WATER SOXHLET EXTRACTION ASSISTED BY FOCUSED MICROWAVES: A CLEAN APPROACH

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Abstract

A prototype of Soxhlet extractor that enables the use of water as extractant and application of focused microwaves on the cartridge zone has been designed and checked. The approach consists of a single unit in which the shortening of the distillation glassware allows reception of the water vapor in a refrigerant connected to the top of the sample-cartridge vessel, its condensation and dropping on the solid sample. When the water into the cartridge has reached a given level, irradiation with focused microwaves during an optimized time accelerates the leaching of the target analytes. Then, a valve is actuated and the extract is driven to the distillation flask where the leached species are concentrated as new vapor is formed and sent to the refrigerant and then to the cartridge. This new hybrid discontinuous-continuous approach has been used for leaching acid herbicides from different types of soil. The time required for total removal of the target compounds was 48 min. A flow injection manifold made posible the integration of the subsequent steps (preconcentration, chromatographic separation and UV detection) for the determination of the analytes, the recoveries of which range from 105.24 to 96.44%.

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INTRODUCTION

The growing trend to automation and/or acceleration of the overall analytical process finds a huge obstacle in the pretreatment of solid samples, which usually requires time-consuming steps based either on dissolution or leaching. The latter type is preferred to the former as a way for achieving a less complex matrix solution.

Soxhlet extraction has been the most used technique for isolation of organic pollutants from environmental samples for years. However, the use of new extraction techniques which overcome the drawbacks associated to Soxhlet is, nowadays, one of the most active research lines in the field of solid sample treatment[1].

The most significant drawbacks of Soxhlet extraction are, the long time required for the extraction and the large amount of organic solvent wasted, which is not only expensive to dispose off but which can cause environmental pollution itself. Moreover, the conventional device is not easily automated.

There are two different ways to circumvent the drawbacks of conventional Soxhlet extraction, namely: a) the use of one of the new alternatives (such as supercritical fluid extraction[2], microwave assisted extraction[3], accelerated solvent extraction[4], etc.); b) the improvement of conventional Soxhlet[5].

Different devices intending to obviate the main shortcomings of the conventional Soxhlet, but keeping its positive characteristics, has been developed. High pressure in Soxhlet devices was achieved by placing the extractor in a cylindrical stainless-steel autoclave or using supercritical fluid-Soxhlet extractors. The main drawback of these approaches is the change from supercritical to liquid state of the extractant, which affects Soxhlet performance. Commercial automated Soxhlet devices (SOXTEC HT and Büchi B811) have the possibility of developing three different steps (namely, boiling, rinsing and recovery of the solvent) by switching a lever, thus obtaining a significant reduction of both time and extractant. Soxwave is a commercial device which operates similarly to Soxtec HT, but using microwave instead of electric heating. The solvent and the sample are irradiated with microwave energy making easier the rupture of the analyte-matrix bonds. The main drawback of Soxwave is its dependence on the extractant dielectric constant, since the interaction of the microwaves is only effective with solvents with high dielectric constant. Thus, efficient extractions

are only obtained with polar solvents and, consequently, this device is not as universal as conventional Soxhlet is. Focused microwave-assisted Soxhlet extraction (FMASE) is an approach developed by Luque de Castro et al. in 1998[6] using a prototype[7] based on the same principles as a conventional Soxhlet extractor, but modified to facilitate accommodation of the sample cartridge compartment in the irradiation zone of a microwave oven. The latter was also modified making an orifice at the bottom of the irradiation zone enabling connection of the cartridge compartment to the distillation flask through a siphon. The use of this device has provided better results than conventional Soxhlet in shorter periods of time for the extraction of different components such as PAHs, dioxins, oil, etc., from different solid matrices such as soil,[8] fly ash[9] and seeds[10], respectively. FMASE maintains the advantages of conventional Soxhlet extraction overcoming limitations such as the long extraction time and non-quantitative extraction of strongly retained analytes, due to the easier rupture of the analyte-matrix bonds by interaction with focused microwaves energy; unavailability for automation, and large volume of organic solvents wasted, as recycling saves 75-85% of the total extractant volume. Moreover, solvent distillation in FMASE is achieved by electrical heating, which is independent of the extractant polarity, thus avoding the principal problem of Soxwave. The main drawback of the commented FMASE device was the difficulty of using water or high boiling point solvents as extractants due to the design of the device which made the evaporation, condensation and dropping on the sample of these solvents very slow.

