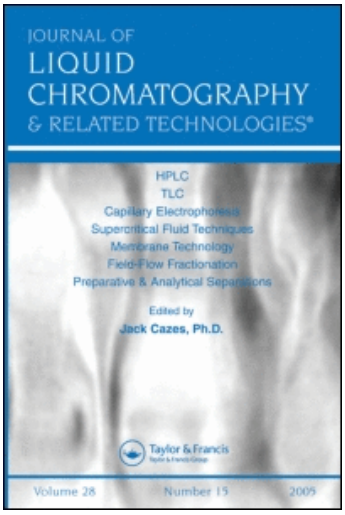

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Simultaneous Separation of Phenylurea-, Triazine- and Phenoxyacid Herbicides by Reverse Phase Ion-Interaction HPLC. Application to Soil Analysis

M. C. Gennaro[a]; D. Giacosa[a]; C. Baglietto[a]; M. Gennari[b]; M. Negre[b]
[a] Dipartimento di Chimica Analitica via P. Giuria, Università di Torino, Torino, Italy [b] Università di Torino - DI.VA.P.R.A. Chimica Agraria via P. Giuria, Torino, Italy

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SIMULTANEOUS SEPARATION OF PHENYLUREA-, TRIAZINE- AND PHENOXY-ACID HERBICIDES BY REVERSE PHASE ION-INTERACTION HPLC. APPLICATION TO SOIL ANALYSIS

M. C. Gennaro,* D. Giacosa, C. Baglietto

Università di Torino - Dipartimento di Chimica Analitica
via P. Giuria 5
10125 Torino, Italy

M. Gennari, M. Negre

Università di Torino - DI.VA.P.R.A. Chimica Agraria
via P. Giuria 15
10126 Torino, Italy

ABSTRACT

An ion interaction method is presented for the simultaneous separation of herbicides belonging to different structural classes. Phenylurea-, triazine- and phenoxyacid derivatives are considered and the method is applied in the analysis of typical Italian soils characterized by different composition.

INTRODUCTION

Phenoxyacids, urea- and triazine- herbicides are widely used for weed control in agriculture and forestry.[1] Because of their low biodegradability and low volatility, the herbicides and their residuals can easily cumulate in soils and become phytotoxic for sensitive crops cultivated in rotation.

Multiresidual methods for soil analysis are therefore required not only to evaluate source and extent of pollution, but also to learn about possible phytotoxicity problems.

Literature reports different methods for herbicide determination which employ immunoanalytical techniques;[2-4] gas-chromatography with ECD,[5-8] NPD[5,9,10] and MS detection;[11-13] HPLC with UV, diode-array,[8,14-16] fluorimetric[17] or MS detection[18,19] and MS-MS[20] technique. Generally the methods concern the separation of herbicides characterized by similar structure and chemical properties.

This paper presents an ion-interaction HPLC method for the simultaneous separation of herbicides belonging to different functional classes and in particular triazinic, ureic and phenoxyacid pesticides. The method is then applied in analysis of soils.

EXPERIMENTAL

Apparatus

A horizontal mechanic shaker (Shaker 309), a centrifuge ALC 4226 and a Rotovapor Heidolph VV 2000, equipped with a thermal bath, were employed in the extraction process.

The chromatographic analysis was performed with a chromatograph Merck-Hitachi (Tokyo, Japan) Lichrograph model L 6200 with a two channel D 2500 chromato integrator interfaced with a UV-Vis detector L 4200 and with a conductivity detector of the same manufacturer.

A spectrophotometer, Hitachi 150-20, was used for absorbance measurements.

pH values were measured with a Metrohm (Herisau, Switzerland) 654 pH-meter provided with a combined glass calomel electrode.

Chemicals and Reagents

o-Phosphoric acid, acetonitrile, dichloromethane, methanol were analytical grade reagents from Fluka (Buchs, Switzerland).

