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# Detection of seven pesticides in cucumbers using hollow fibre-based liquid-phase microextraction and ultra-high pressure liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT

A liquid-phase microextraction (LPME) methodology based on the use of porous polyvinylidene fluoride (PVDF) hollow fibres was developed for extracting seven pesticides from cucumbers. The seven pesticides include propoxur, carbofuran, atrazine, cyanatryn, metolachlor, prometryn and tebuconazole. The PVDF hollow fibre provides higher extraction efficiency due to its higher porosity and better solvent compatibility. A new desorption methodology was developed since some pesticides were absorbed by the wall pore of the PVDF. Ultra-high pressure liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) was used for pesticide analysis. In order to obtain high recoveries and enrichment factors of the analytes, several parameters such as method of sealing, acceptor phase (organic solvents), stirring speed, extraction time, salting out effect, desorption mode and time were optimized. A fast, simple method for closing fibre ends was practiced by using mechanical crimping. Pesticides were extracted from the sample to the organic solvent and then desorbed in a mixture of methanol:water (1:1 v/v) prior to chromatographic analysis. Limits of detection (LOD) for the multi-reaction-monitoring (MRM) mode of the method varies from 0.01 to 0.31 μg/kg with optimized sample preparation. Calibration curves are linear with $R^2 \geq 0.991$. Enrichment factor of the hollow fibre LPME ranges from 100 to 147. Matrix effect has been considered and is in the range of 76–122%. The relative recoveries from cucumber samples are between 63% and 119% with the relative standard deviation (RSD, $n = 6$) lower than 20%.



## 1. Introduction

Pesticides are widely used in agriculture and have played an important role in minimizing weed management costs, killing insects and improving crop production. However, its uncontrolled or improper used can result in adverse impact on food safety and human health. It has aroused a growing concern worldwide. Therefore, various approaches have been developed for identification and quantification of pesticide residues present in agricultural products. Seven pesticides including propoxur, carbofuran, atrazine, cyanatryn, metolachlor, prometryn and tebuconazole were selected based on their extensive use in China for the control of various types of weeds or pests.

Gas chromatography mass spectrometry (GC–MS) [1–4], high-performance liquid chromatography (HPLC) [5,6], particularly reversed-phase liquid chromatography, high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) [7] have been applied to pesticide determination.

Cucumber samples require preparation and clean-up extraction and clean-up steps. The QuEChERS method has many advantages, such as quick, easy, cheap, effective, rugged and safe, and has gained worldwide popularity as a means for sample preparation [8–11]. However, in order to minimize matrix effects, a dispersive solid phase extraction (SPE) clean-up with primary secondary amine (PSA)/graphitized carbon black (GCB)/$C_{18}$ sorbent was applied as complementary to QuEChERS method. SPE [12–14] and solid phase micro-extraction (SPME) [15] are regarded as sensitive and accurate methods for sample preparation. At the same time, the disadvantage of both methods is that they are still relatively expensive. Liquid–liquid extraction (LLE) [16,17] method has been a main traditional extraction method for extracting organic pollutants from aqueous solutions and it is still being widely used. However, LLE requires large amounts of toxic organic solvents.

Compared to methods above, LPME [18–22] is relatively low in cost and requires quite small volume (e.g., microliters of organic solvents) of toxic solvent to extract analytes from aqueous matrices. The use of inexpensive disposable membranes can minimize

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carryover effects. Due to sample-to-acceptor volume ratio, LPME involves a reduction in the consumption of organic solvent and high enrichment factor during the extraction procedure. These features make LPME a very sensitive technique suitable for determination of the pesticides at trace levels. The low solvent consumption also makes LPME an environmental friendly extraction method. In addition, sample clean-up is achieved during LPME due to the 0.2 μm pore size of the hollow fibre, which prevented undesirable large molecules in the donor phase from being extracted.

There are many studies using LPME to determinate pesticide residues in water [23–25], milk [26], beverage [27] and vegetables [28] samples. Generally, porous polyvinylidene fluoride (PVDF) is less used than polypropylene in hollow fibre-based LPME procedures [29]. However, PVDF is also compatible with a broad range of organic solvents and strongly immobilizes the organic solvents. Moreover, 0.18 mm wall thickness provides excellent mechanical stability for the hollow fibre. 0.80 mm inner diameter ensures that the syringe needle can be easily inserted to transfer the acceptor phase into the fibre [30]. 0.82 membrane porosity of PVDF hollow fibre is used which significantly increased velocity of mass transfer and shortened extraction time. In this study, PVDF was used to analysis effect of fibre extraction. Only four pesticides including atrazine, prometryn, metolachlor and carbofuran have been reported with the use of LPME [28,31] employing polypropylene hollow fibres. Propoxur, cyanatryn and tebuconazole have not been reported by LPME.

