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Determination of fluazifop-butyl and fluazifop with the use of disposable solid phase extraction columns for selective clean-up and concentration of Soxhlet soil extracts

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Summary. An analytical procedure based on the solid phase extraction technology has been developed for the clean-up and concentration of Soxhlet soil extracts containing fluazifop-butyl and fluazifop by the use of a phenyl phase cartridge. No liquid-liquid partition has been used; thus the consumption of organic solvents was limited and the use of chlorinated solvents could be avoided. Quantification has been performed by ion-pair HPLC. Despite the large difference in polarity the recoveries of both the compounds from spiked soil samples between 0.1 and 1 μg/g was higher than 90%. The solid-phase adsorption technology resulted in a very effective methodology of clean-up in the case of the polar compound fluazifop, for which a second disposable column with a cyanopropyl phase has been used, and was fairly satisfactory for fluazifop-butyl. The detection limits were less than 0.04 μg/g and 0.10 μg/g, respectively for fluazifop and fluazifop-butyl.

Introduction

The determination of very polar organic chemicals in soil usually presents many difficulties: as a matter of fact humic substances, the most important organic fraction of soil, cause severe interferences in the analytical methods for the quantification of the compounds searched for. Moreover, while polar compounds can be extracted with solvent mixtures containing large proportions of water, very non-polar compounds, on the contrary, can be effectively extracted only with the use of large quantities of organic solvents. Fluazifop-butyl, butyl(RS)-2-[4-(5-trifluoromethyl-2-pyridyloxy-)phenoxy-] propionate, a herbicide belonging to the class of phenoxypropionic acids, undergoes in soil and in plants fast hydrolysis to the free acid, fluazifop, (RS)-2-[4-(5-trifluoromethyl-2-pyridyloxy-)phenoxy-]propionic acid [1, 2]. Between the parent compound and its major degradation product there is a very large difference in polarity: the first has a very low water solubility (about 2 mg/l) and the second has a relatively high acidity (pKa 2.75) and can be retained as a salt by soil minerals. For a simultaneous determination of both acid and ester, the extractant must be a mixture of an organic solvent (generally the major component) and an aqueous solution of an organic or inorganic acid. After extraction, the separation between the organic and the inorganic could be performed by means of liquid-liquid partition with large consumption of organic solvents [3]. Successively, further steps could be necessary to achieve a sufficient clean-up of the sample with abundant expense of time. In this work we have used a particular procedure of filtration together with disposable bonded-phase columns in order to perform the clean-up as well as the concentration of the sample with elimination of the liquid-liquid partition and selective separation of fluazifop-butyl and fluazifop from the extracts. Particular attention was payed to the recovery rates that could be influenced by the large difference in polarities.

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Materials and methods

Chemicals and equipment

1. Solvents: Solvents used were residue analysis grade or HPLC grade (Riedel-de Haën, FRG). Purified water was prepared using a Milli-Q Plus water purification system (Millipore GmbH, FRG).
2. Herbicide standards (99%) were purchased from Ehrenstorfer GmbH, FRG.
3. Membrane filters 0.8 μm pore size, 40 mm effective diameter, and vacuum filtration apparatus 250 ml (Sartorius GmbH, FRG).
4. Celite type 545, 0.020–0.045 mm (Serva, FRG).
5. The disposable bonded phase columns were Phenyl (PH) and Cyanopropyl (CN) solid phase extraxtion columns (500 mg), 3 ml volume, Bond Elut (Analytichem, USA) and the solid phase extraction equipment was formed by

Table 1. Characteristics of soils

Soil code	Particle size distribution (%)				Organic matter (%)	Moisture, max. holding capacity (%)
	Coarse sand	Fine sand	Silt	Clay		
2.1	70.3	21.4	3.2	5.1	0.70	28
2.2	58.2	32.1	3.3	6.4	2.56	47
2.3	47.7	31.6	12.1	8.6	0.96	57

a vacuum waterpump, pointed vacuum flasks, adapters and Analytichem 75 ml reservoirs without use of frits.

