Journal of Chromatography, 479 (1989) 424–429
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 728

Note

Determination of ethirimol, in the presence of some normal soil constituents, by liquid chromatography

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(First received April 10th, 1989; revised manuscript received June 22nd, 1989)

Ethirimol is the ISO common name for 5-butyl-2-ethylamino-6-methylpyrimidin-4-ol. It is a systemic fungicide, effective in controlling mildew in cereals and grasses. Its solubility in water is about 200 ppm.

Ethirimol interactions[1], degradation[2] and distribution[3] in soil have already been studied. Nevertheless, it is interesting to know the adsorption–desorption mechanism of this fungicide on kaolinite, montmorillonite and peat in order to be able to predict its behaviour in different soils and in the environment.

Determinations of ethirimol have been done by gas–liquid chromatography (GLC) of a methyl derivative using a nitrogen-selective thermoionic detector[4] but the method is time consuming and not appropriate for measuring in aqueous phases. Ethirimol has also been determined by liquid scintillation counting [1,2] and direct UV analysis[1] but the isotopic technique does not distinguish between entire and degraded molecules and many substances interfere in the direct UV analysis.

A method is presented in this paper that avoids all those inconveniences.

EXPERIMENTAL

Apparatus

An Hewlett-Packard 1090 liquid chromatograph, equipped with a diode array detector and DPU multichannel integrator, as described in a previous paper[5] 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), montmorillonite from Almería (Spain) and peat from Padul (Spain) were used.

Reagents

Methanol, high-performance liquid chromatography (HPLC) quality, was obtained from Panreac (Madrid, Spain). Water was purified with a Milli-Q water purification system. The eluent was methanol–water (80:20). Ethirimol, as an analytical standard of known purity (98.4%), was obtained from ICI (Yalding, U.K.).

0021-9673/89/$03.50

Calibration solutions

First a solution of an ethirimol standard in water was prepared at $7.26 \cdot 10^{-2}$ g/l and four more solutions were prepared by dilution in water at $4.356 \cdot 10^{-2}$, $1.452 \cdot 10^{-2}$, $0.8712 \cdot 10^{-2}$ and $0.2904 \cdot 10^{-2}$ g/l.

Sample solutions

Approximately 1.0 g of the soil constituent was weighed (to the nearest 0.1 mg). A 20-ml volume of an ethirimol solution at a concentration within the range $0.2904 \cdot 10^{-2}$–$7.26 \cdot 10^{-2}$ 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 225 ± 2 nm *vs.* 450 ± 25 nm, and simultaneously at 297 ± 2 nm *vs.* 450 ± 25 nm; range, automatic; injection volume, 2 µl; spectra from the peak, upslope, apex and downslope; stop time, 2.1 min.

Calibration graph

The calibration graph, see Fig. 1, was constructed with computer software, by the quadratic method, from triplicate injections of the five calibration solutions. Taking into account the low solubility of ethirimol in water (200 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 · 10^{-2}.

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–ethirimol sample, of (b) a

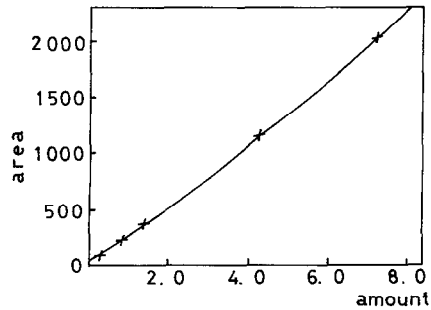

Fig. 1. Calibration graph for ethirimol.

montmorillonite-ethirimol sample and of (c) a peat–ethirimol sample in real time. In the three cases the separation of ethirimol from impurities seems to be adequate, taking into account not only that no peak was observed at the ethirimol retention time when no spiked samples of kaolinite, montmorillonite and peat were chromatographed under the same conditions, but also the purity of the ethirimol peak as will be demonstrated below.

