Analysis of Diflubenzuron by Gas Chromatography/Mass Spectrometry Using Deuterated Diflubenzuron as Internal Standard

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A new and rapid gas chromatographic/mass spectrometric analytical procedure for insect growth regulator diflubenzuron, a phenylurea-based insecticide, has been developed. Diflubenzuron is decomposed by the heat of a gas chromatograph into three reproducible fragments, 4-chlorophenyl isocyanate, 4-chloroaniline, and 2,6-difluorobenzamide, which elute separately from the GC column. The source of the chloroaniline appears to be the thermal breakdown of chlorophenyl isocyanate. No difluorobenzoic acid is generated, confirming that the heat-labile bond of diflubenzuron is different from the bond cleaved during based-catalyzed hydrolysis or microbial degradation. Advantage is taken of this along with the use of dideuterated diflubenzuron as an internal standard, and the selected ion monitoring capability of mass spectrometry, to enable analysis of diflubenzuron in complex extracts of vegetation samples without purification or derivatization. Detectability levels are similar to or better than those of currently used methods. Extraction and analysis of diflubenzuron from dosed leaves demonstrate the selectivity and utility of the technique.

The insect growth regulator diflubenzuron [1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)urea, trade name Dimilin] is a potent insecticide by virtue of its ability to inhibit synthesis of cuticle chitin, thus disrupting normal insect growth and development (Hajjar and Casida, 1978; Mulder and Gijswijt, 1973; Post et al., 1974; Verloop and Ferrell, 1977). Its specificity for those species whose structural integrity depends upon chitin (the arthropod phylum) makes diflubenzuron more selective than the broad-spectrum pesticides (Marx, 1977). However, its effects on nontarget arthropods (Muzzarelli, 1986), its persistence in the environment (Bull and Ivie, 1978; Mansager et al., 1979; Martinat et al., 1987; Mutanen et al., 1988; Nigg et al., 1986; Schaefer and Dupras, 1976; and Van den Berg, 1986), and the carcinogenic activity of 4-chloroaniline, a potential diflubenzuron metabolite (Seuferer et al., 1979; Chhabra, 1989), serve to retain interest in the short- and long-term actions of diflubenzuron in the environment.

Studies of the environmental fate of diflubenzuron have been limited by the analytical methods for quantifying it, primarily high-performance liquid chromatography using ultraviolet detection (Corley et al., 1974). This detection method loses sensitivity when extracts of complex environmental samples, such as leaves, bark, leaf litter, soil, and biological tissues, are analyzed because of interference caused by the UV-absorbing characteristics of many of the coextracted compounds. Several derivatization methods have been used (Smith et al., 1983); however, these require additional derivatization steps, often with long incubation times and usually some purification of the diflubenzuron, manipulations that one would like to avoid when analyzing large numbers of samples.

Gas chromatographic (GC) analysis, especially with mass spectrometric detection, is a highly selective, sensitive, and thus preferred method for analyzing pesticides in environmental samples. However, in the case of diflubenzuron and other phenylurea-based species, the heat of the gas chromatograph causes decomposition of the compound (Tamiri and Zitrin, 1987). For this reason, this method has not been used to date to analyze diflubenzuron (Corley et al., 1974). This paper describes the development of a GC/mass spectrometric method for quantifying diflubenzuron, using the heat-induced fragmentation in conjunction with deuterium-labeled diflubenzuron as an internal standard.

MATERIALS AND METHODS

Materials. Technical grade diflubenzuron was provided by the U.S. Forest Service Experiment Station, Morgantown, WV. Commercial grade diflubenzuron (25% wettable powder) was provided by Alan Miller, WV Department of Agriculture. Standards of diflubenzuron were prepared in acetonitrile and stored at 4 °C.

4-Chlorophenylurea was purchased from Schweizerhall, Inc. 4-Chlorophenyl isocyanate, 2,6-difluorobenzoic acid, 4-chloroaniline, 2,6-difluorobenzamide, benzene, 1,2-dichloroethane, and oxalyl chloride were obtained from Aldrich Chemical Co. The benzene was distilled from calcium hydride under nitrogen and stored under nitrogen. Dichloroethane was distilled from phosphorus pentoxide under nitrogen and stored under nitrogen. Diethyl ether and acetonitrile were obtained from Baker and were used as received. Deuterium oxide (D, 99.9%) was obtained from Cambridge Isotope Laboratories.

The purities of 4-chlorophenylurea, 4-chlorophenyl isocyanate, and 2,6-difluorobenzoic acid were determined by high-performance liquid chromatography using a C-18 column and methanol/water (55:45) as the eluting solvent. In the case of 4-chlorophenyl isocyanate and 4-chlorophenylurea, the appropriate fractions were collected, extracted with methylene chloride, concentrated to dryness by rotary evaporation, and, after redissolving in acetonitrile, rerun on the HPLC to ensure purity prior to GC/mass spectrometry.

All pesticide extraction and sample storage solvents were Fisher Optima grade. Acetone recovered from leaf extractions was redistilled and reused. Due to the toxicity of a number of the compounds and solvents used in the following procedures, gloves were worn where appropriate, and all synthetic work and solvent/extract transfers were done in well-ventilated exhaust hoods.

