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Coupling continuous subcritical water extraction, filtration, preconcentration, chromatographic separation and UV detection for the determination of chlorophenoxy acid herbicides in soils

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Abstract

Subcritical water extraction has been coupled with filtration, preconcentration and chromatographic analysis for the determination of acid herbicides in different types of soil. Two experimental designs were used for the optimization of the leaching step. The use of water as extractant in the continuous mode at a flow-rate of 1 ml/min and 85 °C was sufficient for quantitative extraction of the analytes. A static extraction time was unnecessary for reducing the extraction time to 1 h. A minicolumn containing C_{18}-Hydra as sorbent proved an excellent material for the quantitative preconcentration of the herbicides prior to individual chromatographic separation. A flow-injection manifold was used as interface for coupling the four steps, thus allowing automation of the whole analytical process. Recoveries of the target analytes ranged between 94.2 and 113.1%, and repeatabilities, expressed as relative standard deviations, were between 0.61 and 6.83%. © 2002 Published by Elsevier Science B.V.

Keywords: Subcritical water extraction; Extraction methods; Soil; Chlorophenoxy acids; Phenoxy acids; Pesticides

1. Introduction

Herbicides are common tools for suppressing unwanted species from plants. In the USA, millions of kilograms of herbicides are used annually. Among these, chlorinated acid herbicides constitute a major class that has found wide agricultural applications [1,2].

Quantitative extraction of chlorophenoxy acid herbicides from soil has traditionally constituted a challenge because of their strong binding to soil [3]. The conventional methods for analyzing chlorinated herbicides are labor-intensive, especially for solid samples such as soil where alkaline aqueous extraction followed by protonation of the analytes and liquid–liquid extraction into an organic solvent are mandatory steps. Prior to chromatographic analysis, repeated manual operations of concentration and clean-up can be required, which are both time- and solvent-consuming. In order to both shorten the total analysis time and reduce the volume of solvents which are toxic, flammable, and expensive, extraction techniques such as automated solvent extraction [4], ultrasound-assisted extraction [5,6], microwave-assisted solvent extraction (MASE) [7,8], supercritical fluid extraction [9,10] and pressurized liquid

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extraction (PLE) [11,12] have been proposed as alternatives to the conventional procedure.

Among the new extraction techniques, PLE is the most recent. Methods based on PLE use the solid sample placed into an extraction chamber and the analytes are extracted from the matrix with conventional low-boiling solvents or solvent mixtures at elevated temperatures and pressures high enough to maintain the solvent in the liquid state. In previous works [3,13], PLE has been tested for the extraction of phenoxy acid herbicides from soil and sand using acetone as extractant. However, the analytical performance of the method was not satisfactory, as the recoveries of low levels of the analytes from soil were 68%. The use of a co-extractant such as Na_4EDTA improved the recoveries of the analytes, which ranged between 69 and 117%.

Extraction using liquid water at high temperature and pressure is a promising alternative to other solid sample pretreatments, as water is the ideal solvent for the establishment of clean methods. The most outstanding feature of subcritical water as leaching agent is its capacity for altering its dielectric constant as a function of temperature.

Subcritical water extraction has proved an efficient alternative for the extraction of acid herbicides from soil. However, from an analytical point of view, the most salient negative feature of its use in a continuous extraction mode is the dilution of the analytes in the extract, which calls for a preconcentration step prior to chromatographic analysis of the target compounds making the automation of the analytical process difficult [14,15]. The coupling of a subcritical water extractor with a high pressure liquid chromatograph has recently been developed but using a relatively complicate system involving several shut-off valves [16,17]. In the present work, a fully automated method for the determination of acid herbicides in different types of soil is proposed. The coupling of the steps of the analytical process, namely, subcritical water extraction–filtration–preconcentration–individual chromatographic separation–detection, has been developed using a flow-injection (FI) system as interface between the extractor and the chromatograph, thus allowing an easier to handle and cheaper approach than those reported previously [16,17], and with the possibility of including a filtration step on-line for the removal from the extract of in-suspension particles.

