, especially for complex matrices such as tea, which is rich in polyphenols, flavonoids, and alkaloids[3]. ACN is commonly used for the extraction of residues of neonicotinoid insecticides in tea[3-4,16-17,19]. The main

difference between these reported extraction procedures using ACN is whether the tea was presoaked or not in water before extraction. Our previous study [3,4] had verified that tea sample soaked in water for 30 min before extraction with ACN yielded neonicotinoid insecticide recoveries several times higher than from samples that were not soaked. However, while all neonicotinoid insecticides showed excellent recoveries, the signals were suppressed in UPLC-MS/MS because the coextractives also increased several times in the soaked sample. An SPE methods had been used to clean up the tea extract in our previous studies [3,4] and in several other reports [19,21]. But these SPE cartridges are expensive, time-consuming, and use a large volume of solvent for cleanup procedures. However, these absorbents have not been used to develop a QuEChERS method exclusively for eight neonicotinoid insecticides analysis in tea matrix. In this paper, these absorbents were tested in combination with the inexpensive and excellent absorbent PVPP, which has been used to eliminate polyphenols from tea[3]. In order to save the more expensive absorbents PSA and GCB, different amounts of PVPP were first added to eliminate polyphenols, which cause the main disturbance in tea extract. Spiked tea samples (0.05 mg kg^{-1}) were soaked with water and extracted with ACN. PVPP (100, 200, 300, 400, or 500 mg, six replicates) was added to the extract and processed as described in the methods. The prepared extract test solutions and insecticide standard samples (with three replicates) were analyzed with LC-MS/MS. The matrix effect values were calculated using equation (2). The PVPP pretreatment of the spiked tea samples lowered the matrix effects on the eight neonicotinoid insecticides as evaluated by the quantification ions in MRM

analysis (**Fig. 3)**. The peak response of each insecticide spiked into tea extract increased as the amount of PVPP increased from 100 to 400 mg. For five of the insecticides there was no obvious change as PVPP increased from 400 to 500 mg. The average recoveries of the eight insecticides when treated with PVPP were all higher than 95%. However, increased amounts of PVPP absorbed most of the extract solution, making it difficult to separate enough supernatant for the next step. Therefore, 400 mg of PVPP was used in our developing method.

To further diminish the effects of pigments and polar compounds in tea extract, different amounts of GCB and PSA were tested. Different amounts of GCB (0, 25, 50, 75, 100, 125, 150 mg) were preliminarily tested for pigment absorption in tea extract. When more than 50 mg of GCB was added, the dark green color of the PVPP-treated extract changed to clear (**Fig. S1**). Therefore, the addition of 50, 100, and 150 mg of GCB was further compared by LC-MS/MS analysis. When the amount of GCB was increased from 100 mg to 150 mg, the signals of the eight insecticides was not obviously enhanced, so 100 mg GCB was used in our proposed method **(Fig. 4A)**. PSA was tested in the range of 25 to 125 mg. The addition of 25 mg PSA enhanced the average signals of the eight insecticides **(Fig. 4B)**. The peak response signals did not obviously change as PSA increased from 25 to 125 mg. Therefore 25 mg of PSA was used in our proposed method.

The average recoveries of the eight insecticides in different PSA and GCB treatment groups were all higher than 95%. To further investigate the abilities of

PVPP and PSA to diminish polyphenols in tea extract, different amounts of PVPP and PSA were added to tea extract and the weight of the polyphenols were calculated according to equation (1). The amount of polyphenols in the tea extract decreased when the PSA increased from 25 mg to 500 mg. When 500 mg PSA was added, 15.3 mg polyphenols still remained in the extract **(Fig.S2)**. When more than 200 mg PVPP was added, the polyphenols in tea extract was completely diminished. Because the cost of PVPP is much lower than that of PSA and because a smaller amount of PVPP resulted in better polyphenol removal, PVPP was favored for pretreatment in our proposed method. This study also shows that PVPP could be used in a cleanup procedure for the determination of pesticides containing a P=O group, such as omethoate, which are prone to adsorbing onto PSA [34].

