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Use of Ethylation for the Gas and Liquid Chromatographic Determination of Linuron, Diuron, and Metoxuron and Two of Its Degradation Products: Application to Soil Analysis

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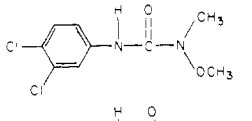
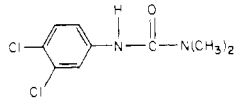
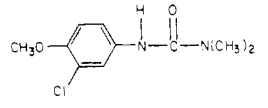
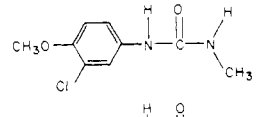
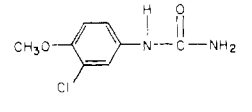
The urea herbicides linuron, diuron, and metoxuron and its metabolites, monomethylmetoxuron and desmethylmetoxuron, were ethylated using ethyl iodide and sodium hydride in dimethyl sulfoxide, with a reaction time of 30 min at room temperature. The products proved to be exceptionally stable and well suited to gas chromatography. The structures were confirmed by gas chromatography–mass spectrometry. Separation of all the test compounds as their ethyl derivatives was achieved by both gas and liquid chromatography. Successful determination of metoxuron and diuron in soil at 1.0 ppm was accomplished by both gas and liquid chromatography. Selective nitrogen–phosphorus detection proved to be superior to electron-capture determination of the two herbicides in soil. Direct liquid chromatographic determination of the two ureas in soil was also possible. The minimum detectable concentrations of metoxuron in the samples was estimated to be less than 50 ppb by gas chromatography with nitrogen–phosphorus detection. The reproducibility of replicate standards carried through the ethylation procedure and analyzed by gas chromatography was 6% relative standard deviation.

The direct gas chromatographic (GC) analysis of many urea herbicides has been reported (Buser and Grolimund, 1974; Katz and Strusz, 1969; McKone, 1969; McKone and Hance, 1968; Spengler and Hamroll, 1970). However, a significant number of these compounds are thermally unstable, decomposing partly to isocyanates and amines (Buchert and Lokke, 1977; Saunders and Vanatta, 1974). The main contributing factor in the decomposition is the N–H moiety of the molecules. Attempts at blocking this group by alkylation (Buchert and Lokke, 1977; Saunders and Vanatta, 1974; Lawrence and Laver, 1975; Tanaka and Wien, 1973), acylation (Saunders and Vanatta, 1974; Ryan and Lawrence, 1977), and silylation (Fishbein and Zielinski, 1965) have been reported. Of these reactions, the alkylated products are the most stable derivatives in the presence of water. The silyl and acyl products tend to hydrolyze back to the original ureas.

Methyl iodide in the presence of sodium hydride has been used successfully for alkylating several urea herbicides for GC determination in a number of foods (Lawrence and Laver, 1975). However, such a derivatization technique cannot distinguish between the N-demethylated degradation products of ureas such as linuron or metoxuron. On the other hand, ethyl iodide as the alkylating agent eliminates this problem and has been successfully applied to the chromatography of some degradation products of linuron (Glad et al., 1978).

In the present study this same alkylation technique with ethyl iodide has been investigated for application to the GC separation metoxuron, monomethylmetoxuron (MMM), desmethylmetoxuron (DMM), linuron, and diuron. The suitability of this technique for analysis of soil spiked with metoxuron and diuron is demonstrated. A comparison is made between this method and direct liquid chromatography (LC) of the ureas (Lawrence, 1976) in terms of sensitivity and suitability for residue analysis.