2,4-D, dichlorprop, 2,4,5-T, diuron, isoproturon, fenuron, 2,4-DB, bromacil, terbumethon, terbutylazine and propazine were from Lab Service Analytica (Anzola dell'Emilia, Bologna, Italy).

Ultrapure water from a Millipore (Milford, MA) MilliQ system was used for the preparation of solutions.

Filters, Merck (Darmstadt, Germany) Anotop 25 Plus (0.22 μm), were employed in the sample preparation.

Chromatographic Conditions

The stationary phase was a reverse phase Phase Separations (Desidee, CLWYD, UK) Spherisorb ODS-2 5S cartridge type column, 250x4.6 mm (5 μm), together with a guard precolumn, Merck (Darmstadt, Germany) LiChrospher 100 RP 18 (5 μm). The experimental mobile phases were 5.0 mmol/L solutions of n-octylammonium o-phosphate prepared in acetonitrile/water at different volume ratios and brought to pH 6.4±0.1 with o-phosphoric acid.

According to proposed models,[21-24] the ion interaction reagent contained in the mobile phase is bound through adsorptive and electrostatic forces onto the surface of the stationary phase, where it gives rise to an electrical double-layer. The interaction properties of the reverse phase packing material are, therefore, modified; the new surface is able to retain anionic and cationic species. About one hour is required for the modification process. Every third day of use, the column is washed (flow-rate 1.0 mL/min) with ultra pure water (20 min), acetonitrile/water 1:1 V/V (20 min) and acetonitrile (10 min).

Dead time was evaluated through conductimetric detection of unretained sodium ion, injected as $NaNO_3$ (15 mg/L).

Retention time reproducibility is within 2% for the same mobile phase preparation and always within 5% for different preparations.

Table 1

Soil Characteristics

Soil	Organic Carbon, %	pH	Clay, %
Fossano	0.91	6.5	10.0
Carpi	0.98	8.0	43.4
Macomer	11.31	5.6	2.9

Preparation of the Standard Solutions

The standard solutions were prepared every 20 days, at concentration of 100.0 mg/L, in acetonitrile from analytical grade standards and stored in brown bottles at 4°C. Working solutions were prepared in water/acetonitrile in the same ratios as in the mobile phase just before the injection into the HPLC system.

Soil Residue Extraction Procedure

An extraction procedure was developed which is able to simultaneously extract from soil all the herbicides investigated. The recovery yields were evaluated for typical Italian soils respectively sampled at Fossano (Piemonte, North-West Italy), Carpi (Emilia, Mid Italy) and Macomer (Sardinia Island, South-West Italy), which are characterized by different compositions (as listed in Table 1).

The method is based on the procedure reported by Meier et al.[25] and modified as follows. Before fortification, the soils were dried to 10% moisture and passed through a 2 mm sieve. 50 g aliquots of soil were then placed into 300 mL screw capped PVC bottles and 2 mL of an aqueous solution of a mixture of the herbicides (5 mg/L each) was added to provide a fortification level of 0.2 mg/Kg. The samples were then added with 50.0 mL of 0.01N NaOH, shaken on a mechanical shaker for 15 min and centrifuged for 10 min at 4000 rpm. The supernatant was transferred to a 300 mL PVC bottle.

Two extractions were performed, each with 50 mL of 0.01 N NaOH and the extracts combined. 10 mL of 1.00 N HCl were then added, the extract centrifuged for 10 min at 4000 rpm and the supernatant transferred to a separatory funnel and shaken three times with portions (50 mL) of dichloromethane. Dichloromethane extracts were dried over anhydrous sodium

sulfate and evaporated to dryness under vacuum on a rotovapor (water bath 30°C). The residue was then dissolved in acetonitrile/water 50/50 V/V and the final volume adjusted to 5.0 mL. The extracts, filtered through 0.22 μm Anotop 25 Plus filters, were analyzed under the HPLC optimized conditions. Further dilutions of the extracts (up to 1/40 V/V) were also performed in order to minimize the matrix effects.