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Synthesis of Deuterated Diflubenzuron. *4-Chloro[2,6-$^2H_2$]aniline.* The hydrochloride salt of 4-chloroaniline was obtained by dissolving 4-chloroaniline in ether and bubbling HCl gas into the solution. The salt was isolated by filtration and dried in a vacuum. The 4-chloroaniline hydrochloride (2.4 g) was dissolved in 30 mL of deuterium oxide and refluxed under nitrogen with stirring for 24 h. The deuterium oxide was then removed by distillation under nitrogen until ~5 mL remained. Deuterium oxide (25 mL) was added to the brown oil and the mixture again refluxed. After 48 h, the deuterium oxide was removed as above, another 30 mL of deuterium oxide was added, and the reflux was continued. This process was repeated until greater than 98% of the 4-chloroaniline was converted to 4-chloro-[2,6-$^2H_2$]aniline. The free base was isolated by extraction with diethyl ether after the pH of the deuterium oxide solution was adjusted to 12 by addition of sodium hydroxide.

1-(2,6-Difluorobenzoyl)-3-(4-chloro-[2,6-$^2H_2$]phenyl)urea (Dimilin) was synthesized by adaptation of the method of Wellinga et al. (1973). 2,6-Difluorobenzamide (2.0 g, 12.7 nmol) was suspended in freshly distilled dichloroethane in an ice bath and stirred under nitrogen while 1.44 mL of oxalyl chloride was added. The reaction was allowed to reach room temperature after addition of oxalyl chloride. After 9 h, the solvent was removed by distillation under nitrogen to leave 2,6-difluorobenzoyl isocyanate as an oil. The oil was dissolved into 25 mL of benzene and added by dropwise addition to 1.6 g of 4-chloro-[2,6-$^2H_2$]-aniline dissolved in 50 mL of benzene that was chilled in an ice bath. After 12 h, the precipitate that was formed was collected by filtration and recrystallized in acetonitrile to yield 980 mg of the title compound.

Extraction of Diflubenzuron from Leaf Surfaces. Leaf samples were dosed with diflubenzuron (25% WP) suspended in water to mimic the situation that would occur in the field. Leaves tested for extraction efficiency were three to six leaves each of fresh black maple, chestnut oak, yellow poplar, black cherry, American beech, and rhododendron and fallen, dried white oak, red oak, and rhododendron. The desired aliquot of constantly stirred 50–500 ppm suspensions of diflubenzuron (200–2000 ppm of wettable powder) was spread as evenly as possible over the leaf surface with a Pipetman. The leaves were dried at room temperature and stored at −23 °C until extraction.

Extraction of diflubenzuron from the leaf surfaces was done by cutting the leaves into 1–2-cm square pieces, weighing, and shaking with 25 mL of acetone for 5 min at room temperature in a stoppered Erlenmeyer flask. (This represents a minimum useful volume; with more leaf material, a volume of 10 mL of acetone/g of leaf material can suffice.) The solvent was decanted into a round-bottom flask and the extraction repeated two times.

Washes were combined, and the acetone was removed by evaporation at 8–10 °C using a rotary evaporator attached to a dual aspirator pump (Buchler) and a submersible pump to circulate the ice-cold water of the trap bath through the evaporator's condenser. The efficient system allows evaporation at low temperatures to avoid any sample loss or decomposition.

The residue, which contains the acetone extract suspended in water, was re-extracted with a few milliliters of methylene chloride after quantitative transfer to a glass conical tube and vortexing for 1 min. After a quick bench-top centrifugation to separate the layers, the lower methylene chloride layer was carefully removed with a Pasteur pipet and passed through anhydrous sodium sulfate into a 50-mL round bottom flask. This methylene chloride extraction was repeated two times, combining the extracts.

After the addition of 50.0 μL of 500 ppm of deuterated diflubenzuron (25.0 μg) as internal standard, the total extract was evaporated to dryness at 8–10 °C on the rotary evaporator. [Because the internal standard is expected to behave in a manner identical with the unknown diflubenzuron during sample workup (stability, solubility, vapor pressure), it can be added to the acetone extract itself to account for any sample losses during workup of the extract. This was not done here because in developing the extraction and concentration procedure the percent overall recovery of diflubenzuron was of interest.] The residue was taken up in 1–2 mL of methylene chloride, solids were removed if necessary by a quick bench-top centrifugation, and the samples were stored at 4 °C in glass vials with Teflon-lined caps until analysis.

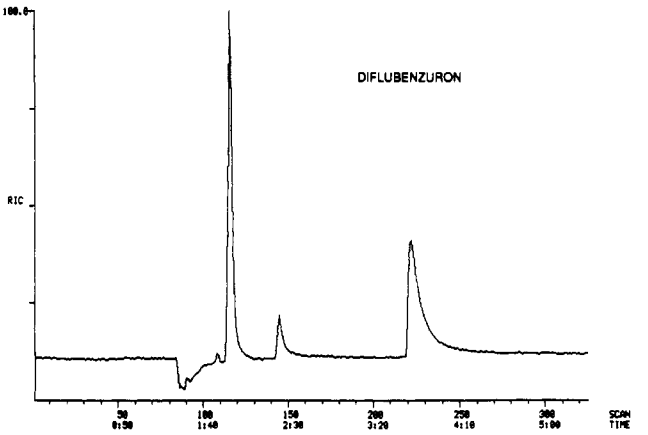


Figure 1. Gas chromatographic profile of diflubenzuron using mass spectrometric detection. Reconstructed (total) ion current (RIC) is plotted as a function of scan number or elution time. All ions from m/z 80 to 550 were monitored, removing solvent interference (acetonitrile, m/z 41). Other conditions are described under Materials and Methods. Sample size was 2.0 μL and concentration 2000 ppm.