In order to enable the use of water as extractant, a new prototype, based on the same principles of the previous FMAS extractor, has been designed consisting of a single unit where the shortening of the distillation glassware allows reception of the water vapor on a refrigerant connected to the top of the sample-cartridge vessel with minimal losses in the way, its condensation and dropping on the solid sample.

With the aim of checking the performance of this new FMASE prototype, the device has been used for leaching acid herbicides (namely, bentazone, 2,4-D, trichlopyr, 2,4,5-T and 2,4,5-Tp) from different types of soil (namely, clayey, slimy, sandy and limy).

The subsequent steps for quantitation of the extracted analytes were performed using a flow injection (FI) manifold that permits the overall automation of these steps (namely, preconcentration, individual chromatographic separation and UV detection). The overall analytical setup is thus a hybrid discontinuous-continuous approach.

EXPERIMENTAL

Instruments and apparatus

The device used for the focused microwave-assisted Soxhlet extraction is a new prototype (SEV^{MR}, Puebla, México) which operates under the same principles as the FMASE used so far. It has been designed in a single unit where the shortening of the distillation glassware allows reception of the water vapor on a refrigerant connected to the top of the sample-cartridge vessel, its condensation and dropping on the solid sample. The sample-cartridge vessel is equipped with a valve which allows the filling of the vessel or its draining to the distillation flask. The device operates at microwave power between 100 to 400 W with irradiation time control ranging from 1 second to 1 hour.

Two Gilson Minipuls-3 low-pressure peristaltic pumps, two Rheodyne 5041 low-pressure injection valves, a home-made minicolumn (4.0 cm in length and 4 mm i.d.) packed with the sorbent material, and Teflon tubing of 0.5 mm i.d. were used to build the flow manifold, which was connected to an HP1100 liquid chromatograph (Hewlett Packard, Avondale, PA, USA) consisting of a G1311A high-pressure quaternary pump, a G1322A vacuum degasser, a Rheodyne 7725 high pressure manual injector valve (20 μL injection loop), and a G1315A diode array detector (DAD). An Ultrabase C18 (250 x 4.6 mm; 5 μm particle size, Scharlau) was the analytical column.

Reagents and samples

The acid herbicides [bentazone, 2,4-dichlorophenoxyacetic acid (2,4-D), trichlopyr, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichlorophenoxy) propionic aid (2,4,5-Tp)] were obtained from Fluka (Buchs, Switzerland). These compounds were used for preparing the stock standard solutions in HPLC-grade methanol (Merck, Darmstadt, Germany). The sorbent used in the preconcentration step was C18-Hydra® (Panreac, Barcelona, Spain).

Four types of soil (namely, clayey, slimy, limy and sandy soil) were selected as matrices to carry out the study. 500 g 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 ethyl 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^{-1} (5 μg g^{-1} 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 ºC for six months. Neither of the soils had detectable levels of the target analytes before spiking. The optimization of the focused microwave-assisted Soxhlet extraction was carried out using the clayey soil.

Procedure

The device used for the leaching step is shown in Figure 1. Five grams of spiked soil containing 25 μg g^{-1} of each herbicide was weighed into a cellulose extraction cartridge which was capped with cotton wool and placed into the sample-cartridge vessel located in the zone of microwave irradiation. 100 mL of Milli-Q water was poured into the distillation flask (two or three boiling glass regulators were also added). The isomantle rheostat was set at 100% in order to reach a continuous flow of water from the distillation flask to the sample-cartridge vessel. The extraction program, which started when this vessel had been filled, consisted of a number of cycles which depended on the extraction kinetics of the target sample. Each cycle involved four steps: (1) filling of the sample-cartridge vessel (vessel valve in load position) by water evaporation from the distillation flask, condensation in the refrigerant, and dropping on the sample; (2) microwave irradiation of the cartridge for a preset interval (irradiation time) at a fixed microwave power; (3) waiting for a preset time (delay time) in which the sample was in contact with the heated water; (4) unloading of the extraction vessel by switching the vessel valve to its unload position thus delivering the vessel content to the distillation flask. After the last cycle, only the firts step was carried out again in order to reduce the volume of the extract contained in the distillation flask to 50 mL.

