On-line supercritical fluid extraction and high performance liquid chromatography for determination of triazine compounds in soil

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On-Line Supercritical Fluid Extraction and High Performance Liquid Chromatography for Determination of Triazine Compounds in Soil

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Introduction

The use of supercritical fluids for analytical extraction of xenobiotics jn environmental samples has received increasing attention [1-4]. It has been reported that supercritical fluid extraction (SFE) offers several advantages over classical liquid solvent processes. In most cases SFE can provide extraction efficiencies comparable to values obtained by conventional Soxhlet extraction. Moreover, SFE is also faster, because of the high diffusivity and low viscosity of supercritical fluids, and also avoids thermal degradation of the extracts.
SFE also has a considerable capacity for off- and on-line inter- facing with analytical chromatographic methods for quantitative and qualitative determination. This reduces sample handling, as well as the possibility of sample loss and contamination. The high sensitivity attainable also proves useful if only small samples are available. Basically, SFE can be easily coupled with supercritical fluid chromatography (SFC) or gas chromatography (GC), and various examples have been recently described [1-4]. Less abundant are applications of on-line coupling with high performance liquid chromatography (HPLC) [5,6]. Yet, HPLC is a chromatographic method preferentially used in the case of thermolabile analytes (such as phenylurea herbicides or triazole fungicides), or when a specific detection (^{14}C) is needed.
We report in this paper an on-line coupling of SFE with HPLC. Application to the extraction and separation of labeled triazine compounds from soil samples is also presented.

Experimental

Chemicals

Solvents and co were analytical grade or SFC quality, respectively. [ring-UL-^{14}C]Atrazine (425 MBq/mmol) was purchased from Sigma (St-Quentin Fallavier, France). Labeled metabolites, namely deethylatrazine, deisopropylatrazine, and hydroxyatrazine were obtained hy fungal biological dealkylation or acidic hydrolysis 171. The radiochemical purity of all triazine compounds was up to 96%.

Apparatus

A schematic diagram of the on-line SFE/HPLC system is presented in Figure 1. The system comprised apparatus for extraction, co)lection, and separation of analytes.

Extraction of samples (contained in a 2.5-mL stainless steel extraction cell) was achieved in dynamic mode by an Isco SFX 2-10 supercritical 0uidexu·actor (Roucaire, Velizy Villacoublay, France) maintained at 50°C, for 30 min. The supercritical CO_2 (density about 0.8 g/mL) was delivered by an lsco 260D syringe pump at a pressure of 23000 kPa [8]. A stainless steel capillary back-pressure restrictor maintained the flow rate of CO_2 at 1.5 mL/min. It was introduced in both a "T' connector and a stainless steel tube (0.51 mm i.d.), and the three elements were heated to 70°C in a separate oven. The "T" connector received also a mixture of MeOH/H_2O 10/90 delivered by a 9001 pump (Varian, Les Ulis, France) al the flow rate of 0.3 mL/min. CO_2 and solvent mixture were passed through a MCH-10 C_{18} HPLC column (3 cm by 4 mm i.d., Varian) fixed on a ten-way valve (Selec-CIL, Varian), and then discarded to waste.

A three-way Rheodyne valve allowed elution of analytes to the analytial column. Analysis was achieved on an ODS-80TM column (25 cm by 4.6 mm i.d., Varian) with a Varian 9010 pump delivering a solvent system composed of acetonjtri1e and water, each acidified with 0.05% H_3PO_4. The solvent system began with 1% acetonitrile for 3 min, then followed by a linear increase to 100% acetonitrile over 15 min. a stationary phase of 10 min and finally a return to the initial composition. Total duration or the solvent program was 40 min. The A220 of the column eluate was monitored with a UV-Vis detector (Varian 9050), whereas the radioactivity was followed by a Berthold HPLC LB 507 A radioactivity system (EG&G, Evry, France).

