Analysis of the Herbicide Diuron in Crops

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An analytical method based on the use of a liquid chromatograph and a photoconductivity detector is described for the determination of diuron in soybeans, carrots, and potatoes. With extraction and cleanup steps, the method provides a means of determining diuron at a level of 10 ng/g (10 ppb). Essentially quantitative recoveries have been obtained from the detection limit (10 ppb) to a fortification level 20 times greater (200 ppb). The method will also detect linuron, neburon, and three diuron metabolites, namely (3,4-dichlorophenyl)-*N*-methylurea, (3,4-dichlorophenyl)urea, and 3,4-dichloroaniline.

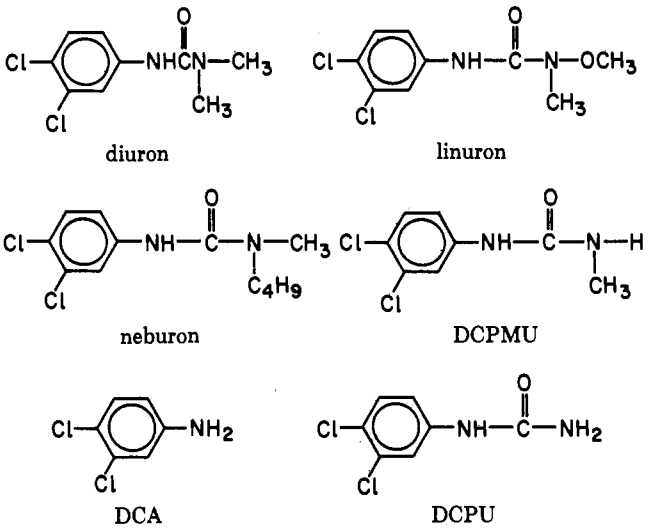
The herbicide diuron is widely used to control a large number of different weed species in a variety of crops. Because of this, it is necessary that methods be available for the analysis of residues in food crops.

Onley et al. (1968) have shown that corn seedlings metabolize diuron successively to (3,4-dichlorophenyl)-*N*-methylurea, (3,4-dichlorophenyl)urea, and 3,4-dichloroaniline. Small quantities of 3,4-dichloronitrobenzene are also formed. After initial extraction with an acetone–water mixture, extensive cleanup procedures are necessary to isolate diuron and its metabolites.

Nash (1968) and Osgood et al. (1972) report similar results with other crops.

Onley and Yip (1969) report a method based on the use of thin-layer chromatography that distinguishes diuron from its metabolites and other urea herbicides. Because of the poor thermal stabilities of the urea herbicides, recent approaches to analysis by gas chromatography have been based on the application of derivatization reactions such as alkylation, reported by Lawrence (1976a), and use of heptafluorobutyric anhydride, reported by Brinkman et al. (1984). Lawrence (1976b), Farrington et al. (1977), and Nielen et al. (1985) describe methods that measure diuron by means of a liquid chromatography system.

A selective and sensitive photoconductivity detector, used in liquid chromatography, has found application to pesticide residue analysis as reported by Büttler and Hörmann (1981), Slates (1983), Walters (1983), Walters and Gilvydis (1983), Walters et al. (1984), and Zahnow (1982, 1985a, 1985b). In this work the application is extended further to the development of a sensitive residue method for diuron in crops without the need for derivatization or extensive sample cleanup. Two urea herbicides of structure similar to diuron and three diuron metabolites are also detected by this procedure, and their structures are shown.



EXPERIMENTAL SECTION

Preliminary Treatment. The entire sample, in a frozen condition, was homogenized with dry ice in a Hobart blender (a toothed-disk mill was used for soybeans) until it reached a powder consistency. The carrots and potatoes were sectioned before blending in the Hobart. The homogenized samples were stored in a freezer until needed.

