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Laura Scrano · Sabino A. Bufo · Franz Menzinger · Philippe Schmitt-Kopplin

Novel degradation products of the herbicide oxasulfuron identified by capillary electrophoresis – mass spectrometry

Abstract Two new intermediates rising from the photolytic reaction of the sulfonylurea herbicide oxasulfuron have been identified in aqueous environment. The higher concentrations of the two derivatives oxetan-3-yl 2-(formilsulfamoyl) benzoate and *N*-(4,6-dimethyl-pyrimidin-2-yl) formamide were reached within 8 h of UV-irradiation. Here we demonstrate that an optimal separation and analysis of such compounds can be achieved by using a novel analytical method based on "non-aqueous" capillary electrophoresis (CE) system joined to an electrospray ionisation-mass spectrometry equipment. Using such a separation method and a particular electrophoretic solution a high reproducibility of migration times and peak areas can be obtained.

Keywords Oxasulfuron · Photolysis · Capillary electrophoresis · Mass spectrometry

Introduction

Capillary electrophoresis (CE) is a technique of separation that has been well developed in the last decade, giving optimal results in terms of sensibility and selectivity as compared to traditional chromatographic techniques (Henry 1996). The analysis is based on the electrophoretic classic effect, although a capillary column of fused silica is used as separation means. The analytes, submitted to an intense electric field, migrate into sharply defined zones of the capillary column as a result of their characteristic mobility and can be revealed by means of an optical detector. The success of such a technique has been also due to the variety of the ways in which the electrophoresis can be realized, and the achieved miniaturization and automation of the instrumentation. A further advantage deriving from the use of the capillaries is that they come diminishing the heating phenomenon during the electrophoretic run.

Recently, CE has been coupled to mass spectrometry (CE–MS) and applied with enormous advantages to the separation and identification of substances of environmental concern, like pesticides (Picó et al. 2003).

Sulfonylureas are herbicides used in pre- and post-emergence for controlling a range of weeds in a variety of crops and have been extremely popular word-wide because of their low mammalian toxicity and unprecedented herbicidal activity. These compounds are subject to pH-dependent hydrolytic degradation and can undergo photolytic degradation both in liquid and sorbed phases (Albanis et al. 2002). Since these herbicides are applied at a very low rate (10–40 g ha^{-1}), the identification and quantitation of their degradation and transformation products in the environment constitute a challenge of shared interest. Numerous articles about the methods of separation and identification of mother substances and their by-products can be found in literature. Vulliet et al. (2002), and Rodriguez and Orescan (1998) have applied liquid chromatography in tandem with mass spectrometry to the separation and identification of several sulfonylureas, and their degradation products. Krynitsky (1997) and Menzinger et al. (2001) report the elevated potentiality of CE, also coupled to a mass spectrometer, for the analysis of numerous classes of pesticides.

This work aims to study the photochemical degradation in aqueous solution of oxasulfuron, a sulfonylurea herbicide, and identify the derivative products by means of

L. Scrano · S. A. Bufo (✉)
Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata,
Viale dell'Ateneo Lucano 10,
85100 Potenza, Italy
e-mail: bufo@unibas.it
Tel.: +39-0971-205232
Fax: +39-0971-206226

F. Menzinger · P. Schmitt-Kopplin
GFS-National Research Centre for Environment and Health,
Institute for Ecological Chemistry,
Ingolstädter Landstrasse 1,
85764 Neuherberg, Germany

non-aqueous capillary electrophoresis tandem electrospray mass spectrometry.

Experimental

Chemicals

Standard of oxasulfuron (purity 98%; molecular weight 406.8), oxetan-3-yl 2-[(4,6-dimethylpyrimidin-2-yl) carbamoyl sulfamoyl] benzoate (CAS RN 144651-06-9), has been supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All solvents (pesticide grade), reagents (analytical grade), and filters (disposable sterilized packet) were purchased from Sigma-Aldrich (St. Louis, MO), and Fluka Chemie (Buchs, Switzerland). Ultrapure water was obtained by means of a Millipore (Billerica, MA) Milli-Q system.