After leaching, the extract obtained was impelled by a peristaltic pump to a dynamic manifold (Figure 2) provided with two injection valves and one confluence point. The extract was merged with a buffer stream (acetic acid/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 located in the loop of an injection valve, thus enabling elution in the direction opposite to

retention with a volume of methanol selected using the third injection valve. 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 fixed time from elution, thus, avoiding sample discrimination problems.

Between samples and during the chromatographic separation, the sorbent in the minicolumn was conditioned by circulating methanol and water through it.

The HPLC separation of the acid herbicides was performed using an isocratic elution program in which a 60:40 1% aqueous solution of H_3PO_4-acetonitrile was used as mobile phase at a flow-rate of 1.7 mL min^{-1}. Photometric detection was performed at 280 nm.

Quantitation of analytes was carried out by running five calibration curves (one for each analyte) using stock standard solutions between 10-50 ppm.

RESULTS AND DISCUSSION

The whole method here proposed involves removal of the target analytes from the soils, preconcentration on an appropriate sorbent, individual chromatographic separation and photometric detection.

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 C18 column. The influence of the flow rate of the mobile phase was studied between 1.0-2.0 mL min^{-1}, and the best separation was obtained for a flow-rate of 1.7 mL min^{-1}. Overlapping of the peaks corresponding to bentazone and 2,4-D occurs using 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 commented under experimental.

Optimization of the preconcentration step

The study of the preconcentration step was performed using 50 mL of a standard solution containing 25 µg of each analyte in order to reproduce the real conditions, in terms of volume of extract and concentration of the analytes, obtained when the extraction step was performed providing quantitative extraction. The order used for optimizing this step was as follows:

(a) *pH of the extract*. Different solutions of HNO_3 and NaOH were used to adjust the pH of the extract between 0.5-8.5. The chromatograms obtained showed an increase of peak height when the pH decreased from 8.5 to 5.0 and a constant value for lower pHs. A pH 4.5 was selected in order to guarantee protonation of all the compounds, which were thus ready for being retained on the sorbent. The use of 1 mol/L acetic acid/acetate buffer (pH=4.5) was mandatory for adjusting the pH of the extract.

(b) *Buffer flow rate*. Flow rates ranging from 0.1 to 0.5 mL min^{-1} were studied in order to adjust the pH of the extract at 4.5. A flow-rate of 0.2 mL min^{-1} was selected for further experiments.

(c) *Retention flow rate*. The flow rate at which the sample passes through the minicolumn was optimized by aspirating the standard solution (the pH was previously adjusted) to the minicolumn at flow rates between 0.5-1.2 mL min^{-1}. The results obtained showed an increase of the retention when the flow rate decreased from 1.2 to 0.7 mL min^{-1}. This fact demonstrated the influence of the retention kinetics. Flow-rates between 0.7 and 0.5 mL min^{-1} provided similar results; so a flow rate of 0.7 mL min^{-1} was selected for subsequent experiments.

(d) *Elution flow rate and volume of eluent*. Both variables 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 eluent carrier was air. Its usage had the aim of dragging away from the system the aqueous phase after passage of the extract through the minicolumn thus avoiding dilution of the eluent. No other carrier can be used without dilution as methanol is miscible with both aqueous and organic media. Elution flow rates (flow rate of the eluent on passage through the minicolumn) between 0.1-1 mL min^{-1} and volumes of eluent between 0.2-2 mL were assayed. Finally, an elution volume of

0.5 mL at 0.15 mL min^{-1} was selected as optimum. The use of 0.5 mL of methanol as eluent entailed a preconcentration factor of 100.

(e) *Breakthrough of the C18-Hydra® minicolumn.* Volumes of sample in the range 10-150 mL containing 25 μg of each herbicide passed through the sorption material were studied. The signal remained constant up to 100 mL and decreased for higher volumes, so the passage of 50 mL of sample through the minicolumn did not surpass its breakthrough volume.

Using the optimal values, quantitative retention and elution of the analytes was achieved.