Extraction Procedure. A 10-g sample of crop was mixed with 75 mL of methanol in a 250-mL glass centrifuge bottle for 1 min on a Tekmar Tissumizer (shaft SDT182EN). The resulting mixture was centrifuged for 15 min at 2000 rpm, and the supernatant liquid was decanted into a 500-mL pear-shaped flask through a funnel plugged with glass wool and 10 g of anhydrous sodium sulfate. The solids remaining in the bottle were extracted two more times in the same manner, and the liquid was combined with the original extraction. The funnel was rinsed with 10 mL of methanol.

The methanol solution in the 500-mL pear-shaped flask was concentrated to 10 mL on a rotary evaporator at 45 °C. Then 90 mL of deionized water was added to the flask while swirling continuously. The extract was poured through a glass wool plugged funnel into a 250-mL separatory funnel, and the flask was rinsed with a 9:1 water–methanol solution (about 10 mL), which also was poured into the separatory funnel.

The water–methanol solution was extracted three times with 75 mL of methylene chloride. The methylene chloride (bottom layer) was drained into a 300-mL pear-shaped flask through a funnel plugged with glass wool and 50 g of anhydrous sodium sulfate.

Note: Since the processing of soybeans caused severe emulsions to form, a soybean extraction mixture was drained into a 300-mL centrifuge bottle from the separatory funnel and centrifuged for 15 min at 2000 rpm. The bottom layer (methylene chloride) was removed with a 50-mL hypodermic syringe and was drained through a funnel plugged with glass wool and 50 g of anhydrous sodium sulfate that emptied into a 60-mL separatory funnel. This solution was drained into the 300-mL pear-shaped flask, making sure that the methylene chloride solution contained no water. The methylene chloride extraction was repeated two more times.

The methylene chloride in the 300-mL pear-shaped flask was concentrated to 2–3 mL at 25 °C on the rotary evaporator. The remaining liquid was then transferred to a 10-mL centrifuge tube with a Pasteur pipet. The 300-mL pear-shaped flask was rinsed with three 2-mL washings of methylene chloride that were also transferred to the 10-mL

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centrifuge tube. The sample was then blown to dryness on a nitrogen evaporator (N-EVAP) at 30 °C or, in the case of soybeans, until all the methylene chloride had evaporated. (With soybeans, there was approximately 0.5 mL of oily liquid that would not evaporate.)

The sample was stored dry in a refrigerator until it was to be analyzed, at which time the volume was brought to 5 mL with HPLC mobile phase. The entire sample was then filtered into a glass vial with a Millex SR 0.5-μm filter unit mounted on a 10-mL hypodermic syringe. These filter units were discarded after each use.

Liquid Chromatography. Details of the liquid chromatography and detector operation have been described previously by Zahnow (1985a).

The column was a Waters μ-Porasil (30 cm × 3.9 mm) controlled at 35 °C. A new column had to be conditioned by pumping a solution that consisted of 10 parts by volume of 2-propanol, 10 parts of methanol, 5 parts of glacial acetic acid, and 1 part of water through it for several hours at a flow rate of 1 mL/min or more. This treatment was also used to clean columns that started to lose their efficiency because of contamination from samples. A contaminated column was characterized by broad peaks that tailed very badly and by shifts to longer retention times. This conditioning solvent had to be thoroughly flushed from the column with the mobile phase. One hour of flushing at 0.5 mL/min was usually sufficient. The mobile phase consisted of the following: 80 parts by volume of isooctane, 10 parts of methanol, 10 parts of 2-propanol, and 1 part of glacial acetic acid. Both the conditioning solvent and the mobile phase were filtered before use through a 0.5-μm Millipore filter (FHUP 04700) held in a Millipore filter apparatus (XX15 04700).

The sample valve was a Valco Model CV-6-UHPa-N60, equipped with a 25-μL loop, for manual injection of standards and samples.