Samples

For preliminary experiments, extraction cells were packed with an inert matrix (hyflo-supercel, 700 mg) or 1-mm sieved loamy sand soil (2.5 g equivalent dry soil packed between two layers of hyflo-

supercel). The matrices were spiked with atrazine (l.5 µg, 3.0 kBq) or metabolites (0.75 µg, 1.5 kBq) dissolved in 10 µL acetone. The solvent was then allowed to evaporate. Soil samples were moistened with 200 µL water prior to extraction.
For application to incubated soils, 2.5 g equivalent dry soil samples (loamy sand) were brought to 60% of moisture-holding capacity by adding water and treated with atrazine (l.5µg, 3.0 kBq, 0.6 ppm). Samples were incubated for two weeks at 25 °C in darkness with $^{14}CO_2$ monitoring. For extraction, the cells were loaded with soil samples between two layers of hyflo-supercel.

Operating Conditions

For sample extraction, the ten-way valve was set in enrichment position to load MCH column A (L_A). The stream of CO_2 was continuously mixed with MeOH/H_2O and solutes were collected onto the MCH-10 column A. After the extraction period (30 min), the outlet valve (E) of the extractor was closed and the solvent mixture rinsed the system for 5 min in order to remove the CO_2 from column A. A start impulse rotated the ten-way valve (K) to backflush position and started the elution of the analytes to the analytical column (Q) by the 9010 pump. At the same time, the MCH-10 column B (L_B) was consequently placed in enrichment and a new sample (loaded during the remaining 5 min) could then he submitted to extraction. During all operations, the analytical column was kept under constant pressure because of the three-way valve (N) which prevented any back low of the solvent mixture into the ten-way valve.

All experiments were performed in triplicate and the mean recoveries were taken. The relative standard deviation was less than 10%.

Results and Discussion

Preliminary experiments were carried out with triazine compounds applied alone on both an inert matrix and soil in order to 1) optimize extraction parameters, and to 2) determine the efficiency of solute trapping on the MCH columns.
First, the capillary restrictor was disconnected from the "T" connector. Extracted triazines were collected by CO_2 bubbling in a MeOH-containing vial and quantified by liquid scintillation counting (LSC). ln the case of hyflo-supercel, all chlorinated compounds (atrazine, deethyl-, and deisopropylatrazine) were extracted with efficiencies greater than 99.5% after 15 min by supercritical CO_2 at 50°C and 23000 kPa. Conversely, hydroxyatrazine was not extracted in similar conditions, nor in the presence of polar modifier (l00 µL MeOH) injected into the cartridge. Hydroxyatrazine was not considered for following assays, because this compound was only poorly

extracted after long-time periods by varying extraction parameters. In soil, atrazinc and deethylatrazine were also totally extracted after 30 min. In contrast, the extraction efficiency was lower for deisopropylatrazine (90.6%). The atrazine concentration in soil was 0.6 ppm and corresponded co levels encountered in agricultural practice. The concentration level for degradation products (deedthyl- and deisopropylatrazine) was half as high (0.3 ppm).

Secondly, the capillary restrictor was connected into the "T' connector, whereas the analytical column was disconnected, in order to assay the efficiency of triazine trapping on the MCH column. The extracted labeled compounds were eluted from the MCH column by pure MeOH, and collected at the outlet of the three-way valve for LSC of the radioactivity. Trapping of each chlorinated triazine was complete in our system whatever the matrix loaded in the extraction cell.

The on-line SFE/HPLC system was also used for extraction and analysis or soil samples incubated with atrazine for two weeks in order to demonstrate its; practical capability for analysis of soil contaminated for a long period. In these experiments, atrazine mineralization (calculated from total CO_2 evolved from the incubations) was less than 2%. ^{14}C extracted by SFE was 90.7% of the initial radioactivity in the samples. Figure 2 shows radio- and UV chromatograms of supercritical extracts obtained with our SFE/HPLC system from 2-week incubations. Only deethylatrazine was formed from atrazine in our soil incubations (Figure 2A). It has heen established elsewhere that the introduction of CO_2 results jn bad chromatographic performance l5J. This was only the case with UV absorbance during the first 5 min of analysis corresponding to the equilibrium time of the analytical column (Figure 28). Then, the cell of the radioactivity detector led to a back pressure at the UV detector and prevented bubble formation responsible for excessive peak broadening. ll is also noteworthy that most compounds interfering with UV detection were eluted after the parent compound atrazine. In fact, under our chromatographic conditions, the retention times of the main known transformation products of atrazine in soils ranged from 11 to 15.0 min. The UV signal obtained for triazines was strong because of the complete transfer of the analytes onto the analytical column, and because of their high UV absorbance.