RESULTS

Development of the Method

As previously mentioned, the present study is devoted to the development of a separation method for herbicides belonging to different functional classes, in consideration that: a) commercial formulations often contain mixtures of different herbicides, b) the different and the generally low herbicide biodegradability can induce cumulating processes, which lead to the presence in soils of herbicides of different structure.

Triazinic, ureic and phenoxyacid herbicides are here considered and namely: propazine (2-chloro-4,6-bis(isopropylamine)-s-triazine), terbutylazine (2-*tert*-butylamino-4-chloro-6-ethylamino-1,3,5-triazine), fenuron (N,N-dimethyl-N-phenylurea), isoproturon (N,N-dimethyl-N'-[4-1-methyl-ethyl)phenyl]urea), diuron (N'(3,4-dichlorophenyl)-N,N-dimethylurea), terbumethon (2-*tert*-butylamine-4-ethylamine-6-methoxy-1,3,5-triazine), 2,4-D ((2,4-dichlorophenoxy)acetic acid), dichlorprop ((±)-2-2-(2,4-dichlorophenoxy)propionic acid), bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid). Their structures suggest the use of ion-interaction chromatographic methods, already developed in this laboratory for the simultaneous separation of anionic species and species which contain protonable nitrogen atoms.[23,24]

The absorbance spectra recorded between 200 and 400 nm for the analytes investigated indicated 228 nm as the wavelength which offers the best average absorbance for all the compounds investigated.

In order to obtain the separation of the largest number of herbicides within reasonable analysis times, the chromatographic conditions of ion-