Preparation of samples

All glassware was heat-sterilized by autoclaving for 60 min at 121°C before use. Aseptic handling materials and laboratory facilities were used throughout the study to maintain sterility. Since the active ingredient is sufficiently soluble in water ($\log P = 0.89$, calculated by PALLAS 3.1 for Windows), the tests have been performed in aqueous solutions. A stock solution of the herbicide (100 mg l^{-1}) in ultrapure water was prepared and kept in the dark at 4°C. Calibration and test solutions were prepared when used by dilution from the stock solution. Solvents and solutions were filtered using sterilized cellulose acetate ultra-filters ($0.2 \mu\text{m}$). The working solution (10 mg l^{-1}) was divided into two parts. One of these portions was used for the irradiation experiment, the other as a control.

Irradiation procedure

UV spectrum of oxasulfuron in water was recorded on a Cary 2300 spectrophotometer (Varian, Harbor City, CA).

The photochemical reaction was performed in a Pyrex reactor (250 ml) at 20°C (water circulation temperature controlled), using a HPK 125W UV-Hg high-pressure lamp from Philips (Eindhoven, The Netherlands) protected with a quartz thimble (total passing wavelength: $240 \text{ nm} < \lambda < 580 \text{ nm}$; irradiance: 79.04 W m^{-2}; irradiating dose per hour of irradiation: 284.5 kJ m^{-2}). Sample was irradiated, and the disappearance of the herbicide at various illumination times was determined. A control sample (having the same initial concentration of the irradiated sample) was kept in the dark at the same temperature, and the herbicide was detected at the same times as the irradiated sample to measure the disappearance due to hydrolysis or to confirm that the degradation process would only be the result of a photochemical reaction. Three replicates were carried out for each experiment.

Analyses

Analyses of samples were performed by non-aqueous CE coupled with an ion trap mass spectrometer. The separation of analytes was obtained through hydrodynamic injections of the samples into a non-coated open fused-silica capillary from Polymicro Technologies (Phoenix, AZ, USA); 75 μm of inner diameter and 87 cm total length, 22 cm to the detector. The front part portion, approximately 65 cm of the capillary, was liquid-cooled and set at a temperature of 20°C, and the remaining portion (about 22 cm) was exposed to ambient air conditions. Before the use, the capillary was washed with a 0.1 N NaOH solution for 1 h, and subsequently it was conditioned for 30 min with the electrophoretic solution. At the end of every analytical run, the capillary was re-equilibrated at least 5 min with the electrophoretic solution. The procedure of washing and conditioning was repeated daily. The introduction of the sample (ca. 55 nl) was obtained with an injection pressure of 3.45 kPa for 10 s and applying an electric potential of 25.5 kV. The electrophoretic buffer was a mixture of 90% acetonitrile/10% methanol (v:v), containing $5 \times 10^{-2} \text{ mol l}^{-1}$ ammonium acetate and 1.2 mol l^{-1} acetic acid (pH 3.4). CE measurements were performed with a model P/ACE 5510 CE system from Beckman Instruments (Fullerton, CA, USA), equipped with an on-column diode-array detector. The CE system was coupled to an ion-trap mass spectrometer model LCQ duo from ThermoElectron (San Jose, CA, USA), furnished of an electrospray ionisation system (ionic source) settled in positive ionisation mode. Instrumental control, data acquisition and data processing were carried out using the Beckman Gold software package for CE and the Thermo *X*Calibur data system version 1.1 for the mass detector. CE-MS was carried out by applying 30 kV to the anode and 4.5 kV to the cathode. The cathode was the electrospray tip on the interface. The standard source conditions were as follows: needle voltage $+ 4.5$ kV; spray current 7 μA; temperature of the heated capillary 180°C, and heated capillary voltage 32 V; sheath gas flow rate (N_2) 0 arbitrary units; coaxial sheath liquid, methanol/water (1:1 v:v) with 1% of acetic acid at a flow rate of 2.5 $\mu\text{l min}^{-1}$.