Optimization of the extraction step

In principle, the variables susceptible to be optimized in FMASE are always the irradiation power, the irradiation time and number of cycles needed for total extraction of the target compounds. However, using the new prototype, another variable can be studied since the unloading of the extract from the sample vessel can be controlled by switching a valve. In this way, a new variable named delay time (interval during which the sample is in contact with the solvent after microwave irradiation and before draining from the irradiation vessel) was optimized jointly with the other variables.

A full two-level factor design would involve an overall of $2^4 = 16$ 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-fractioned 2^{4-1}, type IV resolution design allowing three degrees of freedom, involved eight randomized runs plus three centered points[15]. 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 1a shows 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 1b shows the matrix for this first half-fractioned factorial design and the extraction yield of each analyte in the selected clayey soil.

As can be seen from the Pareto chart in Figure 3, the most important factor is the number of cycles, which is significant for all the compounds. The power of irradiation is not an influential variable for any of the compounds. However, the results show better recoveries with higher power so the maximum value tested, which is the maximum

value provided by the equipment (400 W), was selected for further experiments. Only bentazone was affected by the irradiation and delay times. These effects were positive in all cases, so the maximum values assayed for both factors (100 seconds) were selected as optimal taking into account that in the case of the irradiation time, longer times led water to boil, thus decreasing extraction efficiency owing to the formation of bubbles that prevent solvent-sample contact. Finally, the results indicate an increase in the number of cycles in order to obtain better recoveries, but this was not necessary, since using 5 cycles (the maximum value tested), quantitative recoveries were achieved. However, in order to determine the number of cycles necessary for quantitative recovery of the target compounds from different soils, a study of the extraction kinetics was performed.

Study of the extraction kinetics

Four types of soil were used (namely, clayey, limy, slimy and sandy soil) in order to study the extraction kinetics. In all cases, 5 g of the corresponding spiked soil containing 25 μg g^{-1} of each herbicide was used. Six extractions with different number of cycles ranging from 1 to 6 were performed. The other variables (namely, power of irradiation, irradiation time and delay time) were fixed at their optimal values (400 W, 100 s and 100 s, respectively). As can be seen in Figure 4, the retention of the analytes depend on the type of soil. In all cases, the extraction of the analytes from sandy soil is quantitative after 4 cycles; meanwhile, in the case of slimy soil, where the amount of organic matter and the ionic exchange capacity are higher, the extraction is close but not quantitative for all the herbicides in 6 cycles. 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 (higher than 90%) are obtained for all types of soil, independent of the extraction kinetics. The total time required for complete extraction is 48 min (thus is, 6 cycles at 8 min $cycle^{-1}$). This extraction time is not long as compared with other proposed methods[11-14] taking into account that the soil samples were aged for 6 months in order to make the retention of the analytes similar to that in real contaminated samples.

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[16]. The experiments were carried out using 5 g of clayey soil containing 5 μg g^{-1} of each herbicide. In all experiments the optimal values obtained for all the variables were used. As can be seen in Table 2a, two measurements of each analyte per day were carried out on 7 days. The ANOVA table (Table 2b) 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 as the mean squares within days, represent s_r^2 under repeatability conditions. To determine the variance due to the between-day effect, equations (1) and (2) were used:

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

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

$$s^2_{WR} = s_r^2 + s^2_{between} \qquad (2)$$

As shown in Table 2c, the repeatability, expressed as relative standard deviation, was from 0.51 to 2.58%; meanwhile the within-laboratory reproducibility ranged from 1.53 to 4.50% in the worst case.

CONCLUSIONS

This is the first time that water has been used as extractant in a Soxhlet device with good results concerning both extraction time and efficiency. Both the re-design of the glassware and the application of focused microwaves afford the development of a method which serves as an example of its usefulness. The advantages of the conventional Soxhlet (namely, not increased dilution of the extracted species for longer extraction time, not sample-filtration required, continuous contact of the solid with clean extractant) remain in the new device, which, in addition, is fast an easy to automate.

Nowadays, improvements of the extractor are aimed at avoiding its main drawback: the impossibility of recycling the solvent in the cases that other solvent different from water is used. In addition, an IR-temperature sensor, full automation by programming valve switching, two vessels for simultaneous extraction of two samples are being included in the final, commercial device.

