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Monitoring of phenylurea and propanil herbicides in river water by solid-phase-extraction high performance liquid chromatography with photoinduced-fluorimetric detection☆

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Abstract

The separation and determination of five herbicides, including propanil and the phenylureas diuron, isoproturon, linuron and neburon, has been performed by an HPLC method, using photochemically-induced fluorescence detection. The non-fluorescent herbicides were transformed into fluorescent compounds by post-column photochemical reaction. A 60:40 (v/v) acetonitrile–buffer solution of potassium phosphate dibasic (pH 7, 0.01 M) was used for the chromatographic elution to separate propanil, linuron and neburon. The overlapping of isoproturon and diuron peaks, in the selected conditions, was resolved by changing the initial movil phase composition to 50:50 (v/v) methanol–buffer solution of potassium phosphate dibasic (pH 7, 0.01 M). The procedure was applied with satisfactory results to the analysis of these herbicides in Guadiana river water samples (Badajoz, Spain), allowing the detection of herbicide residues in the order of $\mu g\ l^{-1}$, by using a solid-phase extraction (SPE) pre-concentration step.

Keywords: Herbicides; Phenylureas; Propanil; HPLC; Photo-induced-fluorimetric-detection

1. Introduction

Pesticide residues persist in the environment and may be incorporated and accumulated via food chain, affecting human health. The potential toxicity of these compounds is regulated in the European Community, allowing a maximum of 0.1 $\mu g\ l^{-1}$ of any single pesticide in drinking waters.

Phenylurea herbicides display a selective control of germinating broadleaf weeds and grasses in all kind of crops [1]. As a consequence of their widespread usage, it is vital the control of herbicide residues present in ground and surface water. It has been described in the literature that parts per million amounts of phenylureas may affect embrionic and neonatal development of some fishes and aquatic invertebrates [2].

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Among rice pesticides, propanil is one of the most applied in all the countries, and Spain is not an exception. This herbicide is known to cause acute toxicity in aquatic systems [3], indeed, manufacturer labels warn that it is toxic to fishes. For example, catfish absorb propanil rapidly, distributing it to all its tissues and being its elimination quite low [4]. Hence, animals and humans may be exposed to propanil residues for extended periods of time [5].

All the compounds studied in this work are soil-applied herbicides and, in general, the toxicity for mammals is low. However, the widespread use of these compounds in agriculture has increased the public concern on the presence of their residues in foods and water. Chromatographic techniques have proven to be very robust and the most suitable for multi-analyte pesticide determination. The most commonly sample preparation methods used for aqueous samples are liquid–liquid extraction and solid-phase extraction (SPE). The last one is becoming the most interesting, since it need less solvent and is easily automated. Besides, it is also possible to achieve trace enrichment and clean-up in one step.

In the analysis of herbicide residues, it is not always possible to achieve the desired detection limits with the detection systems used, being necessary the sample manipulation or derivatization. In the present article, UV light is used as the added reagent, producing a post-column photochemical reaction or photoderivatization, which takes place in a photoreactor, converting the non-fluorescent herbicides into strongly fluorescent photoproducts.

2. Experimental

2.1. Apparatus

The studies were carried out on a Waters 600E high-performance liquid chromatograph, equipped with a Waters 610 pump and a Waters 470 scanning fluorescence detector (Waters Millipore, Milford, MA, USA). The system was equipped with a six-way injection valve (Rheodyne), containing a 20 μl loop, and an analytical column Nova-Pak C_{18} (150 × 3.9 mm) (Waters Millipore). A postcolumn photoreactor (Softron, Gynkotek HPLC, Germany), consisting of a PTFE tube network (5 m × 0.3 mm I.D. × 1.6 mm E.D.) knitted around a 4 W xenon lamp, was placed between the column and the detector. Data adquisition and data analysis were performed with the Maxima 825 software package, Version 3.30, supplied by Waters. All the pH readings were taken with a Crison 2001 pH meter.

