Journal of Chromatography, 318 (1985) 144–148
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,210

Note

Reversed-phase high-performance liquid chromatographic method for the simultaneous microquantitative determination of the herbicide isoproturon and its possible degradation products in soil*

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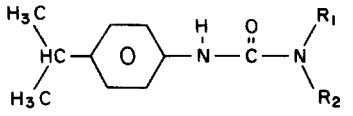
(First received June 25th, 1984; revised manuscript received September 4th, 1984)

Isoproturon [N-(4-isopropylphenyl)-N′,N′-dimethylurea] (I) (Fig. 1) is an effective herbicide for the control of weeds in wheat (*Triticum aestivum* L.) crops[1]. The pre- and post-emergence application of the herbicide is directed to the ground, where it undergoes breakdown[2]. The degradation pattern of isoproturon has not been studied, but it has been established that diuron, another urea herbicide, degrades in the soil by successive demethylation[3]. A similar mode of degradation of isoproturon would give monomethylisoproturon [N-(4-isopropylphenyl)-N′-methylurea] (II) and didesmethylisoproturon [N-(4-isopropylphenyl)urea] (III) as possible metabolites in soil. It was deemed essential to have a convenient method for the simultaneous determination of trace levels of isoproturon (I) and its possible degradation products (II and III) to support reaearch on this aspect.

Literature on the analysis of isoproturon by any method is scarce. Earlier work on residue analysis relied on alkaline hydrolysis of isoproturon to the corresponding aniline, followed by diazotization and reaction with an aromatic amine to form a coloured complex[2,4]. These procedures were time consuming and did not lend themselves to batch analysis. Further, this type of method suffers from the disadvantage that it cannot determine individual residues of the herbicide or its metabolites, but rather the total resicues. In general, compounds belonging to this group of herbicides are thermally unstable and hence their direct analysis by gas–liquid chromatography (GLC) is not feasible[5]. High-performance liquid chromatography (HPLC) has



I , Isoproturon ; $R_1 = R_2 = CH_3$

II , Monomethyl isoproturon ; $R_1 = H$, $R_2 = CH_3$

III , Didesmethyl isoproturon ; $R_1 = R_2 = H$

Fig. 1. Structures of isoproturon and its two possible degradation products.

* Contribution No. 252 from the Division of Agricultural Chemicals, IARI, New Delhi-12, India.

0021-9673/85/$03.30

emerged as an important method for the analysis of pesticides. We report here a convenient and sensitive method for the determination of isoproturon and its possible metabolic products by reversed-phase HPLC. These determinations are useful for studying the persistence of isoproturon and its degradation products in soil.

EXPERIMENTAL

Apparatus

A Spectra Physics (San Jose, CA, U.S.A.) HPLC instrument (Model SP 8000B) equipped with a pump, a UV–visible variable-wavelength detector (4000 B) connected to a loop-injection system and a printer–plotter type recorder was used.

Chromatographic conditions

The stationary phase consisted of LiChrosorb RP-8 packed in a stainless-steel column (25 cm × 4.6 cm I.D.). The mobile phase was acetonitrile–water (1:1) maintained at a flow-rate of 1.5 ml min^{-1}. The UV detector was operated at a wavelength of 240 nm at a sensitivity of 0.04 a.u.f.s. A 10-μl volume of sample was injected each time and chromatograms were recorded on a dual-channel printer split-chart recorder by thermal printing at a chart speed of 1 cm min^{-1}. The instrument had a microprocessor-controlled data system that allowed the automatic calculation of the detector response in terms of peak area. One unit of area was equal to 1 μV sec of analogue input.

Reagents

Isoproturon was obtained from Hoechst (New Delhi, India) and monomethylisoproturon and didesmethylisoproturon from Gharda Chemicals (Dombivilly, Thane, India). All three compounds were recrystallized from benzene to give products with melting points of 150–151°C (I), 120°C (II) and 150°C (III).

Doubly distilled water and distilled analytical-reagent grade acetonitrile and methanol were used. Prior to use, all the solvents were degassed and filtered through Whatman No. 42 filter-paper.

Procedure

Solutions of individual compounds were prepared in acetonitrile–water (1:1). A mixture of the three compounds was first injected and chromatographed at a flow-rate of 1.5 ml min^{-1}, followed by injection of individual compounds for authenticity.

The method was quantitatively validated by separately running solutions of 1, 2, 5, 10 and 25 ppm of I, II and III. Each run was duplicated and the detector response was measured in terms of peak area. Calibration graphs for all three compounds were prepared.

The soil was a fine loam with a composition of 19.0% clay, 21% silt, 60% sand and 0.35% organic carbon and had a pH of 7.2.

A 10-g amount of soil was treated with 1000 μg (or 40 μg) of I, II or III and kept overnight. The moist soil was then extracted with 100 ml of methanol by shaking on a wrist-action shaker for 1 h and then filtered. A 25-ml volume of this extract, corresponding to 2.5 g of soil, was evaporated to dryness and the residue dissolved in 10 ml acetonitrile–water (1:1). A 10-μl volume of this sample was injected and