Analysis of Diflubenzuron and Related Compounds. Gas chromatographic/mass spectrometric analysis was performed on a Finnigan 4500 System GC/mass spectrometer equipped with an Incos Data Station at the WVU Mass Spectrometry Center, Department of Biochemistry. [The WVU Mass Spectrometry Center was established through a Shared Instrumentation Award from the National Institutes of Health (GM 27514) to M.J.W. and an appropriation from the State of West Virginia.] Gas chromatography was run isothermally at 185 °C on a DB-1701 (J&W Scientific, Inc.) 30-m capillary column (0.2 mm i.d.). Two-microliter injections were made in the split mode (split approximately 10:1), and the injector temperature was 280 °C. The ionizing voltage required to produce the maximum number of ions at the m/z values of interest was determined to be 30–40 eV; therefore, 35 eV was used for all samples. Either all ions from m/z 80 to 550 or selected ions were monitored as indicated. Quantifying of specific ions was done in the selected ion monitoring mode using manual integration across each specific peak; in this manner, any concern as to possible variation in peak position due to an isotope effect on retention time is avoided. [In mixtures of deuterated and nondeuterated diflubenzuron species, the H/D isotope ratio is observed to be uniform across the GC peaks, showing that there is no detectable isotope effect on GC retention times.] Calculation of the micrograms of diflubenzuron using deuterated diflubenzuron as internal standard was done as indicated in the text.

RESULTS AND DISCUSSION

Diflubenzuron, a phenylurea-based pesticide, decomposes in the heat of the gas chromatograph, eluting from the column in three distinct and reproducible peaks (Figure 1). Peak I was identified by mass spectrometric analysis as 4-chlorophenyl isocyanate (Figure 2, top), peak II is 4-chloroaniline (Figure 2, middle), and peak III is 2,6-difluorobenzamide (Figure 2, bottom). Gas chromatography of purified 4-chlorophenyl isocyanate results in both the original compound and 4-chloroaniline (M. J. Wimmer, unpublished observations). Therefore, the 4-chloroaniline fragment observed in the diflubenzuron case likely originates from the chlorophenyl isocyanate fragment and not from a second cleavage point in diflubenzuron itself.

The lack of generation of 2,6-difluorobenzoic acid from diflubenzuron during gas chromatography was demonstrated by analyzing a 50:50 mixture of the two compounds (Figure 3). The difference in area ratios of the three di-

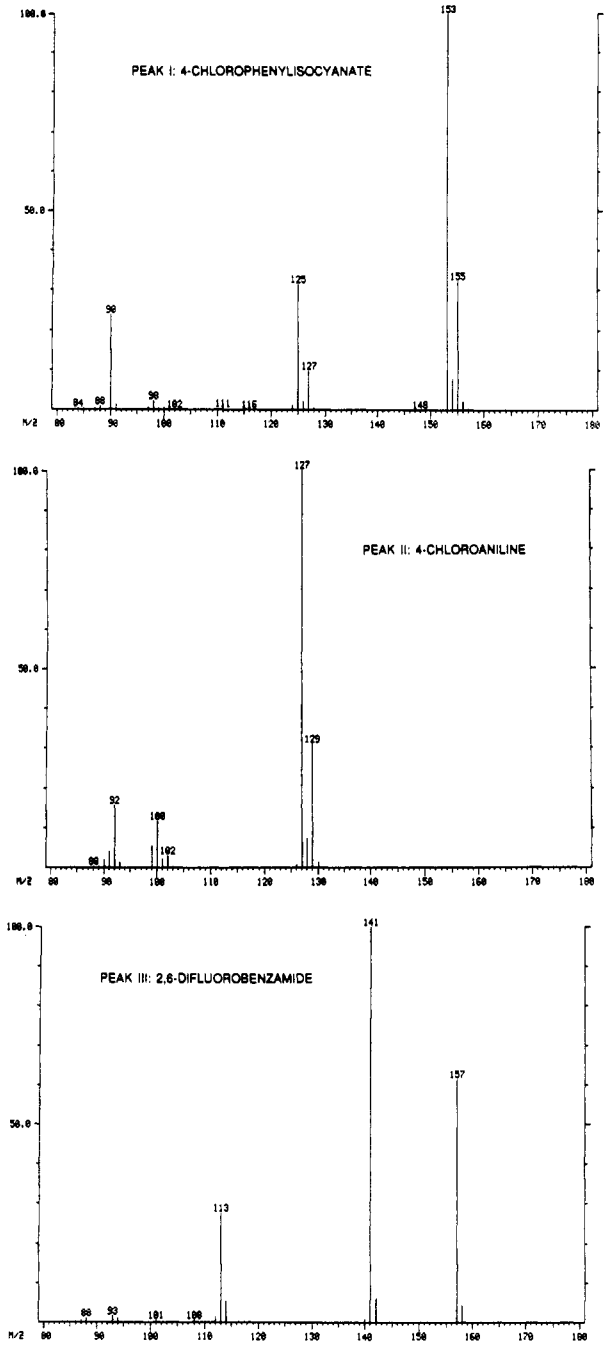


Figure 2. Mass spectra of the diflubenzuron fragments shown in Figure 1. Relative ion intensity is plotted against m/z. (Top) Peak I, 4-chlorophenyl isocyanate; (middle) peak II, 4-chloroaniline; (bottom) peak III, 2,6-difluorobenzamide.