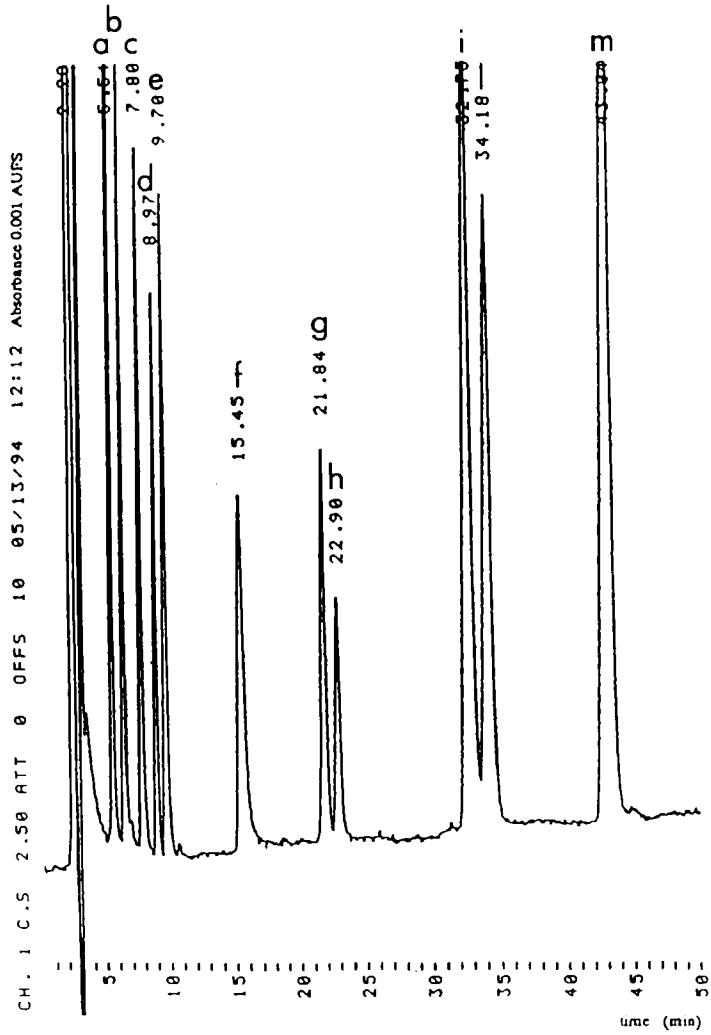

Figure 1. Chromatogram of the standard mixture. a: fenuron (100.0 μg/L), b: 2,4-D (100.0 μg/L), c: dichlorprop (100.0 μg/L), d: bromacil (100.0 μg/L), e: 2,4,5-T (100.0 μg/L), f: 2,4-DB (100.0 μg/L), g: isoproturon (70.0 μg/L), h: diuron (50.0 μg/L), i: terbumethon (100.0 μg/L), l: propazine (50.0 μg/L), m: terbutylazine (100.0 μg/L). Stationary phase: Phase Separations Spherisorb 5S ODS-2 (250x4.6mm; 5 μm); mobile phase: 5.0 mmol/L n-octylamine in water/acetonitrile (65/35) brought to operational pH=6.4 with o-phosphoric acid. Injection volume 100 μL. Flow rate: 1.0 mL/min. Spectrophotometric detection at 228 nm.

interaction reagent (concentration and properties) and organic modifier concentration in the mobile phase were optimized. The concentration of octylammonium o-phosphate (used as the ion interaction reagent) was varied between 1.0 and 10.0 mmol/L with acetonitrile concentrations in the mobile phase ranging between 25 and 40%. A pH value of 6.4 was chosen, which permits the formation of ionized species from both basic (urea and triazine derivatives) and acidic (phenoxyacids) pesticides.

Because, due to their more lipophilic properties, the triazine- derivatives generally show, under these conditions, higher retention with respect to phenoxyacids, gradient elutions were investigated. It is worth mentioning that the use of gradient elution in ion-interaction chromatography is a very controversial point, because some authors advantageously use gradient elutions while others affirm that the gradient elution mode is not suitable. Our results agree with the latter opinion; just when the gradient conditions were imposed, high baseline noise together with uncontrolled and non-reproducible conditions were obtained.

Very likely, when the ion-pair reagent is present in the mobile phase at concentrations which are high enough to assure to the analyte more lipophilic properties but not to induce a surface modification of the stationary phase, gradient elution can be advantageously used. When, on the contrary, as in our conditions, the ion interaction concentration is high enough to cause the dynamic modification of the surface, the increasing concentration of the organic modifier disturbs the conditions of dynamic equilibrium which have been established between the moiety adsorbed onto the stationary phase surface and the mobile phase.

Therefore, the best conditions for the separation of the mixture turned out to be: isocratic elution, 5.0 mmol/L n-octylammonium o-phosphate, acetonitrile concentration 35%, pH=6.4, flow-rate 1.0 mL/min, UV detection at 228 nm.

As an example, Figure 1 shows the separation obtained under these conditions for a mixture containing the following eleven herbicides: fenuron, dichlorprop, 2,4-D, bromacil, 2,4,5-T, isoproturon, diuron, 2,4-DB, terbumethon, propazine and terbutylazine, at concentration of 100.0 μg/L (or less) each.