6. GPC: column 90 × 0.9 cm i.d., filled with Bio-Beads SX-3 (Bio-Rad, FRG), eluent ethylacetate/n-hexane (1:1), flow rate 1 ml/min.
7. Tetrabutylammonium hydrogensulfate (TBAHSO4) in ion-pair chromatography grade (Fluka, FRG).
8. HPLC: Pump 300 CS and gradient former 250 B (Gynkotek GmbH, FRG), variable wavelength UV/VIS-detector Knauer model 87.00 (Knauer GmbH, FRG), integrator HP 3390 (Hewlett Packard), columns 25 cm × 4 mm i.d. (Bischoff, Leonberg, FRG), Hypersil-Phenyl 5 µm (Shandon) and 12.5 cm × 4.6 mm i.d. (Bischoff, Leonberg, FRG), Hypersil MOS (C8) 5 µm (Shandon), in both the cases the precolumn was 2 cm × 4 mm i.d., Hypersil MOS (C8) 5 µm (Shandon).

Soils

The three standard soils used in the study have been provided by Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer (LUFA), FRG. The soils were adjusted to 40% of their moisture holding capacities at zero suction (MHC). The physical and chemical characteristics of the soils are shown in Table 1.

Preparation and extraction of spiked soil samples

Soil samples corresponding to 50 g dry weight were weighed into round-bottomed flasks (250 ml) and acetone solutions (5 ml) of each of fluazifop and fluazifop-butyl were applied dropwise to obtain the desired concentrations in soil for both the chemicals. The flasks were then shaken for 5 min, treated in an ultrasonic bath for 30 s and then the acetone was removed with a vacuum evaporator.

The soil samples were filled into cellulose extraction thimbles, placed in a Soxhlet extractor and wetted with 5 ml of a 0.2 mol/l solution of citric acid. The extractions were carried out with 150 ml acetone for 18 h, starting 10 – 15 min after the application of the acidic solution.

Clean-up and concentration procedure

After addition of 10 ml of 1-propanol the Soxhlet extracts were transferred into a pointed vacuum flask (250 ml) and the acetone was evaporated in a rotary evaporator. The residual mixtures were made up to 75 ml with the addition of 0.004 mol/l hydrochloric acid (about pH 2.2) and poured into the vacuum filtration apparatus equipped with a membrane filter covered with 200 mg of celite. Just before dryness of the filtration residue, 25 ml 0.004 mol/l HCl were added to mobilize the water soluble substances (including fluazifop) possibly retained by the celite and the filter. The combined filtrates were mixed and passed through a phenyl bonded phase column activated before with 3 ml methanol and 3 ml methanol/water (1:3). The column was washed with 10 ml 0.004 mol/l hydrochloric acid; then fluazifop was eluted with 5 ml of methanol/water (60:40) and the eluate set aside. At this point one fraction of fluazifop-butyl has been retained by the column and another fraction remained in the filtration equipment. In order to increase the recovery of fluazifop-butyl avoiding break-through in the extraction cartridge, the column was firstly dried in vacuum for 10 min and cleaned with 3.5 ml n-hexane/acetone (95:5): this eluate was collected in a pointed flask. Then the column was dried and reactivated with 3 ml methanol and 3 ml methanol/water (1:3). The fraction of fluazifop-butyl retained in the filtration apparatus was remobilized by washing the filter first with 10 ml 1-propanol and then with 35 ml water; the filtrates were collected, mixed and then passed through the phenyl phase column used in the previous step. Then the cartridge was washed with 10 ml water and finally eluted with 3.5 ml of n-hexane/acetone (95:5) that were collected in the pointed flask containing the first fraction of fluazifop-butyl. The solvent was evaporated in a rotary evaporator and the residue was dissolved and made up to 5 ml with methanol prior to HPLC analysis.

The previously collected methanolic eluate containing fluazifop (5 ml) was diluted with 30 ml 0.004 mol/l hydrochloric acid, mixed and then passed through a cyano-propyl column that has been activated before with 3 ml methanol and 3 ml methanol/water (1:3). After washing with 10 ml 0.004 mol/l HCl and drying the column, the fluazifop residue was recovered with 3 ml n-hexane/acetone (65:35). For the HPLC determination the eluate was dried in a rotary evaporator and re-dissolved in 5 ml of acetonitrile/water (1:1). The clean-up and concentration procedure for the determination of fluazifop-butyl and fluazifop is shown in Fig. 1.

