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Short communication

# High-performance liquid chromatographic determination of phenylureas by photochemically-induced fluorescence detection ☆

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**Abstract**

A HPLC method, using photochemically-induced fluorescence detection, is described for the separation and determination of four phenylurea herbicides including diuron, isoproturon, linuron and neburon. A post-column photoreactor, consisting of a reactor knitted around a 4 W xenon lamp, has been included between the column and the detector, in order to transform the non-fluorescent herbicides into fluorophors. The influence of mobile phase composition, flow-rate, pH, and buffer concentration has been studied. An acetonitrile–buffer solution of potassium phosphate dibasic of pH 7 and 0.01 *M* concentration (60:40, v/v), was selected as optimum. For the fluorimetric detection, optimal excitation/emission wavelengths 324/403, 301/433, 335/411 and 326/385 nm were selected for the determination of diuron, isoproturon, linuron and neburon, respectively. The detection limits ranged between 0.07 and 0.46 μg/ml, according to the compound.


*Keywords:* Photochemically induced fluorescence detection; Fluorescence detection; Detection, LC; Pesticides; Phenylureas

## 1. Introduction

Phenylureas are water soluble compounds. They can easily migrate from the soil to crops, persist in the environment and become incorporated and accumulated via the food-chain, affecting human health. The herbicides can also reach ground waters where, if there is no microbial activity, degradation processes are very slow and accumulation phenomena can easily lead to toxic levels. Because of that, the European Union regulates their use and allows a maximum of 0.05 mg/kg of any single pesticide in agricultural foodstuffs [1–3].

European and UK drinking water regulations [4] require that potable water contain less than 0.1 μg/l of an individual pesticide, and less than 0.5 μg/l of total pesticides. Consequently, it is important to study and provide methods for determining pesticides at the levels required [5].

Monitoring pesticides at their low concentrations requires selective and sensitive methods. Sometimes, these methods are sophisticated, expensive and complicated. The object of our paper is to describe a simple and easy method to separate and determine

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phenylureas using an equipment available in most laboratories: a high-performance liquid chromatography (HPLC) system with fluorimetric detection. Although phenylureas are non-fluorescent, the existence of a photoreactor placed between the column and the detector, transform them into fluorescent compounds [6].

## 2. Experimental

### *2.1. Apparatus*

The studies were carried out on a Waters 600E high-performance liquid chromatograph equipped with a 610 pump and a 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 post-column 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 acquisition and data analysis were performed with a 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 acquisition was performed by use of 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 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.

### *2.2. Chemicals and reagents*

The diuron, isoproturon, linuron and neburon pesticide standards were obtained from Riedel-de Haën (Seelze, Germany). Potassium phosphate monobasic was obtained from Panreac (Barcelona, Spain) and potassium hydroxide pellets were purchased from Merck (Darmstadt, Germany). HPLC-grade solvents, including acetonitrile and methanol, were obtained from Merck and 2-propanol from Panreac. HPLC-grade water was obtained from a Milli-Q System (Waters Millipore). All other chemicals were of analytical-reagent grade.

### *2.3. Chromatographic conditions*

The flow-rate ranged from 0.4 to 0.7 ml/min. Potassium phosphate buffer solutions of analytical concentrations 0.002, 0.005, 0.01 and 0.02 *M*, were prepared by dissolving different amounts of potassium phosphate dibasic in deionized water. Acetonitrile was mixed with this solution in a 60:40 proportion and the apparent pH of the solution was adjusted by addition of 1.0 *M* potassium hydroxide to 6, 6.5, 7, 7.5 and 8. The mobile phase was filtered through a 0.45 μm nylon filter and degassed for 5 min in an ultrasonic bath.

Stock standard solutions (400 μg/ml) of each herbicide were freshly prepared by weighing 4 mg of each solute and dissolving in HPLC-grade acetonitrile. 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 aluminum foil.

## 3. Results and discussion

### *3.1. Optimization of the mobile phase and flow-rate influence*

Different mobile phases were tested such as methanol, 2-propanol and acetonitrile mixed with different proportions of phosphate or Tris buffers. The organic solvents were chosen because they have been used previously for the determination of phenylureas and they provided the highest fluorescence signals [7,8]. The best results were obtained by using acetonitrile–phosphate buffer in a 60:40 pro-

portion. Initially, the buffer analytical concentration was 0.005 *M* with an apparent pH of 7.