Evaluation of matrix effect

Matrix effects are common problems that occur when using LC-MS or MS/MS and have an adverse effect on the analytical results. The response of the target compound can be enhanced or suppressed due to the interfering matrix components, which is commonly known as signal suppression/enhancement effect (SSE). The matrix effects from different kinds of tea on the 8 neonicotinoids (spiked level, 0.05 mg kg^{-1}) are shown in **Fig. 5**. The signal suppression effect was prominent for six of the insecticides, with suppression as high as 44-61% for nitenpyram, clothianidin and imidaclothiz, in three matrices. Analyte/solute combinations resulting in moderate MEs were acetamiprid and thiacloprid in all three kinds of tea, imidacloprid in black and oolong tea, and thiamethoxam in black tea. Imidacloprid showed the highest SD

of matrix effect values (21%), while the MEs of the other insecticides had SDs lower than 7%. This result might indicate that imidacloprid is differentially affected by different matrices, although this would need to be investigated further.

The tea matrix effect was evaluated with six different spiked levels of each neonicotinoid according to the equation (3) (**Table 2**). The MEs for the eight insecticides showed a similar signal suppression, with a stronger suppression by the three kinds of tea on nitenpyram, clothianidin and imidaclothiz. Ion suppression of insecticide samples was also reported in tea samples extracted with QuEChERS approach[2]. Since a selective sample preparation to eliminate most of the matrix components is rather difficult and may risk significant losses of some trace analytes, it is best to be avoided. Alternatively, an isotopically labeled standard (IS; imdicloprid-d4) could been used to correct for the recovery rates of these insecticides [17,19]. However, a single IS cannot compensate for the encountered matrix effects, as it would be different with each analyte in each kind of tea, especially for imidacloprid. In addition, previous studies showed that the ME might not be completely eliminated and that ESI is more prone to ME than atmospheric pressure chemical ionization (APCI)[7]. Therefore, to compensate for these significant MEs and to improve the linearity, reliability and accuracy of the analytical results, matrix matched calibration (MMC) curves were used.

Linearity, LOQ and recovery

MMC curves developed on different blank tea matrices were linear over the working concentration ranges of the eight insecticides. Calibration curves fitted by

linear regression showed coefficients of determination (R^2) ranging from 0.9957 to 0.9975 in green tea, 0.9954 to 0.9979 in oolong tea, and 0.9926 to 0.9977 in black tea. The LOQs of tea were below 0.01 mg kg^{-1} (**Table 2).** The LOQs were quite satisfactory when compared to the regulatory limits of daily exposure in tea [3].

Method accuracy and recovery were evaluated by addition of standard solutions in blank (green, black and oolong) tea samples. Six aliquots of tea matrix were spiked with target compounds at three concentration levels: 0.01, 0.05 and 0.5 mg kg^{-1}. Except that the mean recovery of nitenpyram 0.5 mg kg^{-1} in black tea were 60%, the recoveries of all insecticides in three kinds of tea matrix were all above 70%, with relative standard deviations (RSDs) of 0~15% (Table 3). The method allows to simultaneously analyze eight insecticides at a reasonable sensitivity while maintaining simplicity and cost-effectiveness. Improving the method sensitivity further may be unwarranted.

Analysis of commercial tea samples.

The developed method of sorbent pretreatment was used to analyze the neonicotinoid insecticides in 29 commercially available tea samples (13 green tea, 13 black tea and 3 oolong tea samples). Only three of the neonicotinoid insecticides - thiamethoxam, imidacloprid and acetamiprid - were detected from these samples (data shown in **Table S1**). In the positive samples, the concentration of imidacloprid were 0.025 and 0.042 mg kg^{-1} in 2 green tea samples, 0.032 mg kg^{-1} in 1 black tea samples and 0.013 mg kg^{-1} in 1 oolong tea sample, all of which were below the maximum residue limits (MRLs) for imidacloprid in tea set by Japan (10 mg kg^{-1}) and the EU

MRL (0.05 mg kg^{-1}). The concentration of acetamiprid were 0.016 and 0.089 mg kg^{-1} in 2 green tea samples, 0.052~0.126 mg kg^{-1} in 3 black tea samples and 0.012 mg kg^{-1} in 1 oolong tea sample, also below the acetamiprid MRL set by Japan (50 mg kg^{-1}). However, the concentration of acetamiprid in 3 black tea samples (0.052, 0.125 and 0.126 mg kg^{-1}) was above the EU MRL (0.05 mg kg^{-1} for acetamiprid). Thiamethoxam was detected in just one oolong tea sample (0.014 mg kg^{-1}) and was below the EU MRL (20 mg kg^{-1} for thiamethoxam), Japanese MRL (15 mg kg^{-1}) and Chinese MRL (10 mg kg^{-1}). The MRM chromatography of several neonicotinoid insecticides in representative positive samples are shown in **Fig. 6.**

Conclusion

The method as optimized herein is effective, simple and accurate. It is also the first reported investigation using PVPP to diminish the main interfering compounds of tea matrix (polyphenols) in an UPLC-MS/MS method developed to determine multiple pesticide residues. In addition, it is the first verification of neonicotinoid insecticide analysis in tea by UPLC-MS/MS with ammonium formate in the mobile phase, which strongly enhanced the signal of neonicotinoid insecticides. These additions resulted in a robust method for the simultaneous detection of eight neonicotinoid insecticides in tea samples. Furthermore, this modified QuEChERS method could be used in the determination by LC-MS or LC-MS/MS of other classes of pesticide residues that are disrupted by the matrix effect of tea samples.