During normal operation the mobile phase was pumped through the column at 0.5 mL/min, which was judged to be the minimum practical rate. At this flow rate diuron eluted from the column in approximately 13 min, depending on the extent of column deactivation. This rate was selected because the detector response increased with decreasing flow rate due to the longer residence time of the sample in the quartz reactor coil. Further slowing of the flow is not desirable because the retention times would increase and peaks would broaden. Also, pump stability may become a problem at very low flows.

Standardization. A standard stock solution was prepared by weighing out 10.0 mg of the diuron, dissolving it in methylene chloride, and diluting it to 100 mL in a volumetric flask. This solution was quite stable and could be used for many months.

The working standards used for liquid chromatography as well as for the fortification of recovery samples were prepared by pipetting 1.0 mL of the stock solution into a clean, dry, 100-mL volumetric flask, evaporating the methylene chloride with a gentle nitrogen stream, dissolving the residue in the mobile phase and diluting to volume with the mobile phase. Standards of lower concentration were prepared from the 1.0 μg/mL standard by appropriate dilution with the mobile phase. This set of standards was replaced with a fresh set every month. Over this time period little change in detector response was observed. All standards were stored in a refrigerator when not in use.

RESULTS AND DISCUSSION

The average sensitivity of the detector for diuron was 200–300 mm/ng, depending on column condition, and the response was linear over the diuron concentration range (0.10–1.0 μg/mL) used in this study. A 1-mV recorder with a chart width of 25 cm was used as the readout device.

Due to the fact that the background of most crop extracts produced a base line that was irregular, quantitation was based on peak height measurements.

The amount of diuron in a given sample can be calculated from the following equation if the quantities described in this procedure are used:

$$\text{ng/g (ppb)} = 20H/\text{RF}$$

H is the peak height (mm) normalized to range 1 and attenuation 1, and RF is the response factor (mm/ng).

Soybeans, carrots, and potatoes were chosen for the crop recovery study. These crops were fortified at four levels: the detection limit (10 ppb), 20 ppb, 50 ppb, and a level 20 times greater than the detection limit (200 ppb). The results of the measurements are shown in Table I, and from this it can be seen that recovery is not dependent on the type of crop. All three crops show some tendency toward lower recoveries at the detection limit, and the increased difficulty in obtaining accurate results is indicated by the relatively high standard deviations at this level.

Typical chromatograms of extracts of soybeans, carrots, and potatoes can be seen in Figures 1–3. The average recoveries were 90% for soybeans, 93% for carrots, and 83% for potatoes.

These results demonstrate that diuron can be effectively measured in crops such as soybeans, carrots, and potatoes. The limit of detection for this method is 10 ppb when a normal-phase HPLC separation is used with the highly sensitive and selective photoconductivity detector. The

Table I. Recoveries of Diuron from Crops[a]

crop	fortifcn, ppb	recovery, % mean	SD	range
soybeans	10	90	9	83–105
	20	107	5	100–113
	50	92	5	85–100
	200	108	5	103–117
carrots	10	93	18	73–111
	20	99	11	89–119
	50	108	10	94–118
	200	106	3	102–109
potatoes	10	83	10	65–95
	20	102	4	98–109
	50	105	7	99–116
	200	103	7	95–113

[a] $N = 6$ for each crop at each fortification level.

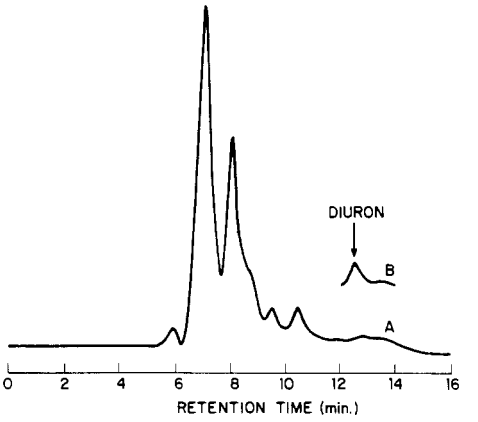

Figure 1. (A) Soybean control. (B) Soybeans fortified with 10 ppb diuron. Detector sensitivity, 1 × 10.