ACKNOWLEDGEMENTS

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Table 1. Data for the haf-fractioned factorial design.

a) Factor levels in the half-fractioned factorial design

Factor	Key	Units	Low level (-)	High level (+)
Power of irradiation	A	W	200	400
Irradiation time	B	second	20	100
Number of cycles	C	-	1	5
Delay time	D	second	20	100

b) Design matrix and response values in the half-fractioned factorial design (recoveries are expressed as percent)

Run	A	B	C	D	bentazone	2,4-D	Trichlopyr	2,4,5-T	2,4,5-Tp
1	+	-	-	+	39.80	33.86	28.45	20.23	11.12
2	-	-	+	+	61.85	86.01	88.53	104.05	97.24
3	0	0	0	0	59.53	72.95	72.45	96.90	74.95
4	0	0	0	0	59.06	73.24	73.27	95.99	73.03
5	-	-	-	-	21.29	16.97	18.90	14.88	6.46
6	+	+	-	-	30.41	32.06	30.38	26.47	19.13
7	-	+	-	+	73.76	29.63	20.17	14.29	4.95
8	0	0	0	0	59.25	73.84	70.01	96.82	72.69
9	-	+	+	-	103.46	101.38	94.63	103.92	97.14
10	+	-	+	-	71.99	71.10	77.39	101.97	84.38
11	+	+	+	+	97.94	102.68	97.33	101.68	87.01

Table 2 . Experimental set-up and results obtained from the evaluation of the precision of the proposed method

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

Day	Bentazone		2,4-D		Trichlopyr		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	99.65	100.02	101.27	100.08	99.54	101.22	98.55	99.25	96.44	99.74
2	98.98	99.35	102.88	99.88	99.77	100.00	100.47	98.45	102.45	100.25
3	100.25	101.24	97.54	98.44	105.24	103.37	102.43	101.69	99.58	96.53
4	104.51	102.22	98.66	99.05	101.01	99.44	99.68	99.44	103.47	102.33
5	98.58	97.99	99.57	101.25	98.66	97.58	98.78	97.58	98.49	100.07
6	99.65	98.54	101.65	103.38	99.35	100.49	100.11	100.26	99.25	99.64
7	97.88	96.99	106.05	104.55	97.84	98.63	101.81	102.54	100.78	98.11

b) ANOVA table

Compound	Source	SS[a]	Df[b]	MS[c]
Bentazone	Between days	44.7611	6	7.4602
	Within days	4.4040	7	0.6291
	Total	49.1651	13	-
2,4-D	Between days	70.0845	6	11.6808
	Within days	9.7218	7	1.3888
	Total	79.8063	13	-
Trichlopyr	Between days	50.4685	6	8.4114
	Within days	5.9636	7	0.8519
	Total	56.4321	13	-
2,4,5-T	Between days	28.1151	6	4.6859
	Within days	3.5855	7	0.5122
	Total	31.7006	13	-
2,4,5-Tp	Between days	30.2106	6	5.0351
	Within days	18.0548	7	2.5793
	Total	48.2654	13	-

[a]Sum of squares; [b]degrees of freedom; [c]Mean of squares

c) Repeatability relative standard deviation and within-laboratory reproducibility relative standard deviation obtained for each analyte

Parameter	Bentazone	2,4-D	Trichlopyr	2,4,5-T	2,4,5-Tp
$\%s_r$	0.63	1.37	0.85	0.51	2.58
$\%s_{WR}$	1.96	2.63	4.50	1.53	2.81

$\%s_r$, repeatability relative standard deviation; $\%s_{WR}$, within-laboratory relative standard deviation