2. Experimental

2.1. Instruments and apparatus

Subcritical water extraction (SWE) was performed using the following assembly: (1) a Shimadzu LC10AD pump with digital flow-rate and readouts. (2) An extractor consisting of a stainless steel cylindrical extraction chamber (Análisis Vínicos, Ciudad Real, Spain) (10 cm×10 mm I.D.), closed with screws at either end. The screw caps contained stainless steel filter plates (2 μm in thickness and 1/4 in. I.D.; 1 in.=2.54 cm) to ensure that the soil sample remained in the extraction chamber. (3) A gas chromatograph (Varian, Spain) oven where the extraction chamber, together with a stainless steel preheater, was located. (4) A cooler system (consisting of a coil coupled to an Ultraterm 6000383 P-Selecta (Barcelona, Spain) recirculation bath. (5) A needle valve (Análisis Vínicos) acting as a restrictor coupled at the outlet of the cooler. (6) A collector made from a glass test-tube (9.5 cm×1.3 mm I.D.), with an outlet PTFE tube (0.5 mm I.D.) fitted at the bottom. This device acted as interface between the extractor and the flow manifold.

A Gilson Minipuls-3 low-pressure peristaltic pump, three Rheodyne 5041 low-pressure injection valves, a laboratory-made minicolumn (4.0 cm×4 mm I.D.) packed with the sorbent material, a 0.25-μm nylon filter (Technichon) and PTFE tubing of 0.5 mm I.D. were used to build the flow manifold (Fig. 1B), which was connected to the Rheodyne 7725 high-pressure injection valve (20-μl injection loop) of an HP1100 liquid chromatograph (Hewlett-Packard, Avondale, PA, USA) consisting of a G1311A high-pressure quaternary pump, a G1322A vacuum degasser, and a G1315A diode array detection (DAD) system. An Ultrabase C_{18} (250×4.6 mm; 5-μm particle size, Scharlau) was the analytical column.

2.2. Reagents and samples

The acid herbicides [bentazone, 2,4-dichlorophenoxyacetic acid (2,4-D), triclopyr, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-Tp)] were obtained from Fluka (Buchs, Switzerland) and used for preparing the stock standard solutions in HPLC-grade