Table II. Chromatographic Properties/Standard Mobile Phase

compound	ret time, min	resp factor, mm/ng	compound	ret time, min	resp factor, mm/ng
neburon	10.0	254	diuron	13.1	288
linuron	10.9	254	DCPU	13.5	380
DCPMU	12.4	307	DCA	18.4	131

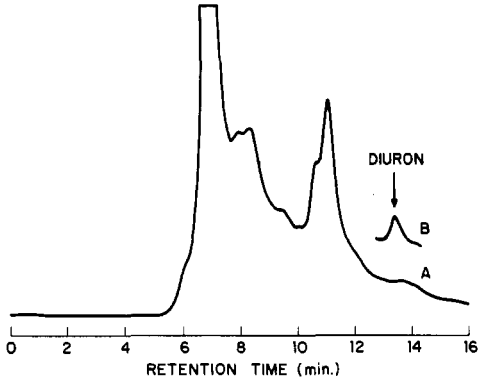

Figure 2. (A) Carrot control. (B) Carrots fortified with 10 ppb diuron. Detector sensitivity, 1 × 10.

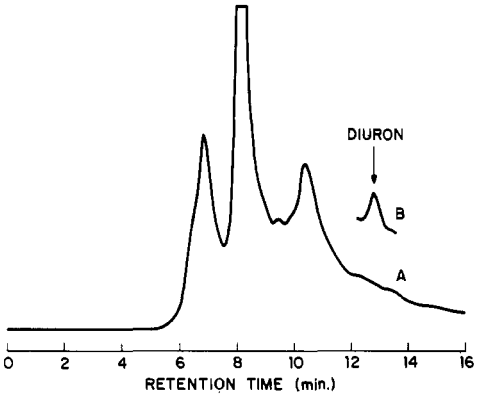

Figure 3. (A) Potato control. (B) Potatoes fortified with 10 ppb diuron. Detector sensitivity, 1 × 10.

average diuron recoveries for all of the crops were near 100% at each of the three higher fortification levels. The average diuron recoveries at a 10 ppb fortification level for all of the crops were near 90%. It is apparent that, at the 10 ppb fortification level, replicate measurements of individual samples are required to ensure reasonable accuracy.

This method will also detect the following compounds: linuron, neburon, and three diuron metabolites, namely (3,4-dichlorophenyl)-*N*-methylurea (DCPMU), (3,4-dichlorophenyl)urea (DCPU), and 3,4-dichloroaniline (DCA). All of these compounds can be isolated and measured by the diuron method, and of the five compounds, only DCPU cannot be resolved chromatographically from diuron. Of the remaining four, only DCPMU does not show base-line resolution, but reasonably good quantitation of all four compounds is still possible. Retention times and response factors for all of the compounds can be found in Table II. It should be noted that DCA is not stable for more than several days in the mobile phase, and its retention time is very sensitive to the condition of the column.

The diuron plant residue method was applied to soybeans, carrots, and potatoes at 50 ppb fortification levels of diuron, linuron, neburon, DCPU, DCPMU, and DCA. The experimental data for these recoveries can be found in Table III. Generally speaking, recoveries from carrots were good for diuron and linuron, and recoveries from potatoes were good for diuron, neburon, and DCPMU.

Table III. Recovery Data[a]

	recoveries, % at 50 ppb fortification					
crop	diuron	linuron	neburon	DCPU	DCPMU	DCA
soybeans	95 (2)	91 (2)	83 (2)	100 (1)	102 (1)	84 (1)
carrots	102 (1)	94 (1)	52 (1)	63 (1)	67 (2)	0 (1)
potatoes	106 (1)	75 (1)	90 (1)	44 (1)	100 (2)	27 (2)

[a] Key: (1) one crop sample; (2) averge of two crop samples.

