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# Determination of chlorophenols in soil samples by microwave-assisted extraction coupled to headspace solid-phase microextraction and gas chromatography–electron-capture detection

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**Abstract**

Microwave-assisted extraction coupled to headspace solid-phase microextraction was studied and applied for one-step in-situ sample preparation prior to analysis of chlorophenols (CPs) in soil samples. The CPs in soil sample were extracted into the aqueous solution and then directly onto the solid-phase microextraction (SPME) fiber in headspace under the aid of microwave irradiation. After being desorbed from SPME fiber in the GC injection port, CPs were analyzed with a GC–electron-capture detection system. Parameters affecting the extraction efficiency such as the extraction solutions, the pH in the slurry, the humic acid content in the soil, the power and the irradiation time of microwave as well as the desorption parameters were investigated. Experimental results indicated that the extraction of a 1.0 g soil sample with a 6-ml aqueous solution (pH 2) and a polyacrylate fiber under the medium-power irradiation (132 W) for 9 min achieved the best extraction efficiency of about 90% recovery and less than 10% RSD. Desorption was optimal at 300 °C for 3 min. Detection limits were obtained at around 0.1–2.0 μg/kg levels. The proposed method provided a simple, fast, and organic solvent-free procedure to analyze CPs from soil sample matrix.


*Keywords:* Soil; Extraction methods; Environmental analysis; Chlorophenols; Phenols; Organochlorine compounds

## 1. Introduction

Chlorophenols (CPs) are well known for their toxicity and persistence in the environment [1,2]. They are introduced from the chloro-bleaching process of pulp pesticide and pharmaceutical manufacture [3,4]. Thus, they are commonly found in contaminated soil as well as the surface water.

Many methods for CPs analysis are based on chromatographic techniques, such as liquid chromatography [5,6], gas chromatography [7,8] and capillary electrophoresis [9,10]. Before chromatographic measurement, appropriate sample pretreatments are usually required to clean up or to enrich the target species. Conventionally, the liquid–liquid extraction and solid-phase extraction were applied for the pretreatment of CPs in soil samples, whereas the sonication and the Soxhlet methods were mainly employed in the analysis of pentachlorophenol in wood. Although these extraction methods offer effi-

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cient and precise results, they are relatively time-consuming, hazardous to health due to the usage of organic solvents, and highly expensive with respect to the disposal of solvents. Therefore, pretreatments with short time and less or free use of organic solvents have led to the recent development of new extraction approaches. Solid phase micro-extraction (SPME) was developed to resolve some of mentioned problems [11–13]. It accomplishes the sampling, extraction, and enrichment in a single-step operation.

The immerse SPME coupled to GC analysis was first used for the analysis of phenolic compounds in environmental samples [7,14–16]. The technique was found being significantly influenced by sample matrix [7]. To avoid matrix effects, the headspace (HS) SPME (HS-SPME) was then developed and applied successfully to eliminate interference problems [12,17,18]. However, HS-SPME has been reported to be efficient only for the analytes with high and medium Henry coefficients [19]. For semi-volatile compounds in the mid-boiling range, the HS method did not perform well at room temperature in reasonable time [20]. Doong et al. [21] indicated that for quantitative analysis of semi-volatiles in soils it is necessary to promote the partition of the compounds into the headspace and speed up the mass transfer process.

In this decade, microwave energy was investigated and widely applied in analytical chemistry such as accelerating sample digestion, extraction, and also in chemical reactions [22–24]. Through the dipole rotation and ionic conductance of polar substances or ionic species under the microwave irradiation, the temperature of system rises within a very short-time period. Therefore, microwave heating has the potential to improve the SPME sampling for organic compounds.

In our previous study, with the described advantages of SPME and microwave assistance, we succeed to use microwave assisted HS-SPME as one-step in situ headspace sampling in the determination of CPs in water and dichlorov in vegetable samples by irradiating the aqueous matrix to increase the volatility of analytes [25,26]. Because the matrix of the soil sample is much different from that of the aqueous sample, the influence factors or the influential levels on the sampling efficiency are required to re-evaluate. In this study, the microwave assisted extraction (MAE) on-line HS-SPME (MAE–HS-SPME) coupled to GC–electron-capture detection (ECD) was investigated to develop a simple, fast, and solvent-free analytical process to analyze CPs in soil sample.