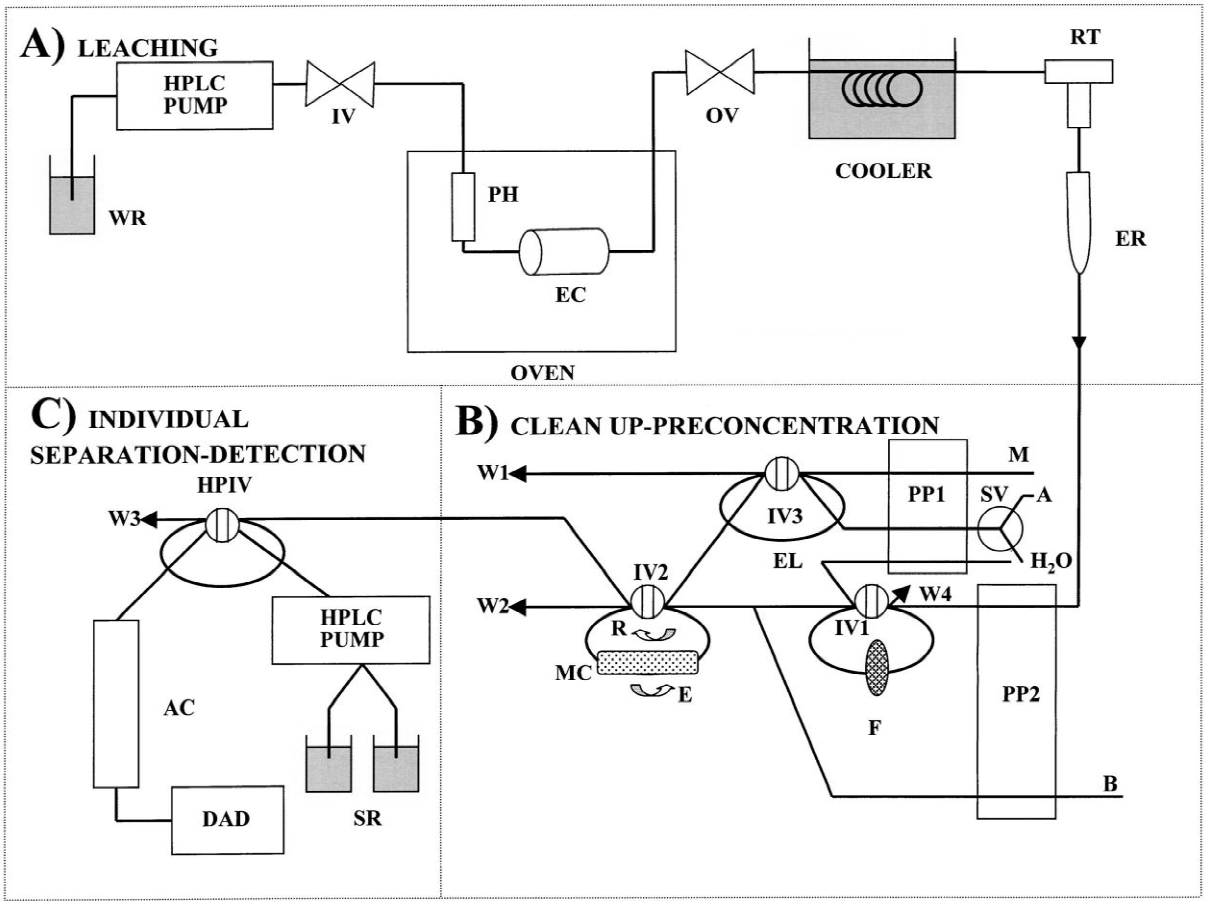

Fig. 1. Scheme of the approach used for the automated development of the overall process with division of the parts involved in each step. (A) Leaching step: WR, water reservoir; IV, inlet valve; PH, pre-heater; EC, extraction chamber; OV, outlet valve; RT, restrictor; ER, extract reservoir. (B) Clean-up/preconcentration step: M, methanol; A, air; B, buffer; PP, peristaltic pump; F, filter; EL, elution loop; MC, minicolumn; IV1, IV2 and IV3, injection valves; E, elution sense; R, retention sense; W, waste; SV, selection valve. (C) Individual separation–detection step: HPIV, high-pressure injection valve; AC, analytical column; DAD, diode array detector; SR, solvent reservoirs.

methanol (Merck, Darmstadt, Germany). The sorbent used in the preconcentration step was C_{18}-Hydra (Panreac, Barcelona, Spain).

Four types of soil (namely, clayey, slimy, limy and sandy soil) were selected as matrices to be studied, and their features (namely, pH, CO_3^{2-}, ion-exchange capacity, % organic matter, % sand, % silt and % clay) are shown in Table 1. The sandy soil was used

Table 1
Features of the soils used for the study

Soil	pH	CO_3^{2-} (%)	IEC[a] (mmol/kg)	OM[b] (%)	Sand (%)	Silt (%)	Clay (%)
Clayey	7.60	27.71	102.3	1.23	3.9	32.5	63.6
Slimy	8.06	50.40	267.6	1.24	15.0	42.2	42.8
Limy	8.50	19.51	136.0	0.51	43.4	44.9	11.7
Sandy	4.60	0.12	10.2	0.72	89.2	4.6	6.2

[a] Ion-exchange capacity.
[b] Organic matter.

as inert matrix. A 500-g amount of each soil was sieved to a size smaller than 1 mm and spiked with the acid herbicides by adding to the soils 150 ml of diethyl ether (Panreac, Barcelona, Spain) containing the necessary volume of stock standard solutions of the acid herbicides to obtain a final total concentration in the dry soil of 25 μg/g (5 μg/g of each herbicide). Then the slurries were shaken for 72 h and, after evaporation of the solvent, the soils were completely dried under an N_2 stream and finally stored at 25 °C for 6 months. None of the soils had detectable levels of the target analytes before spiking. The clayey soil was used for optimization of the extraction process.

2.3. Procedure

The method was developed using the assembly shown in Fig. 1, based on the coupling of leaching, filtration, preconcentration and chromatographic separation and detection units. A 5-g amount of the corresponding spiked soil was weighed and placed in the extraction chamber. After assembling the extraction cell with the dynamic system in the oven, the cell was pressurized with 20 bar of Milli-Q water impelled by the HPLC pump by opening the inlet needle valve from the pump. The valve was then closed and the oven was brought up to the desired temperature as quickly as possible. The inlet and outlet valves were then opened and the water pumped through the extraction chamber at a given flow-rate between 10 and 70 min. The leachate was cooled at 25 °C and the effluent from the restrictor at the end of the dynamic system was connected to the filtration–preconcentration manifold.

