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Determination of a metabolite of the herbicide pyridate in drinking and groundwater using high-performance liquid chromatography with amperometric detection

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ABSTRACT

The main metabolite of the herbicide pyridate is 3-phenyl-4-hydroxy-6-chlorpyridazine (CL9673). A high-performance liquid chromatographic method with amperometric detection is described for determining CL9673 at residue levels in water samples. Sample preconcentration is performed by passage through a C_{18} extraction cartridge. A recovery study using tap water samples spiked with CL9673 at a concentration of 0.1 μg/l showed a recovery of 84.8% (coefficient of variation 6.2%). The method is suitable for the determination of CL9673 in drinking and groundwater.

INTRODUCTION

In the case of residue analysis of the herbicide pyridate {*O*[3-phenyl-6-chlorpyridazinyl-(4)]-S(*n*-octyl)-thiocarbonate}, which is used in the agricultural management of cereals, maize and rape, attention has primarily focused on the determination of its metabolite 3-phenyl-4-hydroxy-6-chlorpyridazine (CL9673, Fig. 1). CL9673 is physiologically active in plants and is the first metabolite of pyridate, which acts only as a carrier [1]. The occurrence of residues of pyridate and CL9673 are to be expected in treated plants and have previously been investigated in detail [1–5]. Leaching of CL9673 residues from topsoils may pollute groundwater. Until now, the determination of trace amounts of CL9673 in water samples has not been reported.

Analytical methods for the determination of pyridate residues use high-performance liquid chromatography (HPLC), gas chromatography (GC) and tandem mass spectrometry (MS–MS). Two-dimendional HPLC with UV detection has been applied to the determination of CL9673 in plant matrices [1,2]. Owing to its low volatility and low thermal stability, CL9673 cannot be determined by GC without derivatization. Pentafluorbenzylchloride has been used to form stable and volatile derivatives of CL9673 [3,4]. Jaklin *et al.* [5] have reported the use of MS–MS with direct probe insertion to determine CL9673 in crude plant extracts without derivatization. Buchberger *et al.* [6] have used HPLC with both UV and electrochemical detection to determine the phenolic metabolites bromoxynil and ioxynil of a herbicide formulation containing bromoxyniloctanoate, ioxyniloctanoate and pyridate. Al-

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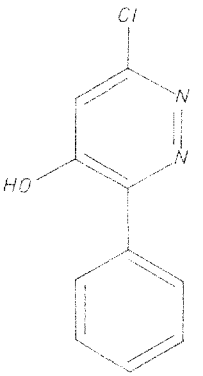


Fig. 1. Structure of 3-phenyl-4-hydroxy-6-chlorpyridazine (CL9673)

though this work focuses on sample preparation for the simultaneous determination of the metabolites of this herbicide formulation, it is not clear why CL9673 was excluded from these investigations.

Several recent papers have described the electrochemical behaviour of phenolic compounds [7–10]. The chemical structure of CL9673 encouraged these authors to investigate the application of amperometric detection for the determination of CL9673.

This paper describes a procedure for the determination of CL9673 in drinking and groundwater using solid-phase extraction and reversed-phase HPLC with amperometric detection.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was a HP1090, Series M (Hewlett-Packard, Avondale, PA, USA) system fitted with a reversed-phase 5 μm ODS Hypersil column (100 × 2 mm I.D.). Elution of the solutes was monitored with a programmable electrochemical detector (Model HP1049A, Hewlett-Packard) in amperometric mode. The detector was equipped with a glassy carbon working electrode and a solid state Ag/AgCl reference electrode. The working electrode was set at + 1.25V.

Data integration was carried out using a series 300 HP 9000 computer (Hewlett-Packard).

Reagents

CL9673 was obtained from Chemie Linz (Linz, Austria). Working standard solutions of CL9673 of various concentrations were obtained by diluting a standard stock solution (1 mg/ml in methanol) with HPLC-grade water. The concentrations used were 0.4, 0.2, 0.04, 0.02 and 0.004 μg/ml. The working standard solutions were freshly prepared every 2 weeks.

Analytical-reagent grade acetic acid, acetone and sodium chloride were purchased from Merck (Darmstadt, Germany). HPLC-grade water and methanol used

for the preparation of the mobile phase were purchased from Promochem (Wesel, Germany) and J. T. Baker (Phillipsburg, NJ, USA), respectively. Bond-Elut C_{18} solid-phase extraction cartridges containing 1 g of sorbent were purchased from Analytichem International (Harbor City, CA, USA). The cartridges were activated by washing with methanol (5 ml) followed by water (5 ml) prior to use.

Millex-SLCR004NB 0.5-μm membrane filters were purchased from Millipore (Bedford, MA, USA).

Chromatographic conditions

Separations were effected under isocratic conditions at 40°C using a pre-mixed mobile phase of methanol–water (15:85) containing 3.5 m*M* sodium chloride and 0.3% acetic acid at a flow-rate of 0.5 ml/min. The mobile phase was continously degassed by purging with helium.