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Figure captions

Fig. 1. Peak responses of eight neonicotinoid standards (0.05 mg/kg, n=3) isolated using a mobile pha

se of pure water or water with the addition of ammonium formate, formic acid or a combination of the two.

Fig. 2. The MRM chromatograms of eight neonicotinoid standards with a mobile phase consisting of 5 mM ammonium formate in water.

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Fig. 5. The matrix effect (%) of different kinds of tea (green, black or oolong) on the different neonicotinoid insecticides (spiked level 0.05 mg kg^{-1}, n=3); 1-dinotefuran，2-nitenpyram，3-thiamethoxam，4- clothianidin，5- imidacloprid，6-imidaclothiz，7-acetamiprid，8-thiacloprid.

Fig. 6. Representative LC-MS/MS chromatograms of some of the positive samples. A. Oolong tea contaminated with thiamethoxam at 0.013 mg kg^{-1} (thiamethoxam transitions: A1, 292.0→211.0; A2, 292.0→181.0); B. Black tea sample that was positive for imidacloprid at 0.007 mg kg^{-1} (imidacloprid transitions: B1, 256.0→209; B2, 256→175.0); and C. Black tea sample with acetamiprid at 0.008 mg kg^{-1} (acetamiprid transitions: C1, 223.0→126.0; C2, 223.0→56.0).

Table 1. LC-MS/MS conditions for detection of neonicotinoid insecticides and their fragments.

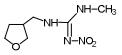
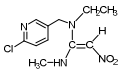
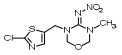
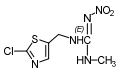
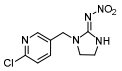
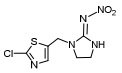
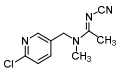
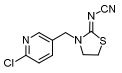
Insecticide	Chemical Structure	Retention Time (min)	MRM1	MRM2	Fragmentor Voltage (V)	Collision Energy (eV)	Cell Acceleration Voltage (V)
dinotefuran		2.958	203/129	203//157	75	5	7
nitenpyram		3.671	271/237	271/224	95	12	7
thiamethoxam		5.004	292/211	292/181	80	4	7
clothianidin		6.097	250/169	250/132	85	6	7
imidacloprid		6.630	256/209	256/175	100	9	7
imidaclothiz		7.103	262/181	262/180	80	5	7
acetamiprid		7.405	223/126	223/56	115	12	7
thiacloprid		9.174	253/126	253//186	120	13	7

Table 2. LC-MS/MS coefficients of determination (R^2) for Matrix-Matched Standards and ME_S of insecticide.

