High-Performance Liquid Chromatography Column Switching Applied to the Trace Determination of Herbicides in Environmental and Drinking Water Samples

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Abstract

A selective and sensitive coupled-column high-performance liquid chromatographic method is developed for the simultaneous determination of 5 phenylurea herbicides (monuron, linuron, isoproturon, monolinuron, and diuron) in environmental and drinking water samples. Sample clean-up is performed automatically by means of a column switching technique. Using 2 octadecyl silica columns connected via two programmable 6-port valves and ultraviolet detection at 244 nm, the aforementioned compounds can be determined at the low concentration levels required for pesticide residue analysis in water samples. A mobile phase consisting of a mixture of methanol–water (55:45, v/v) is pumped at 1 mL/min. For the 5 phenylureas, high recoveries ranging from 94.9 to 101.6%, good reproducibility with relative standard deviations lower than 5%, and wide linear ranges up to 20 µg/L are observed with determination limits of 0.05 µg/L. The method is successfully applied to the screening of different environmental water samples such as surface, ground, rain, and drinking water.

Introduction

Pesticide residue analysis is an important and complex field of environmental analytical chemistry. These compounds may be transformed by chemical and biological processes or transported from the application site by runoff, leaching, volatilization, transport on soil particles, and wind erosion. Local transport over distances of several miles may be responsible for adverse effects on nontarget species. The contamination of water by pesticide residues is a matter of concern with the contamination of Earth's atmosphere. The pollution of soil, ground, and surface water involves a serious risk to the environment and also human health by way of direct exposure or through residues in food and drinking water (1–3). The regular control of possibly harmful xenobiotic concentrations in agricultural products and different environmental matrices is therefore an important task during the evaluation of a chemical intended for agricultural use, under field circumstances, and in finished agricultural products or environmental samples.

Monuron, monolinuron, isoproturon, diuron, and linuron are members of the substituted urea class of pesticides (Table I). These chemical compounds are selective systematic herbicides that are applied to the soil as a pre-emergence treatment. They are absorbed principally by the roots but also by the leaves with translocation and inhibition of the photosynthetic electron transport (4).

The European Union (EU) provides directives and regulations concerning the maximum residue levels of pesticides in foodstuffs and water in accordance with the recommendations of the Codex Committee on Pesticide Residues. In the case of drinking water, the EU directive declares that the amount should not exceed the level of 0.1 µg/L for individual compounds and 0.5 µg/L for total pesticides (5). This essentially means that methods for water analysis must present detection limits 1000 times lower than those for foodstuffs.

In general, pesticide residue analysis is performed by gas chromatography (GC) (6–8) with different types of detection, such as electron capture (8), nitrogen–phosphorus selective (9), and mass spectrometric (MS) (10,11) detection methods. The high polarity and low volatility of the phenylureas makes the use of a preliminary chemical derivatization necessary. It adds an additional sample treatment step, making the methods more laborious and time consuming (apart from the possible sample loss due to excessive manipulations or uncompleted reactions). For all of these reasons, techniques capable of performing the separation in the liquid phase are preferred.

Thin-layer chromatography (12–15) and potentiometry (16) have been used for the identification and determination of phenylurea herbicides in chemical formulations, whereas HPLC has been the most employed technique for the quantitative analysis in different biological and environmental

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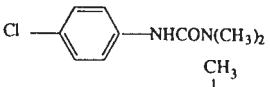
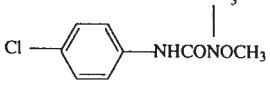
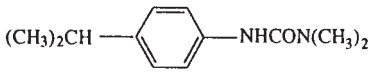
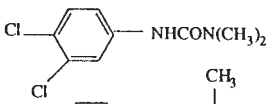
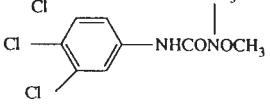
Table I. Names and Chemical Structures of the 5 Phenylurea Herbicides Used in this Study

Common name	Molecular structure	IUPAC name*	Molecular weight
Monuron		3-(4-chlorophenyl)-1,1-dimethylurea	198.6
Monolinuron		3-(4-chlorophenyl)-1-methoxy-1-methylurea	214.6
Isoproturon		3-(4-isopropylphenyl)-1,1-dimethylurea	206.3
Diuron		3-(3,4-dichlorophenyl)-1,1-dimethylurea	233.1
Linuron		3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea	240.1

* IUPAC, International Union of Pure and Applied Chemistry.