flubenzuron fragment peaks compared to those of Figure 1 is due to the switch to selected ion monitoring in contrast to all ions being measured. Difluorobenzoic acid, monitored at m/z 158 and 141, elutes closely following the chloroaniline fragment (m/z 127) and well before difluorobenzamide (m/z 157 and 141). No difluorobenzoic acid has been detected in any diflubenzuron elution profile, confirming the location of a thermally labile bond within the urea moiety of diflubenzuron and the lack of production of 4-chlorophenylurea.

The heat-induced decomposition, illustrated in Chart I, was also observed by Ivie et al. (1980) in heated aqueous media, although 4-chlorophenyl isocyanate apparently was not monitored. The thermally sensitive bond of diflubenzuron is different from the one broken during base-catalyzed hydrolysis (Ivie et al., 1980), also shown in Chart

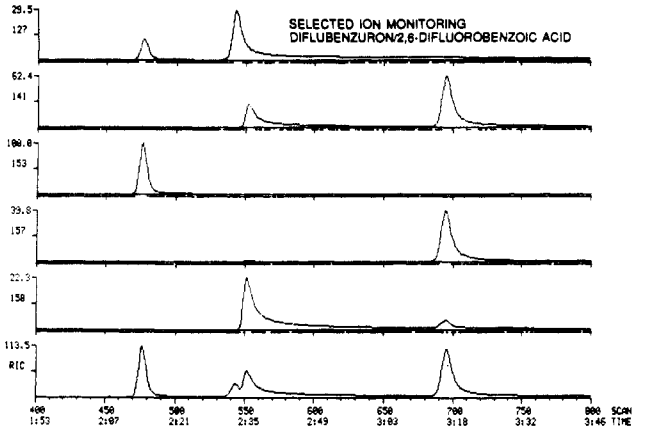
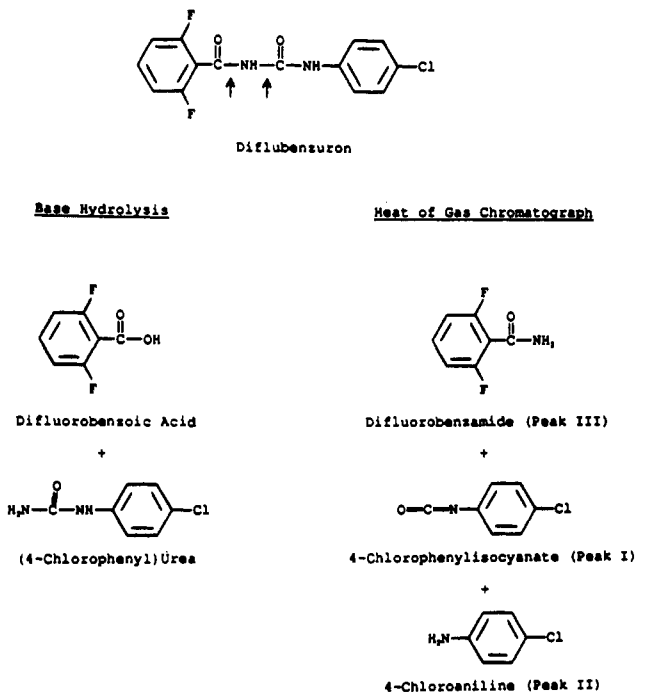


Figure 3. Gas chromatographic profile of a mixture of diflubenzuron and 2,6-difluorobenzoic acid, 250 ppm each. Selected ion monitoring (relative intensity) at m/z 127, 141, 153, 157, and 158, along with RIC, is plotted as a function of scan number or time.

Chart I



I, which generates difluorobenzoic acid. [Hydrolysis cleaves the same bond that soil microorganisms have been found to break (Seuferer et al., 1979), and this is likely the major mechanism for breakdown of the pesticide in the environment (Metcalf et al., 1975).] Therefore, one can take advantage of the different heat-induced cleavage points to develop a GC/mass spectrometric analysis for diflubenzuron in environmental samples.

The major advantage of using a mass spectrometer as the gas chromatograph detector for such analysis is that interference by coextracted compounds can be prevented without the diflubenzuron being isolated. This is accomplished by using selected ion monitoring (SIM) in which only those ions from the compounds of interest are monitored. The identity of the species to be quantified is confirmed by the ion profile as well as the retention time on the gas chromatographic column, avoiding the double gas chromatographic runs for peak identification found in many standard GC methods. Sensitivity is also high. The ultraviolet absorption monitoring of high-performance liquid chromatographic procedures cannot

achieve this ease of analysis and maintain sensitivity, as many compounds coextracted with diflubenzuron also absorb in the UV range.