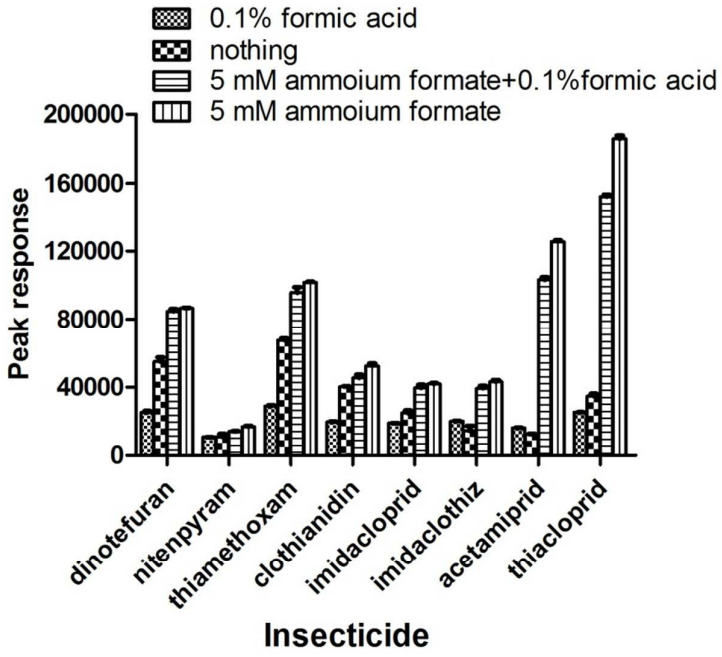
Insecticide[a]	LOQ (mg kg^{-1})	Matrix	R^2	Matrix Effect (ME)
dinotefuran	0.01	green tea	0.9975	-37
		oolong tea	0.9959	-32
		black tea	0.9944	-37
nitenpyram	0.01	green tea	0.9963	-49
		oolong tea	0.9954	-51
		black tea	0.9926	-46
thiamethoxam	0.01	green tea	0.9975	-23
		oolong tea	0.9978	-32
		black tea	0.9957	-19
clothianidin	0.01	green tea	0.9977	-58
		oolong tea	0.9976	-47
		black tea	0.9977	-43
imidacloprid	0.01	green tea	0.9973	-39
		oolong tea	0.9961	-13
		black tea	0.9942	-16
imidaclothiz	0.01	green tea	0.9978	-53
		oolong tea	0.9979	-66
		black tea	0.9976	-57
acetamiprid	0.01	green tea	0.9972	-11
		oolong tea	0.9976	-23
		black tea	0.9941	-14
thiacloprid	0.01	green tea	0.9957	-10
		oolong tea	0.9965	-12
		black tea	0.9960	-5

[a]Spiked from 0.001~0.2 μg mL^{-1}, 6 Calibration Data Points at Different Concentrations

Table 3. Recoveries and Relative Standard Deviations (RSDs) of eight neonicotinoid insecticides in spiked tea samples (n = 6).

	[a] Mean% (RSD%)								
	green tea			oolong tea			black tea		
Insecticide	0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.5 mg kg^{-1}	0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.5 mg kg^{-1}	0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.5 mg kg^{-1}
dinotefuran	87.2(4.9)	84.5(4.0)	79.2(3.5)	85.1(4.6)	89.3(0.3)	81.6(2.0)	79.0(3.5)	74.9(14.2)	70.8(4.4)
nitenpyram	94.0(5.9)	81.2(4.0)	79.4(2.0)	93.3(9.3)	83.4(1.2)	77.5(2.5)	78.5(3.7)	71.3(3.2)	60.0(9.3)
thiamethoxam	86.0(2.4)	84.9(3.7)	80.4(1.4)	95.1(2.2)	91.4(0.8)	85.9(3.0)	83.2(2.7)	76.3(15.4)	72.6(4.7)
clothianidin	80.3(13.7)	74.6(6.0)	71.9(1.9)	70.9(4.5)	79.0(1.2)	78.3(2.7)	72.2(2.1)	74.3(10.1)	72.0(3.5)
imidacloprid	96.0(4.5)	89.7(7.7)	78.4(2.9)	91.1(2.6)	87.1(1.4)	82.6(1.9)	109.4(2.6)	90.1(10.7)	71.7(5.4)
imidaclothiz	83.5(2.5)	84.9(7.1)	76.6(1.3)	81.2(3.5)	80.5(0.8)	79.4(1.6)	70.0(3.3)	76.7(3.6)	73.7(4.3)
acetamiprid	89.1(14.5)	70.1(4.2)	85.2(1.3)	92.3(3.0)	86.1(0.2)	83.6(1.3)	104.7(3.7)	92.1(8.3)	74.4(5.4)
thiacloprid	83.2(2.0)	80.4(0.7)	79.8(1.7)	76.7(3.4)	78.8(1.0)	78.2(1.8)	70.7(3.4)	70.8(4.8)	76.3(6.0)

[a] Average of six replicate.