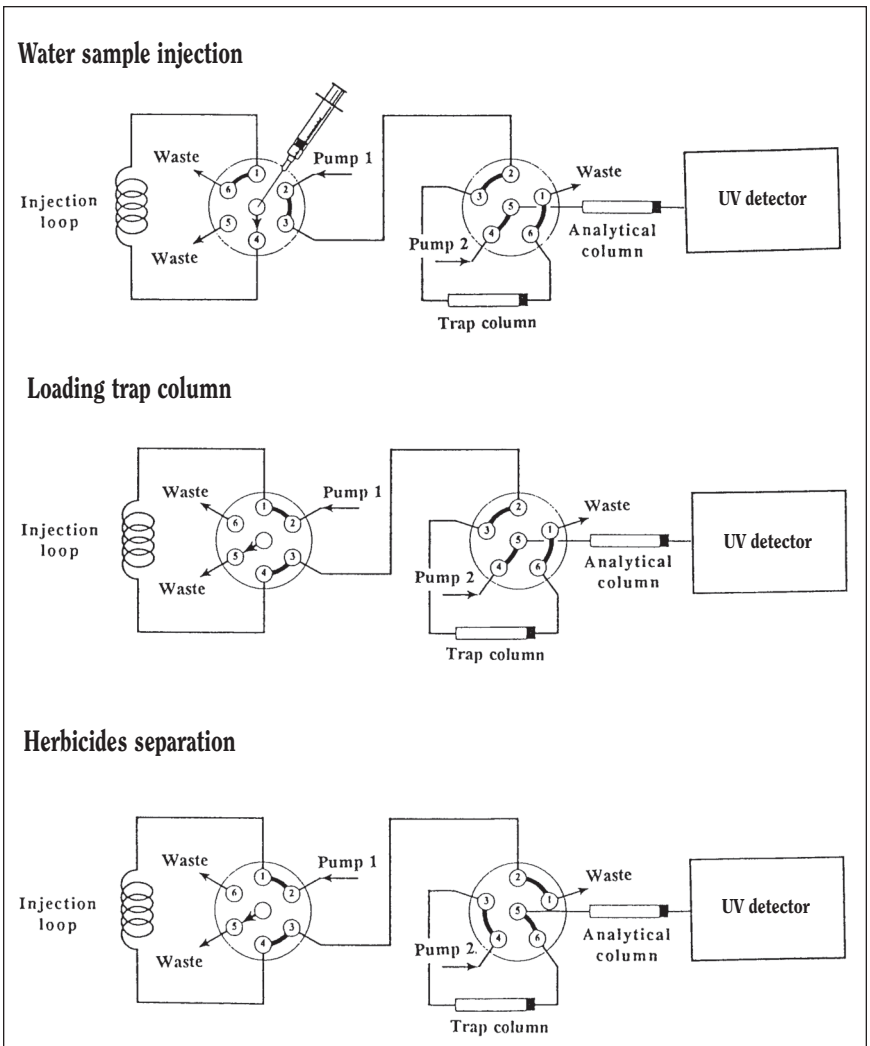


Figure 1. Scheme of the HPLC column switching system.

matrices (17–20). For their determination in water samples where high sensitivity is required, the use of liquid chromatography (LC) coupled with MS or with ultraviolet (UV) detection using a preconcentration step have been reported (21–27). Recently, papers about the application of capillary electrophoresis to the separation but not the quantitative determination of some phenylureas have been published (28,29).

The use of HPLC combined with column switching is an appropriate analytical technique that can provide the required sensitivity and selectivity in the analysis of polar pesticides at low concentration (30,31). The ability to perform online sample clean-up avoids laborious sample preconcentration steps, making the methods easily automizable and applicable to routine analysis.

The objective of this study was to develop a sensitive and selective column-switching procedure for the simultaneous determination of 5 phenylurea herbicides (monuron, monolinuron, isoproturon, diuron, and linuron) in environmental and drinking water samples.

Experimental

Chemicals, reagents, and standards

Monuron, monolinuron, isoproturon, diuron, and linuron (all with a purity higher than 99%) were obtained from Sigma

Aldrich Kft. (Hungary, Budapest). HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). HPLC-grade water was obtained using a Milli-Q system (Millipore, Bedford, MA). Stock standard solutions of each pesticide (1000 µg/mL) were prepared by dissolving the required amount of each pesticide in acetonitrile and were kept under refrigeration. Dilutions were made with Milli-Q water to the desired final concentrations.

