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Article

Determination of Acidic Herbicides in Water Samples by *In Situ* Derivatization, Single Drop Microextraction and Gas Chromatography-Mass Spectrometry

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Um método para a determinação de quantidades traços de herbicidas ácidos em amostras de água foi desenvolvido. O procedimento analítico envolve derivatização *in situ* dos analitos para seus ésteres metílicos com sulfato de dimetilo, amostragem usando microextração com única gota (SDME) e cromatografia gasosa-espectrometria de massa (GC-MS). Os efeitos do pH, força iônica, tempo de extração, solvente de extração assim como condições de derivatização foram estudados. Ésteres metílicos foram extraídos com 2 μL de *n*-heptano. A resposta foi linearmente dependente da concentração na faixa de 0,05-10,0 ng mL^{-1}. Limites de detecção foram obtidos no intervalo de 1,8-3,0 ng L^{-1}. A análise por derivatização-SDME/GC-MS rendeu boa precisão (RSD entre 7,0 e 15,2%). O método foi validado pela análise de amostras enriquecidas.

A method for the determination of trace amounts of acidic herbicides in water samples was developed. The analytical procedure involves *in situ* derivatization of analytes to their methyl esters with dimethyl sulfate, sampling using single drop microextraction (SDME) and gas chromatography-mass spectrometry (GC-MS). The effects of pH, ionic strength, extraction time, solvent of extraction as well as derivatization conditions were studied. Methyl esters were extracted using 2 μL of *n*-heptane. The response was linearly dependent on the concentration in the range 0.05-10.0 ng mL^{-1}. Limits of detection were achieved at the level of 1.8-3.0 ng L^{-1}. Derivatization-SDME/GC-MS analysis yielded good precision (RSD between 7.0 and 15.2%). The method was validated by analysis of spiked matrix samples.

Keywords: acidic herbicides residues, direct derivatization-SDME, GC-MS

Introduction

The use of pesticides yields increased agricultural outputs. However, the slow degradation of pesticides in the environment and the improper usage by farmers could lead to environmental contamination of water and other environmental systems.[1] Considering the acidic herbicides, an important group of pesticides are mainly chlorophenoxy acids and related compounds which are widely used in agriculture and forestry for weed control. They are relatively inexpensive and potent even at low concentrations. After application, the acidic herbicides can easily enter into different water bodies due to their relatively good solubility in water.[2-5] Acidic herbicides are toxic for many living organisms. Some are mutagenic, teratogenic and carcinogenic and some can cause toxic effects such as cytotoxicity and DNA structure damage. Due to their biological activity, their determination in aqueous samples is important.[6-8]

Suitable methods exist for quantitative measurement of acidic herbicides in water samples, using gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography-mass spectrometry (HPLC-MS), but in general the preconcentration step is extensive, requiring large sample volumes (between 0.5 and 2.0 L), sample clean-up or the use of organic solvents.[9-14] There is a need for fast and simple methods to supply this analysis.

The single drop microextraction (SDME) is a two-step process that is conducive to the simultaneous extraction and preconcentration of organic compounds. Since its introduction, SDME has gained popularity as a simple, inexpensive, reliable and flexible tool for the sampling of a variety of volatile and semivolatile compounds. SDME

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requires less sample volume than solid phase extraction or liquid extraction. On the other hand, the GC analysis of acidic herbicides is complicated because a derivatization step is necessary prior to the analysis. Generally, prior to the determination of acidic herbicides in water, the analytes are transferred to another phase in which the derivatization takes place.[15-18] However, few studies consider the option of performing the derivatization directly in the water matrix.[19,20] To date, the acidic herbicides have not been investigated by the direct derivatization-SDME procedure. By *in situ* methylation, the acidic herbicides are converted to less polar methyl esters, improving the extraction into the SDME drop.

In this work, a new method for analysis of three acidic herbicides: 4-(4-chloro-2-methylphenoxy) butanoic acid (MCPB), 2-(2-methyl-4-chlorophenoxy) propionic acid (MCPP) and [(3,5,6-trichloro-2-pyridinyl)oxy] acetic acid (triclopyr) in water samples using SDME/GC-MS is proposed. The analytical procedure involves *in situ* derivatization of acidic herbicides to their methyl esters with dimethyl sulfate.

Experimental

Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Water was purified with a Nanopure system (Barnstead, USA). MCPB, MCPP and triclopyr were supplied by Chem Service (West Chester, PA, USA). A stock standard solution of 1000 μg mL^{-1} of each compound was prepared in methanol. Working solutions were obtained by appropriated dilutions with methanol. The derivatization reagent dimethyl sulfate (DMS) was purchased from Riedel-de Haen. Tetrabutylammonium hydrogen sulfate (TBA-HSO_4) was obtained from Fluka.

