Journal of Chromatography, 447 (1988) 426–431
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 621

Note

Liquid chromatographic method for the determination of cyanazine in the presence of some normal soil constituents

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(Received April 26th, 1988)

Cyanazine is the ISO common name for 2-chloro-4-(1-cyano-1-methylethylamino)-6-ethylamino-1,3,5-triazine. It is an especially active herbicide, from the triazine group, with pre- and post-emergence activity by photosynthesis inhibition. It is soluble in water at 171 ppm and Sirons *et al.*[1] found significant quantities of it and its metabolic amide, in soils, 12 months after application.

Because of the possible toxicity of cyanazine for man, through contaminated plants and waters, it was decided to study the adsorption–desorption process of cyanazine on soil and its constituents. For this purpose it was necessary to develop an analytical method for the determination of cyanazine in the presence of soil constituents. This study was mainly done on peat since this is the soil constituent that releases the greatest quantity of interfering substances in the presence of aqueous solutions.

To avoid the partial degradation of cyanazine in gas–liquid chromatography (GLC)[2] an high-performance liquid chromatographic (HPLC) method was developed. A reversed phase was chosen, instead of a normal phase, as recommended[3], to eliminate organic solvents of low polarity which would make difficult the interpretation of the adsorption–desorption process of cyanazine on soil.

EXPERIMENTAL

Apparatus

An high-performance liquid chromatograph Hewlett-Packard 1090, equipped with a 4.5-μl spectrometer cell, HP-85 personal computing system, HP-7470A graphics plotter, HP-ThinkJet printer, HP-9121 discs unit, automatic variable-volume injector, diode-array detector and DPU multichannel integrator, was used. The column (Hewlett-Packard 799160D-552) was 100 mm × 2.1 mm I.D., stainless steel, packed with ODS-Hypersil (5 μm).

The Millex filters (Millipore, Bedford, MA, U.S.A.) used were type HV_4, 4 mm, pore size 0.45 μm.

Soil constituents

Kaolinite from Lage (Spain) and Peat from Padul (Spain) were used.

0021-9673/88/$03.50

Reagents

Methanol, HPLC quality, was obtained from Panreac (Madrid, Spain). Water was purified with a Milli-Q water purification system. The eluent was methanol–water (60:40). Cyanazine, as an analytical standard of known purity, was obtained from Shell Research (Sittingbourne, U.K.).

Calibration solutions. First a solution of a cyanazine standard in water was prepared at $10.1988 \cdot 10^{-2}$ g/l and two more solutions were prepared by dilution in water at 1/2.5 and 1/25.

Sample solutions. Approximately 1.0 g of the soil constituent was weighed (to the nearest 0.1 mg). A 20-ml volume of a cyanazine solution at a concentration within the range $0.4 \cdot 10^{-2}$–$10 \cdot 10^{-2}$ g/l was added and shaken mechanically for X min (the time necessary for the study on adsorption–desorption). The solution was then centrifuged at 12 062 *g* for 20 min and an aliquot of the supernatant was filtered through a Millex HV_4 filter into a small vial fitted with a cap.

Chromatography

The chromatographic conditions were as follows: flow-rate, 0.2 ml/min; column temperature, 40°C; wavelength readings at 219 ± 2 nm *vs.* 350 ± 25 nm, and simultaneously at 225 ± 2 nm *vs.* 350 ± 25 nm; range, automatic; injection volume, 2 μl; spectra from the peak, upslope, apex and downslope; stop time, 2.75 min.

Calibration graph

The calibration graph, see Fig. 1, was constructed with a computer software, by the quadratic method, from triplicate injections of the three calibration solutions. Taking into account the low solubility of cyanazine in water (171 ppm), a wider range of concentrations is not feasible.

Quantitation

Triplicate injections of each sample solution were made and the results directly obtained in $g/l \cdot 10^{-2}$.

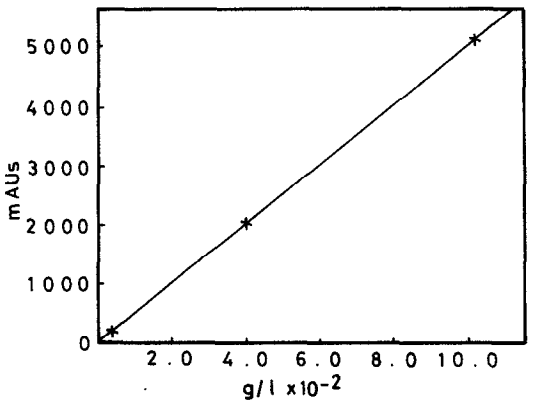

Fig. 1. Calibration graph for cyanazine.