The spectra were recorded with an SLM Aminco-Bowman series 2 luminescence spectrometer. The excitation and emission band widths were of 4 nm. Data adquisition was performed by use of the Aminco-Bowman AB_2 program, running under OS/2. An unfiltered Osram 200 W HBO high-pressure mercury lamp with an Oriel Model 8500 power supply was utilized for the photolysis reactions. The photochemical set-up included a light-box consisting of a fan, a mercury lamp and a quartz lens. A standard Hellma 1-cm pathlengh quartz fluorescence cuvette was placed on an optical bench at 30 cm from the mercury lamp. The solutions were magnetically stirred during the UV irradiation.

The photochemical reactor is the instrument used to performed the photoreaction. It consist of a suitable lamp around which is wrapped a teflon tube that carries the HPLC effluent around the irradiator. To avoid the samples overheating a fan is included in the system. A scheme of this instrument is presented in the Fig. 1.

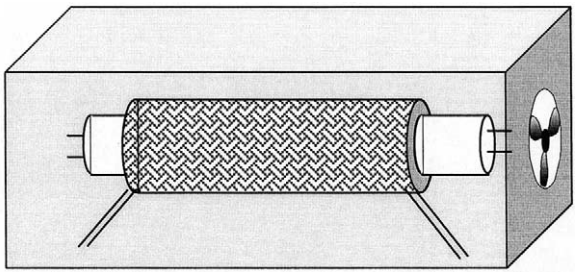

Fig. 1. Scheme of the photoreactor used in the experiments.

2.2. Chemicals and reagents

The standard pesticides diuron, isoproturon, linuron and neburon were obtained from Riedel-de Haën (Seelze, Germany) and propanil from Chem Service (Birkenhead, England). Potassium phosphate dibasic was obtained from Panreac (Barcelona, Spain) and potassium hydroxide pellets were obtained from Merck (Darmstadt, Germany). HPLC grade solvents, including acetonitrile and methanol, were obtained from Merck (Darmstadt, Germany). HPLC-grade water was obtained from a Milli-Q System (Waters Millipore, Milford, MA, USA). All other chemicals were of analytical-reagent grade.

Stock standard solutions (400 μg ml^{-1}) of each herbicide were freshly prepared by weighing 4 mg of each solute, and dissolving in chromatographic-grade acetonitrile or methanol. Then, they were stored at −20 °C. Solutions of different concentrations were prepared by dilution of the standard solutions with the mobile phase. These standard solutions were filtered before injection through a Millipore syringe adapter, containing a 0.45 μm regenerated cellulose membrane filter. The solutions were protected against light with aluminium foil.

2.3. Chromatographic conditions

2.3.1. Quaternary mixtures of isoproturon–propanil–linuron–neburon or diuron–propanil–linuron–neburon

The mobile phase was prepared by mixing a buffer of potassium phosphate dibasic pH 7, 0.01 M, with HPLC-grade acetonitrile in a 60:40, v/v proportion. The flow rate was of 0.4 ml min^{-1} and a 20 μl volume of sample was injected each time. Potassium phosphate dibasic buffer solutions of analytical concentrations 0.01 M were prepared by dissolving potassium phosphate dibasic in deionized water. Acetonitrile was mixtured with this solution in a 60:40 proportion, and the pH of the solution was adjusted by addition of 1.0 M potassium hydroxide to 7. The mobile phase was filtered through a 0.45 μm nylon filter and degassed for 5 min in an ultrasonic bath [6].

2.3.2. Binary mixture of isoproturon–diuron

The mobile phase used for the resolution of the binary mixture was composed by methanol and 0.01 M potassium phosphate dibasic solution in a 50:50, v/v, proportion. The flow rate was 0.8 ml min^{-1} and a 20 μl volume of sample was injected each time. The pH was adjusted by addition of 1.0 M potassium hydroxide to 7. The mobile phase was filtered through a 0.45 μm nylon filter and degassed for 5 min in an ultrasonic bath.

2.4. Sampling treatment

The river water samples were collected in 5 l pre-cleaned amber glass bottles, previously rinsed with ultrapure water. The samples were filtered through a Whatman No. 1 filter paper to remove sand and other suspended solid matter, and then stored at 4 °C in the dark. Before analysis, the samples were filtered first through Millipore 0.45 μm, and then through 0.22 μm nylon filters. A pH value of around 8.3 was measured for the river water samples.