Initially viewed as a problem in quantifying diflubenzuron, the heat-induced decomposition during gas chromatography results in fragments that can be used in quantitative analysis. Any variability, however, in the decomposition reaction would preclude use of a non-diflubenzuron internal standard. To overcome this potential problem, deuterated diflubenzuron itself was chosen as the internal standard.

Dideuterodiflubenzuron [1-(2,6-difluorobenzoyl)-3-(4-chloro-2,6-dideuterophenyl)urea] was synthesized with deuterium at the 2- and 6-positions of the chlorophenyl ring. The C–D bonds are stable under conditions used in the sample workup and analysis. Because the deuteriums are placed three bonds away from the bond broken during the gas chromatographic run, no isotope effect is expected in the heat-induced cleavage reaction (see below). Therefore, any variability in thermal decomposition during GC runs should be the same for both protio and dideutero species.

Dideuterodiflubenzuron behaves as expected upon gas chromatography, resulting in the same three heat-generated fragments (Figure 4). The chlorophenyl fragments produce molecular ions which are increased by 2 m/z units in mass; the deuterium positions are well over 99% enriched, avoiding correction for contaminating undeuterated compound in subsequent analyses. The lack of a detectable isotope effect in the thermal decomposition reaction is suggested by observing identical ratios of fragment peak areas between the protio and deutero species; the same result is found by integration of either the reconstructed ion current (RIC) or selected ions for each peak. GC/mass spectrometry of a 50:50 mixture of diflubenzuron and dideuterodiflubenzuron (Figure 5) also shows that the deuterated species fragments and behaves chromatographically identical with the protio species, critical for its use as an internal standard. (The difference in retention times of the three peaks compared to Figure 1 is due to use of a new column.)

Diflubenzuron can be quantitatively analyzed by using the deuterated species and the thermal decomposition reaction (Chart II). Because of the replacement of deuterium in the chlorophenyl ring, two of the heat-generated fragments of diflubenzuron contain deuterium, the 4-chlorophenyl isocyanate and the 4-chloroaniline. Isotope ratios of the undeuterated to deuterated major ions as indicated allow quantitation of the undeuterated species if a known amount of dideuterodiflubenzuron is added as the internal standard. Having two fragments that contain the deuterium provides an internal check of the result, for the two ratios (m/z 127/129 and 153/155) should be equal if diflubenzuron is the starting material.

Analysis of a series of known standards, each of which contained 50.0 μg of dideuterodiflubenzuron and variable amounts of the protio species, confirms the analytical rationale. Selected ion monitoring of the most abundant ion of each fragment (protio and dideutero) is used to monitor the gas chromatographic runs (Chart II): m/z 153 and 155 for peak I, the 4-chlorophenyl isocyanate fragment; m/z 127 and 129 for peak II, 4-chloroaniline; and m/z 141 for peak III, difluorobenzamide. (The third fragment contains no deuterium and, therefore, is not used in the quantitation.)

The H/D ratios (m/z 153/155 and 127/129) are used to quantify the protio species. The following calculation determines the amount of protiodiflubenzuron in the sample, taking into account the natural abundance contribution of chlorine isotope of mass 37 to the 129 and 155 peaks from the 127 and 153 peaks, respectively (chlorine-37 is found at 32.5% of chlorine isotope 35):

$$\mu\text{g of diflubenzuron} = m/z\ 127 \div [m/z\ 129 - (0.325 \times m/z\ 127)] \times 50.0\ \mu\text{g of internal standard}$$

The calculation using the 153/155 ratio is analogous.

Excellent agreement is seen between the H/D ratios of the two fragments (Table I), and the calculated amount of protiodiflubenzuron agrees very well with the actual amount over the wide range of concentrations used. This agreement over a 250-fold range of ratios of deutero/protio species implies that the thermal decomposition reaction does not change with concentration, important for ease of use and flexibility of the method.