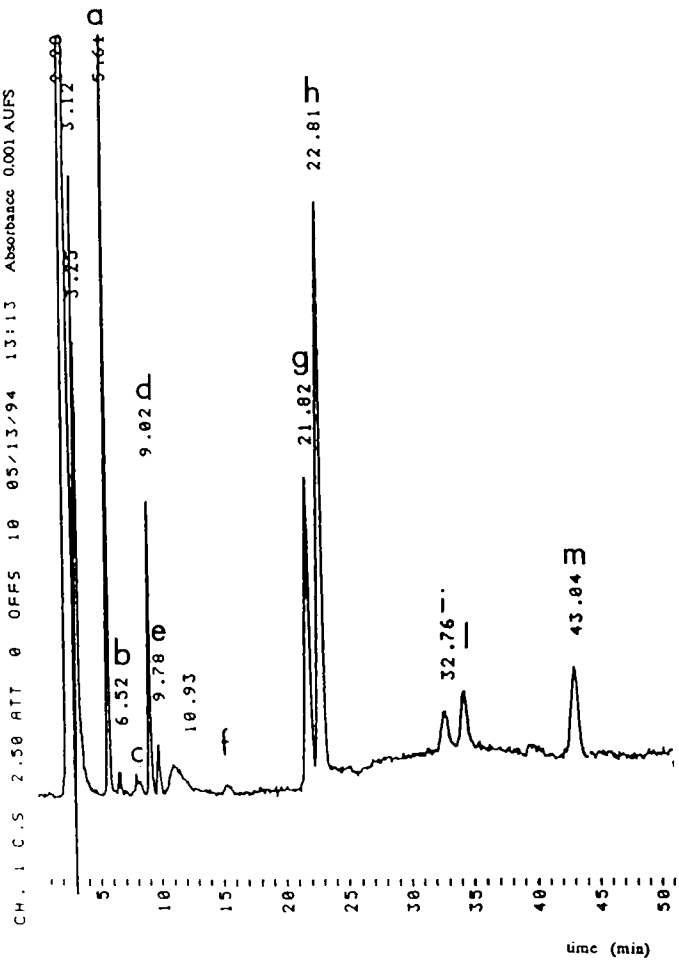

Figure 2. Elution of the standard mixture of Figure 1 in the same chromatographic conditions. Spectrophotometric detection at 254 nm.

Taking into account the different absorbance spectra of the components of the mixture, detection at different wavelengths was also investigated, in order to obtain conditions of partial spectral selectivity and for identification purposes. Figures 2 and 3 show, as an example, chromatograms recorded for the same mixture as in Figure 1 under the same chromatographic conditions, except for the detection wavelengths which, respectively, are 254 and 288 nm. At 254 nm (Figure 2) the urea derivatives and bromacil can easily be resolved

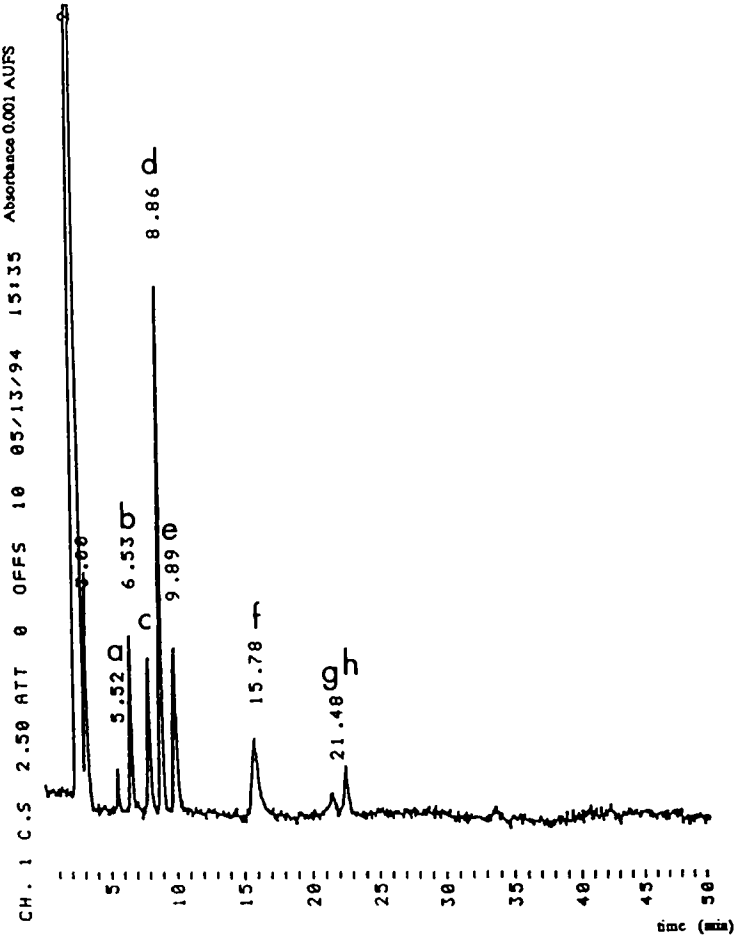

Figure 3. Elution of the standard mixture of Figure 1 in the same chromatographic conditions. Spectrophotometric detection at 288 nm.

from phenoxyacids and triazines which do not absorb significantly and, in turn, at 288 nm (Figure 3), only bromacil is clearly detected because triazine derivatives do not absorb and phenoxyacids and ureic derivatives show a very low absorbance.