Prior to the extration, the Sep-Pak Plus C_{18} bonded phase was aconditionated with 8.0 ml of acetonitrile, followed by 8.0 ml of Milli-Q water and the disk was not allowed to dry. To achieve a 100-times pre-concentration for the quaternary mixtures assayed, the following procedure was performed. A 300-ml water sample containing the herbicide was mixed and allowed to pass through the Sep-Pak at a flow-rate of 5 ml min^{-1}. After sample extraction, it was necessary to wash the Sep-Pak with 2×5 ml of Milli-Q water followed by 2×5 ml of acetonitrile–Milli-Q water (1:4) v/v. The herbicides trapped in the disk were collected by using 2.0 ml of acetonitrile as eluting solvent. The final sample volume was of 3.0 ml, which was injected in the column. In the binary mixtures of isoprouron and diuron, methanol was used for the elution in the place of acetonitrile, in the above described conditioning steps, and the final sample volume was of 5.0 ml, reaching a pre-concentration step of 60 times. A control blank was prepared without any pesticide presence.

3. Results and discussion

3.1. Resolution of the binary mixture of isoproturon and diuron

In a preliminary study, we have shown the influence of the mobile phase, flow-rate, as well as pH and buffer solution concentration in the resolution of two ternary mixtures of phenylurea herbicides composed of isoproturon–linuron–neburon or diuron–linuron–neburon. The best results were obtained when working with acetonitrile–potassium dibasic phosphate buffer (60:40) v/v, 0.01 M, pH 7 and a flow-rate of 0.4 ml min^{-1} [6].

In the conditions above mentioned, the peaks corresponding to isoproturon and diuron were completely overlapped. Therefore, a new method has been developed to perform the resolution of both peaks. Methanol–potassium phosphate dibasic buffer in a 50:50, v/v, proportion was chosen as the optimal mobile phase. The buffer analytical concentration was of 0.01 M and an apparent pH of 7. The flow-rate was varied between 0.4 and 1.0 ml min^{-1}, finding that a flow-rate of 0.8 ml min^{-1} provided higher fluorescent signals in a relatively short period of time, below 20 min.

In the selected conditions, photolysis reactions were performed by irradiating with UV light a 4 ml volume of the working solutions placed in a quartz cuvette, which were magnetically stirred at room temperature. Maximum excitation and emission wavelengths of the formed herbicide photoproducts were determined at 275/333 nm, respectively, for diuron, and two pairs of wavelengths for isoproturon, 295/428 and 274/331 nm. As a compromise value, for further experiments, 275/333 nm will be selected as the excitation and emission wavelengths fixed in the fluorescent detector to monitor both herbicide photoproducts. The obtained chromatogram is shown in Fig. 2.

For the chromatographic procedure, an aliquot of sample containing the herbicide mixture was injected through the injection valve into the carrier stream. The fluorescence detector was programmed to monitor, at the selected excitation and emission wavelengths, the photoproducts by measuring the peak area. The photolysis reaction

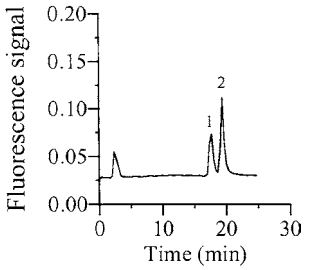

Fig. 2. Chromatograms obtained from standard solution samples at 8.0 μg ml^{-1} of: (1) isoproturon; and (2) diuron. Mobile phase methanol–phosphate buffer (50:50) v/v, 0.01 M, pH 7, at a flow-rate of 0.8 ml min^{-1}, $\lambda_{exc}/\lambda_m = 275/333$ nm.

was achieved by irradiating with the xenon lamp the injected solution in the photoreactor. The excitation and emission wavelengths, retention times, capacity factors and peak resolutions of both compounds are summarized in Table 1. In these conditions, the other herbicides were eluted using times higher than 20 min.

Calibration plots, with three replicates for each concentration value and three injections for each concentration level, obtained in the ranges shown in Table 2, indicate a good linearity. The analytical sensitivity (γ^{-1}) [7] ranged from 0.24 to 0.40 μg ml^{-1}, and the relative standard deviations (%) have been calculated for the concentrations given in parentheses. Herbicides detection limits were determined according to the criterium of Clayton et al.[8].