Results and discussion

Analytical method

In spite of several constraints that must be considered when CE and MS are coupled on-line, especially regarding the joining of the electrophoretic capillary with the capillary entering the ionisation source equipment, using the separation method and the electrophoretic buffer selected for this work an optimal separation of oxasulfuron and its photo-products was obtained. Figure 1 illustrates the electropherogram recorded in total ion current mode injecting the solution of the herbicide after 8 h of UV-irradiation. For clarity, in the abscissa of Fig. 1, values of the running ionic mobility 'μ' ($\text{cm}^2 \text{ V}^{-1} \text{ min}^{-1}$) have been transformed in
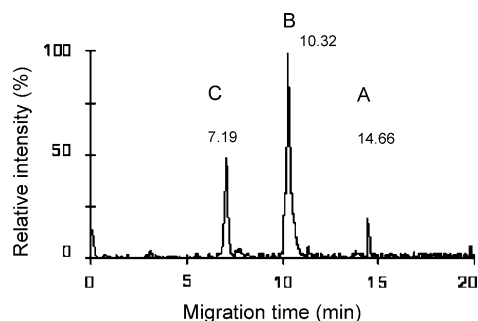
Fig. 1 Electropherogram "non-aqueous capillary electrophoresis – electrospray ionisation-mass spectrometry (NACE-ESI-MS)", obtained injecting the solution resulted after 8 h of UV-irradiation: **A** oxasulfuron; **B** and **C** photo-degradation products

time unit (min); values of the capillary section and applied voltage were used in the calculation.

Three injections of each sample were repeated for the three replicate experiments obtaining satisfactory repeatability of analytical results either in terms of migration times or peak areas. The determination coefficient of the calibration curve (0.1–10 mg l^{-1}; $n = 3$ injections $\times$ 8 concentrations) was 0.9916. Considering a signal/noise ratio of 3, the detection limit for oxasulfuron was 0.12 mg l^{-1}. The relative standard deviations ($n = 3$ injections $\times$ 3 replicate samples) of migration times for the (A) mother molecule and (B, C) photo-products, obtained with 8 h of UV-irradiation, were 0.84 % (A); 0.79 % (B); 0.96 % (C). The relative standard deviations of peak areas, calculated using the scale of times, were 4.5 % (A); 5.9 % (B); 1.7 % (C). The relative standard deviations of peak areas, obtained using the scale of mobility, were 3.6% (A); 3.9% (B); 3.0% (C). These results are in agreement with literature findings (Menzinger et al. 2001; Hernández-Borges et al. 2004) and close to those obtained by Rodriguez and Orescan (1998) and Clowers et al. (2001), which used liquid chromatography coupled with electrospray ionisation mass spectrometry for the analytical investigation. Figure 2 shows mass spectra of substances identified in the solution of oxasulfuron after 8 h of UV-irradiation.

Photo-products

The aqueous solution of oxasulfuron kept in the dark at 20°C evidenced a slightly hydrolysis reaction: after 50 h the concentration of the herbicide underwent a reduction of 13% respect to the initial value (0.02458 mM). On the contrary, in the first 10 h the concentration of the irradiated solution diminished up to 10% of the initial value, showing a half-life of 4.1 h and following a first-order kinetics.