Chromatography

The chromatographic conditions for the determination of fluazifop were the following: column Hypersil Phenyl, flow rate 1 ml/min, isocratic, mobile phase 54% acetonitrile – 46% water (v/v), ion-pair reagent tetrabutylammonium hydrogensulfate (TBAHSO4) added and maintained at a concentration of 0.5 mmol in the eluent. In the case of fluazifop-butyl the stationary phase was Hypersil MOS (C_8), the flow rate was 1 ml/min and the gradient was the following: 60% acetonitrile – 40% water isocratic for 20 s, linear gradient to 70% acetonitrile – 30% water for 3 min, then isocratic. In both cases the detection was performed at 222 nm and the quantification by linear comparison of peak areas and calibration with external standards.

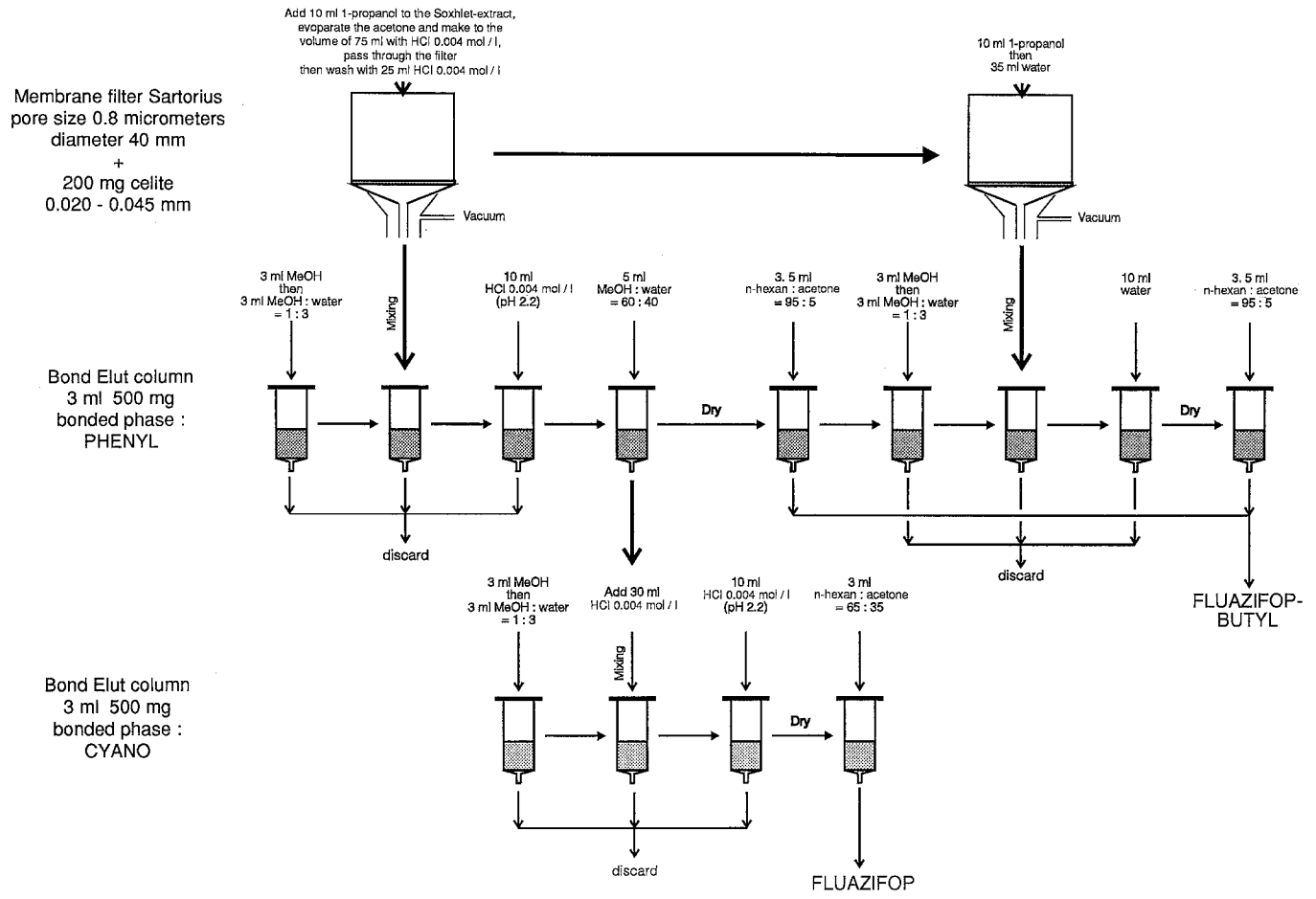

Fig. 1. Clean-up flow scheme for soil extracts with fluazifop and fluazifop-butyl residues