The overall flow-injection manifold includes three injection valves and one confluence point. The effluent was firstly cleaned from particles by passage through a filter (F), which was located in the loop of the injection valve IV1; then merged with a buffer stream (acetic acid–sodium acetate buffer at pH 4.5) in order to protonate the acid herbicides. After merging, the effluent was driven to a minicolumn where the analytes were retained. The minicolumn was also located in the loop of an injection valve (IV2), thus enabling elution in the direction opposite to retention with a volume of methanol selected using the third injection valve (IV3). The eluate was driven to the loop of the high-pressure injection valve of the chromatograph using an air stream as carrier. The injection of the volume (20 μl) selected by the high-pressure injection valve of the chromatograph was performed at a fixed time from elution, thus avoiding sample discrimination problems.

Between samples and during chromatographic separation, the filter was cleaned by passing water in the opposite direction to filtration at a high flow-rate, and the sorbent in the minicolumn was conditioned by circulating methanol and water through it.

The HPLC separation of the acid herbicides was carried out using an isocratic elution program in which a 1% aqueous solution of H_3PO_4–acetonitrile (60:40) was used as mobile phase at a flow-rate of 1.7 ml/min. Spectrophotometric detection was performed at 280 nm.

Quantitation of the analytes was carried out by running five calibration curves (one for each analyte) using standard solutions at concentrations between 10 and 50 μg/ml.

3. Results and discussion

The overall method proposed here involves removal of the target analytes from the soils, separation of suspended particles by filtration, preconcentration on an appropriate sorbent, chromatographic separation and photometric detection. The order used for optimizing the steps was as follows: first, the chromatographic separation of the target analytes using photometric detection was optimized for checking the earlier steps; then the variables affecting the preconcentration step were studied and the extraction itself was optimized in depth. Finally, a filtration step was inserted between extraction and preconcentration, when, in the overall coupling, the presence of solid particles in the cooled extract was detected.

3.1. Optimization of the chromatographic separation

The experimental variables, optimized in order to obtain appropriate separation of the analytes, were the composition of the mobile phase, the flow-rate and injection volume. Different mixtures of 1%

aqueous H_3PO_4–acetonitrile and different gradients were used for separation of the acid herbicides by the Ultrabase C_{18} column. The influence of the flow-rate of the mobile phase was studied in the range 1.0–2.0 ml/min, and the best separation was obtained for a flow-rate of 1.7 ml/min. Overlapping of the peaks corresponding to bentazone and 2,4-D occurs at higher flow-rates. An injection volume of 20 μl was selected in order to obtain a quantifiable photometric signal. Complete separation of the analytes was achieved with the isocratic program discussed under the Experimental section. Thirty minutes were necessary for complete separation of the target compounds.

3.2. Optimization of the preconcentration step

The study of the preconcentration step was carried out using the flow-injection manifold in Fig. 1B. A 50-ml aliquot of a standard solution containing 25 μg of each analyte was used in all instances.

In order to obtain the best pH of the extract for retention, different solutions of HNO_3 and NaOH were used to adjust the pH between 0.5 and 8.5. The chromatograms obtained after elution showed an increase in the peak height when the pH decreased from 8.5 to 5.0 and remained constant for lower pH values. A pH of 4.5 was selected in order to guarantee that all the compounds were protonated and, consequently, ready for being retained on the sorbent. A 1 mol/l acetic acid–sodium acetate buffer (pH 4.5) was used for adjusting the pH of the extract.

The buffer flow-rate was selected as a compromise between the adjustment of the pH and dilution of the extract. Flow-rates between 0.1 and 0.5 ml/min were studied. The required pH was obtained using a flow-rate of 0.2 ml/min, which was selected for further experiments.

The retention flow-rate was optimized by aspirating the standard solution (the pH was previously adjusted) to the minicolumn. Flow-rates ranging from 0.5 to 1.2 ml/min were tested. The results obtained showed an increase in the recovery when the flow-rate decreased from 1.2 to 0.7 ml/min, thus demonstrating the influence of the retention kinetics. Flow-rates between 0.7 and 0.5 ml/min provided similar results; so a flow-rate of 0.7 ml/min was selected for subsequent experiments.

The elution flow-rate and the volume of eluent (methanol) were optimized jointly taking into account that a minimum volume of eluent was the most important aspect in order to obtain the highest preconcentration factor. The carrier into which the methanol was injected was air. Its usage had the aim of dragging away from the system the aqueous phase before passage of the eluent through the minicolumn thus avoiding dilution. No other carrier can be used without causing dilution as methanol is miscible with both aqueous and organic carriers. Elution flow-rates from 0.1 to 1 ml/min and volumes of eluent from 0.2 to 2 ml were assayed. Finally, an elution volume of 0.5 ml at 0.15 ml/min was selected as optimum. The use of 0.5 ml of methanol as eluent resulted in preconcentration factors ranging from 20 to 150, depending on the volume of extract (from 10 to 75 ml).