The elevated reactivity of the herbicide under light irradiation can be explained considering the frequencies and intensities of maximum absorptions for this compound (Fig. 3) in the range of Hg-UV arc emissions. According to the method of calculation proposed by Scott (2001), taking into account that the overlapping of lamp emissions and herbicide absorptions is restricted to the UV-
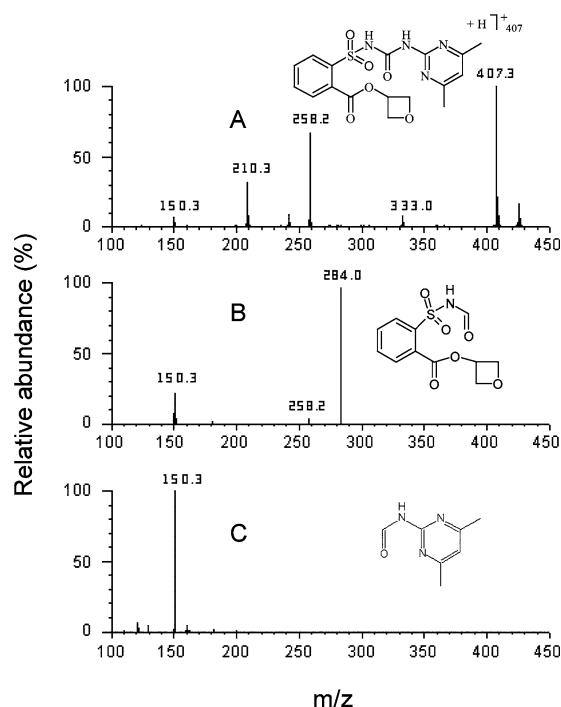

Fig. 2 Electrospray positive ionisation-mass spectra of (**A**, m/z 407) oxasulfuron and (**B**, m/z 284; **C**, m/z 150) two photo-degradation products obtained after 8 h of UV-irradiation

interval 240–300 nm, we calculate an effective irradiance of 14.33 W m^{-2} and an effective UV-dose of 51.6 kJ m^{-2} per hour of irradiation (the 18% of the total hourly dose of radiations attributable to the HPK lamp). These values indicate that there is an adequate interaction between the UV-radiations and the herbicide molecules with a consequent high reactivity of the oxasulfuron and its capacity to give rise to photolytic derivatives.

During the photolysis reaction, it was possible to reveal the formation of two by-products identified as (1) oxetan-3-yl 2-(formilsulfamoyl) benzoate (Fig. 2b), and (2) N-(4,6-dimethyl-pyrimidin-2-yl) formamide (Fig. 2c). Both derivatives detected arise from the breakdown of the sulfonylurea bridge. The concentration of these intermediates increased during the first 8-h of irradiation after which it decreased rapidly in the following hours (data not shown).
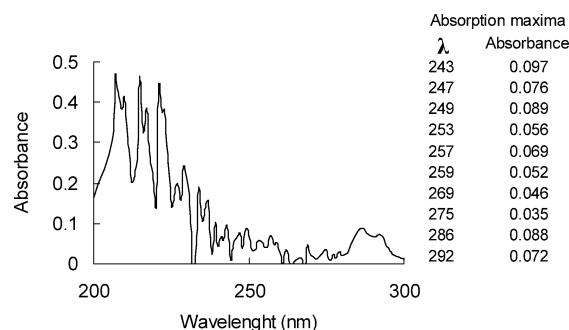

Fig. 3 UV-spectrum of oxasulfuron in aqueous solution, and absorption maxima detected in the range of UV-radiations emitted from the Hg-Philips HPK lamp

Conclusion

The adopted system was very effective in the separation of the active ingredient and its derivatives. The repeatability and sensibility of the analytical method allowed the limit of detection to be reached as adequately low as required by the particular application realized in this work. For the nature of the analytes we were going to separate, the choice of a "non-aqueous" electrophoretic solution was of remarkable aid. Oxasulfuron, while showed a sufficient resistance to the hydrolytic reaction, was particularly sensible to the UV-radiations as well as its photolytic intermediates, which were degraded in turn by lighting treatment. This behaviour lets us suppose that in aqueous environment the herbicidal residues can get accumulated only in the absence of light.

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