Table 1
Excitation and emission wavelengths, retention times (t_R), capacity factors (K') and peak resolutions (R) for the herbicide mixtures

Mixture	$\lambda_{exc}/\lambda_{em}$ (nm)	t_R (min)	K'	R
Isoproturon	275/333	16.6	6.2	–
Diuron	275/333	18.5	7.0	1.6
Diuron	324/403	6.4	0.6	–
Propanil	368/455	7.9	1.0	2.2
Linuron	335/411	8.8	1.3	1.4
Neburon	326/385	12.7	2.3	5.3
Isoproturon	301/433	6.3	0.6	–
Propanil	368/455	7.9	1.0	2.5
Linuron	335/411	8.8	1.2	1.3
Neburon	326/385	12.7	2.2	5.7

Table 2
Analytical parameters for the phenylureas and propanil determination

Compound	Curve equation	R^2	Analytical sensitivity[a] ($\mu g\ ml^{-1}$)	LOD[b] ($\mu g\ ml^{-1}$)	RSD[c] (%)	Concentration range ($\mu g\ ml^{-1}$)
Isoproturon	$A = 0.42C - 0.23$	0.998	0.28	0.80	2.0 (3.0 $\mu g\ ml^{-1}$)	4.0–20.0
Propanil	$A = 0.39C - 0.24$	0.998	0.24	0.70	2.0 (3.0 $\mu g\ ml^{-1}$)	4.0–20.0
Diuron	$A = 2.15C - 0.03$	0.992	0.09	0.23	1.6 (2.0 $\mu g\ ml^{-1}$)	0.4–3.2
Propanil	$A = 9.54C - 0.03$	0.996	0.03	0.08	1.0 (1.0 $\mu g\ ml^{-1}$)	0.2–1.6
Linuron	$A = 2.56C - 0.08$	0.990	0.10	0.26	1.6 (2.0 $\mu g\ ml^{-1}$)	0.4–2.8
Neburon	$A = 1.90C - 0.34$	0.990	0.14	0.35	1.7 (3.0 $\mu g\ ml^{-1}$)	0.6–4.8
Isoproturon	$A = 4.05C - 0.06$	0.992	0.06	0.16	1.4 (1.7 $\mu g\ ml^{-1}$)	0.5–2.4
Propanil	$A = 9.38C - 0.01$	0.992	0.03	0.07	1.6 (0.7 $\mu g\ ml^{-1}$)	0.2–1.0
Linuron	$A = 2.63C - 0.34$	0.994	0.04	0.13	1.2 (1.7 $\mu g\ ml^{-1}$)	0.5–2.4
Neburon	$A = 1.61C - 0.32$	0.994	0.04	0.13	1.1 (1.7 $\mu g\ ml^{-1}$)	0.5–2.4

[a] Analytical sensitivity (γ^{-1}): residual mean/slope of calibration curve [7].
[b] Calculated by Clayton's method ($\alpha = \beta = 0.05$) [8].
[c] The concentration used to calculate the RSD (%) is indicated in parentheses.

3.2. Resolution of quaternary mixtures of isoproturon–propanil–linuron–neburon or diuron–propanil–linuron–neburon

Propanil is an herbicide that has been applied together with phenylureas in the control of broad-leaf weeds and this is the reason of studying the separation of a mixture composed by three phenylureas and propanil, in the same conditions that the ternary mixtures [6].

The results summarized in Table 1 show the optimal excitation and emission wavelengths as well as the retention times, capacity factors and peak resolution of the two possible quaternary mixtures. The separation of the four herbicides is achieved in a relatively short time, about 13 min (Fig. 3).

The stablished calibration curves were linear over the range of interest given in Table 2. The determination coefficients, using the peak area as the analytical signal, were in all cases higher than 0.99, indicating good performance of the chromatographic method. As it is shown in Table 2, detection limits were in the range of 0.07–0.35 $\mu g\ ml^{-1}$.

With the objective of resolving a mixture of five herbicides including isoproturon, diuron, linuron, neburon and propanil, the next procedure should be followed.