Figure 1

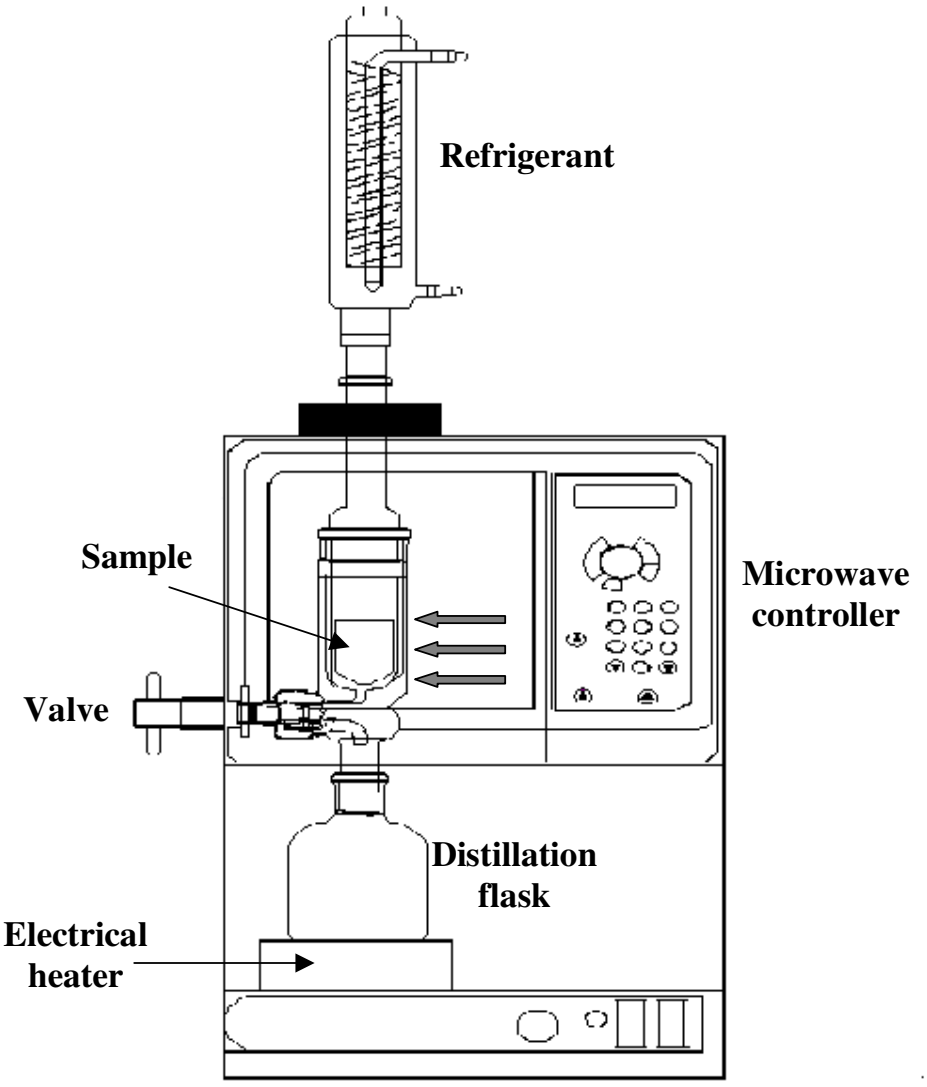
Refrigerant
Sample
Microwave controller
Valve
Distillation flask
Electrical heater

Figure 2

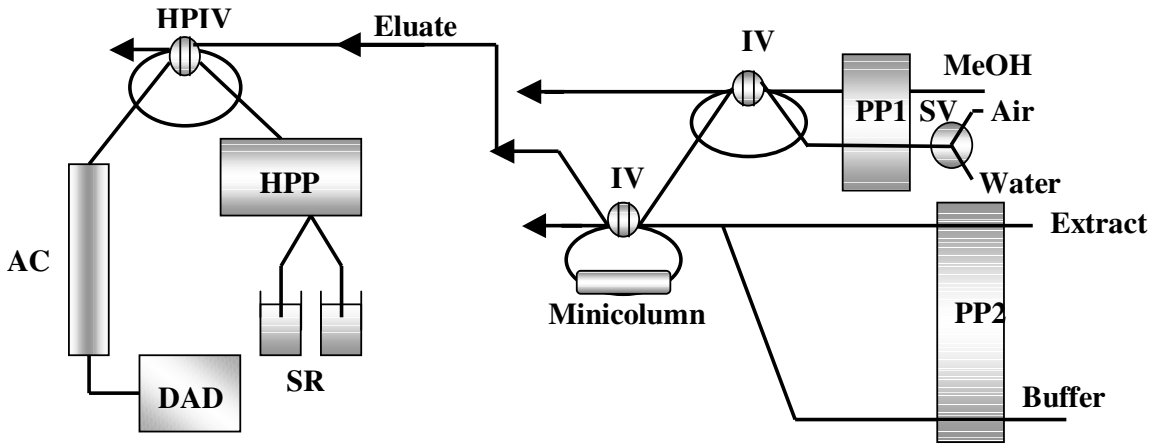
HPIV
Eluate
IV
MeOH
PP1
SV
Air
HPP
IV
Water
AC
Extract
Minicolumn
PP2
SR
DAD
Buffer