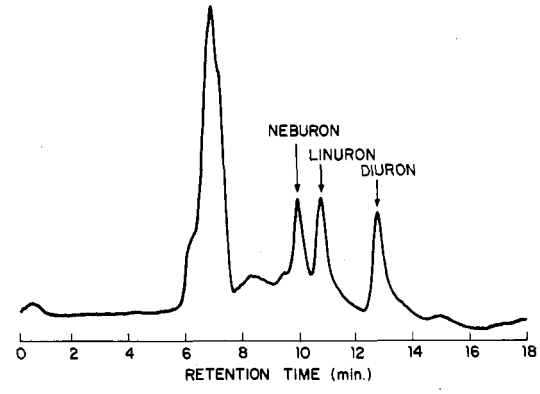

Figure 4. Soybeans fortified with 50 ppb neburon, linuron, and diuron. Detector sensitivity, 1 × 10.

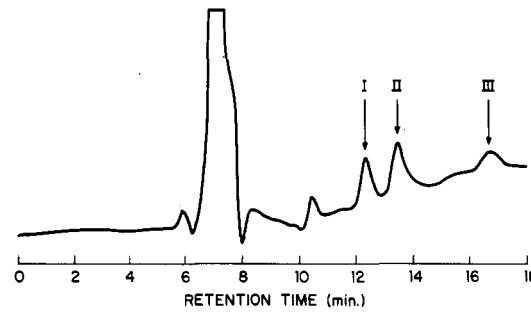

Figure 5. Soybeans fortified with 50 ppb DCPMU (I), DCPU (II), and DCA (III). Detector sensitivity, 1 × 10.

The recoveries from soybeans were good for all of the compounds.

The chromatograms of extracts of soybeans are shown in Figures 4 and 5. The recoveries of diuron, linuron, and neburon were 98%, 106%, and 87%, respectively, whereas with DCPMU, DCPU, and DCA the recoveries were 100%, 102%, and 84%, respectively.

Diuron can be resolved from DCPMU and DCPU by using a weaker mobile phase. The following mobile phase was tested: 900 mL of isooctane, 50 mL of methanol, 50 mL of 2-propanol, 3 mL of glacial acetic acid. However, when this mobile phase was used, the response factors of all the compounds dropped dramatically and the retention times increased. Retention times and response factors for all of the compounds in the weaker mobile phase are as follows: neburon, 13.6 min, 3.0 mm/ng; linuron, 14.8 min, 2.8 mm/ng; DCPMU, 20.3 min, 2.9 mm/ng; diuron, 21.4 min, 3.2 mm/ng; DCPU, 23.9 min, 3.4 mm/ng; DCA, 48.8 min, 1.0 mm/ng. With these response factors, recoveries are still possible at levels as low as 50 ppb. If a lower level must be analyzed, a slightly stronger mobile phase can be selected to increase the response of the photoconductivity detector. Also, larger samples can be injected onto the

HPLC column. A third alternative is to process larger samples, and a fourth is to concentrate the sample extracts to volumes less than 5 mL.

CONCLUSION

Residues of diuron can be detected and measured in soybeans, carrots, and potatoes with good accuracy, and it is likely that the method is applicable to other crops also. Two related urea herbicides, linuron and neburon, as well as three metabolites of diuron, can be detected and measured at 50 ppb in soybeans. DCA, in particular, presents problems in carrots and potatoes.

Registry No. DCPMU, 3567-62-2; DCA, 95-76-1; DCPU, 2327-02-8; diuron, 330-54-1; linuron, 330-55-2; neburon, 555-37-3.