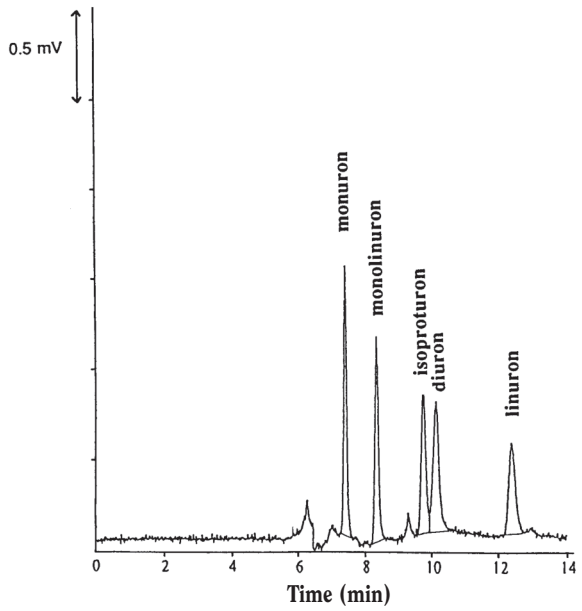


Figure 2. Typical chromatogram of 5 phenylurea herbicides obtained in the column-switching HPLC system with UV detection. Operating conditions: two Beckman Ultrasphere ODS columns, 50 × 4.6-mm i.d., 5-µm particle size; eluents, methanol–water (55:45, v/v); flow rate, 1 mL/min; UV detection, 244 nm; sample volume, 3.5 mL; concentration of the standard mixture, 0.5 µg/mL for each compound.

Instrumentation

The HPLC system was a JASCO LC (JASCO, Kyoto, Japan) equipped with two JASCO PU-850 pumps, an AS-950 autosampler, and a UV-975 UV/visible detector. Two programmable six-port valves were used for the column switching. The injection loop volume was 3.5 mL. Data acquisition and processing were accomplished by means of a Waters (Milford, MA) Maxima 820 data station using an IBM (White Pines, NY) PC/AT 486 computer. Two Beckman (Palo Alto, CA) Ultrasphere octadecyl silica (ODS) columns (50 × 4.6-mm i.d., 5-µm particle size) were used as trap and analytical columns.

Column-switching system and HPLC operating conditions

The column switching arrangement used for the clean-up of the water samples is illustrated schematically in Figure 1. Surface, rain, ground, and drinking water; blank and fortified surface water samples; and standard solutions prepared in Milli-Q water were subjected to analysis after filtration.

The methodology involved the injection of a large volume (normally in the milliliter range) of uncleaned water sample with the system in loading position. The mobile phase was flushed through the first (trap) column for the removal of the most polar interferences. Just before the first analyte of interest started to elute, the trap column was switched on-line in series with the second (analytical) column (loading position). The same mobile phase was used to transfer the fraction containing the herbicides from the first to the second column (herbicides separation position).

After the direct injection of 3.5 mL of water sample on the first column, a clean-up was performed with 6.1 mL of methanol–water (55:45, v/v) pumped at a flow rate of 1 mL/min. The transfer of the retained analytes was performed with 3.25 mL of the same mobile phase. Separation on the second column took place under the same isocratic conditions. UV detection of the separated herbicides was carried out at a wavelength of 244 nm. Every injection was made in triplicate. An

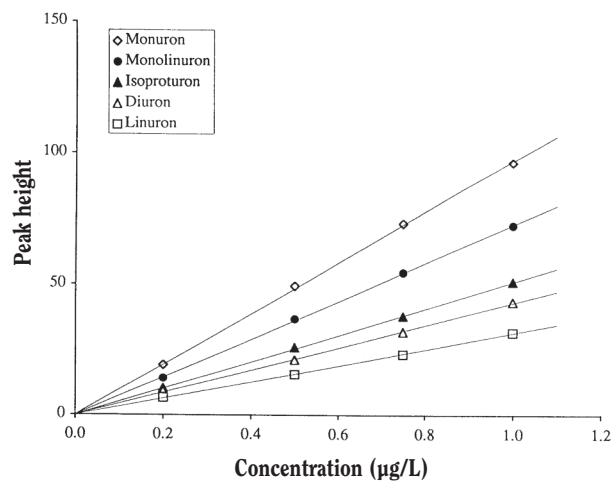


Figure 3. Calibration curve for the 5 phenylurea herbicides in the concentration range 0.2–1.0 µg/L.