Figure 3

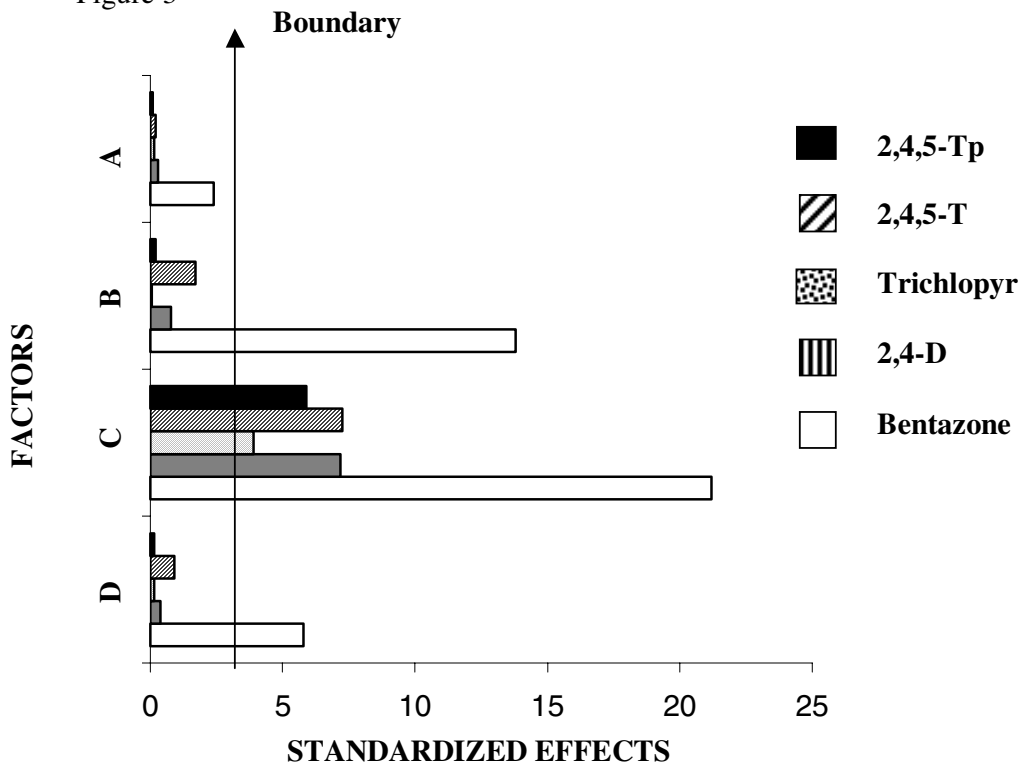
Boundary
FACTORS
A
B
C
D
0
5
10
15
20
25
STANDARDIZED EFFECTS
2,4,5-Tp
2,4,5-T
Trichlopyr
2,4-D
Bentazone