Table I. Structures of the Ureas

name	chemical structure
linuron	
diuron	
metoxuron	
monomethylmetoxuron (MMM)	
desmethylmetoxuron (DMM)	

EXPERIMENTAL SECTION

Reagents. The chemical structures of the ureas studied are shown in Table I. All were obtained from Sandoz Ltd. (Basel, Switzerland). Sodium hydride was obtained from J. T. Baker (Deventner, The Netherlands) as a 50% oil dispersion. About 2 g of the material was washed with hexane and then stored in a small, tightly sealed, screw-capped vial when not in use. The soil sample was obtained

Food Research Division, Food Directorate, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada (J.F.L.), and the Analytical Chemistry Department, Free University of Amsterdam, 1083 De Boelelaan 1081 HV, Amsterdam, The Netherlands (C.V.B., U.A.T.B., R.W.F.).

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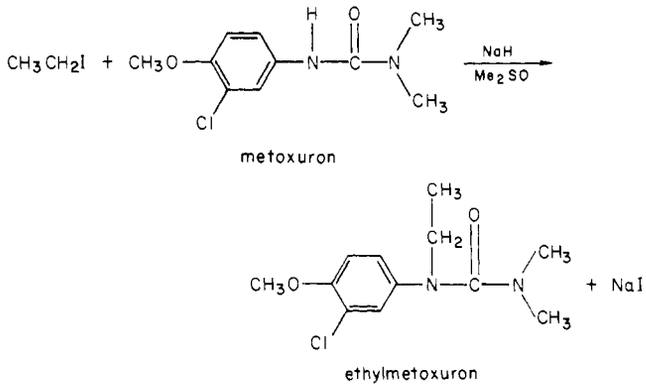

Figure 1. Reaction scheme for the ethylation of metoxuron.

locally. All solvents were analytical or reagent grade materials.

Gas Chromatography. For electron-capture detection a Pye Unicam 104 chromatograph equipped with a ^{63}Ni EC detector was employed. Separations were accomplished using a 2 m × 2 mm (i.d.) glass column packed with 4% OV-210 on 100/120 mesh Chromosorb W(HP) at a temperature of 175 °C and a nitrogen flow rate of 15 mL/min. Hydrogen (30 mL/min) was used as the make-up gas. Injection volumes were 1.0 μL.

Selective nitrogen detection was carried out on a Hewlett-Packard Model 5730A chromatograph with a dual NP-FID detector. Separations were achieved using a 50 m × 0.3 mm (i.d.) open tubulular column wall-coated with SE-30, at a temperature of 200 °C and a helium flow rate of 5 mL/min. Injection volumes were 1.0 μL.

Liquid Chromatography. A Varian Model 8500 pump and Variscan variable wavelength UV detector operated at 255 nm and 0.1 absorbance units (maximum sensitivity) were used. Chromatography was carried out with a 25 cm × 2 mm i.d. Micropak Si-10 column using 15% ethanol (v/v) in hexane as the mobile phase for the underivatized ureas. A solvent composition of 1% ethanol (v/v) in hexane was used for separation of the ethylated products. Mobile phase flow rate was maintained at 1.0 mL/min throughout. Injection volumes were 10.0 μL.

Ethylation. To a test tube containing the urea (5–50 μg) were added 0.5 mL of dimethyl sulfoxide and 0.5 mL of ethyl iodide, followed by 20–50 mg of sodium hydride. The test tube was capped, shaken gently, and then permitted to stand for 30 min at room temperature. After this time, 3 mL of hexane was added, and the contents were shaken. Then 7–10 mL of water was added dropwise with care to destroy excess sodium hydride. (Note: sodium hydride reacts rapidly with water to produce hydrogen gas.) When evolution of hydrogen ceased the test tube was capped and shaken vigorously for about 1 min. The organic layer was then rinsed with a second 10-mL volume of water. The hexane layer was dried with a small quantity of anhydrous sodium sulfate, then evaporated just to dryness, and dissolved in 1 mL of fresh hexane for chromatography.

Soil Extraction. Soil samples were extracted with methanol and cleaned up using Florisil column chromatography as described elsewhere (Scholten et al., 1979). The fraction from the column cleanup containing the ureas was evaporated to dryness in a test tube and then carried through the ethylation procedure described above.

