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In situ derivatization combined to automated microextraction by packed sorbents for the determination of chlorophenols in soil samples by gas chromatography mass spectrometry

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ABSTRACT

A method based on the coupling of *in situ* extraction and derivatization of chlorophenols (CPs) (2-chlorophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol) from soils, accomplishing their preconcentration by means of automated microextraction by packed sorbent (MEPS), is proposed. After extraction and acylation of the chlorophenols in aqueous medium, the liquid phase obtained is subjected to the MEPS procedure. The QuEChERS (*Quick*, *Easy*, *Cheap*, *Effective*, *Rugged and Safe*) and MEPS techniques were compared and the results confirmed the preconcentration carried out with MEPS.

The existence of a matrix effect was checked and the analytical characteristics of the method were determined in a soil sample. The method provided good linearity (from 1 to 12 $\mu g\,kg^{-1}$), together with good repeatability and reproducibility values (RSD equal to or less than 10%). The limits of detection were in the 0.118–0.894 $\mu g\,kg^{-1}$ range. A certified reference material was applied to validate the proposed methodology.

1. Introduction

Among chloroorganic contaminants, CPs form a group of chemicals widely used as biocides, wood preservative and pesticides. They can enter the environment during their manufacture or during their use. They are also produced during the bleaching of wood pulp with chlorine in paper production. The use of these compounds as a wood preservative has resulted in the soil contamination around the sawmills where these compounds have been used [1]. Owing to their physico-chemical properties, CPs tend to sorb onto solid material and accumulate in soils, sediments, sludge and ash samples [2]. Spanish legislation regarding soil pollution has established Generic Reference Levels (GRL) for a list of substances and different soil uses. The classification of soils as polluted or non-polluted is based on these GRL and takes soil use into account [3,4].

CPs are moderately polar compounds that tend to give broad, tailed peaks, largely compromising chromatographic separation, peak integration, and method reliability if they have not been derivatized previously [5]. Analyte derivatization is employed in many analytical methods that employ gas chromatography (GC) as the separation step. Derivatization increases analyte volatility, improves the chromatographic characteristics of an analyte by decreasing its polarity, and/or increases the detector sensitivity of the target analytes [6]. In the case of phenolic compounds, the reaction most widely used is acylation, since this can be carried out *in situ* in aqueous medium in just a few minutes, with high yield and low reagent cost [7]. The reagent most widely employed is acetic anhydride in basic medium, fixed with sodium or potassium carbonate [8–10] or sodium or potassium hydrogen carbonate [5,11,12]. Other alternatives to the basic medium used are sodium or potassium hydrogen phosphate [13–15] or sodium hydroxide [16].

Derivatization is applied together with the techniques used to extract CPs from soil matrices: mainly ultrasonic extraction [17–19], microwave (MW) extraction [20–22], steam distillation extraction (SDE) [23], solid phase extraction (SPE) [24], and solid phase microextraction (SPME) [25–27]. The latter two techniques can be used after extraction with solvents. QuEChERS has also been used to extract CPs from soil and further derivatization of the extracts [28]. In soils, a simplified version of the original QuEChERS method for the extraction of chlorinated compounds has been developed [29]. This version eliminates the dispersive solid-phase

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extraction (d-SPE) cleanup step after extraction owing to the non-fatty characteristics of soil matrices and hence involves fewer sample treatment steps.

Microextraction by Packed Sorbent (MEPS) is a technique for sample preparation introduced by Abdel-Rehim in 2004 [30] and is based on a miniaturization of conventional solid-phase extraction (SPE) in which a sorbent packing material is placed between the barrel and needle of a syringe as a cartridge. In comparison with the conventional SPE method, this technique reduces both sample volume and that of the organic solvent for elution of the compounds; additionally, analysis costs are lower than with SPE. Moreover, the small amount of sorbent used in MEPS cartridges (1–4 mg) enables them to be reused more than 100 times by optimizing the cleaning conditions [31]. The MEPS technique can be completely automated, allowing on-line coupling with gas and liquid chromatography. MEPS applications include the analysis of diverse compounds in biological matrices, such as human plasma, blood, urine and saliva [32,33], the analysis of environmental samples, mainly water and wastewater [33–40], and the analysis of food samples [41,42].

Here we propose a new analytical method based on the coupling of the *in situ* extraction and derivatization of chlorophenols from soils with the preconcentration of the compounds by means of on-line MEPS. A comparison of MEPS with simplified QuEChERS was carried out. The acylation is preferred for the derivatization of CPs present in water solutions [7]. Moreover, the simplicity in coupling MEPS with GC separation facilitates the automation of the procedure. In both cases, derivatization was carried out directly in soil and the derivatized compounds were extracted. In both procedures, a programed temperature vaporizer (PTV) operating in solvent-vent mode is used to inject the extracts in the gas chromatographic system. The existence of a matrix effect was checked with two types of soils and the analytical characteristics of the method were determined in a spiked soil sample (soil 1). A certified reference material (CRM) was analyzed to validate the proposed methodology.