Instrumentation

Chromatography analysis was performed using a 6890N series gas chromatograph equipped with a split-splitless injector for the HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and 5973 quadrupole mass selective detector (Agilent Technologies, USA). The split/splitless injector temperature was set at 250 °C and all injections were made in splitless mode with the split closed for 3 min. The oven temperature was held at 65 °C for 1 min, and then heated to 250 °C at a heating rate of 20 °C min^{-1}. Temperature was held at 250 °C for 4.0 min. The carrier gas was helium (purity 99.999 %) at a flow rate of 1 mL min^{-1}. The mass spectrometer detector was tuned by maximum sensitivity auto-tune. The following *m/z* values were acquired in the electron impact ionization mode by single ion monitoring and used for quantification of the analytes: 169-228 for MCPP, 210-269 for triclopyr and 101-242 for MCPB.

Procedure

A volume of 3.0 mL of standard solution or sample was placed in a 5 mL screw-cap glass vial. Phosphate buffer solution (pH 8.0, 0.75 mol L^{-1}, 0.4 mL) and Na_2SO_4 (1.2 g) were then added and the sample was agitated with a 1.8 cm long PTFE-coated stir bar. After addition of the ion-pairing reagent (TBA-HSO_4, 0.1 mol L^{-1}, 12.0 μL), the vial was closed. A volume of 12.0 μL of derivatization reagent (DMS) was then injected through the septum. After 1 min at 25 ºC, a 10 μL Hamilton syringe (Reno, USA) with a bevel-needle tip (bevel 22º) containing 2 μL of *n*-heptane was clamped above the vial containing the water sample. Then, the microsyringe was lowered and its needle passed through the septum until the tip of the needle was immersed in the sample. The plunger was depressed and the 1 μL drop of the extractant phase was exposed to the sample. The samples were agitated with a magnetic stirring bar at 400 rpm during both derivatization and extraction. The analytes were allowed to partition between the aqueous sample and *n*-heptane droplet at room temperature for 25 min. After the extraction, the organic drop was drawn back into the microsyringe and the needle was removed from the vial and immediately transferred into the GC injection port for analysis.

Aqueous standards were prepared and analyzed for calibration. Real water samples were taken from Zipayara River (Zulia,Venezuela) and of the aqueduct of the Maracaibo City.

Results and Discussion

Optimization of the microextraction with in situ derivatization

It has been reported that DMS reacts with carboxylic acids in water to produce the corresponding methyl ester.[21,22] Although the reaction mechanism is not completely understood, it is proposed that TBA salts (in conjunction with pH control) allow the stabilization of the carboxylate anions and the subsequent methylation with dimethyl sulfate.

Preliminary experiments were carried out to optimize the main parameters affecting both the derivatization and SDME of the investigated analytes. In this study, deionized

water samples that were spiked with the appropriate amount of the standard solution were used.

In order to set an optimal volume of the derivatization reagent for the complete esterification of the acidic herbicides, a range of 2.0-24.0 μL of DMS volumes was tested. The increase of the DMS volume in the range of 2.0-12.0 μL slightly improved the methyl ester yield, while the response remained constant at higher DMS amounts. For this reason, a volume of 12.0 μL was used for the rest of the experiments.

It was investigated the TBA-HSO_4 volume effect on the amount of the extracted acidic herbicide from the sample. The TBA-HSO_4 volume profile was studied by monitoring the GC area counts as a function of TBA-HSO_4 volume. The response increased when the TBA-HSO_4 volume increased from 0-12.0 μL, while larger TBA-HSO_4 volumes did not produce significant increase in the response. The TBA-HSO_4 addition of 12.0 μL ensured the maximum response for acidic herbicide methyl esters.

The pH effect was investigated by changing the pH in the range of 4-9. As shown in Figure 1, no significant effect was observed in the range of 4-6. At higher pH, an increase was observed in the response. From these results, it was decided to adjust the pH of the water samples to 8.0. A 0.75 mol L^{-1} concentration of the phosphate buffer (pH 8.0) was selected to obtain an adequate buffering capacity.

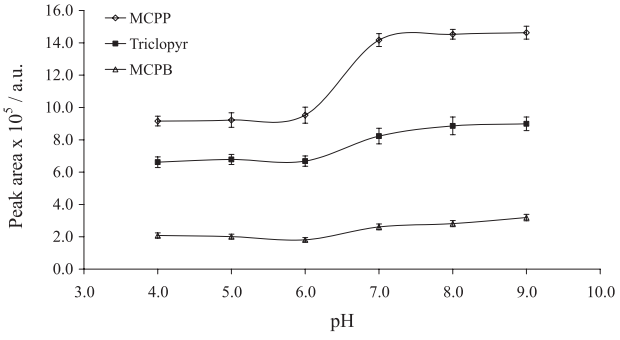

Figure 1. pH effect on the sample for *in situ* methylation-SDME of the acidic herbicides.