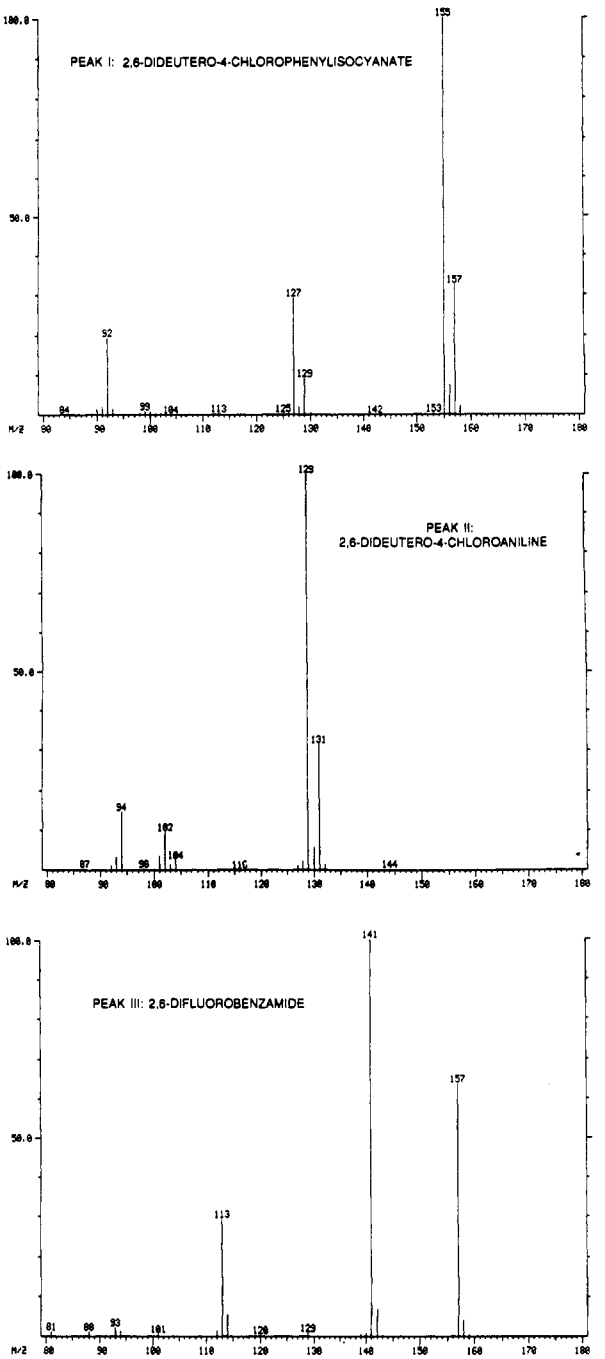


Figure 4. Mass spectra of gas chromatographic fragments generated from diflubenzuron deuterated in the 2- and 6-positions of the chlorophenyl ring. The column profile is identical with Figure 1, and peak identification is the same as Figure 2.

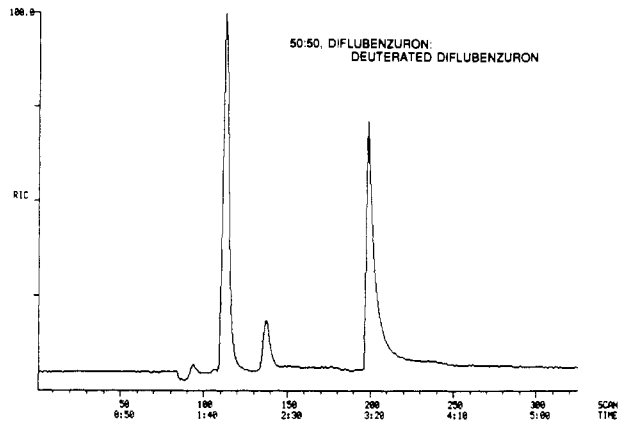


Figure 5. Gas chromatographic profile of a mixture of protio- and dideuterodiflubenzuron, 1000 ppm each. Conditions are the same as Figure 1, except for use of a new column that slightly changed the retention times.

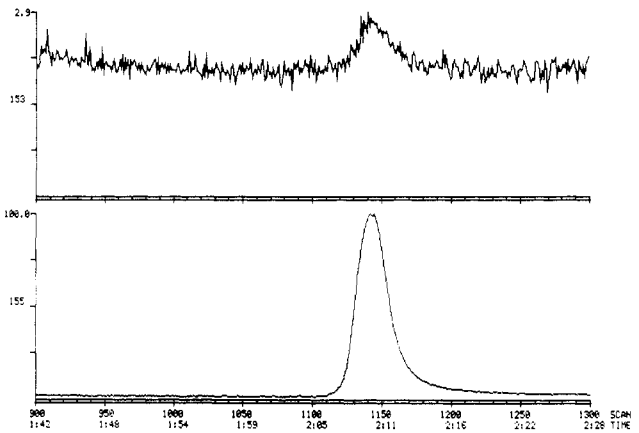
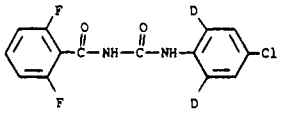


Figure 6. Gas chromatographic profile of a mixture of 50 ppb of protiodiflubenzuron and 50 ppm of dideuterodiflubenzuron using selected ion monitoring mass spectrometric detection at m/z 153 and 155. Injection volume was 2.0 μL.

Chart II



Internal Standard: 1-(2,6-difluorobenzoyl)-3-(4-chloro-[2,6-$^2H_2$]phenyl)-urea

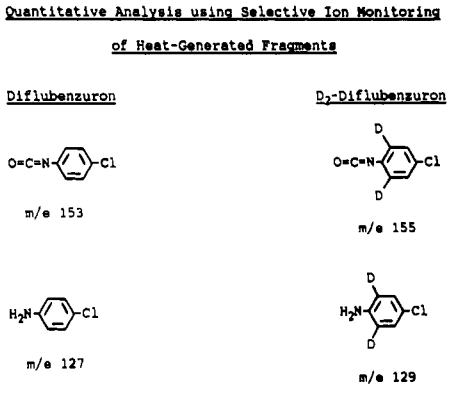


Table I. Analysis of Diflubenzuron Using Deuterated Diflubenzuron as Internal Standard

diflubenzuron added, μg/mL	m/z 127	m/z 129	m/z 153	m/z 155	diflubenzuron calcd, μg/mL 127/129	diflubenzuron calcd, μg/mL 153/155
250	100	51.6	100	52.5	262	250
100	100	84.5	100	84.7	96	96
50.0	71.0	100	71.6	100	46	47
20.0	33.0	100	33.8	100	18	19
5.0	8.6	100	9.1	100	4.4	4.7
1.0	1.8	100	2.2	100	0.93	1.1