## 2. Experimental

### 2.1. Chemicals and reagents

Deionized water was produced using a Barnstead Nanopure water system (Barnstead, NY, USA) for all aqueous solutions. All chemicals and solvents were of ACS reagent grade. 2,4-Dichlorophenol (2,4-DCP) and pentachlorophenol (PCP) were obtained from Aldrich (Milwaukee, WI, USA), 2,4,6-trichlorophenol (2,4,6-TCP) was from TCI (Tokyo, Japan), and 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) was from Riedel-de Häen (Hannover, Germany). Their standard stock solutions (1.0 mg/ml) were prepared by dissolving 0.100 g in 100 ml methanol and stored in silanized brown glass bottles with Teflon-lined cap, and kept at 4 °C. Fresh working solutions were prepared by the appropriate dilution of the stock solutions with methanol (Mallinckrodt, KY, USA). Humic acid was obtained from Fluka (Fluka Chemie, Switzerland).

### 2.2. GC–ECD system

The GC used in this work was Hewlett-Packard 5890 system equipped with an electron-capture detector ($^{63}$Ni), and a split/split-less injector. Separations were done through a fused-silica DB-5 capillary column (30 m×0.25 mm I.D., 1.0 μm film thickness), (J&W Scientific, Folsom, CA, USA). The temperature program used was as follows: 60 $^{0}$C hold for 1 min, rising temperature at 20 °C/min to 300 °C and held for 8 min. The injector was held isothermally at 300 °C for CPs desorption (3 min). The ECD system was maintained at 320 °C. The carrier gas was nitrogen at the flow-rate of 1.0 ml/min, and the make-up gas was at 56 ml/min with nitrogen, the flow-rate for the purge gas was 5 ml/min. A Chem-Lab (Taipei, Taiwan) data system

was used to obtain the chromatogram and perform data calculations.

### 2.3. MAE–HS-SPME system

The microwave oven used in this work was a modified version of the home-used TMO-2030P system (2450 MHz, Tatung, Taipei, Taiwan) with a maximum power of 650 W, equipped with a cooling condenser connecting to tap water. After the modification, the powers of microwave were 11, 132, 160, and 210 W for weak, medium, medium-high, and high irradiation, respectively. In order to keep the volume of headspace as small as possible, a glass tube was used to seal and guide the vapor through the SPME fiber. The sampling system was set-up as shown in Fig. 1. In order to keep from the leak of microwave irradiation, aluminum foils was tacked on the inner-wall and the outer-wall of microwave body in the interface part. A microwave leak detector (MD-2000, Less EMF, NY USA) was used to check the safety aspects before the running.

The SPME device consisting of the holder and fiber assembly for manual sampling was obtained from Supelco (Bellefonte, PA, USA) and used without modification. The fibers selected in this study were 1-cm length coated with polyacrylate (85 μm film thickness). The fibers were conditioned under nitrogen in the hot injection port of the GC at 300 °C for 2 h prior to use. The needle on the SPME manual holder was set at its maximum length of 4-cm in the GC injector port. A desorption temperature of 300 °C for 3 min was set to produce the highest sensitivity of CPs. All the analyses were performed with a 50-ml ground bottle containing 1 g of soil sample and 6 m1 of extraction solvent. In order to find the optimal parameters of the microwave system and SPME, 1 g soil containing 0.2 μg/g for DCP, 0.02 μg/g for other CPs was added into 6 ml aqueous solutions adjusted at pH 2.0.

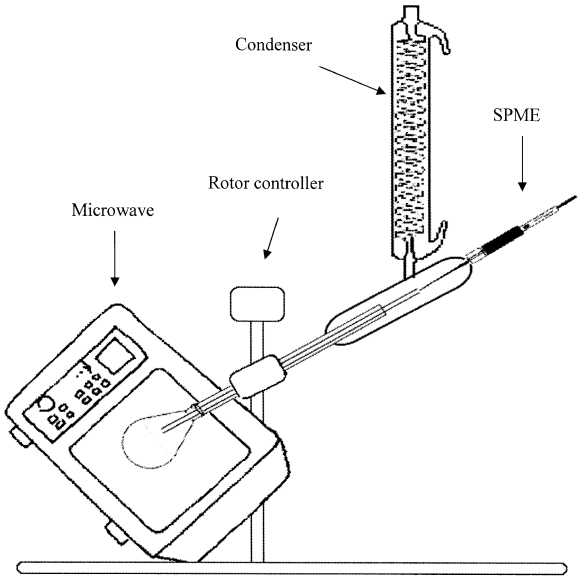


Fig. 1. The assembly of the MAE–HS-SPME apparatus.