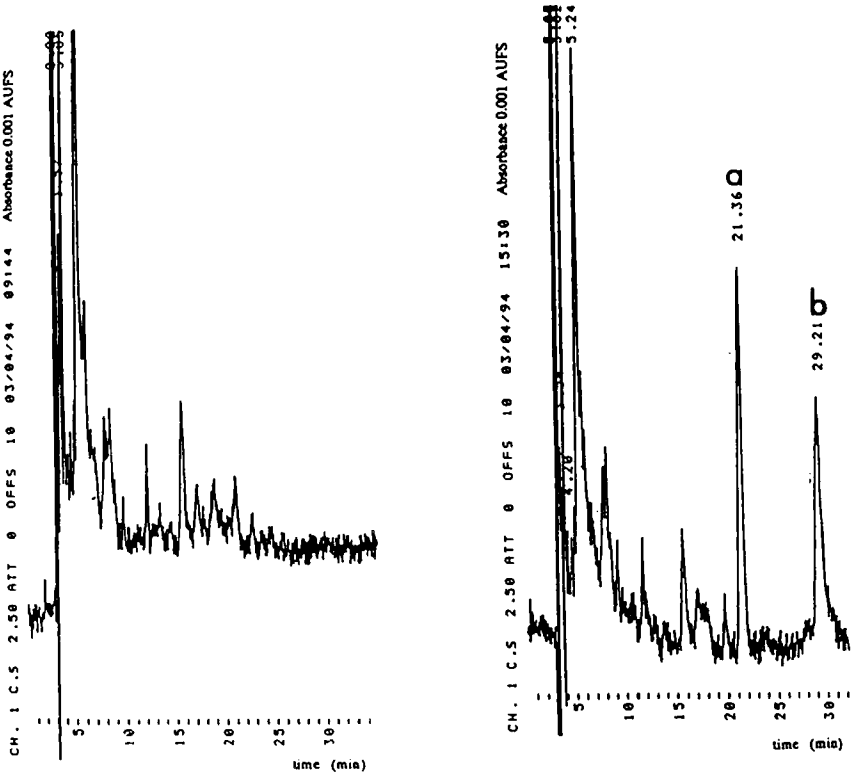

Figure 4. Chromatographic analysis of Fossano soil sample. (A): native sample extracted and diluted to final volume of 100 mL; (B): same treatment on sample added of a: 0.200 mg/Kg of 2,4-D and b: dichlorprop. Stationary phase: Phase Separations Spherisorb 5S ODS-2 (250x4.6mm; 5 μm); mobile phase: 5.0 mmol/L n-octylamine in water/acetonitrile (73/27) brought to operational pH=6.4 with o-phosphoric acid. Injection volume 100 μL. Flow rate: 1.0 mL/min. Spectrophotometric detection at 228 nm.

Application to Soils

The method was then applied for the analysis of three samples of soils representative of the Italian geopedology and, namely, from Fossano (North Italy), Carpi (Mid-Italy) and Macomer (Sardegna Island) and characterized by a different content of organic carbon and clay (see Table 1).