In order to determine the breakthrough of the C_{18}-Hydra minicolumn, the volume of sample containing 25 μg of each analyte passed through the sorption material was studied in the range 10–150 ml. The signal remained constant up to 100 ml and decreased for higher volumes, so in subsequent experiments, volumes higher than 100 ml were not used so as not to surpass the breakthrough volume of the minicolumn. Under these conditions, quantitative retention and elution of the analytes was achieved.

3.3. Optimization of the extraction step

Preliminary experiments were aimed at selecting the optimum extractant. Distilled, acidified and basic water solutions were assayed as extractants under the same experimental conditions. Similar results were obtained using distilled water and basic water solution; however, the latter extractant removed more undesirable components from the matrix and corrosion of the chamber and/or tubing in the extractor was detected. For these reasons, distilled water was selected as the optimum extractant.

The overall recovery of acid herbicides from soil samples achieved by SWE could be influenced by a number of variables, namely: temperature (A), water flow-rate (B), static (C) and dynamic extraction times (D). A full two-level factor design would

Table 2
Factor levels in the first (half-fractioned) factorial design

Factor	Key	Low level (−)	High level (+)
Temperature (°C)	A	100	200
Water flow-rate (ml/min)	B	1.0	2.5
Static extraction time (min)	C	0	15
Dynamic extraction time (min)	D	10	30

involve an overall $2^4=16$ experiments, in addition to the replicates for statistical evaluation of the coefficients for the fitted model and the degree of coincidence of the hyperplane obtained. The selection of a half-fraction 2^{4-1}, type IV resolution design allowing three degrees of freedom, involved eight randomized runs plus three centered points [18]. This design possesses an alias structure such as that the main effects are clearly different from the two-factor interactions but the latter are partially confounded with other two-factor interaction effects. Table 2 lists the upper and lower values given to each factor. Such values were selected from the available data and experience gathered in the preliminary experiments. Table 3 shows the matrix for this first factorial design and the extraction yield of each analyte in the selected clayey soil.

The conclusions are that 2,4-D and triclopyr are the compounds most affected by the experimental conditions, mainly by temperature and dynamic extraction time. The water flow-rate seems to be significant only for 2,4-D; meanwhile, the static extraction time does not seem to be influential for any of the target analytes.

In view of these results, since static extraction time is not an influential variable, the lower value of the design was selected. As this value was 0 min, static extraction was not carried out, thus saving the time required for this operation. In the case of the water flow-rate, the lower value was also selected as 2,4-D and triclopyr, the compounds most affected by this factor, showed a higher recovery in the experiments at 1 ml/min water flow-rate (the lower value tested). Concerning other factors, the response surface tended to minimum and maximum values for temperature and dynamic extraction time, respectively. Because the results suggested that these are the most significant factors, a new factorial design with longer dynamic extraction times and lower temperature was carried out. A three-level 3^2 factorial design, involving nine runs using the low, medium and high levels depicted in Table 4, was conducted. Table 5 summarizes the results obtained, together with the corresponding design matrix. Only the dynamic extraction time appears as statistically significant for all analytes; meanwhile, temperature is only significant for 2,4,5-T and 2,4,5-Tp. A maximum of the response surface was observed for

Table 3
Design matrix and response values in the first (half-fractioned) factorial design

Run	A	B	C	D	Bentazone	2,4-D	Triclopyr	2,4,5-T	2,4,5-Tp
1	+	–	–	+	42.0	30.9	46.2	61.2	52.0
2	–	–	–	–	52.8	37.6	57.5	61.2	50.9
3	0	0	0	0	53.8	53.0	68.1	65.8	54.8
4	+	+	+	+	50.3	63.5	72.4	77.8	65.5
5	+	+	–	–	49.6	57.3	67.8	69.0	61.8
6	–	–	+	+	94.1	95.0	89.8	97.7	78.5
7	+	–	+	–	68.9	47.3	65.4	87.4	75.5
8	–	+	+	–	59.0	53.4	57.4	48.3	38.8
9	–	+	–	+	93.4	92.8	94.4	101.5	84.3
10	0	0	0	0	55.0	52.0	68.3	66.0	53.8
11	0	0	0	0	54.4	52.4	68.7	65.2	54.0

Recoveries are expressed as percent.