### 2.4. Preparation of soil sample

The CPs-free soil sample was collected from a sub-surface soil of garden. After removing twigs and extraneous material it was dried in an oven at 120 °C for 24 h and then homogenized by crushing in a mortar and screened to a particle size of 20 mesh. The organic carbon content was 1.37% (relevant to 2.35% humus) by elemental analyzer. The soil sample was confirmed as CPs-free by liquid–liquid extraction (LLE) coupled to GC–MS.

About 100 g soils were weighted out in a jar, to which various CPs standard mixtures dissolved in 100 ml of acetone were added to give varied CPs-content soils. After mixed thoroughly, the slurry was allowed to stand, loosely covered to protect it from dust, and stirred occasionally until acetone completely evaporated (approximately 2 days). The soil was then capped and kept in a desiccator for the following studies. A real soil sample was collected from a contaminated industrial park in Tainan city (Southern Taiwan).

## 3. Results and discussion

In order to optimize the MAE–HS-SPME sampling technique for CPs in soil, factors affecting the sampling efficiency, such as the power of microwave and its irradiation time (same as fiber absorption time), extraction solution (water addition and polarity modifier), humic acid in the soil as well as the desorption conditions were studied thoroughly.

### 3.1. Optimization of microwave irradiation conditions

In this study MAE combined with HS-SPME is employed for collecting the semi-volatile CPs from soil sample. The influence of the irradiation power and irradiation time of microwave on the extraction is investigated. Fig. 2 shows the recovery of CPs on SPME fiber during various irradiation time of medium power in the MAE–HS-SPME process. The results show the recovery of CPs increases with time and go to flatness after 9 min irradiation for 2,4,6-TCP, 2,3,4,6-TeCP, and PCP whereas it reaches the optimum at 9 min for 2,4-DCP. It indicates that the 2,4-DCP might be lost in longer microwave irradiation due to its more volatility in CPs. Compared to the optimization at 5 min for the MAE–HS-SPME extraction of CPs on the same fiber [25], the optimization for soil samples has taken a longer time than in aqueous sample. It indicates that the interaction force occurred between the soil matrix and the CPs. When examining the effect of power, the medium power irradiation offers a higher recovery compared to those with weak, medium-high, and high powers. It also depicted that irradiation under the medium-high power and the high power would cause the loss of DCP based on their relative volatility. Therefore, microwave irradiation with medium power (132 W) for 9 min was recommended to assist the extraction and HS-SPME sampling.

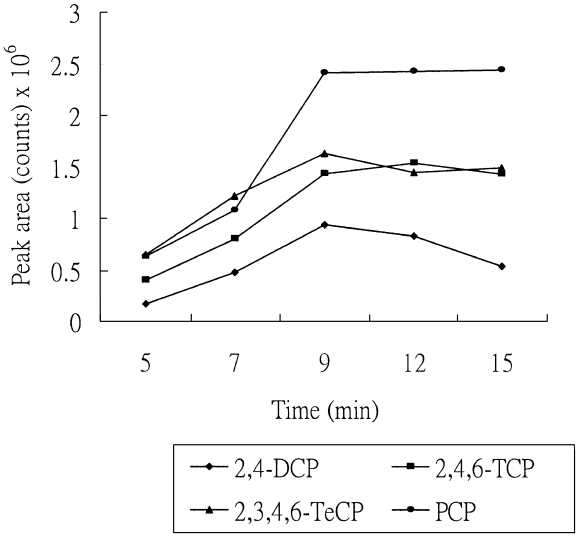


Fig. 2. The recovery of CPs on SPME fiber in various irradiation times in the MAE–HS-SPME process.

### 3.2. Thermal desorption conditions

For better separation efficiency and resolution, thermal desorption requires a possible minimum time. In our previous study for aqueous sample [25], the optimal desorption temperature and desorption time in hot GC injector for CPs were obtained as desorbed at 300 °C for 3 min. Because many organic species might exist in the soil matrix and then affect the fiber regeneration, the thermal desorption temperature and desorption time was thus re-examined. After series tests, the results show that the desorption efficiency and the regeneration of fiber are not significant difference from that for aqueous sample. Thus, desorption condition is set at 300 °C for 3 min for each run. After this, no significant blank values were observed for the re-injection. Thus, no further regeneration mode for the fiber was necessary.