RESULTS AND DISCUSSION

Reaction Conditions. Figure 1 illustrates the ethylation reaction for metoxuron. The reaction was complete for all ureas in 30 min at room temperature. The additional N–H positions of MMM and DMM were also ethylated. Thus, linuron, diuron, and metoxuron produced monoethylated derivatives, while two ethyl groups were added to MMM and three to DMM. These results were confirmed by GC–MS. The ethylation was found to be reproducible (6% relative standarized deviation) in the range of concentrations studied (5–50 μg of urea).

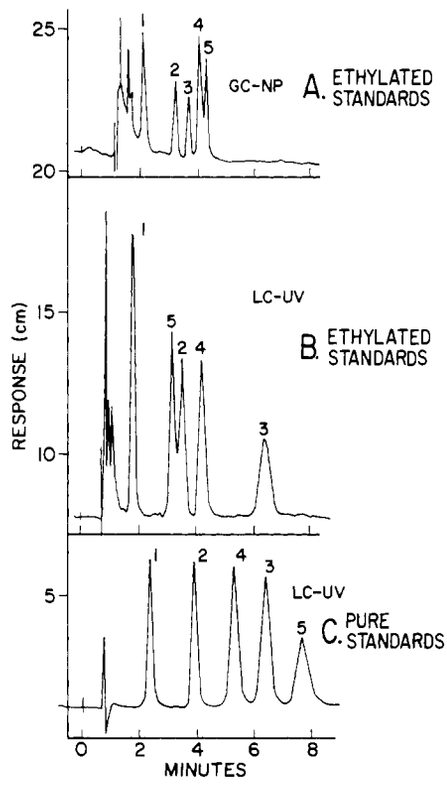

Figure 2. Separation of the test compounds. (A) GC separation of the ethylated ureas, operating conditions are as described in the text for NP detection. (B) LC separation of the ethylated ureas, mobile phase 1% ethanol in hexane. (C) LC separation of the pure ureas (not ethylated); mobile phase 10% ethanol in hexane. Other LC conditions are as described in the text. Compound 1 = linuron, 2 = diuron, 3 = metoxuron, 4 = MMM, and 5 = DMM.

Table II. Estimated Detector Responses to Ureas[a]

compound	detector response		
	GC-EC	GC-NP	LC-UV
ethylated metoxuron	1.3	6.0	0.02
ethylated linuron	21.0	18	0.07
ethylated diuron	17.0	14.0	0.04
metoxuron			0.02
linuron			0.08
diuron			0.05

[a] Peak height (cm) per nanogram at 64× for GC-EC and GC-NP and at 0.1 absorbance unit for LC-UV at 255 nm.

Chromatography. Figure 2 compares chromatographic results obtained by both GC and LC for the ethylated ureas. Both systems were adequate for separating the five derivatives. The retention of metoxuron, MMM, and DMM in GC was directly related to the molecular weight of the derivatives, while by LC the separation was based on polarity, thus providing a reversal in elution order compared to GC. In the case of the LC separation of the pure standards, MMM eluted before metoxuron, while DMM eluted later. Linuron and diuron eluted in the same order for the three chromatography systems investigated.

Table II compares the responses of the three detectors under the chromatographic conditions described in the