[a] The indicated micrograms of diflubenzuron were added from 100 and 5.0 ppm stock solutions along with 50.0 μg of deuterated diflubenzuron in a total volume of 1.0 mL/sample. The solvent was acetonitrile. Two microliters of each sample, run in duplicate, was analyzed by using selected ion monitoring at m/z 127, 129, 153, and 155. The micrograms of diflubenzuron were calculated as described under Results and Discussion. The "100" figure in the m/z columns represents the value to which the other data are normalized and enables calculations to at least three significant figures as shown.

By use of SIM at only m/z 153 and 155 for added sensitivity, the detection limit was investigated. The profile of a 2-μL injection of 50 ppb of diflubenzuron/50 ppm of dideuterodiflubenzuron (Figure 6) shows that the peak representing 100 pg of the protio species is clearly visible above the baseline. The limit of detection for the instrumentation and conditions used is in the 50-pg range injected in the split mode, less with splitless injection. In a standard 1-L water sample, with extract concentrated to 0.5 mL, this represents a detection limit of around 12 ppt, equal to or better than other diflubenzuron methods used. In more complex samples, such as leaf or litter extracts, the detection limit may be less if coextracted compounds interfere. However, in the SIM mode, only those compounds that have ions of the m/z values monitored (127, 129, 153, 155) will be seen, and this will lower that interference (see below).

The analysis of extracts of several types of fresh and fallen, dried leaves dosed with known amounts of diflubenzuron successfully demonstrates the technique on environmental samples where interfering compounds are expected. After extraction and addition of internal standard, the samples, ranging from buff to dark green in color, were analyzed by GC/mass spectrometry using SIM (Table II). The close agreement seen between the two H/D ratios involving the deuterated fragments, along with the accuracy in the quantitation itself, demonstrates the lack of interference from coextracted compounds in the analysis. The extraction efficiency over a 40-fold concentration range appears to be virtually 100%, lending further utility to the overall procedures used herein for the processing of vegetation samples containing diflubenzuron. The same results were obtained after storage of two of the samples at 4 °C for 3 weeks, reflecting on the stability of diflubenzuron in the biological extracts in acetonitrile.

The sensitivity level in complex extracts of vegetation is sufficiently low to enable analysis of diflubenzuron under field conditions. The 5-μg sample of Table II (10 ng of diflubenzuron injected into the GC) was extracted from approximately 1 g of leaf material and was easily detected. To more accurately determine the sensitivity in actual field samples, an 11-g sample of oak leaf material was analyzed from a tree in an area sprayed with diflubenzuron under normal field operations. With this much leaf material, the coextracted compounds having ions at the m/z values of interest (127, 129, 153, and 155) are numerous (Figure 7). However, by use of the chlorophenyl isocyanate fragment of diflubenzuron (Figure 7, top, peak A) along with the chloroaniline fragment (Figure 7, bottom, peak B), the H/D ratios between the protio and deuterated species remain in close agreement (7.94/100 and 7.96/

Table II. Quantitative Analysis of Diflubenzuron Extracted from Dosed Leaves[a]

sample	appld diflubenzuron, μg	m/z 127/129	calcd diflubenzuron, μg	m/z 153/155	calcd diflubenzuron, μg
fresh black maple	200	100/44.1	216	100/44.1	217
	100	100/57.8	99	100/57.9	98
	10	29.4/100	8.1	32.2/100	9.0
	5.0	18.9/100	5.0	19.8/100	5.3
fallen, dried white oak	100[b]	100/83.9	97	100/85.1	95
	100	100/58.9	95	100/58.3	97
fallen, dried red oak	10	31.8/100	8.9	32.7/100	9.1
fresh chestnut oak	50	100/82.6	50	100/78.1	55
	25	76.4/100	25	82.3/100	28
fresh yellow poplar	50	100/86.8	46	100/81.1	51
fresh black cherry	75	100/68.9	69	100/65.0	77
	25	63.2/100	20	68.1/100	22
fresh American beech	75	100/72.8	62	100/70.3	66
	25	70.1/100	23	75.0/100	25
fresh rhododendron	100	100/57.4	100	100/56.9	102
dried rhododendron	100	100/56.1	106	100/56.7	103

[a] The "100" figure in the m/z data is explained in the Table I legend. The data represent the average of duplicate runs. [b] 50.0 μg of deuterated internal standard added instead of 25.0 μg.