### 3.3. Effect of water–soil ratio on extraction

Because the partition ratio of semi-volatile compounds between the soil and the headspace is very low, the headspace sampling of semi-volatile species at room temperature is thus largely limited. Therefore, the partition ratio tended to become enhanced by microwave irradiation. However, low recovery of CPs was obtained in our studies for the microwave assisted desorption to soil sample directly and in-situ headspace SPME sampling. It depicted the energy absorbed by the soil matrix was not enough to desorb the CPs from soil matrix into headspace. Thus, quantity of polar water was added into the soil to enhance the absorption of energy from microwave irradiation. Although water vapor in the headspace is un-favor to the HS-SPME efficiency [27], however, a favorable effect of water addition to the soil samples is observed in our experiments. For this, Zhang and Pawliszyn [28] had indicated that the addition of small amounts of water can facilitate the desorption and vaporization of analytes from soil. The effect of water–soil ratio on extraction was thus investigated. Because the aqueous solution with pH 2.0 could

offer the optimal extraction efficiency in our previous study [25], the extraction slurry was adjusted to pH 2.0 prior to the MAE–HS-SPME sampling. Fig. 3 shows the responses (peak area) obtained after adding water from 0 to 15 ml into 1.0 g of soil by the proposed MAE–HS-SPME process. As can be seen, this plot exhibits a maximum response for 6 ml of water added to the system. The results clearly demonstrated that the addition of water to the samples is necessary to release the semi-volatiles into the gas phase. It indicates that water is not only as extraction solution but also to absorb microwave energy to increase the volatility of CPs. Therefore, in the subsequent studies 6-ml of water was added to extract 1.0 g of soil sample for CPs in the MAE–HS-SPME process.

### 3.4. Effect of the polarity modifier on extraction

For increase the extraction efficiency of organic species from soil sample, organic solvents are always proposed. However, the conventional organic solvents are unsuitable to extract CPs from soil sample in the proposed MAE–HS-SPME system due to the competition for the adsorption site with CPs. Thus, ethylene glycol and glycerol were tested as the modifier of the extraction solution to increase the solubility of CPs in the slurry, and then increased the volatility of CPs into headspace due to their low volatility and high dissipation factors to microwave irradiation. After a series of tests, the addition of both polarity modifiers got worse in the extraction efficiency for 2,4-DCP and 2,4,6-TCP, and being insignificant to 2,3,4,6-TeCP and PCP. A broaden tailing peak related to ethylene glycol or glycerol was observed in the chromatogram. It depicts that the competition for the adsorption site still occurred, and the addition of polarity modifier solvents (glycerol, ethyl glycol) increased the solubility of CPs in extraction solution which might decrease the evaporation of CPs into headspace although they increased the extraction efficiency of CPs from soil matrix to slurry solution. Thus, in the propose method, no polarity modifier was recommended to add into the extraction solution.

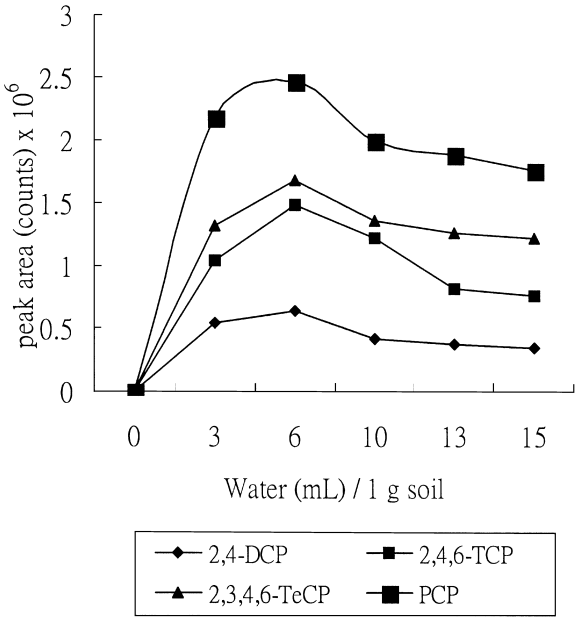


Fig. 3. The effect of the water–soil ratio on extraction efficiency.

