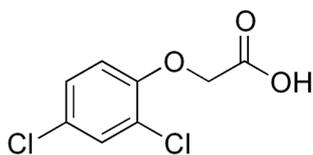


Supplementary Materials

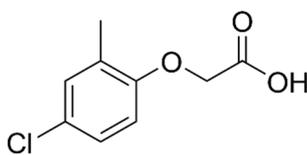
Hollow-Fiber Liquid-Phase Microextraction (HF-LPME) Coupled On-Line to Liquid Chromatography for the Determination of the Herbicides 2,4-Dichlorophenoxyacetic Acid and 2-Methyl-4-chlorophenoxyacetic Acid and Their Main Metabolites in Soil Samples

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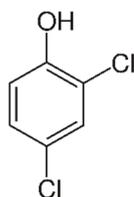
Chemical structures of the separated compounds



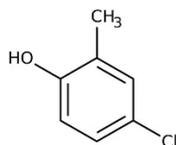
2,4-dichlorophenoxyacetic acid (2,4-D)



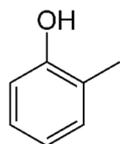
2-methyl-4-chlorophenoxyacetic acid (MCPA)



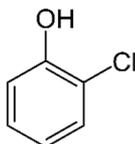
2,4-dichlorophenol (2,4-DCP)



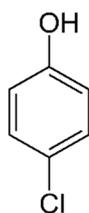
4-chloro-2-methylphenol (4-C-2-MP)



2-methylphenol (2-MP)



2-chlorophenol (2-CP)



4-chlorophenol (4-CP)

Quality parameters of the on-line HF-LPME method

The use of conventional ordinary least squares (OLS) is not appropriate for this study as this simple regression calculation procedure can yield highly biased results at low concentration levels [1,2]. It was found that applying OLS the relative errors at the smaller concentration of the working ranges assessed ($1 \mu\text{g}\cdot\text{l}^{-1}$) were $>40\%$, which is above the commonly accepted value of $<20\%$ at this level [3]. Moreover, the relative standard error (RSE) [2] determined for all the calibrations ranged from 22% to 55%, when the accepted value in our laboratory is set at $\leq 15\%$. For these reasons, weighted least squares (WLS) were applied. This regression calculation gave acceptable relative errors at low levels ($<20\%$) and the RSE values of the calibrations measured were in the range from 7% to 15%, which confirmed the goodness-of-fit of the WLS regression parameters determined. Linearity was also assessed through the residuals distribution obtained for the calibration curves, which always gave random distribution of the residuals, with standardized residual values <2 . Therefore, the proposed methodology showed linear responses, with accurate and precise determinations being obtained in a working range between 1 and $30 \mu\text{g}\cdot\text{l}^{-1}$ for each analyte (Table SM1), which corresponds to a range in soil samples between 2 and $60 \mu\text{g}\cdot\text{kg}^{-1}$ soil.

The method detection limits (MDL) for the target compounds were between 0.1 and $0.3 \mu\text{g}\cdot\text{kg}^{-1}$ soil (determined from spiked soils at a content that yielded a signal-to-noise ratio ≥ 3). These detection limits were equivalent to those obtained in other studies using HF-LPME procedures for the extraction of phenoxyacetic acids [4,5]. Figure SM1 shows the chromatogram of a spiked soil sample at $0.5 \mu\text{g}\cdot\text{kg}^{-1}$ level (between the determined limits of detection and quantification) analyzed with the on-line HF-LPME/HPLC-UV method. The comparison between the limits of detection of the HPLC-UV method and the on-line HF-LPME/HPLC-UV method shows that enrichment factors between 100 and 200 are obtained with the on-line microextraction method.