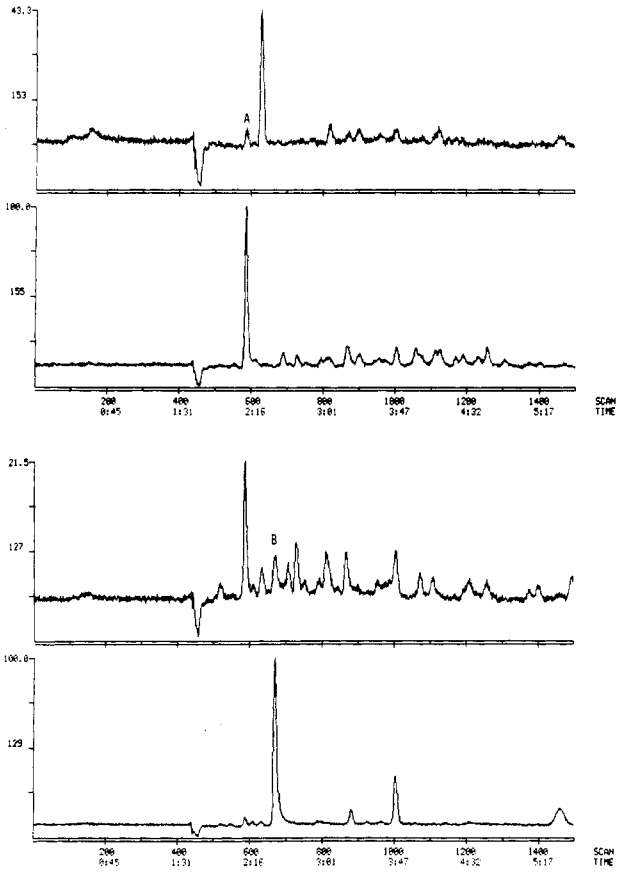


Figure 7. Gas chromatograhic profile of an extract of oak leaf material from a tree sprayed in the field with diflubenzuron. Selected ion monitoring (relative intensity) is plotted as a function of scan number or time. (Top) SIM at m/z 153 and 155 for the protio and deutero 4-chlorophenyl isocyanate fragments, the two peaks under (A). (Bottom) SIM at m/z 127 and 129 for the protio and deutero 4-chloroaniline fragments, the two peaks under (B). Injection volume was 2.0 μL.

100, respectively). This implies that the coextracted compounds are effectively separated from the diflubenzuron fragments, enabling accurate analysis of the pesticide using either one, although the isocyanate fragment shows less potential interference. The 2.0-μg level of pesticide detected in the sample, or 180 ppb on the leaf material, is appreciably above the detectability limit, and this tree showed one of the lowest concentrations of diflubenzuron among seven trees studied (S. R. Toney, D. L. Wellings, and M. J. Wimmer, unpublished results).

Further work is ongoing to test this analytical method for diflubenzuron in other environmental media (leaf litter, bark, biological tissue) and to extend the method to the environmental breakdown products of the pesticide. The analysis becomes more complicated if the extract of an environmental sample contains certain contaminating species from the breakdown reactions. There is no report to date that the 4-chlorophenyl isocyanate fragment used in analysis is produced in the environmental degradation of diflubenzuron; the major products appear to be 4-chlorophenylurea and 4-chloroaniline (Mansager et al., 1979; Metcalf et al., 1975; Seuferer et al., 1979). If 4-chloroaniline alone is present with diflubenzuron, the m/z 127/129 ratio will appear higher than the m/z 153/155. The chlorophenyl isocyanate fragment can then be used to quantify the diflubenzuron, and the difference in the two fragment ratios can actually be used to quantify the 4-chloroaniline.

Another complication arises in the presence of 4-chlorophenylurea. Pure 4-chlorophenylurea decomposes to 4-chlorophenyl isocyanate and 4-chloroaniline during gas chromatography, with no detectable level of the urea molecule apparent (M. J. Wimmer, unpublished observation). Thus, if 4-chlorophenylurea is present in the environmental extract, then the diflubenzuron level will appear higher than the actual value. This is likely to be a problem with litter samples where most of the breakdown of diflubenzuron is postulated to occur, in contrast to leaf surfaces on which no detectable breakdown has been seen (Bull and Ivie, 1978; Mansager et al., 1979).

The 4-chloroaniline and 4-chlorophenylurea interferences can be overcome in one of two ways: (a) a straightforward separation of the protio- and deuterodiflubenzuron from these breakdown products by HPLC prior to GC/mass spectrometric analysis (S. R. Toney and M. J. Wimmer, unpublished observations) or (b) use of a diflubenzuron internal standard that is deuterated in the difluorobenzamide, rather than the chlorophenyl, moiety. Both options are currently being pursued. Furthermore, monitoring of environmental samples for difluorobenzoic acid as well as difluorobenzamide may reflect the presence of 4-chlorophenylurea.

In conclusion, a new method for quantifying low amounts of diflubenzuron in environmental samples has been developed. The technique turns the problem of thermal decomposition during gas chromatography into an advantage. By use of deuterated diflubenzuron as an internal standard, and selected ion monitoring in the mass spec-

trometric analysis, no derivatization or purification of diflubenzuron from leaf extracts is required.

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Registry No. Diflubenzuron, 35367-38-5; 4-chlorophenyl isocyanate, 104-12-1; 4-chloroaniline, 106-47-8; 2,6-difluorobenzamide, 18063-03-1; 4-chloroaniline hydrochloride, 20265-96-7; deuterium oxide, 7789-20-0; 4-chloro[2,6-$^{2}H_2$]aniline, 35749-94-1; oxalyl chloride, 79-37-8; 2,6-difluorobenzoyl isocyanate, 60731-73-9.