Table 4
Factor levels in the second (three-level) factorial design

Factor	Key	Low level (-)	Medium level (0)	High level (+)
Temperature (°C)	A	50	75	100
Dynamic extraction time (min)	D	30	40	50

Table 5
Design matrix and response values in the second (three-level) factorial design

Run	A	D	Bentazone	2,4-D	Triclopyr	2,4,5-T	2,4,5-Tp
1	+	–	39.0	67.8	76.0	67.3	54.8
2	–	0	79.8	73.2	74.0	68.7	56.8
3	0	0	105.9	85.0	86.3	89.4	75.7
4	+	+	82.8	84.7	90.6	99.4	80.3
5	–	+	104.6	95.7	89.1	93.0	78.6
6	+	0	79.8	87.4	92.7	97.4	82.0
7	–	–	10.4	9.0	12.5	5.6	4.6
8	0	+	84.6	92.4	94.7	102.5	85.8
9	0	–	69.5	87.7	76.5	72.2	57.6

Recoveries are expressed as percent.

temperature in all cases, but not for the dynamic extraction time, which tends to values longer than the maximum tested. Analyzing the design only for temperature, a second-order polynomial equation was obtained for each analyte (Table 6), the optimal temperature of which was obtained by equalizing to zero the first derivative of the polynomial, solving the resulting equation system, and decodifying the results. As can be seen in Table 6, different but close optimal temperatures were obtained for the analytes. However, a weighted average was performed taking into account the standardized effect of temperature for each herbicide, thus obtaining a final optimal value of 85 °C. This behavior was foreseeable due to the facts that: (a) the analytes are polar; (b) the dielectric constant of water decreases with increasing temperature.

Finally, in order to both optimize the dynamic extraction time and study the kinetics of the extraction process, seven extractions at different dynamic extraction times ranging from 10 to 70 min were performed. The other variables (namely, temperature, water flow-rate and static extraction time) were fixed at their optimal values (85 °C, 1 ml/min and 0 min, respectively). This final step was carried out using the four types of soil in order to study the different behaviors of the target analytes depending on the matrices in which they are retained. As can be seen in Fig. 2, the retention of the analytes depends on the type of soil. In all cases, the extraction of the analytes from sandy soil is quantitative in 30 min; meanwhile in the case of slimy soil, where both the amount of organic matter and the ion-exchange capacity are higher (Table 1), the extraction is not

Table 6
Second-order polynomial equations and optimal temperature obtained for each analyte

Compound	Second-order polynomial equation	Codified value	Decodified value (°C)	Standardized effect
Bentazone	$\text{Bentazone} = 94.18 + 4.88T - 24.32T^2$	6.8×10^{-4}	75.00	0.65
2,4-D	$\text{2,4-D} = 240.2 + 32.22T - 54.75T^2$	0.294	82.35	1.40
Triclopyr	$\text{Trichlopyr} = 550.733 + 102.62T - 98.72T^2$	0.520	88.00	2.60
2,4,5-T	$\text{2,4,5-T} = 236.93 + 42.85T - 60.08T^2$	0.356	83.90	3.80
2,4,5-Tp	$\text{2,4,5-Tp} = 190.37 + 35.45T - 37.15T^2$	0.477	86.92	3.60