Sample preparation

Water samples (300 ml) were adjusted to pH 2 with 6 *M* hydrochloric acid. For dynamic solvatisation of the sorbent material, methanol (5 ml) was added and the sample applied to an activated Bond-Elut C_{18} cartridge under suction at a flow-rate of about 10 ml/min. The extraction column was allowed to dry prior to elution with acetone (6 ml). The eluate was concentrated to approximately 0.2 ml under a gentle stream of nitrogen. The sample was diluted with water to 0.5 ml. The exact volume was measured with a microlitre syringe and filtered through a 0.5-μm membrane filter. Sample aliquots (25 μl) were injected into the HPLC system.

RESULTS AND DISCUSSION

A chromatogram of Vienna tap water fortified with 0.1 μg/l CL9673 is shown in Fig. 2. CL9673 was eluted from the column with a retention time of 8.05 min. A sufficient separation of the peak from other constituents present in the water sample

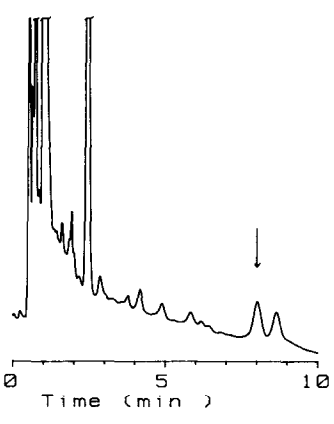
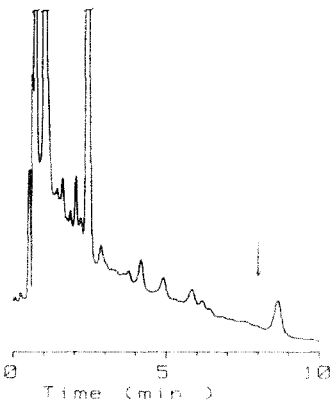


Fig. 2. HPLC chromatogram of Vienna tap water fortified with 0.1 μg/l CL9673. Arrow indicates retention time of CL9673.



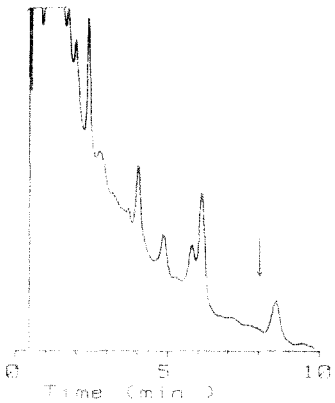


Fig. 3. HPLC chromatogram of Vienna tap water (blank). Arrow indicates retention time of CL9673.

Fig. 4. HPLC chromatogram of an untreated water sample (blank) from a well near Vienna. Arrow indicates retention time of CL9673.

was obtained. As an example, a chromatogram of Vienna tap water (blank) is shown in Fig. 3 and the chromatogram of an untreated groundwater sample (blank) obtained from a well near Vienna is presented in Fig. 4.

The effect of the applied potential on the peak area of CL9673 was determined by changing the potential from +1.0 V to +1.4 V. The relationship between the potential of the working electrode and the recorder response is shown in Fig. 5. An applied potential of +1.25 V was considered to be optimum as a lower potential resulted in a considerable loss of sensitivity and a higher potential resulted in unacceptable background noise. The detection limit was 0.1 ng per injected volume at a

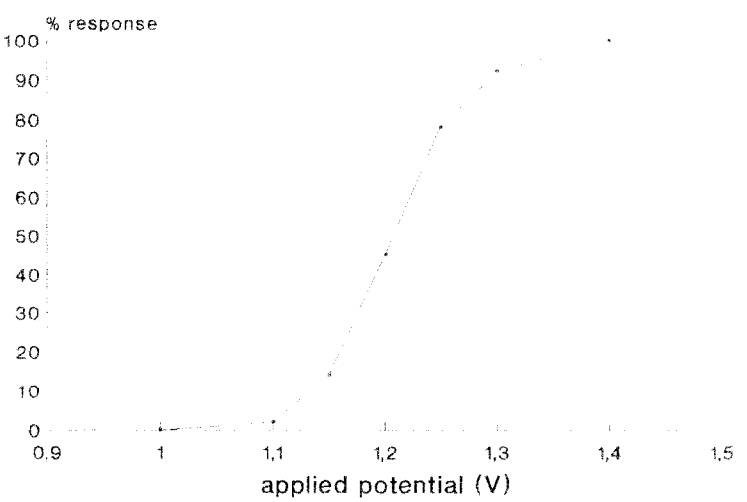


Fig. 5. Effect of applied potential on the response of CL9673.

signal-to-noise ratio of 5. The linearity of the detector response was obtained on the injection of 0.1–10 ng of CL9673 (coefficient of correlation 0.9992).

A recovery study using six tap water samples spiked with CL9673 at a level of 0.1 μg/l showed a recovery of 84.8% with a coefficient of variation of 6.2%. The results were calculated via peak areas using an external calibration graph.

From the results obtained it can be concluded that the proposed method is suitable for the determination of trace amounts of CL9673 in drinking and groundwater.

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