Fig. 3 shows the replots of the previous chromatograms (a), (b) and (c), with special annotation: (d), (e) and (f), where baselines, retention times, tick marks and hatched shadings of integrated areas are shown. This is an adventage of the chromatograph used and reveals just how correct the integration process is. On the other hand, it shows how the information obtained in a chromatographic development can be used for further calculations and/or representations without repeating the chromatographic steps.

Fig. 4, the signal plus spectra plot of the same chromatogram (c, Fig. 1), shows the purity of the chromatographic peak. To do this, the detector performs three scans at three points (times) in every chromatographic peak: prior to, at and after every maximum. These spectrochromatograms are shown separately (1–3) and overlaid (4) (upper left, Fig. 4). If the three spectra are similar, the peak corresponds to a pure substance, in this case, ethirimol. This demonstration of the purity of a chromatographic peak is possible only with the use of a diode array detector. The identical shape of the spectrum for the analytical standard and an unknown sample is a confirmatory test of identity.

The ethirimol spectrum shows a maximum absorbance at 225 nm and another at 297 nm which are the two wavelengths chosen for simultaneous integration. Fig. 5 shows the ratio of the signals obtained at these two wavelenghts *vs.* time for the ethirimol peak shown in chromatogram (c), Fig. 2. The linear relationship is a second demonstration of peak purity and is another advantage of the diode array detector and the multichannel integrator.

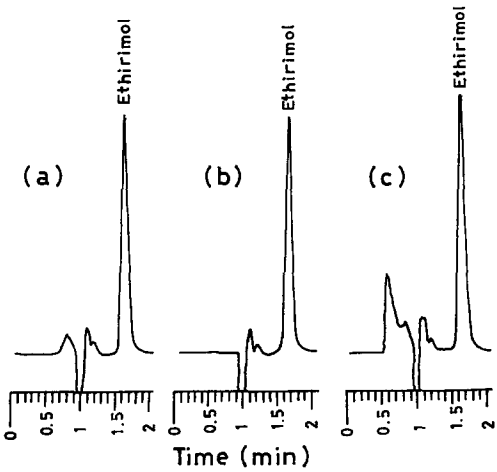

Fig. 2. Chromatography of (a) a kaolinite–ethirimol sample, (b) a montmorillonite–ethirimol sample and (c) a peat–ethirimol sample in real time.

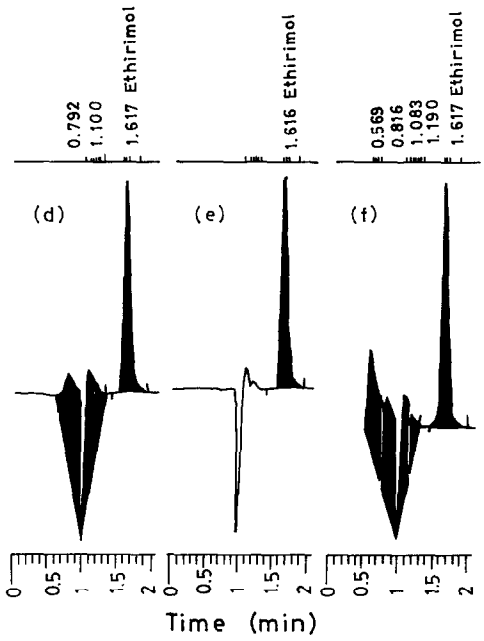

Fig. 3. Replots (d), (e) and (f) of the chromatograms in Fig. 2a, b and c, respectively.