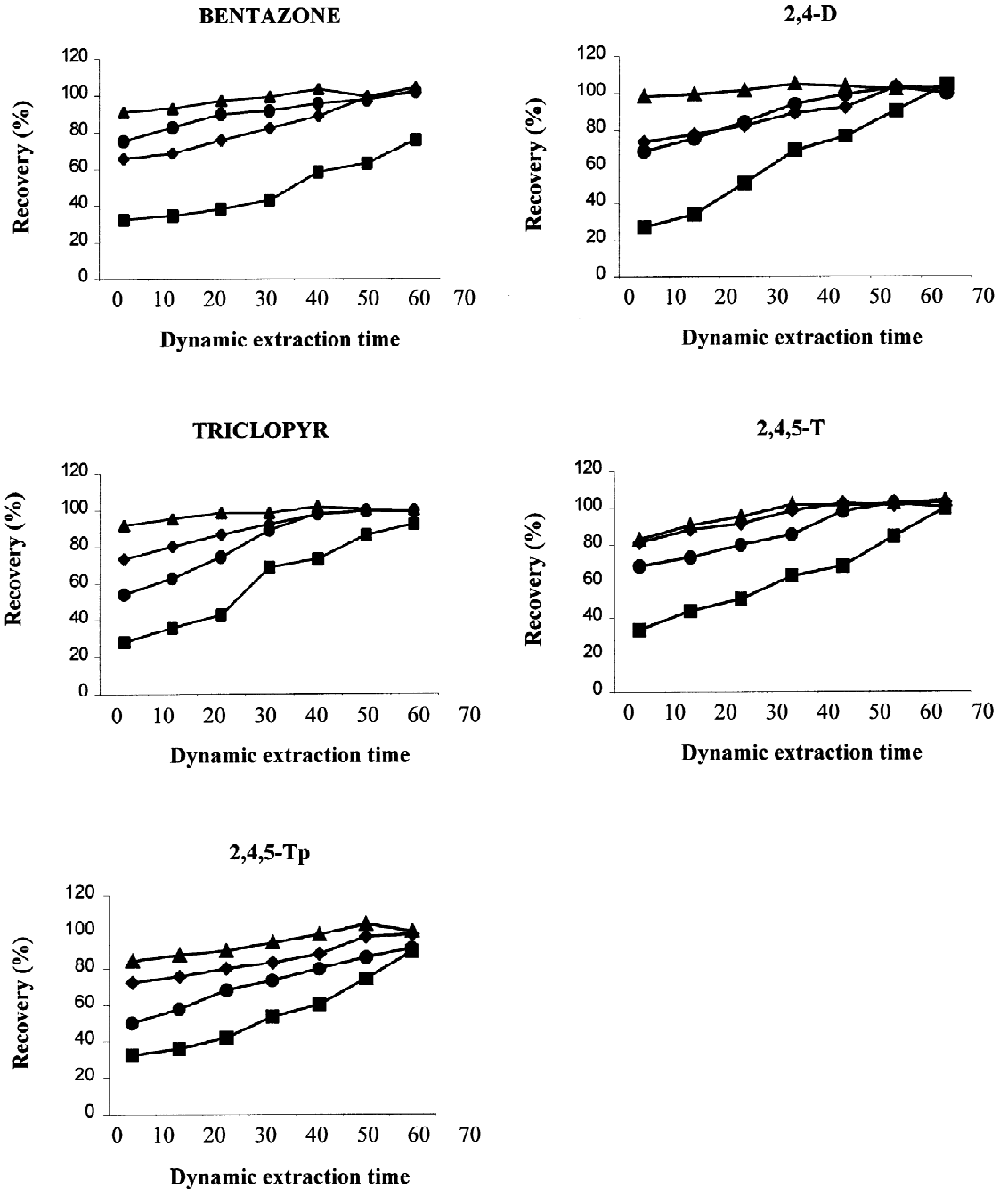

Fig. 2. Kinetics of extraction of the target herbicides from different soils. ♦, clayey soil; ■, slimy soil; ▲, sandy soil; ●, limy soil.

quantitative for all the herbicides in 70 min. The behavior of the herbicides in limy soil is similar to that in clayey soil; however, it must be concluded that using the optimal conditions obtained for clayey soil, good recoveries are obtained for all types of soil, independent of the kinetics of the extraction.

3.4. Optimization of the filtration step

Solid particles formed in cooling the extract reached the preconcentration minicolumn and caused a deterioration effect on the packed sorbent. For this reason, a continuous filtration system was coupled between the extractor and the preconcentration minicolumn (see Procedure); 0.25-μm nylon filters with different diameters ranging from 14 to 29 mm were tested. Filters with low diameter gave rise to overpressure in the system, so the filter of higher diameter was selected for further experiments. In addition to avoiding overpressure, this filter has a higher area and thus an increased capacity for retention of the undesirable particles.

The location of this device in the loop of an auxiliary injection valve, as IV3 in Fig. 1, allowed cleaning of the filter after use by passage of distilled water at high flow-rate in the direction opposite to filtration. The inclusion of the filter in the dynamic approach dramatically extended the sorbent minicolumn life, which did not deteriorate during the subsequent experiments.