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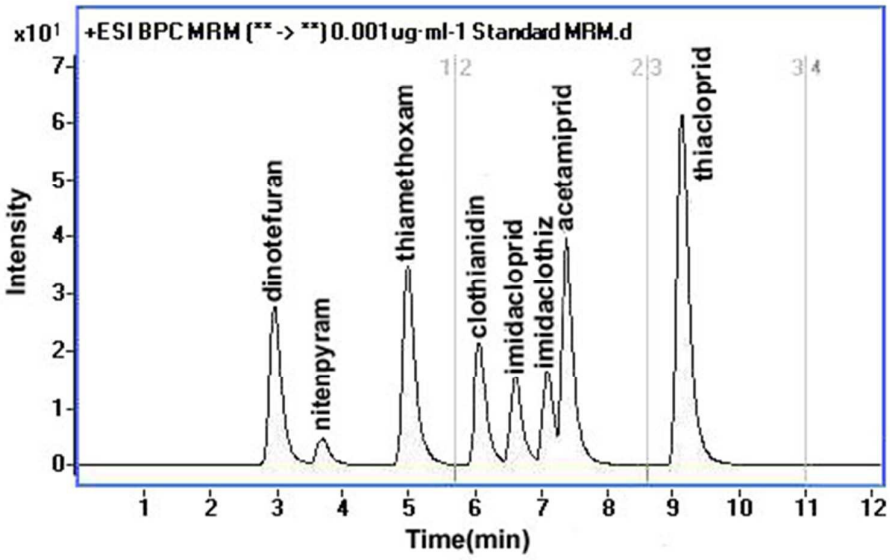

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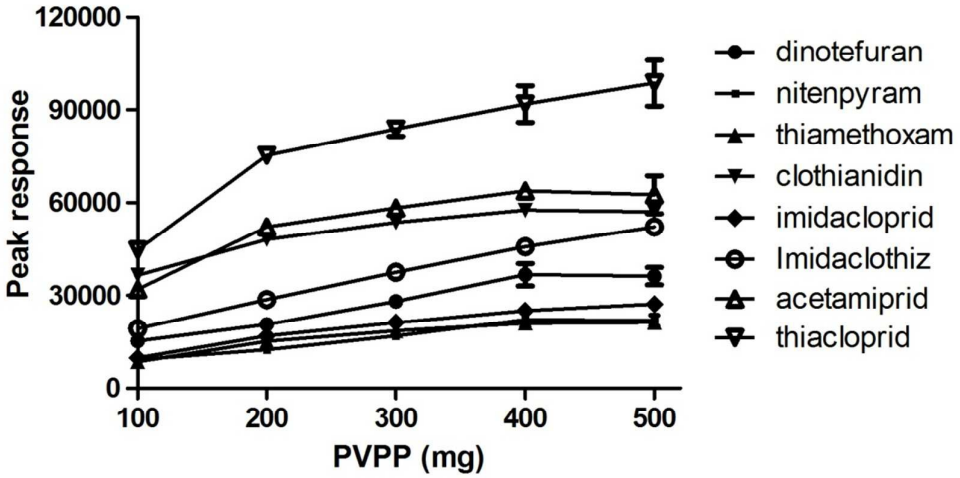

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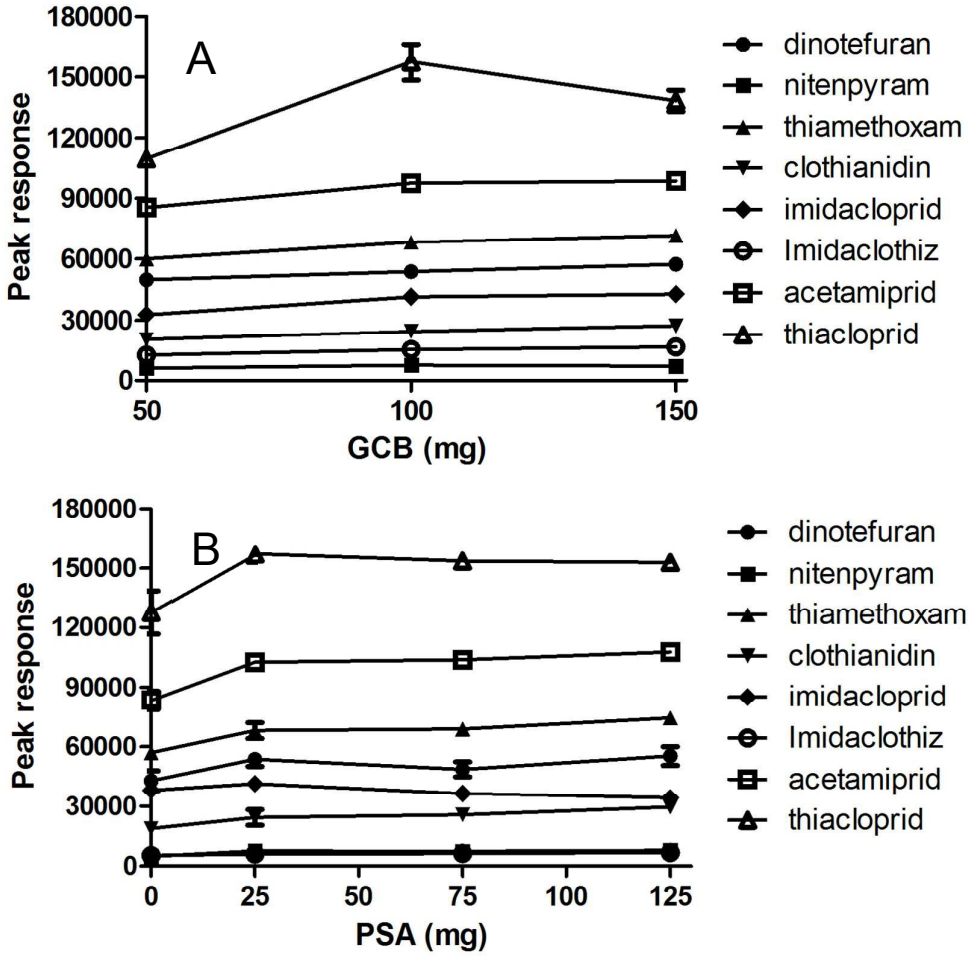