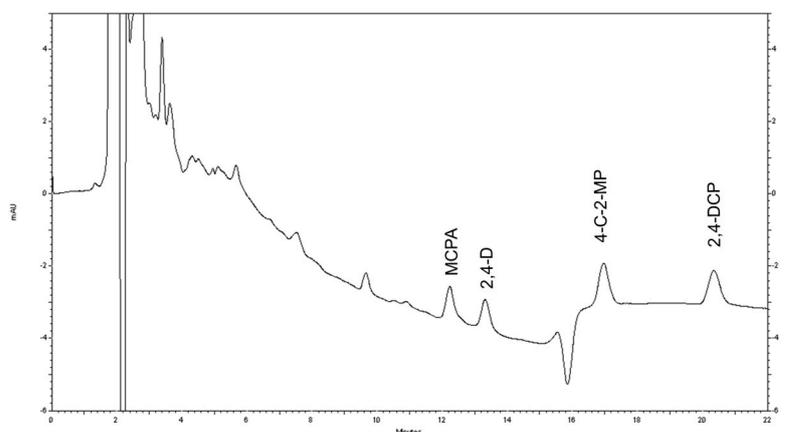


Figure S1. Chromatogram obtained after the HF-LPME/HPLC-UV analysis of a non-contaminated soil sample, spiked at $0.5 \mu\text{g}\cdot\text{kg}^{-1}$ with four target analytes.

Table S1. Results obtained in the validation of the on-line HF-LPME/HPLM-UV methodology proposed. (WLS: weighted least squares; RSE: relative standard error; MDL: Method Detection Limit; SE: Standard Error).

	Calibration Curves (WLS)			MDL ($\mu\text{g}\cdot\text{kg}^{-1}$)	Working Range ($\mu\text{g}\cdot\text{L}^{-1}$)	
	Intercept (SE)	Slope (SE)	RSE (%)		($\mu\text{g}\cdot\text{L}^{-1}$)	($\mu\text{g}\cdot\text{kg}^{-1}$)
2-MP	371 (30)	140 (4)	7.2	0.3	1-30	2-60
2-CP	889 (71)	200 (12)	15.0	0.3	1-25	2-50
4-CP	1280 (218)	892 (34)	9.9	0.2	1-30	2-60
MCPA	865 (156)	640 (26)	9.8	0.1	1-25	2-50
2,4-D	1319 (116)	442 (19)	10.4	0.1	1-25	2-50
4-C-2-MP	899 (190)	621 (26)	12.5	0.2	1-30	2-60
2,4-DCP	575 (190)	615 (26)	9.0	0.1	1-25	2-50

Three independent replicates of the first and last standard of the chosen working range were analyzed to have information about the precision within this range. For all the target compounds, an F-test confirmed that the standard deviations obtained at these concentration levels were equivalent ($p > 0.05$). Therefore, the precision can be taken as constant in the selected working ranges (homoscedasticity). For this reason, the precision of the method was only assessed at one level, and three replicate analyses of a soil sample, fortified at $10 \mu\text{g}\cdot\text{kg}^{-1}$ soil, were analyzed with the HF-LPME/HPLC-UV method, yielding relative standard deviations $< 15\%$, which were considered acceptable.

Trueness was determined through the evaluation of three soil samples that were previously analyzed with a validated method based on SPE (with MDL ranging from 5 to $10 \mu\text{g}\cdot\text{kg}^{-1}$ soil) [6]. The results obtained (Table SM2) gave equivalent results between the two methods for those compounds that were detected with both methods ($p > 0.05$). The lower method detection limits of the proposed HF-LPME procedure allowed to detect some compounds in these samples that were not detected with the SPE method.

Table S2. Concentrations detected ($\mu\text{g}\cdot\text{kg}^{-1}$ soil) for three soil samples evaluated with the proposed on-line HF-LPME method and a previously validated method using SPE [6] ($n = 3$; values in brackets are the sample standard deviation, d: detected; nd: not detected, $< \text{MDL}$).

Soil	Method	Analyte						
		MCPA	2,4-D	2-MP	2-CP	4-CP	4-C-2-MP	2,4-DCP
1	SPE	nd	nd	nd	55 (4)	nd	61 (3)	nd
	HF-LPME	d	d	nd	61 (4)	nd	58 (3)	nd
	<i>p</i> -value	--	--	--	0.123	--	0.473	--
2	SPE	d	nd	nd	nd	nd	225 (6)	nd
	HFSLM	7 (1)	nd	nd	2 (0.3)	nd	209 (11)	nd
	<i>p</i> -value	--	--	--	--	--	0.084	--
3	SPE	nd	nd	nd	131 (7)	nd	135 (6)	nd
	HFSLM	d	d	4 (0.6)	136 (5)	nd	121 (11)	nd
	<i>p</i> -value	--	--	--	0.340	--	0.125	--

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