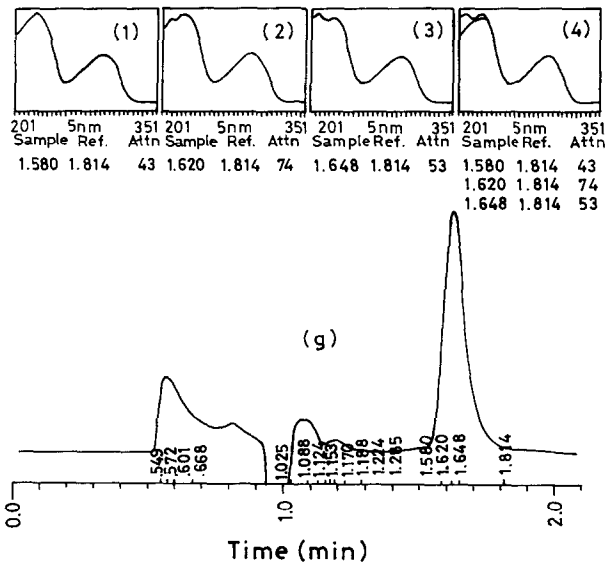

Fig. 4. Signal plus spectra plot of the chromatogram in Fig. 2c. (g) chromatographic signal; (1), (2) and (3) spectrochromatograms of the ethirimol peak, prior to, at and after its absorption maximum; (4) the three previous spectrochromatograms overlaid.

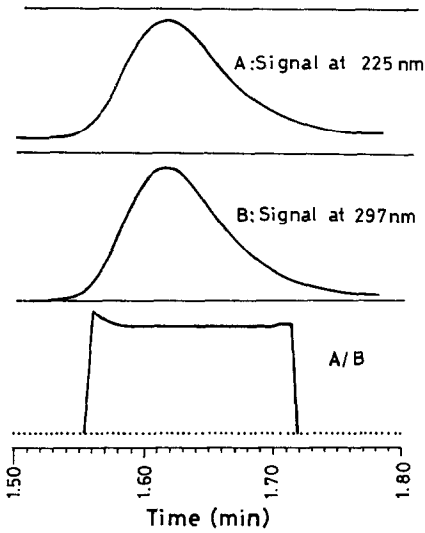

Fig. 5. Ratio of signals in the chromatogram in Fig. 2c between 1.5 and 1.8 min.

The standard addition technique was used to test the ability of the HPLC system to accurately determine added ethirimol in a peat–ethirimol supernatant. Five 2-ml aliquots of a peat–ethirimol supernatant at a concentration of $5.5185 \cdot 10^{-2}$ g/l were added with 0, 1, 2, 3 and 4 ml of an ethirimol solution at a concentration of $11.037 \cdot 10^{-2}$ g/l and 4, 3, 2, 1 and 0 ml of water respectively. The detector response to ethirimol in the presence of coextracted constituents of the peat soil ranged from 97.68 to 104.35% of theoretical. Details are given in Table I. A peat–ethirimol sample was chosen for this experiment because peat extracts are dirtier than those of kaolinite and montmorillonite, as is seen in Fig. 2.

The relative standard deviations for nine repeated injections of two ethirimol samples at $1.96 \cdot 10^{-2}$ and $7.42 \cdot 10^{-2}$ g/l were respectively $S_r = 3.28$ and 0.40.

The detection limit was 0.4 ng of ethirimol, equivalent to 2 μl of a solution at a concentration of $0.02 \cdot 10^{-2}$ g/l.

TABLE I

RECOVERY FOR ETHIRIMOL

C.L. = Confidence limit ($P = 0.05$).

Ethirimol added (ng/2 µl)	*Ethirimol found (ng/2 µl ± C.L.)*	*Response (%)*	S_r[a]
36.79	38.39 ± 2.01	104.35	1.22
73.58	74.55 ± 2.72	101.32	0.85
110.37	110.04 ± 2.49	99.70	0.53
147.16	143.75 ± 2.89	97.68	0.47

[a] Relative standard deviation for three determinations.

The method described is specific, accurate and precise and it presents a detection limit comparable to that of the Edwards method[4] using GLC with nitrogen-selective thermoionic detection. Other advantages of this method, due to the use of a microbore column, a 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 ethirimol in water, 200 ppm, the concentration range studied, $(0.2904–7.26) \cdot 10^{-2}$ g/l, is the most suitable for adsorption–desorption studies of ethirimol on soil and soil constituents.

ACKNOWLEDGEMENTS

We gratefully acknowledge support for this work from grant PR84-0160-C04-02 from the Comision Asesora de Investigación Cientifica y Técnica (CAICYT). The valuable technical assistance of Mª.D. Maroto is gratefully acknowledged.

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