RESULTS AND DISCUSSION

The linear calibration graph (Fig. 1) shows that Beer's law is followed at the tested concentrations.

Fig. 2 shows the chromatography of (a) a kaolinite–cyanazine sample and of (b) a peat–cyanazine sample. In both cases the separation of the cyanazine from impurities seems to be adequate. Fig. 3 shows the chromatograms in Fig. 2a and b plotted in different ways.

Fig. 4 shows the signal plus spectra plot of the chromatogram in Fig. 2b, and indicates the purity of the chromatographic peaks eluted. For this last purpose, the detector performs three scannings at three points (times) of every chromatographic peak: prior to at and after every maximum by reference to the base; (a), (b), and (c) show these spectrochromatograms separately and (d) all three overlaid. The purity of the cyanazine peak is indicated by the similarity of the three spectra which show a maximum at 219 nm and strong absorbance at 225 nm. These are the two wavelengths chosen for simultaneous integration. The ratio of the two signals is shown in Fig. 5 for the chromatogram in Fig. 2b between 0.5 and 2.4 min. The linear plot of the signal ratio, A/B, for the cyanazine peak, is a second demonstration of its purity.

The advantages of this kind of detectors have been pointed out by Lázaro *et al.*[4].

The standard addition method was used to test the accuracy of the method. Four aliquots of a peat–cyanazine supernatant, prepared according to *Sample solutions*, were spiked with cyanazine. Details are given in Table I. Recoveries ranged from 95.14 to 105.17%, with a relative standard deviation of 0.19–2.96.

The relative standard deviation for seven repeated injections of a cyanazine sample at $2.53 \cdot 10^{-2}$ g/l was $S_r = 0.55$.

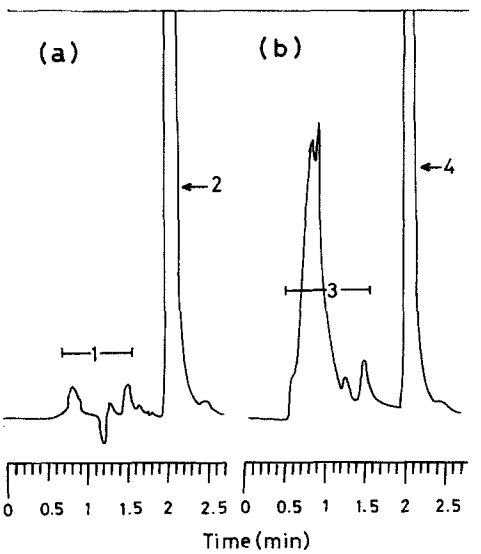

Fig. 2. Chromatography of (a) a kaolinite–cyanazine sample and (b) a peat–cyanazine sample. Peaks: 1 = kaolinite components; 2, 4 = cyanazine; 3 = peat components.

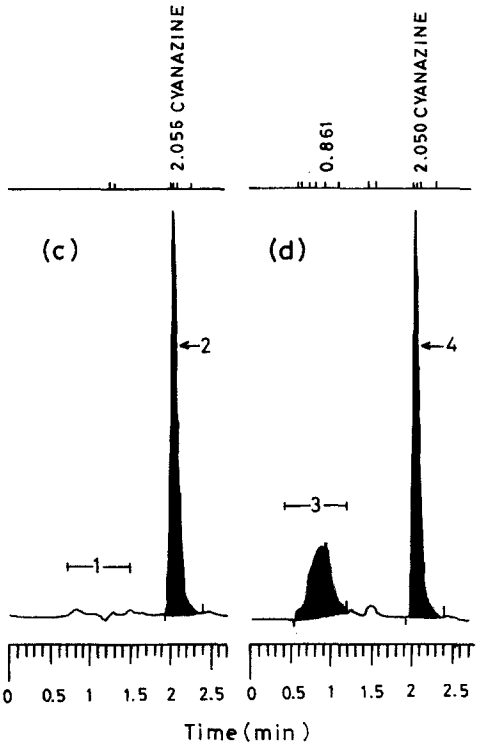

Fig. 3. Replots (c) and (d) of the chromatograms in Fig. 2a and b, respectively.