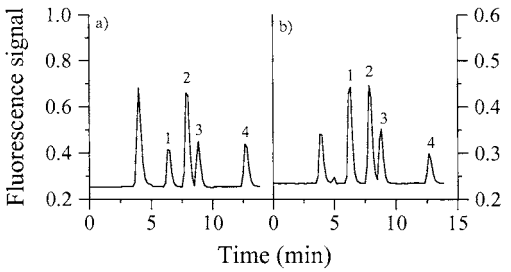

Fig. 3. Chromatographic separation of two mixtures composed by four herbicides. Mobile phase acetonitrile–phosphate buffer (60:40) v/v, 0.01 M, pH 7, at a flow-rate of 0.4 ml min^{-1}: (a) Compounds: (1) diuron, (2) propanil, (3) linuron, and (4) neburon, containing 2.0, 1.0, 2.0 and 3.0 $\mu g\ ml^{-1}$, respectively; (b) Compounds: (1) isoproturon, (2) propanil, (3) linuron, and (4) neburon, containing 1.2, 0.5, 1.2 and 1.2 $\mu g\ ml^{-1}$, respectively.

In the conditions given for the analisis of quaternary mixtures (acetonitrile–potassium phosphate buffer (60:40) v/v, 0.01 M, pH 7 and a flow-rate of 0.4 ml min^{-1}) a chromatogram containing four peaks will be obtained. The first one would correspond to a mixture of isoproturon and diuron or one of themselves, and the others would correspond to propanil, linuron and neb-

uron, respectively. This method is not able to separate isoproturon and diuron, but using the conditions described for the binary mixtures (methanol–potassium phosphate buffer (50:50) v/v, 0.01 M, pH 7 and a flow-rate of 0.8 ml min^{-1}) in a second run, two peaks would appear, the first one corresponding to isoproturon and the second one to diuron.

3.3. Analytical applications

Recovery experiments of the phenylurea herbicides under study were performed on spiked Guadiana river water samples (Badajoz, Spain), as described under Section 2.

A preliminary chromatographic run, performed on filtered river water samples under the optimal chromatographic conditions, permits us to exclude the presence of the analyzed compounds at a concentration close to or higher than the detection limit of both methods. On the other hand, it shows no matrix interference in the time range considered.

In order to improve the sensitivity of water analysis, some authors use pre-concentration steps [9–11]. The phenylurea herbicides are mainly extracted from water with dichloromethane [12–16] or, in some cases, with chloroform [17]. Nevertheless, SPE is frequently used and C_{18} is the phase normally employed [18,19]. With SPE it is also possible to achieve trace enrichment and clean-up in one step.

In the present work, the SPE technique was utilized 1 or 2 days after the collection of water samples as reported in the Section 2.

In order to evaluate the usefulness of the HPLC-photochemically-induced fluorescence proposed method for both herbicide mixtures, recovery experiments were performed on spiked Guadiana river water samples. The herbicides were spiked in 300 ml of water, and after that, all the compounds were extracted by SPE. The recoveries were above 80%, in the binary mixtures and ranged from 81 to 94%, for the mixture composed by diuron–propanil–linuron–neburon and from 86 to 100% when separating isoproturon–propanil–linuron–neburon, being comparable to those reported in the literature [20]. In all cases, the peak area was used as the analytical signal as it produced the best results.

Standard deviations were between 1 and 9%, below 30%, according to the US Environmental Protection Agency (EPA) specifications, that stipulate acceptable recovery values in the range from 70 up to 130%, with a maximum relative standard deviation of 30% [21].

4. Conclusions

As a conclusion, the chromatographic method developed for the separation and determination of phenylurea herbicides and propanil is very suitable for the analysis of river water samples. The obtained results show that the pre-concentration steps are worthy because they allow a 100-fold pre-concentration. Thanks to the SPE it was possible to detect herbicide residues in the order of ppb (μg l^{-1}) in river water samples. The residue concentrations reported for ground water range between 0.1 and 30 μg l^{-1} [22], and the proposed methods allow the determination of such concentrations. It must take into account that light is a cheap derivatizing agent which requires simple, flexible and clean instrumentation [23]. Besides, there are some advantages of using light as a reactive such as photons do not produce interfering residues or decomposition products; there is no need for additional pumps; there is not analyte dilution and, finally, light sources do not exhibit stability problems.

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