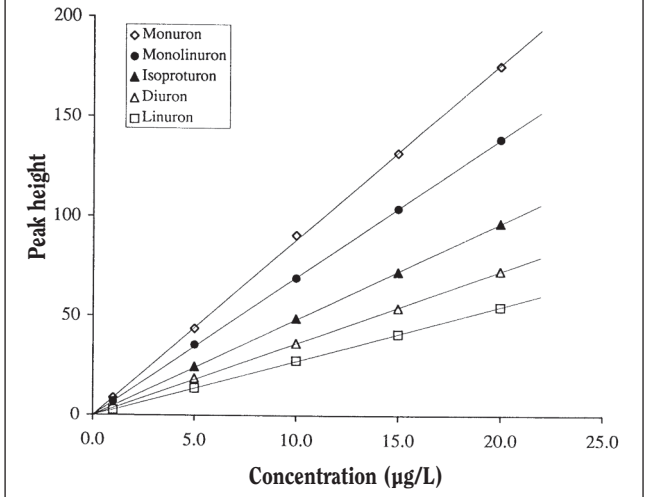


Figure 4. Calibration curve for the 5 phenylurea herbicides in the concentration range 1.0–20.0 µg/L.

Table II. Validation Parameters for Surface Water Samples Spiked at 0.5 µg/L*

Compound	Regression line equation	Correlation coefficient	Recovery (%)	RSD (%)
Monuron	$y = 96.20x + 0.52$	0.9996	96.4	4.8
Monolinuron	$y = 72.48x + 0.06$	0.9997	94.9	2.9
Isoproturon	$y = 50.74x + 0.21$	0.9998	97.4	3.8
Diuron	$y = 42.94x + 0.11$	0.9997	101.6	2.4
Linuron	$y = 31.44x + 0.15$	0.9998	99.2	3.4

* The surface water samples were spiked at 0.5-µg/L levels for each compound ($n = 5$). The concentration range of calibration was 0.2–1.0 µg/L.
† y represents peak height; x represents concentration.
‡ RSD, relative standard deviation.

Table III. Validation Parameters for Surface Water Samples Spiked at 10.0 µg/L*

Compound	Regression line equation	Correlation coefficient	Recovery (%)	RSD (%)
Monuron	$y = 8.77x + 0.15$	0.9996	101.4	2.8
Monolinuron	$y = 6.89x + 0.70$	0.9999	99.0	1.9
Isoproturon	$y = 4.80x + 0.55$	0.9999	96.8	3.4
Diuron	$y = 3.59x + 0.65$	0.9999	99.6	2.4
Linuron	$y = 2.71x + 0.35$	0.9999	97.2	4.9

* The surface water samples were spiked at 10.0-µg/L levels for each compound ($n = 5$). The concentration range of calibration was 1.0–20.0 µg/L.
† y represents peak height; x represents concentration.
‡ RSD, relative standard deviation.

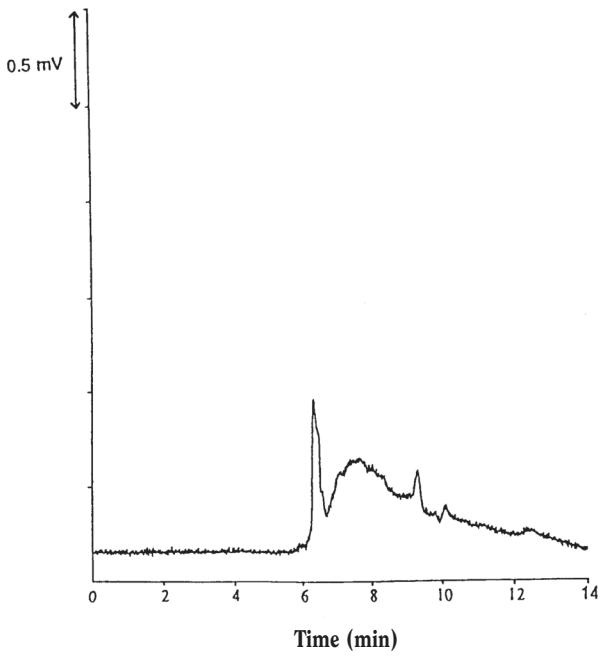


Figure 5. Typical chromatogram of surface water blank sample. Operating conditions were the same as in Figure 3.

analysis from the injection of the sample to the elution of the chromatographic peaks was less than 15 min.

Results and Discussion

Pesticide residue analysis is an important field in environmental analytical chemistry; recently, several new developments have been reported. Each technique possesses advantages and limitations that must be considered when identifying the most suitable procedure for applications. When evaluating the different alternatives, several features must be taken into account, such as the limit of detection required, possible matrix interferences, the number of samples to be analyzed, etc. The increasing availability of LC methods for the analysis of pesticides is mainly the result of their applicability to thermolabile and polar compounds for which GC would require previous derivatization.