Results and discussion

The large difference in polarities between fluazifop-butyl and fluazifop causes difficulties in the application of the sorbent extraction technology for the simultaneous determination of both compounds from soil extracts. Retention of the more polar organic substance on solid reversed-phase extraction columns can be achieved by exploitation of non-polar interactions. This means that we have to dissolve the sample in a solvent mixture containing a quantity of water as large as possible [4]. But in this case the possibility to obtain large recoveries of non-polar compounds could be reduced considerably. Coagulation of soil colloids and adsorption of low water soluble compounds onto the surface of glasses may occur and then filtration is indispensable to prevent clogging of the pores of the column. In performing filtration, adsorption of the analysed compounds onto the solids to be removed and onto the filter medium cannot be avoided [4]. The simple application of the common polyethylene frits to the reservoirs did not allow an effective flow through the disposable extraction columns, even not in the case of addition of celite as filtration aid and consequently recoveries of non-polar compounds were decreasing. Therefore, in the handling of soil extracts containing fluazifop-butyl and fluazifop, we have developed a combination of the techniques of membrane filtration and of adsorption onto phenyl silica bonded phase in order to replace the liquid-liquid partition and to achieve large recoveries of both the compounds. The use of frits in the reservoirs was also avoided. 1-Propanol has been found very useful as a solvent in some clean-up steps for two main reasons: I) the high boiling point of 97.4° makes possible (after addition of a convenient quantity of this alcohol to the extract) the removal of acetone in vacuum without complete consumption of the organic phase and therefore the settling of fluazifop-butyl on the walls of the flask is prevented; II) the relatively high viscosity of 1-propanol facilitates the transfer of the low water soluble compounds from the filter to the filtrate that successively should be passed through the bonded-phase column; also, we have experimentally observed an easier flow through the bonded-phase (a liquid with an intermediate dynamic viscosity can more easily reduce the retained interfering materials onto the column). Indeed, with the soil 2.2 (which has the higher organic matter content) breakthrough of substances in the 500 mg phenyl cartridge almost occurred and the requirement of time to pass through by vacuum suction the additional 10 ml of water (necessary to wash the column before desorption) was rather large; but this problem could be avoided by analysing a smaller aliquot of the soil extract or employing a column containing a larger quantity of stationary phase (1 g or more) [5].

Two fractions were collected after concentration and clean-up with the phenyl phase cartridge: a methanolic solu-

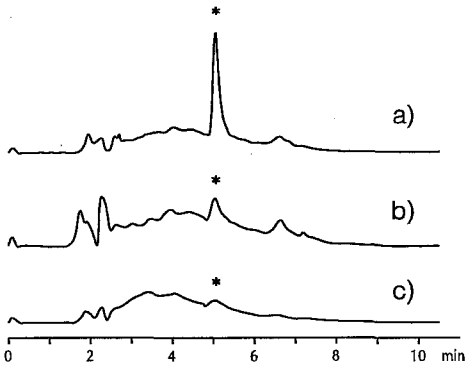

Fig. 2. HPLC chromatograms of fluazifop from soil extracts; spiking levels: *a* 1 μg/g; *b* 0.1 μg/g; *c* 0.04 μg/g

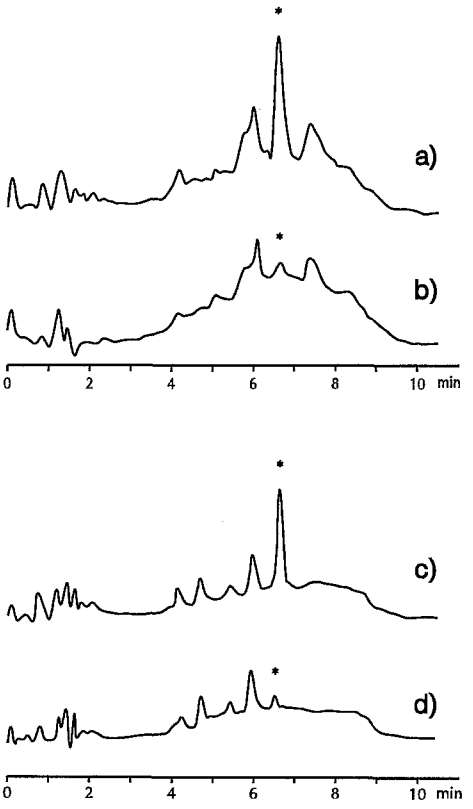

Fig. 3. HPLC chromatograms of fluazifop-butyl from soil extracts: *a* and *b* spiking levels 1 μg/g and 0.1 μg/g without further GPC clean-up; *c* and *d* the same samples after clean-up by GPC