3.5. Evaluation of the precision of the method

In order to evaluate the precision of the proposed method, within-laboratory reproducibility and repeatability were estimated in a single experimental set-up with duplicates [19]. The experiments were carried out using 5 g of clayey soil containing 5 μg/g of each herbicide. In all experiments, the optimal values obtained for all the variables were used but 60 min was set as the dynamic extraction time. As can be seen in Table 7, two measurements of each analyte per day were carried out over 7 days. The analysis of variance (ANOVA) table (Table 8) shows the sum of squares (SS), the degrees of freedom (df) and the mean squares (MS) between and within days. The residual mean squares, which are termed the mean squares within days, represent s_r^2 under repeatability conditions. To determine the variance due to the between-day effect, Eqs. (1) and (2) were used

$$s^2_{\text{between}} = (MS_{\text{between}} - MS_{\text{within}})/n_j \quad (1)$$

Table 8
ANOVA table for the within-laboratory reproducibility and repeatability study

Compound	Source	SS[a]	df[b]	MS[c]
Bentazone	Between days	278.36	6	46.39
	Within days	17.85	7	2.55
	Total	296.21	13	
2,4-D	Between days	417.69	6	69.62
	Within days	49.18	7	7.03
	Total	466.87	13	
Triclopyr	Between days	252.62	6	42.10
	Within days	4.37	7	0.62
	Total	256.99	13	
2,4,5-T	Between days	325.15	6	54.19
	Within days	4.40	7	0.63
	Total	329.54	13	
2,4,5-Tp	Between days	158.89	6	26.48
	Within days	47.47	7	6.78
	Total	206.37	13	

[a] Sum of squares.
[b] Degrees of freedom.
[c] Mean square.

Table 7
Experiment for the determination of within-laboratory reproducibility and repeatability from a single experimental set-up

Day	Bentazone		2,4-D		Triclopyr		2,4,5-T		2,4,5-Tp	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	98.7	99.3	103.4	102.2	99.9	100.3	111.4	112.5	108.5	97.9
2	95.4	97.4	111.0	109.9	108.7	109.5	103.1	103.9	97.5	99.7
3	111.1	109.0	101.5	102.0	107.6	109.3	99.8	99.9	98.5	98.5
4	98.6	99.2	97.0	95.0	100.0	101.2	100.2	103.3	104.6	108.5
5	101.2	102.3	94.2	103.1	98.6	99.5	94.5	95.7	95.9	96.9
6	96.6	94.7	99.0	99.6	97.4	98.3	99.7	99.0	100.1	99.8
7	104.1	99.4	113.1	109.7	99.5	100.9	102.3	103.0	99.7	100.0

Recoveries are expressed as percent.

Table 9
Repeatability relative standard deviation and within-laboratory reproducibility relative standard deviation obtained for each analyte

Parameter	Bentazone	2,4-D	Triclopyr	2,4,5-T	2,4,5-Tp
s_r (%)	2.54	6.83	0.61	0.62	6.75
s_{WR} (%)	5.30	10.58	4.50	5.11	7.44

s_r, repeatability relative standard deviation; s_{WR}, within-laboratory relative standard deviation.

where n_j is the number of replicates per day. The within-laboratory reproducibility, s^2_{WR}, is equal to:

$$s^2_{WR} = s^2_r + s^2_{between} \quad (2)$$

As shown in Table 9, the repeatability, expressed as relative standard deviation, was from 0.61 to 6.83%; meanwhile the within-laboratory reproducibility ranged from 4.50 to 10.58% in the worst case.

4. Conclusions

A fully automated approach for leaching, filtration, preconcentration and individual separation–detection of acid herbicides bentazone, 2,4-D, triclopyr, 2,4,5-T and 2,4,5-Tp from different types of soil has been developed. The average recoveries of the five compounds ranged from 94.20 to 113.10% with a repeatability, expressed as relative standard deviation, from 0.61% for triclopyr to 6.83% for 2,4-D and a within-laboratory reproducibility, expressed as relative standard deviation, from 4.50% for triclopyr to 10.58% for 2,4-D.

The use of water as extractant in the continuous approach avoids the use of organic solvent and/or a co-extractant such as Na_4EDTA, apart from the reduction of the time for quantitative extraction of the target analytes from the different types of soil.

Novel aspects of the proposed approach are: (a) the use of an in-line filter for particle retention. Its location in the loop of an injection valve enables easy automated removal of the retained material. (b) The use of air as carrier for the eluent, thus avoiding dilution.

This is also the first time that these four separation steps (leaching, filtration, preconcentration and chromatographic separation) have been sequentially developed in a continuous method.

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