In the present study, PVDF hollow fibre was selected. Hollow fibre LPME combined with UHPLC–MS/MS was used to determine seven pesticides in cucumber samples. In order to obtain high recovery and enrichment of analytes, several parameters such as the method of sealing, acceptor phase (organic solvents), stirring speed, extraction time, salting out effect and desorption mode and time were optimized. In addition, a fast and simple method for closing the ends of the fibre was practiced by using a pair of mechanical crimping and sealed with a tweezer. Pesticides were extracted from the sample to the organic solvent and they were desorbed in a mixture of methanol:water (1:1 v/v) prior to chromatographic analysis. To evaluate the practical applicability of the HF-LPME method, specificity, limits of detection, linearity, enrichment factor, matrix effect and recovery were investigated under optimal extraction conditions.

## 2. Experimental

### 2.1. Materials and reagents

Cucumbers were purchased from several supermarkets in Beijing, China. The selected pesticides are listed in Table 1. All pesticide analytical standards are also obtained from Beijing Modern Oriental Fine Chemicals Co., Ltd. Toluene, n-hexane, dichloromethane and chloroform were purchased from Beijing Chemical Plant. 1-Octanol was from West Long Chemical Co., Ltd. Ethyl acetate was from Beijing Wide Fine Chemical Company. HPLC grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, USA). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water. The porous PVDF hollow fibre (PVDF, 1.15 mm external diameter, 0.80 mm inner diameter, 0.16 μm pore size, 0.82 membrane porosity) was purchased from Institute of Biological and Chemical Engineering in Tianjin Polytechnic University. Mechanical crimping (1.30 mm inner diameter) was from Yueqing Baoxing Electrical Factory (Zhejiang, China).

### 2.2. Instrumentation

Waters Acquity™ ultra-high performance liquid chromatography (Waters Acquity BEH $C_{18}$ column, autosampler, binary solvent management system) and Waters Micromass Quattro Premier XE triple quadrupole spectrometer (Waters, USA) equipped with an electrospray ion source were used in this study and operated by using MassLynx 4.1 software. The sample was separated on a Waters Acquity™ BEH $C_{18}$ column (50 mm × 2.1 mm, 1.7 μm) with gradient elution using acetonitrile (A) and water (B, containing 0.2% formic acid) at the flow rate of 0.20 mL/min. The initial composition was 5% A and then a linear elution gradient was programmed from 5% to 95% for 6 min, finally, 3 min was used for equilibrium of the column to the initial conditions. The mass spectrometer was operated in the MRM mode with positive electrospray ionization ($ESI^+$) source. The highest abundant ion was used for the quantitative ion. The other ion was selected as confirmation of individual analytes (Table 1). The nebulization gas was set at 600 L/h at a temperature of 350 °C, the cone gas was set at 50 L/h, and the source temperature was 100 °C. The capillary voltage was 3.5 kV.

### 2.3. Extraction procedure

Stock solutions (1000 μg/mL) of seven pesticides as listed in Table 1 were prepared in pure methanol and further diluted with methanol to obtain standard solutions with various concentrations. The standards were stored and refrigerated at −18 °C. Subsequently, residues of seven pesticides present in cucumber samples were detected using HF-LPME coupled to UHPLC–MS/MS. The HF-LPME approach was used for sample pretreatment to enrich pesticide residues in the cucumber samples. The cucumber sample (2.0 ± 0.2 g) which had been previously homogenized was weighed in a 10 mL vial. 5 mL pure water was added, then the vial was vigorously shaken for 1 min and ultrasonically stirred for 5 min. The extract was centrifuged at 12,000 rpm for 3 min. The supernatant was taken with a pipette, transferred to a 10 mL vial and enriched by HF-LPME.

Hollow fibre and mechanical crimping were cleaned in acetone for 3 min in order to remove any contaminants. Before using, the hollow fibre was cut manually into 8 cm segment for each extraction and impregnated with chloroform for 10 s to open up membrane pores. After impregnation, 32 μL of chloroform in the syringe was injected to flush the hollow fibre to avoid air bubble formation. The syringe needle was tightly fitted with an 8 cm length of fibre so that the fibre was completely filled with organic solvent.

**Table 1**
Parameters of MS/MS detection.

| Compound | Confirmation ion | CV/CE | Quantification ion | Retention time | CV/CE |
|---|---|---|---|---|---|
| Propoxur | 210.1 > 110.9 | 22/8 | 210.1 > 167.9 | 3.77 | 22/5 |
| Atrazine | 216.1 > 96.0 | 27/20 | 216.1 > 173.9 | 3.95 | 27/15 |
| Carbofuran | 222.1 > 122.9 | 27/11 | 222.1 > 165.0 | 3.79 | 27/11 |
| Cyanatryn | 241.1 > 131.9 | 33/20 | 241.1 > 214.0 | 3.55 | 33/15 |
| Prometryn | 242.1 > 116.1 | 33/20 | 242.1 > 158.0 | 3.84 | 33/20 |
| Metolachlor | 284.2 > 176.1 | 25/14 | 284.2 > 252.1 | 5.02 | 25/10 |
| Tebuconazole | 308.1 > 124.7 | 30/15 | 308.1 > 70.0 | 4.79 | 30/17 |

CV, cone voltage; CE, collision energy.