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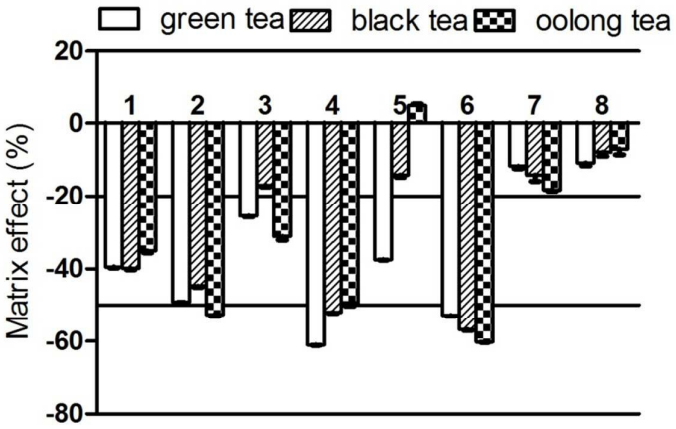

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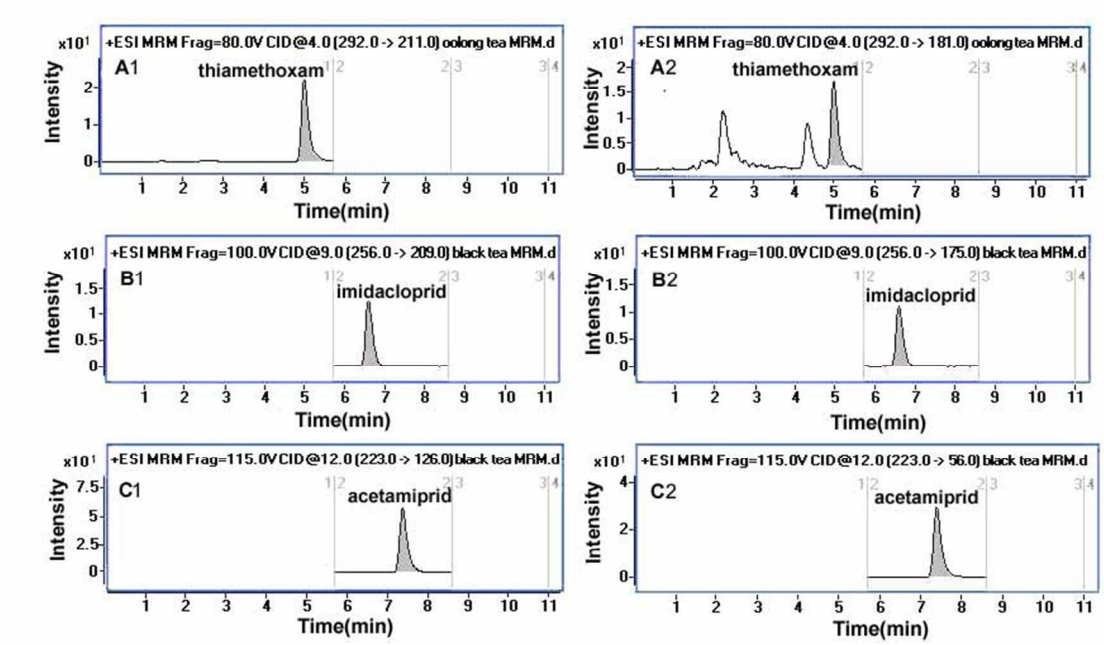

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Graphic Abstract