The minimum residue levels allowed in general water samples are low (0.1 µg/L per individual pesticide) and make the use of a preconcentration step necessary in most of the analytical techniques. Another additional problem is the high concentration of interferences present in environmental water samples. The ground and surface water samples contain a relatively high concentration of anions and humic and fulvic acids that produce a high UV response in the early part of the chromatogram, causing interferences at the retention time when the analytes are eluted.

Most of the HPLC methods reported in the literature use solid-phase extraction with C_{18} cartridges for the simultaneous sample clean-up and preconcentration. Although this alternative is effective, it always imposes an additional analysis step; is laborious, time consuming, and sometimes expensive; and involves sample manipulations with probabilities of analyte losses.

To avoid these problems, it is better to use a column-switching arrangement. In this way, using simple instrumentation, a practical and fast online clean-up and a preconcentration necessary to reach the concentration required for UV detection are achieved. In Figure 2, typical chromatograms obtained from the injection of a standard mixture of the 5 phenylurea herbicides are shown.

After the optimization of the most significant parameters (eluents, clean-up, and transfer volume), a calibration curve was constructed by injecting different concentrations of standard solutions of the 5 herbicides. Different attenuation values had to be used in the detector to cover the entire concentration range studied; therefore, the calibration was divided into two parts, the last point of the low concentration range curve

(1.0 µg/L) being the first one of the second calibration. As observed in Figures 3 and 4, the calibration curves were linear from 0.2 µg/L to 20.0 µg/L. In Tables II and III, the corresponding correlation equations and regression coefficients are listed. The limit of detection was 0.05 µg/L for each compound as determined according to the American Society of Testing and Materials ASTM D4210 standard. The reproducibility was evaluated in terms of percent relative standard deviation (n = 5), which was lower than 5% in all the cases. Quantitation was performed by peak height comparison with the calibration curve.

The procedure was validated by analyzing two series (n = 5) of surface water samples spiked with the 5 herbicides at 2 different concentration levels (0.5 µg/L and 10.0 µg/L). Figures 5 and 6, corresponding to the chromatograms of a surface water blank and the fortified surface water (at 0.5 µg/ L concentration level), respectively, demonstrate the performance of the system.

The developed method was successfully applied to the analysis of different environmental samples of surface, rain, ground, and drinking water.

Conclusion

The column-switching method has been demonstrated to be a powerful technique for the mono- and multiresidue analysis of phenylurea herbicides in environmental and drinking water samples. The validation and recovery parameters fulfilled the requirements of the European Community Directive for pesticide residue analysis in water samples. The developed column-switching procedure using two identical ODS columns possesses a high separation power and increased selectivity. Application of the cutting technique results in high sensitivity, because large-volume injections can be performed for very diluted samples. The simplicity and ruggedness of the system allows for easy automation and high sample throughput, making it applicable to the routine monitoring of polar phenylurea herbicides in environmental and drinking water samples. This HPLC column switching arrangement may be coupled with MS for the positive identification and confirmation of these herbicides.

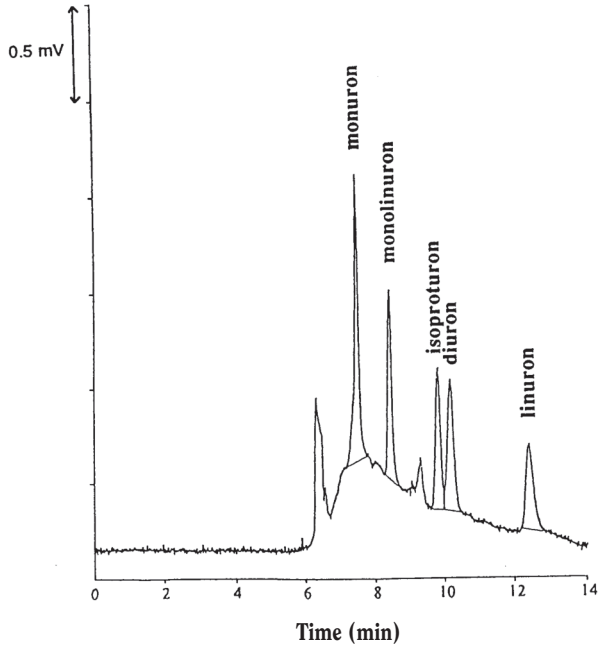


Figure 6. Typical chromatogram of fortified surface water sample. The fortification level was 0.5 µg/L for each analyte. Operating conditions were the same as in Figure 3.

Acknowledgments

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