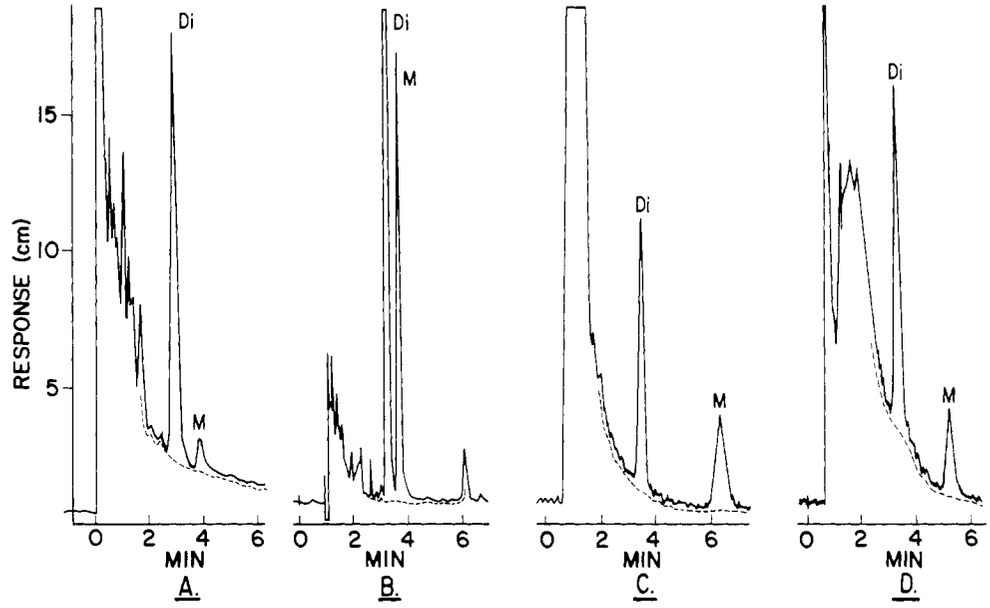

Figure 3. Soil sample analysis (1.0 ppm): comparison of chromatographic results. (A) GC–EC, (B) GC–NP, (C and D) LC–UV. A, B, and C are after ethylation. D represents direct LC analysis. Di = diuron and M = metoxuron. All chromatography conditions are as described in the text. For EC analysis, 1 mg of sample was injected. NP = 2-mg sample; LC–UV = 200-mg sample.

experimental section. The tenfold difference in sensitivity of metoxuron and linuron or diuron to EC detection is partly due to the lower halogen content of metoxuron. This has also been observed for the methylated derivatives (Buchert and Lokke, 1977). With both NP and UV detectors, metoxuron appears to be about twofold less sensitive than linuron or diuron.

Soil Analysis. Figure 3A–C compares chromatograms obtained from the same spiked soil extract, containing 1.0 ppm of diuron and metoxuron, after ethylation. LC–UV is seen to be superior to GC–EC for metoxuron determination, while the results for diuron are similar. The selective NP detector gives the best overall performance in terms of sensitivity and selectivity for the ethylated compounds. Estimated detection limits for this detector were about 2–50 ppb for the compounds studied, in soil.

Chromatogram D of Figure 3 shows the direct LC–UV determination of diuron and metoxuron in the same soil extract, before ethylation. The results are similar to those obtained by LC–UV after ethylation and, thus, direct determination would be preferred since the ethylation step is not required. Ethylation could still serve as a useful confirmatory test in a similar fashion as methylation of ureas has been used (Lawrence, 1976). Recovery of diuron and metoxuron from the spiked soil (four samples) was higher than 60%. Systematic studies to improve this yield were not carried out.

CONCLUSION

Ethylation of urea herbicides with subsequent GC–NP, GC–EC, or LC–UV analysis provides a sensitive and selective technique for their determination in complex substrates such as soils at levels down to 50 ppb or less. The ethylation procedure is especially useful for the separation of the N-demethylated degradation products of metoxuron [and other ureas (Glad et al., 1978)]—a separation which cannot be achieved by methylation, which yields the same derivative for metoxuron and both its degradation products.

Regarding the assessment of the relative merits of the various methods of analysis, one should be rather cautious since very different types of equipment and only one type of substrate were used. Still, it can be safely concluded that GC–NP combines good selectivity with high sensitivity, while LC–UV offers other possibilities for residue analysis, for example, as shown in Figure 3D for the direct analysis of urea herbicides without derivatization.

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