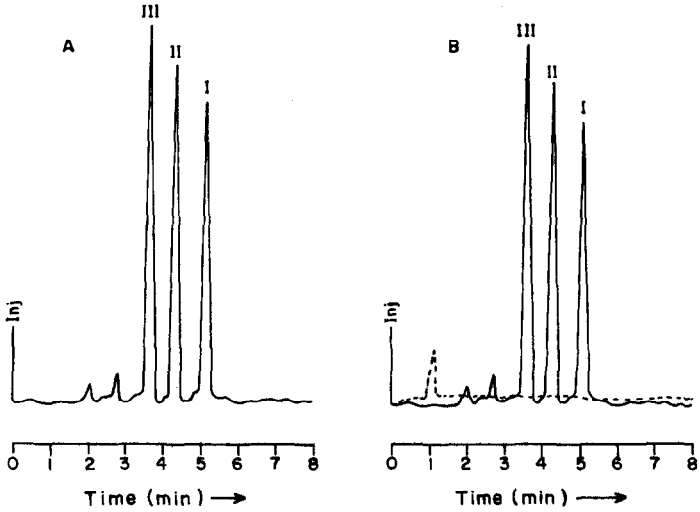


Fig. 2. Liquid chromatograms of a mixture of I, II and III (5 ppm of each) using a LiChrosorb RP-8 column, acetonitrile–water (1:1) as the mobile phase at a flow-rate of 1.5 ml/min and UV detection at 240 nm (0.04 a.u.f.s.). A, Standard solution; B, extract from treated soil (solid line) and soil blank (broken line).

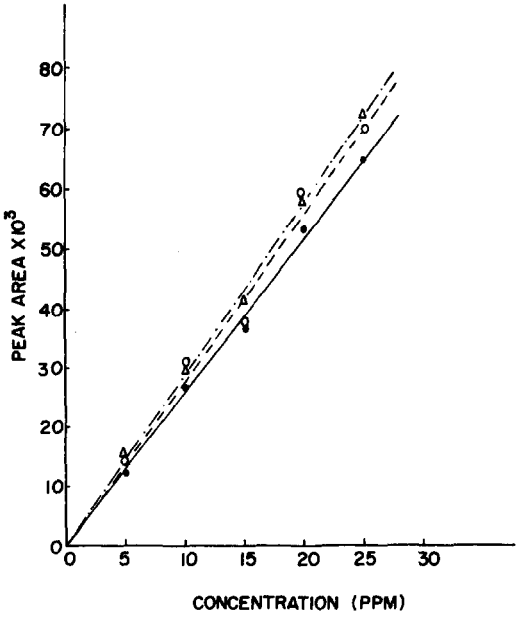


Fig. 3. Calibration graphs for I (●), II (○) and III (△).

chromatographed. This was preceded by the injection of authentic samples of known concentrations of each component (standard). Similarly, soil fortified with a mixture of I, II and III was also extracted and chromatographed.

The concentration of the analyte in the extracted sample of soil was calculated by comparing the peak area of the sample with that of the standard, as follows:

$$C_i = \frac{A_i}{K}$$

where C_i is the concentration of component i, A_i is the area of the peak corresponding to i and K = peak area of the standard / concentration of the standard.

RESULTS AND DISCUSSION

Mobile phases consisting of methanol–water and acetonitrile–water in various proportions were tried, and it was found that acetonitrile–water (1:1) gave the best resolution of the mixture of I, II and III. Under these conditions, the three components eluted at 3.6 (III), 4.3 (II) and 5.1 min (I).

The identity of each component was established by injecting authentic samples of the individual compounds. A chromatogram obtained by injecting a mixture of 10 μl of a standard solution containing 10 ppm each of I, II and III is shown in Fig. 2A. The relative retention times are 1.0 (I), 0.84 (II) and 0.70 (III). It is evident that excellent peak resolution for the three compounds was obtained.

The calibration graphs obtained by plotting concentrations against peak areas were linear over the range 1–25 ppm (Fig. 3) and had correlation coefficients (r) of 0.970, 0.939 and 0.988 for I, II and III, respectively.

After optimizing the HPLC conditions, the method was extended to the analysis of soil. The soil blank did not give any peak interfering with these compounds (Fig. 2B). The results of analyses of the three compounds in soils are given in Table I. The recovery ranged between 100.1 and 103.0%. Five-fold injections of 25 and 1

TABLE I

ACCURACY AND PRECISION OF HPLC DETERMINATION OF ISOPROTURON AND ITS TWO DEGRADATION PRODUCTS

n = 5.

Compound	*Conc. (ppm)*	*Mean result (ppm)*	*Standard deviation (ppm)*	*95% Confidence interval (ppm)*	*Relative mean error (%)*
I	25.00	25.18	0.25	25.18 ± 0.22	0.88
	1.00	1.07	0.10	1.07 ± 0.09	8.56
II	25.00	25.12	0.18	25.12 ± 0.16	0.55
	1.00	1.11	0.11	1.11 ± 0.10	9.17
III	25.00	25.04	0.16	25.04 ± 0.14	0.56
	1.00	1.03	0.07	1.03 ± 0.06	6.26

ppm of I, II and III were used to determine the standard deviation. It was found that duplicate injections of each sample were optimum for operating in a 95% confidence interval to obtain the desired precision. Hence the accuracy and precision of the HPLC method for the residue analysis of I, II and III were adequate for the intended purpose.

The limits of detection were also determined. It was found that all three compounds could be analysed satisfactorily at levels down to 0.5 ppm.

It can be concluded that the described HPLC method is simple and specific for isoproturon and its two possible degradation products. It permits analysis at room temperature, thus avoiding possible thermal degradation under GLC conditions. As the total run time for each sample is less than 6 min, this method is suitable for batch analysis.

ACKNOWLEDGEMENTS

We are grateful to Dr. N. K. Roy, Head, Division of Agricultural Chemicals, IARI, New Delhi, for providing facilities and giving suggestions. We also thank Hoechst, New Delhi, and Gharda Chemicals, Dombivilly, Thane, for providing the herbicide and its metabolites respectively.

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