The flow-rate was varied between 0.4 and 0.7 ml/min. It was found that the flow-rate affected the fluorescent signal of all the herbicides. As they must be irradiated to be converted into fluorophors, an increase in the flow-rate makes the compounds to be irradiated for a shorter period of time, so the intensity will be lower than when the flow-rate is lower, as is shown in Fig. 1. A flow-rate of 0.4 ml/min was selected and under these conditions all the herbicides were eluted in less than 12 min, as shown in Fig. 2. The retention times, capacity factor values and resolution were calculated as the mean values of the results obtained from 10 identical replicate analyses (Table 1).

### *3.2. Influence of pH and buffer solution concentration*

The herbicides under study are known to be hydrolyzed in acid and basic media. With a view on applications to environmental samples, the pH and buffer concentration effects on signal intensity were investigated. Tris and phosphate buffer solutions of pH from 6 to 8 were tested, as well as buffer

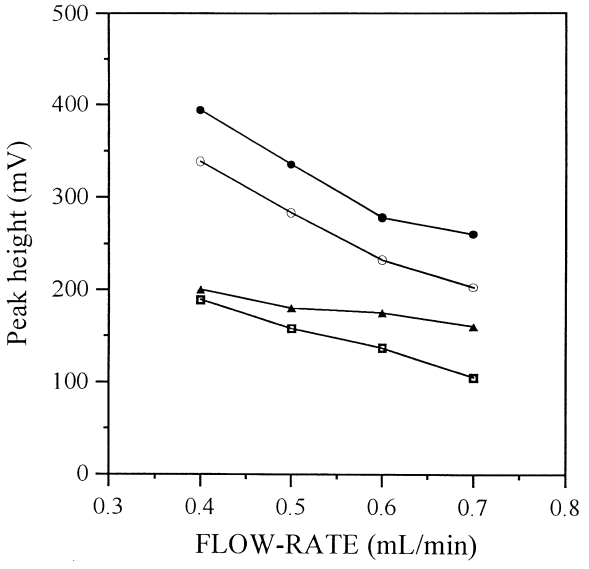


Fig. 1. Effect of the flow-rate on the peak height of the herbicides studied. Diuron (○), isoproturon (▲), linuron (●), neburon (□).

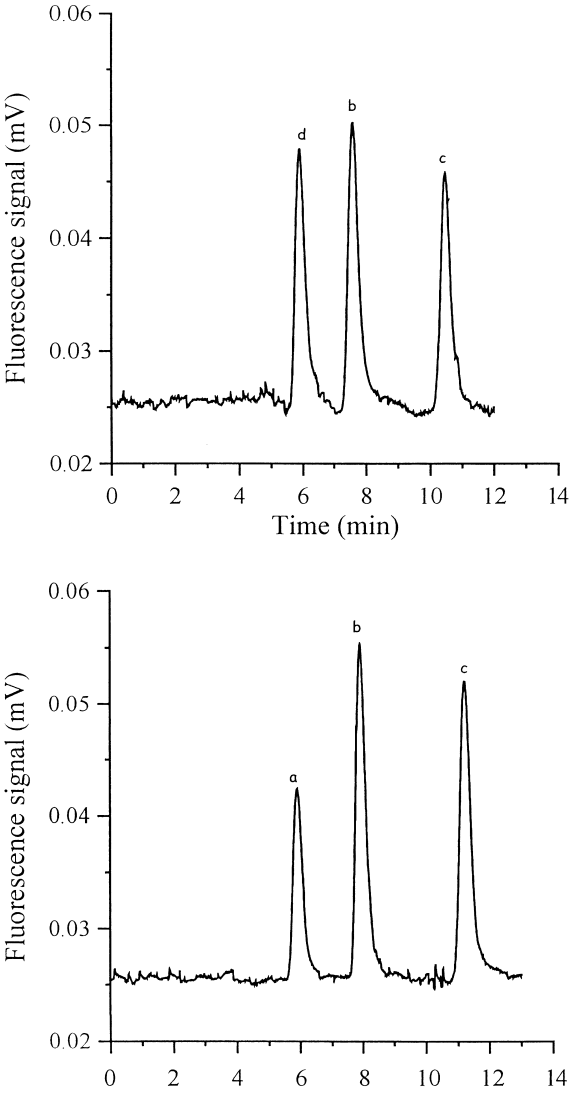


Fig. 2. Chromatograms obtained from standard solutions of (a) diuron, (b) linuron, (c) neburon and (d) isoproturon, containing 1, 2, 3 and 2 μg/ml, respectively. Mobile phase acetonitrile–0.01 *M* phosphate buffer, pH 7 (60:40), at a flow-rate of 0.5 ml/min.

analytical concentrations ranging from 0.002 to 0.02 *M*. Phosphate buffer was selected because the peaks were better defined than with Tris buffer.