The role of the ionic strength of the matrix was investigated by using sodium sulfate. For many organic analytes, aqueous solubility decreases with increasing ionic strength, and thus, the partitioning from the aqueous solution to the organic solvent is improved. The tested concentrations for a sample volume of 3 mL varied from 0 to 0.4 g mL^{-1} of Na_2SO_4. The obtained results show a strong increment in the signal for all analytes when Na_2SO_4 concentration is increased. Higher concentrations of salt could not be used since it is not solubilized at room temperature. Thus, 0.4 g mL^{-1} of Na_2SO_4 was selected to obtain an adequate salting-out effect.

The selection of an appropriate solvent of extraction is of major importance for the optimization of the SDME process. In this study, three water immiscible solvents (namely *n*-hexane, *n*-heptane and isooctane) were tested. Among the different tested extracting solvents, the use of *n*-heptane resulted in the best extraction efficiency and drop stability. Thus, *n*-heptane was chosen as an extracting solvent in this investigation.

The agitation effect on the pesticide extraction was studied. The sample agitation is assumed to reduce the required time to establish the partition equilibrium between the aqueous and the organic phase. The optimum stirring rate was determined in the range of 200-500 rpm. Extraction increases with the increase of the stirring speed. However, a high stirring speed increases the risk of drop dislodgement.[23] To ensure the formation of a stable and reproducible microdrop, a stirring speed of 400 rpm was used in this work.

The time that analytes are in contact with the drop is significant for SDME sampling.[24] Extraction time profiles were studied extracting samples of 10 ng mL^{-1} of acidic herbicides and monitoring the GC area counts as a function of the exposure time (Figure 2).

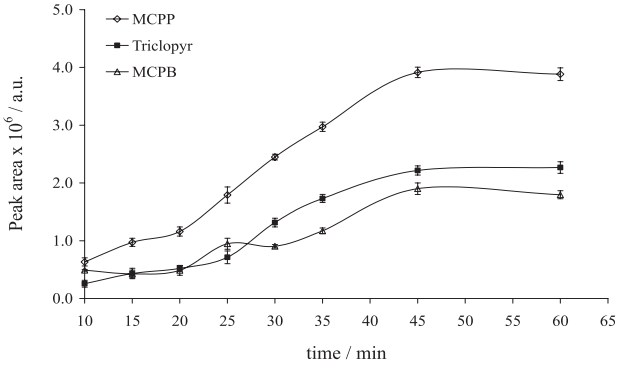

Figure 2. Absorption time profile of 10 ng mL^{-1} of acidic herbicides in deionized water.

The equilibrium was obtained after 45 min. In SDME as discussed for Jeannot and Cantwell,[25] a direct relationship exists between the concentration of the extracted analyte by SDME, the extraction time and the initial analyte concentration in the sample. This relationship indicates that SDME quantification is feasible before reaching partition equilibrium once the SDME conditions and the extraction time are held constant.[26,27] An extraction time of 25 min was selected as a compromise between the analyte response and the time of analysis.

Application and validation of the proposed method

The calibration graphs for the deionized water samples that were treated according to the previously described

procedure (with monitoring of the SIM mode) were linear for the concentration range 0.05-10.0 ng mL^{-1}. This range agreed with environmental levels in water currently reported in the literature[17] for these compounds. The calibration levels were 0.05, 1.0, 2.5, 5.0 and 10.0 ng mL^{-1}. Two replicates were used for each of the five prepared standards to obtain the calibration graphs. The results for regression coefficient are summarized in Table 1. The repeatability was measured by performing 8 independent determinations. The precisions ranged from 7.0 to 15.2% (relative standard deviation, RSD) (Table 1), which should be satisfactory for determining the acidic herbicides in water matrix.

Table 1. Analytical parameters

	Analytes		
	MCPB	MCPP	Triclopyr
Correlation coefficient	0.998	0.997	0.998
Limit of detection / (ng L^{-1})	2.8	1.8	3.0
Limit of quantification / (ng L^{-1})	9.6	6.0	10.0
Repeatability (RSD / %), n = 8			
0.25 ng mL^{-1}	7.7	15.2	13.5
1.00 ng mL^{-1}	7.0	8.6	8.7
3.00 ng mL^{-1}	8.6	9.0	9.4

RSD: relative standard deviation.