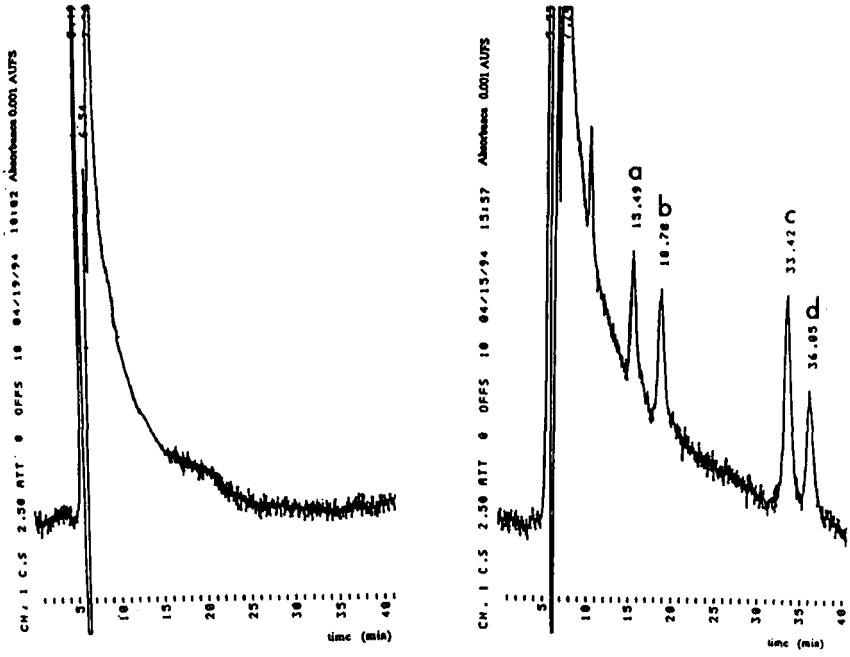

Figure 5. Chromatographic analysis of Macomer soil sample. (A): native sample extracted and diluted to final volume of 200 mL; (B): same treatment on sample added of 0.200 mg/Kg of a: 2,4-D, b: dichlorprop, c: isoproturon and d: diuron. Stationary phase: Phase Separations Spherisorb 5S ODS-2 (250x4.6mm; 5 μm); mobile phase: 5.0 mmol/L n-octylamine in water/acetonitrile (65/35) brought to operational pH=6.4 with o-phosphoric acid. Injection volume 100 μL. Flow rate: 0.5 mL/min. Spectrophotometric detection at 228 nm.

In order to consider matrix interference as well as the variability of the sample composition, a blank of each soil was extracted according to the described procedure and a series of chromatograms was recorded by sequential dilutions of the extract until acceptable baselines were obtained. Fortunately, the chromatograms showed that the time windows corresponding to the investigated pesticides can be considered free from matrix interference.

As an example, Figure 4 shows (A) the chromatogram recorded for the extract of native Fossano soil and (B) the chromatogram of the extract of the same sample after fortification with 2,4-D and dichlorprop (0.2 mg/Kg each).

Table 2

Pesticide Recoveries

Herbicide	Fortification Level (mg/Kg)	Number of Assays	Mean Recovery, %	S.D. ±
2.4-D	0.20	8	74.8	8.9
Dichlorprop	0.20	7	69.4	10.6
2,4,5-T	0.20	3	63.7	10.2
Isoproturon	0.20	3	57.7	10.1
Diuron	0.20	3	55.0	8.5

Figure 5 shows the chromatograms of the extracts of Macomer soil before (A) and after (B) contamination with 2,4-D, dichlorprop, isoproturon and diuron (0.2 mg/Kg each).

The residue fortification and the extraction steps were performed for all the soils considered and the standard addition method was employed in order to evaluate the percentual recovery yields. All the experiments were repeated three times and acceptable linearities were always obtained (regression correlation factors r^2 always > 0.90).

The results obtained are reported in Table 2 and show that the recovery percent yields range around 64%. The yield is not very high, but these values are of the same order as those obtained by Meier et al.[25] for phenoxyacid herbicides and depend on the complexity of the matrix.

In conclusion, the method presented here permits the simultaneous determination, in soils, of herbicides belonging to different chemical classes and, in particular, of herbicides with cationic properties (like triazine- and phenylurea- derivatives) and anionic (like phenoxyacids) at the natural pH of water and soil.

The method, moreover, can be used for soils of different origin and composition.

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