2. Experimental

2.1. Chemicals

The chlorophenols employed (2-chlorophenol, 2,4-dichlorophenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol) were supplied by Sigma-Aldrich (Steinheim, Germany). Ethyl acetate was HPLC grade (CHROMASOLV® Plus) and was purchased from Sigma–Aldrich (Steinheim, Germany). Methanol was purchased from Merck (Darmstadt, Germany) and acetic anhydride, potassium hydrogen carbonate and dehydrated disodium hydrogen phosphate were from Scharlau (Barcelona, Spain). The ultrapure water used was obtained with an Elgastat UHQ water purification system. Abbreviation of the acetylated derivatives are as follow: 2-chlorophenol (2-CPA); 4-chloro-e-methylphenol (4C-3MPA); 2,4-dichlorophenol (2,4-DCPA); 2,4,6-trichlorophenol (2,4,6-TCPA). The CRM employed was a clay soil (RTC-CRM136) and purchased from LGC Promochem (Barcelona, Spain).

2.2. Standard solutions and samples

A stock standard solution of each chlorophenol (2000 mg L^{-1}) was prepared in methanol. These were kept in a refrigerator at 4 °C and diluted to the desired concentration when used. These solutions were used to optimize the method, to perform the calibration standards, and to spike the soil samples at different concentrations.

Soil matrices were used for the optimization of the sample pretreatment step to check the existence of a matrix effect and to determine the analytical characteristics of the method. Two different soils from Badajoz (Spain) were used: a sandy soil (soil 1) and a clay-loam soil (soil 2) taken from agricultural cropland. Table 1 shows the physical and chemical properties of these soils.

Table 1
Physical and chemical properties of the two soils studied.

	Soil 1	Soil 2
pH	5.62	6.47
% Organic matter	0.64	2.03
% Carbon	0.37	1.18
C.E.C.[*] (cmol kg^{-1})	2.94	31.54
% Sand	90.83	39.78
% Silt	6.21	29.66
% Clay	2.95	30.56
Texture	Sand	Clay loam

[*] C.E.C.: cation-exchange capacity.

The procedure used to spike the samples was as follows: 20 g of soil was placed in a 100 mL flask and 1 mL of a chlorophenol solution in ethyl acetate was added (at a suitable concentration for each case). The flask was sealed hermetically and shaken vigorously for 15 min to achieve perfect homogenization of the compounds in the matrix. The samples were stored in a refrigerator (4 °C) for 5 days to allow the interaction between the compounds and the matrix to take place. After 5 days, the flask was opened to allow the solvent to evaporate off (6 h). Then, the flask was closed and stored at 4 °C again for a further two days.

2.3. Apparatus

MEPS was carried out automatically with an MPS2 Multi Purpose Sampler (Gerstel, Mühlheim an der Ruhr, Germany). The method was fully automated across the whole set of samples. Once they had been placed in the autosampler tray, the different steps of the analysis were performed automatically for each sample. In the optimized method, the analytes were retained in a BIN (Barrel Insert and Needle) assembly containing a packing of 4 mg of a solid-phase silica-C18 material (volume 8 μL, mean particle size 45 μm, pore size 60 Å), inserted into a 100 μL gas-tight MEPS syringe. This syringe had a modified front fitting and retaining nut to accommodate the MEPS BIN. The BIN assemblies and MEPS syringe were from SGE Analytical Science (Griesheim, Germany). A VT 32-10 tray, with positions for 10-mL vials, was used. The software used to control sample processing was Maestro, from Gerstel.

Analyses of the chlorophenols were performed on a GC-MS instrument (Agilent Technologies, Santa Clara, CA, USA) consisting of an Agilent 7890A series gas chromatograph equipped with a 6890 Agilent Technologies programmed temperature vaporizer (PTV) injector and an Agilent 5975C inert XL MSD. A glass wool liner (71 mm × 2 mm) was used in the PTV injector. Cooling was accomplished with liquid CO_2. Chlorophenols were separated on a DB-VRX column (20 m × 0.18 mm × 1.00 μm) (J&W Scientific, Folsom, CA, USA). The carrier gas was helium N50 (99.005% pure; Air Liquide). The detector was a quadrupole mass spectrometer. Data acquisition was performed with MSD ChemStation, v. E.02.00.493 software from Agilent Technologies. The compounds were identified using the NIST_98 database (NIST/EPA/NIH Mass Spectral Library, version 2.0) for confirmation.

2.4. Analytical procedure

2.4.1. In situ extraction and derivatization reaction

2.5 g of soil sample was weighed in a 15-mL glass centrifuge tube with a screw cap, which kept the tube closed for most of the sample preparation step, thus preventing losses of volatile compounds during this stage as much as possible. 6 mL of 0.4 M potassium hydrogen carbonate was added in order to provide a suitable medium for the derivatization reaction. Then, 120 μL of