tion containing fluazifop residues and a solution in n-hexane/acetone of less polar compounds (including fluazifop-butyl). Further clean-up with a cyanopropyl Bond Elut column has been necessary for fluazifop. This has been retained in the column by non-polar interactions and then, after drying the catridge in vacuum, the desorption has been carried out with a solvent mixture of calibrated polarity (65% n-hexane −35% acetone). Adsorption of fluazifop was favoured on either the phenyl- or the cyano propyl phase by a slight acidic pH (about pH 2.2) due to the formation of the undissociated acid. The proposed clean-up procedure

Table 2. Recovery of fluazifop-butyl and fluazifop from soil

Soil	n	Spiking level (μg/g)	Recovery (%) ± S.D.	
			Fluazifop-butyl	Fluazifop
2.1	3	1.0	104.1 ± 0.8	97.9 ± 1.5
2.1	3	0.1	95.2 ± 5.4	102.8 ± 3.5
2.2	3	1.0	103.2 ± 1.2	88.7 ± 4.2
2.2	3	0.1	90.1 ± 7.0	95.9 ± 5.1
2.3	3	1.0	99.7 ± 0.4	97.0 ± 1.2
2.3	3	0.1	98.7 ± 5.3	103.7 ± 3.6

was found very effective in the case of fluazifop: in Fig. 2 three HPLC chromatograms are presented, for the spiking levels of 1 μg/g (*a*), 0.1 μg/g (*b*) and 0.04 μg/g (*c*), respectively, in soil 2.1; the latter level corresponds to our detection limit for fluazifop. In the case of fluazifop-butyl, clean-up was fairly satisfactory for HPLC determination: in Fig. 3a and 3b, two chromatograms are shown for the spiking levels of 1 μg/g and 0.1 μg/g, always in soil 2.1. In order to evaluate the possibility of further clean-up, the same samples corresponding to the two chromatograms 3a and 3b were submitted to GPC after removal of methanol and dissolution of the residues in ethylacetate/n-hexane (1:1). The resulting chromatograms are reported in Fig. 3c and 3d. Non-aqueous GPC resulted in a very effective clean-up methodology for non-polar compounds such as fluazifop-butyl, but a reduction of about 20% in recovery rates was detected which might be caused by problems concerning the homogeneity of the column filling and subsequent losses during fractionation.

The recovery rates of both fluazifop-butyl (without clean-up by GPC) and fluazifop from spiked soil samples are reported in Table 2.

The recovery rates were always over 90% except in the case of fluazifop residues in soil 2.2 at the spiking level of 1 μg/g, where the recovery was 88.7%. In this case some fluazifop was presumably retained in the filter as a consequence of deposition of relatively large amounts of organic matter on the celite in the first step of the filtration procedure. Larger recoveries of fluazifop could also be obtained for soil 2.2 with a slighly different clean-up procedure but at the expense of the recovery of fluazifop-butyl, as tests during the development of the presented procedure have shown (partial results are not reported).

Conclusions

The results shown in this paper indicate that concentration of soil extracts without liquid-liquid partition can be successfully performed by means of solid-phase extraction columns, also in the case of the simultaneous determination of chemicals characterized by a very large difference in polarities as shown with fluazifop-butyl and fluazifop. In this way the consumption of organic solvents was considerably reduced. The column breakthrough of interfering materials can be prevented by the use of an appropriate filtration technique. The selection of proper sorbent phases also allowed a very good clean-up in the case of the polar compound fluazifop.

For the non-polar fluazifop-butyl further clean-up could be done by GPC. In the latter case the preliminary desorption of more polar substances from the solid phase extraction columns considerably reduced the quantity of coloured interfering substances in the sample with a possible increase of the life-time of the GPC column. A very effective demineralization of the samples also allowed an increase in the performance of HPLC measurements. Finally, it could be shown that high recoveries can be obtained by careful exploitation of the selectivity of bonded phases.

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