### 3.5. Effect of humic acid in soil on extraction

Fromberg et al. [29] indicated that matrix effects mainly depended on the organic carbon content in the soil. In order to investigate the effect of humic acid in soil matrix, fortified soil samples (adding 0–2% of humic acid to the soil with 2.35% humus) were analyzed three times with the proposed procedure. The results show that there was no significant influence on extraction efficiency by humic acid. It depicts that the interaction force between the CPs and humic acid was removed under the microwave irradiation.

### 3.6. Validation of the methods

In order to test the applicability of the proposed method for quantitative determination of CPs in soil matrix, standard spiked-soil samples were used for calibration after they were subjected to overall treatment procedure, i.e. MAE–HS-SPME and thermal desorption from the fiber into the chromatographic system. An ECD chromatogram of CPs standards spiked in soil under chromatographic condition described in the experimental section is showed in Fig. 4. Calibration plots were built-up over the concentration ranges of 0.5–25, 1.0–25,

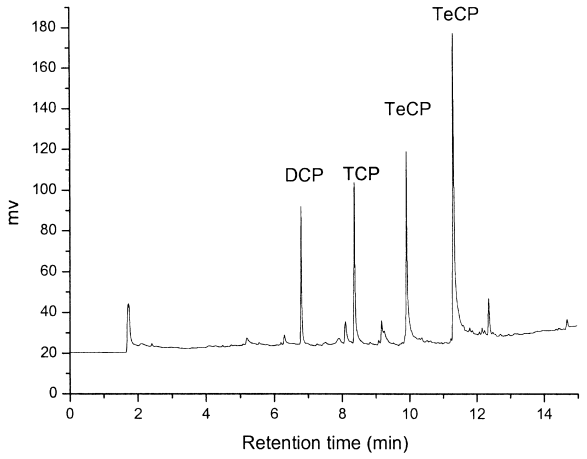


Fig. 4. Chromatogram of CPs for spiked soil sample. Concentration: 0.2 μg/g for DCP and 0.02 μg/g for other CPs.

1.0–25, and 5–300 μg/kg, for PCP, 2,3,4,6-TeCP, 2,4,6-TCP, and 2,4-DCP, respectively. The linear relationships between the peak area and the injected quantity were in good agreement with the correlation coefficients being 0.9927, 0.9959, 0.9963, and 0.9955 for PCP, 2,3,4,6-TeCP, 2,4,6-TCP, and 2,4-DCP, respectively. Compared the slope of the calibration plot of CPs in the spiked-soils and that by direct injection of standard CPs in methanol, the percentage of CPs extracted from the soil sample to the fiber were around 3–6% depending on the volatility of species. The detection limits were calculated based on three times the average background noise divided by the detection sensitivity (slope of calibration plot), which were 0.1, 0.2, 0.2, and 2.0 μg/kg for PCP, 2,3,4,6-TeCP, 2,4,6-TCP, and 2,4-DCP, respectively. The precision of this method was estimated by performing 8 extractions of pH 2.0 sample solutions spiked all studied CPs at concentrations displayed in experimental section. The precisions ranged between 5 and 10% RSD, which should be satisfactory for determining the CPs in soil matrix. In order to examine the applicability of the method to determine CPs in real samples, soil sample was collected from contaminated industrial areas in Tainan city and analyzed by the proposed method. Because the concentrations of CPs in real soil sample were found in relative high levels and over the calibration ranges, thus 25 mg of real soil sample was diluted with 975 mg of CPs-free soil, mixed thoroughly, prior to the analysis. The results are listed in Table 1. It can be seen around 560 μg/g of PCP was found in the soil sample. It greatly exceeds the permitted levels in Taiwan. It indicated the soil has been polluted by the nearby past-PCP factory. When spiking two concentrations of each CPs (within their linear dynamic ranges) in the CPs-free soil sample, and with the propose MAE–HS-SPME–thermal desorption–GC–ECD determination, results are also listed in Table 1. The recoveries were 86.1–98.4% with 2.25–7.29% RSD for various CPs. The accuracy and precision are acceptable in environmental analysis of complicated matrix samples. Fig. 5a is the chromatogram of CPs in real soil sample with split-less injection. It can be seen the 2,4-DCP and the 2,4,6-TCP can be evaluated, with the 2,3,4,6-TeCP and the PCP being in over-scale. Fig. 5b shows the chromatogram with split injection