The limit of detection was calculated by comparing the signal-to-noise ratio (*S/N*) of the lowest detectable concentration to a *S/N* ration of 3. A *S/N* ration of 10 was applied for the calculation of the limit of quantification. The limits of detection were between 1.8 and 3.0 ng L^{-1} (Table 1) being in accordance with other methods that are based on GC or HPLC techniques.[17,20,26-28]

The optimum SDME sampling conditions for deionized water were applied to the tap water and river water matrices.

It was tried to find acidic herbicides in tap water samples and river water samples. It was not found acidic herbicides above our limit of detection. This is probably due to the fact that in Venezuela, the triclopyr is a herbicide of little use for the control of weeds in rice and pastures, while MCPB and MCPP are not used. Samples were fortified with different levels of acidic herbicides. External calibration was used in the evaluation of acidic herbicides because matrix effects were not observed. The identity of these compound was confirmed by using a peak retention time window within 1.5% and ratios of two of the most intensive and characteristic ions of the mass spectrum. Table 2 shows the results of the recovery study and a representative chromatogram of the spiked tap water sample with 1.0 ng mL^{-1} of each analyte is depicted in Figure 3.

Table 2. Results of assays to check the accuracy of the proposed method for acidic herbicides in spiked tap water and river water samples

Analyte	Sample	Spiked / (ng mL^{-1})	Found[a] / (ng mL^{-1})	Recovery[b] / %
MCPB	tap water	0.25	0.26 ± 0.01	102.4
		1.00	0.94 ± 0.06	93.7
		3.00	3.09 ± 0.27	103.0
	river water	0.25	0.22 ± 0.01	87.2
		1.00	0.86 ± 0.09	86.3
		3.00	3.19 ± 0.18	106.3
MCPP	tap water	0.25	0.26 ± 0.01	102.4
		1.00	1.08 ± 0.01	107.9
		3.00	2.77 ± 0.02	92.5
	river water	0.25	0.24 ± 0.01	93.8
		1.00	0.98 ± 0.05	97.6
		3.00	3.02 ± 0.09	100.8
Triclopyr	tap water	0.25	0.25 ± 0.03	98.9
		1.00	0.99 ± 0.02	98.8
		3.00	2.92 ± 0.15	97.3
	river water	0.25	0.22 ± 0.01	85.8
		1.00	0.85 ± 0.04	84.8
		3.00	2.89 ± 0.28	96.4

[a]Average value ± standard deviation of four determinations; [b]recovery (%) refers to the determined acidic herbicide concentrations rather than the actual percent of extracted analytes by the SDME analysis.

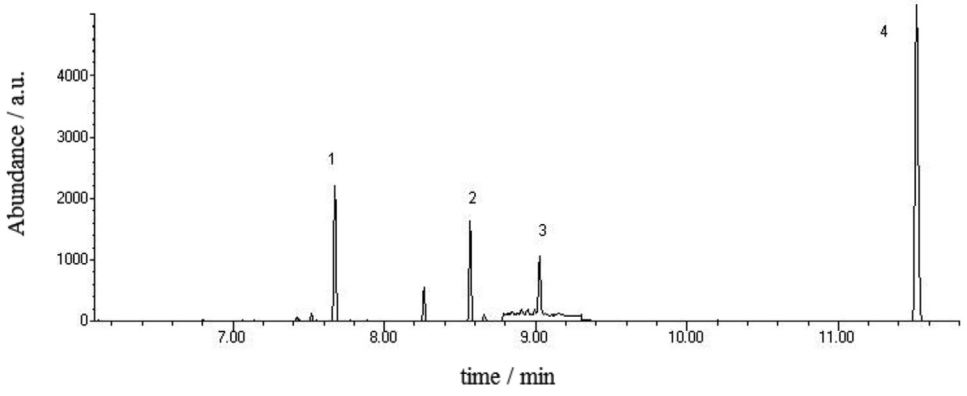

Figure 3. Typical chromatogram obtained in SIM mode of a spiked tap water sample with 1.0 ng mL^{-1} of each analyte: (1) MCPP, (2) triclopyr, (3) MCPB and (4) triphenyl phosphate (internal standard).

The recoveries were greater than 84.8% and no interfering peaks were observed for the blank samples. These results demonstrate the usefulness of the proposed method for this application.

Conclusions

A simple and practical GC-MS method in combination with *in situ* derivatization SDME for the determination of the acidic herbicides MCPB, MCPP and triclopyr in water samples is presented. Sensitive responses were obtained using 12.0 μL of DMS, 12.0 μL of 0.1 mol L^{-1} TBA-HSO_4, 1.0 μL *n*-heptane drop, 0.4 g mL^{-1} Na_2SO_4, 25 min of extraction time and pH 8.0 in combination. Non-equilibrium conditions were adopted in order to reduce the extraction time. In view of its simplicity and sensitivity, the proposed method is applicable for the quantification of residues of acidic herbicides in tap water and river water samples.

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