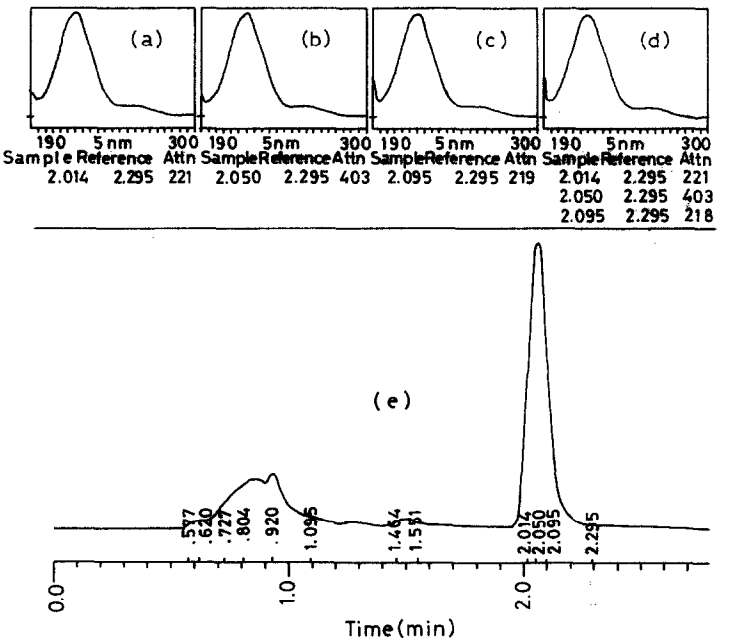

Fig. 4. Signal plus spectra plots of the chromatogram in Fig. 2b. (e) Chromatographic signal; (a), (b) and (c) spectrochromatograms of the cyanazine peak, prior to, at and after its absorption maximum; (d) the three previous spectrochromatograms overlaid.

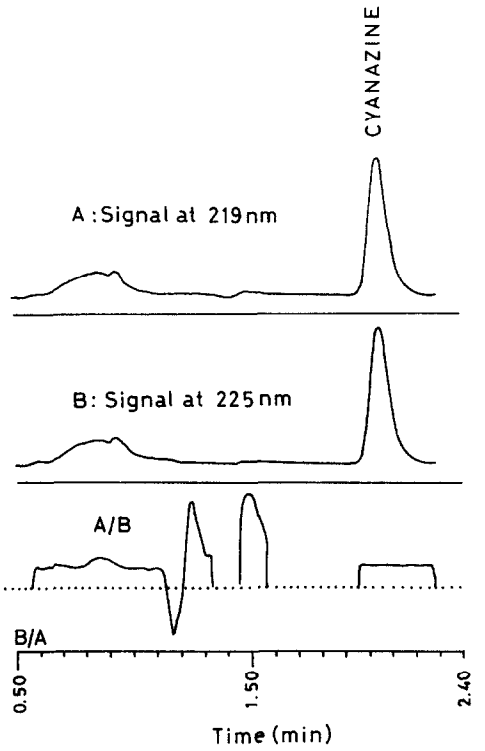

Fig. 5. Ratio of signals in the chromatogram in Fig. 2b between 0.5 and 2.4 min.

The detection limit was 0.253 ng of cyanazine, equivalent to 2 μl of a solution of this chemical at a concentration of $1.265 \cdot 10^{-4}$ g/l.

The method described is specific, accurate and precise and it presents a detection limit comparable to that of the Zweig method[5] using GLC with electron-capture and alkali flame ionization detection.

Other advantages of this method due to the use of a microbore column, a

TABLE I

RECOVERY TEST FOR CYANAZINE

C.L. = confidence limit (P = 0.05).

Cynazine added (ng/2 µl)	*Cyanazine found (ng/2 µl ± C.L.)*	*Recovery (%)*	S_r*
37.48	35.66 ± 1.49	95.14	1.29
74.96	74.32 ± 6.70	99.15	2.96
112.44	118.25 ± 0.69	105.17	0.19
149.92	151.80 ± 2.32	101.25	0.50

* Relative standard deviation for three determinations.

diode-array detector and a multichannel integrator are: an enormous saving in operating costs, valuable information on the integration process and different tests of the purity or otherwise of every chromatographic peak.

In view of the limited solubility of cyanazine in water, 171 ppm, the concentration range studied, $(0.4–10.20) \cdot 10^{-2}$ g/l, is the most suitable for adsorption–desorption studies of cyanazine on soil and soil constituents.

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