Table 1
Analytical results of CPs in contaminated soil sample and spiked samples

| | Concentration of the spiked sample[a] (μg/g) | Recovery (%) | RSD (%, $n=3$) | Contaminated soil sample (μg/g) |
|---|---|---|---|---|
| 2,4-DCP | 0.2 | 90.2 | 2.25 | 1.82 |
| | 0.01 | 86.1 | 7.21 | |
| 2,4,6-TCP | 0.02 | 98.4 | 6.27 | 4.22 |
| | 0.002 | 88.6 | 3.52 | |
| 2,3,4,6-TeCP | 0.02 | 90.8 | 4.28 | 10.3 |
| | 0.002 | 92.5 | 7.29 | |
| PCP | 0.02 | 93.3 | 2.78 | 560 |
| | 0.001 | 89.2 | 6.21 | |

[a] Spiked in CPs-free soil sample described in experimental section.

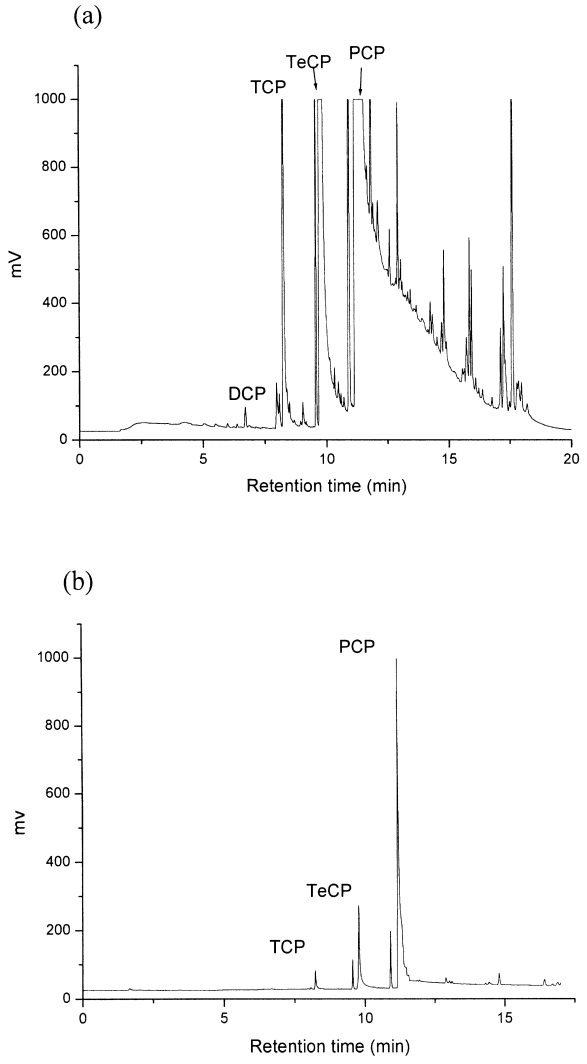


Fig. 5. Chromatogram of CPs in real sample (a) with splitless injection; (b) with split injection.

(ratio of 100:1). Obviously, the 2,4,6-TCP, the 2,3,4,6-TeCP and the PCPs are well separated, but the signal of 2,4-DCP is non-detectable due to the split of sample.

### *3.7. Comparison of the proposed MAE–HS-SPME method with other SPME methods*

All the SPME methods have the advantage of being fast, low-cost and solving the organic solvent problems in sample pretreatment. The conventional immersed SPME has been solvent-less and usually takes 40–70 min to achieve a sampling for phenols in water sample [7,30,31], but it suffers the matrix effect in complicate samples [7]. Although the HS-SPME method is free to matrix effect, it takes 1–2 h to collect most phenols and over 4 h for PCP [31]. As described previously, the MAE–HS-SPME is proposed to shorten the sampling time. It takes only 9 min to complete the sample pretreatment for CPs. With the proposed method, the SPME fiber can be used for over 100 samplings.

## 4. Conclusion

In this paper, the determination of CPs in soil sample by MAE–HS-SPME with GC–ECD has been described, and the optimal conditions have been established. From the results, it has proved the applicability of the proposed method provides a simple, fast, convenient, free from organic solvent, and free sample matrix containment procedure to collect CPs from complicated soil matrix.

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