Conclusion

The on-line SFE/HPLC system presented in this paper allows extraction and analysis of labeled triazine compounds in soil samples in minimum time with minimum sample preparation, It is thus useful for laboratory experiments with radiochemicals. Nevertheless, both complete automation of the system and additional data including detection limits and influence of UV-interfering compounds are required for routine analysis of unlabeled pesticide residues in soils.

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Figure 1. Schematic diagram of on-line super critical-fluid extraction/high performance liquid chromatography system: (A) CO_2 tank; (B) supercritical fluid pump; (C) extractor; (D) extraction cell; (E) outlet valve; (F) oven; (G) capillary restrictor; (H) "T' connector; (I) stainless steel tube; (J) 9001 HPLC pump; (K) ten-way valve; (L_A) MCH column A; (L_B) MCH column B; (M) 9010 HPLC pump; (N) three-way valve; (O) analytical column; (P) UV detector; (Q) radioactivity detector; (R) waste. The ten-way valve is set in enrichment position of MCH column A and the three-way valve allows elution of column B.

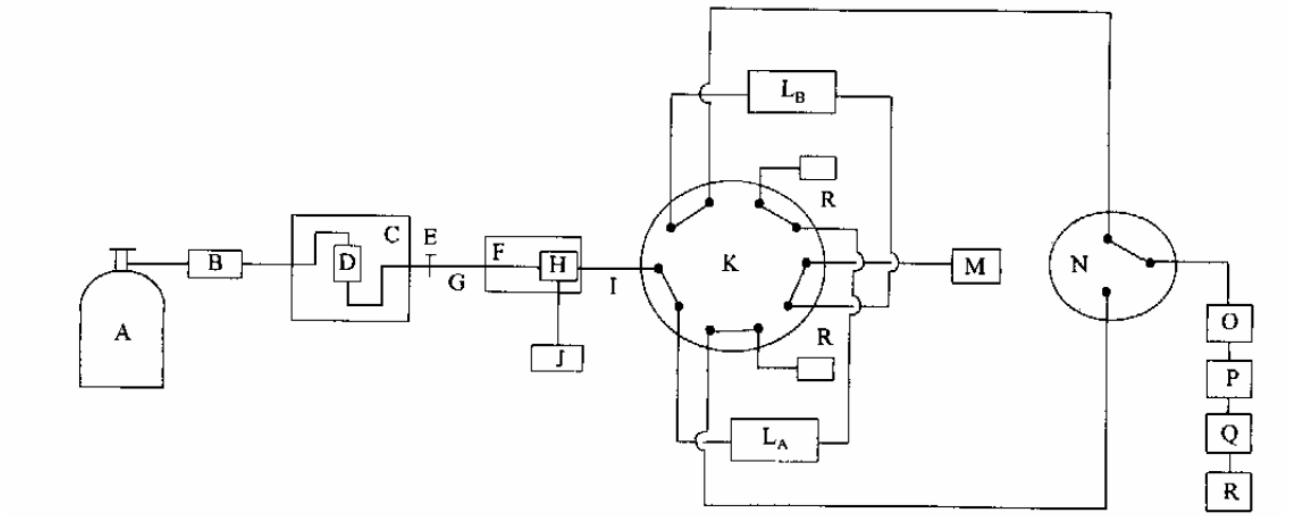

Figure 2. Radiochromatogram (A) and UV chromatogram (B) of triazine compounds obtained with the SFE/HPLC system from incubated soil.