The best peaks were obtained when using pH 6–7 for diuron and linuron; for isoproturon and neburon the best signals were obtained with pH 7; pH higher than 7 provided asymmetric peaks. Consequently, pH 7 was chosen for further experiments. All buffer

Table 1
Retention times, capacity factors and resolution of chromatographic peaks

| Compound | Retention time, $t_R$ (min) | Capacity factor | Resolution, $R_{2/1}$ |
|---|---|---|---|
| Diuron | 5.9 | 0.48 | – |
| Linuron | 7.6 | 0.9 | 1.5 |
| Neburon | 10.5 | 1.6 | 2.3 |
| Isoproturon | 5.9 | 0.48 | – |
| Linuron | 7.9 | 1.0 | 1.8 |
| Neburon | 11.2 | 1.8 | 2.4 |

concentrations provided good signals, but concentrations of 0.01 and 0.005 *M* were the ones that provided slightly higher signals. A 0.01 *M* concentration was selected as optimum.

### 3.3. Excitation and emission maxima

The photochemically-induced fluorescence (PIF) excitation and emission wavelengths of the formed photoproduct of each herbicide were determined, under the selected conditions, in order to program the fluorescence detector. The excitation/emission wavelengths obtained after 15 min of irradiation were 324/403, 301/433, 335/411 and 326/385 nm for diuron, isoproturon, linuron and neburon, respectively.

### 3.4. Analytical figures of merit

In order to evaluate the analytical usefulness of the method, analytical figures of merit were determined in the optimal conditions. Calibration curves were constructed by preparing samples in triplicate, containing increasing concentrations of each herbicide. The peak area or height values were plotted against concentration. The study was performed with concentration ranges of 0.6–3.6 μg/ml for diuron, 0.4–2.0 μg/ml for isoproturon, 0.6–3.6 μg/ml for linuron and 0.9–4.8 μg/ml for neburon. The results are summarized in Table 2, which also includes the detection limit values, calculated by the method proposed by Clayton et al. [9], the correlation coefficients and the precision of the method as relative standard deviation (RSD).

## 4. Conclusions

Ternary mixtures of diuron–linuron–neburon or isoproturon–linuron–neburon were analyzed by HPLC and by post-column derivatization using UV

Table 2
Analytical parameters for the phenylurea determination

| Compound (concentration range) | Signal | Curve equation | $R^2$ | Analytical sensitivity[a] (μg/ml) | LOD[b] (μg/ml) | RSD[c] (%) |
|---|---|---|---|---|---|---|
| Diuron | Height | $H=137.77C-40.49$ | 0.994 | 0.08 | 0.20 | 2.3 (1.6 μg/ml) |
| (0.6–3.6 μg/ml) | Area | $A=2.90C-0.88$ | 0.994 | 0.08 | 0.20 | 1.9 (1.6 μg/ml) |
| Linuron | Height | $H=152.75C-41.05$ | 0.994 | 0.08 | 0.19 | 2.5 (1.4 μg/ml) |
| (0.6–3.6 μg/ml) | Area | $A=3.37C-0.88$ | 0.993 | 0.09 | 0.22 | 2.4 (1.4 μg/ml) |
| Neburon | Height | $H=92.32C-53.92$ | 0.990 | 0.14 | 0.35 | 3.0 (3.0 μg/ml) |
| (0.9–4.8 μg/ml) | Area | $A=2.03C-0.92$ | 0.983 | 0.18 | 0.46 | 2.0 (3.0 μg/ml) |
| Isoproturon | Height | $H=191.2C-22.17$ | 0.997 | 0.03 | 0.07 | 2.3 (1.6 μg/ml) |
| (0.4–2.0 μg/ml) | Area | $A=4.07C-0.50$ | 0.996 | 0.03 | 0.09 | 2.2 (1.6 μg/ml) |
| Linuron | Height | $H=175.3C-47.47$ | 0.998 | 0.04 | 0.12 | 3.1 (1.6 μg/ml) |
| (0.8–3.6 μg/ml) | Area | $A=3.81C-1.09$ | 0.996 | 0.06 | 0.16 | 1.7 (1.6 μg/ml) |
| Neburon | Height | $H=111.9C-66.70$ | 0.995 | 0.08 | 0.24 | 2.5 (2.4 μg/ml) |
| (1.2–4.2 μg/ml) | Area | $A=2.61C-1.64$ | 0.996 | 0.07 | 0.20 | 2.6 (2.4 μg/ml) |

[a] Analytical sensitivity: residual mean/slope of calibration curve.
[b] Calculated by Clayton et al.'s method ($\alpha=\beta=0.05$) [9].
[c] The concentration used to calculate the RSD (%) is indicated in parentheses.

light. The proposed method is more sensitive than those described in the literature, using absorptiometric detection for the phenylurea analysis.

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