Figure 4

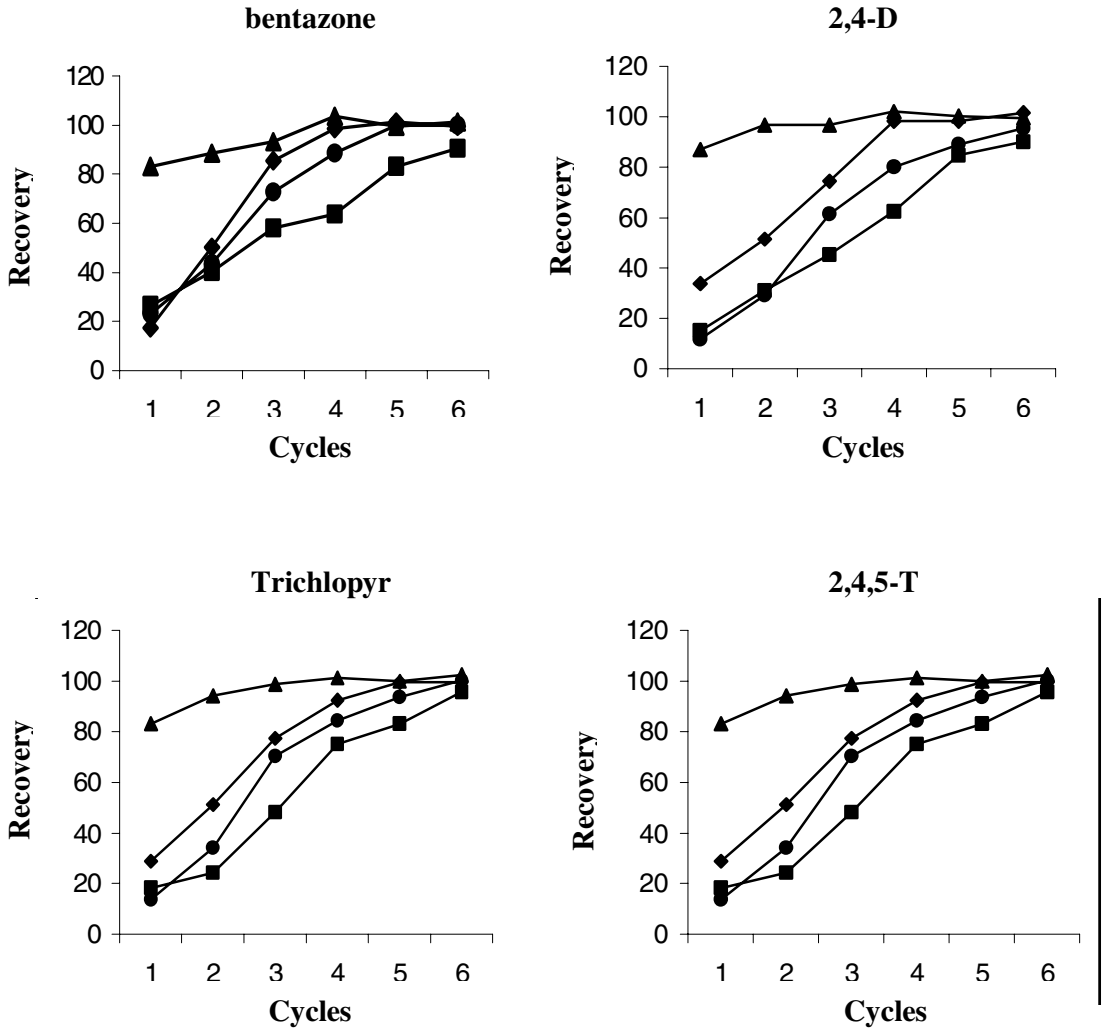
bentazone
2,4-D
Trichlopyr
2,4,5-T
Recovery
Cycles
0
20
40
60
80
100
120
1
2
3
4
5
6

Figure Captions

Figure 1. Scheme of the improved FMAS extractor.

Figure 2. Scheme of the manifold used for coupling preconcentration-individual chromatographic separation-UV detection. PP, peristaltic pump; SV, selection valve; IV, injection valve; HPIV, high pressure injection valve; HPP, high pressure pump; AC, analytical column; DAD, diode array detector; SR, solvent reservoirs.

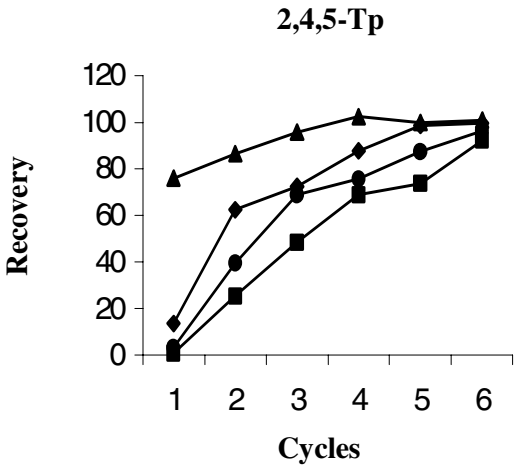

Figure 3. Pareto chart for the standardized main effects in the half-fractioned factorial design. The vertical line indicates the statistical significance bound for the effects.

Figure 4. Kinetics of extraction for the acid herbicides from different soils.

◆ clayey soil ■ slimy soil ▲ sandy soil ● limy soil