LITERATURE CITED

Brinkman, U. A. Th.; De Kok, A.; Geerdink, R. B. *J. Chromatogr.* **1984**, *283*, 113.
Büttler, B.; Hörmann, W. D. *J. Agric. Food Chem.* **1981**, *29*, 257.
Farrington, D. S.; Hopkin, R. G.; Ruzicka, J. H. A. *Analyst* **1977**, *102*, 377.
Lawrence, J. F. *J. Assoc. Off. Anal. Chem.* **1976a**, *59*, 1061.
Lawrence, J. F. *J. Assoc. Off. Anal. Chem.* **1976b**, *59*, 1066.
Nash, R. G. *Agron. J.* **1968**, *60*, 177.
Nielen, M. W. F.; Koomen, G.; Frei, R. W.; Brickman, U. A. Th. *J. Liq. Chromatogr.* **1985**, *8*, 315.
Onley, J. H.; Yip, G. *J. Assoc. Off. Anal. Chem.* **1969**, *52*, 526.
Onley, J. H.; Yip, G.; Aldridge, M. H. *J. Agric. Food Chem.* **1968**, *16*, 426.
Osgood, R. V.; Romanowski, R. R.; Hilton, H. W. *Weed Sci.* **1972**, *20*, 537.
Slates, R. V. *J. Agric. Food Chem.* **1983**, *21*, 113.
Walters, S. M. *J. Chromatogr.* **1983**, *259*, 227.
Walters, S. M.; Gilvydis, D. M. *LC Mag.* **1983**, *1*, 302.
Walters, S. M.; Westerby, B. C.; Gilvydis, D. M. *J. Chromatogr.* **1984**, *317*, 533.
Zahnow, E. W. *J. Agric. Food Chem.* **1982**, *30*, 854.
Zahnow, E. W. *J. Agric. Food Chem.* **1985a**, *33*, 479.
Zahnow, E. W. *J. Agric. Food Chem.* **1985b**, *33*, 1206.

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Linear Furocoumarin Accumulation in Celery Plants Infected with *Erwinia carotovora* pv. *carotovora*

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Four linear furocoumarins (psoralens), xanthotoxin, psoralen, bergapten, and isopimpinellin, were isolated from celery (*Apium graveolens* L.) infected with *Erwinia carotovora* pv. *carotovora* (Ecc). High levels of the four compounds were found in rotted areas (22 ppm of fresh weight) and also in leaves (5 ppm) and healthy portions of stalks of diseased plants (17 ppm), whereas the total linear furocoumarin content of healthy plants was 0.7 ppm for stalks and 2.3 ppm for leaves. Psoralen accumulation was induced only when Ecc was inoculated on metabolizing celery plants. The bacterium itself did not produce any linear furocoumarin.

Sclerotinia sclerotiorum diseased celery (pink rot) was found to cause a bullous dermatitis in people working in celery (*Apium graveolens* L.) fields (Birmingham et al., 1961). The skin disorders (erythema followed by blistering and peeling of the skin) were caused, in association with ultraviolet irradiation (320–370 nm), by certain photosensitizing agents named psoralens (linear furocoumarins) accumulated in the tissues of diseased plants (Scheel et al., 1963).

Three linear furocoumarins were isolated from diseased celery: 8-methoxypsoralen (xanthotoxin) (Floss et al., 1969; Scheel et al., 1963; Wu et al, 1972); 4,5′,8-trimethoxypsoralen (trisoralen) (Floss et al., 1969; Scheel et al., 1963); 5-methoxypsoralen (bergapten) (Wu et al., 1972). Traces of 5,8-dimethoxypsoralen (isopimpinellin) and psoralen, in addition to xanthotoxin and bergapten, were also found in healthy celery (Beier et al., 1983; Musajo et al., 1964; Innocenti et al., 1976). Moreover, a linear furocoumarin phytoalexin response was observed in stressed celery (Beier and Oertli, 1983).

Recently, we reported on a bullous dermatitis suffered by workers handling celery plants infected by a plant pathogenic bacterium: *Erwinia carotovora* pv. *carotovora* (Ecc) (Varvaro et al., 1984; Surico et al., 1985). By the extraction procedure of Scheel et al. (1963) two phototoxins were isolated from artificially or naturally diseased tissues: xanthotoxin and bergapten. To obtain more information on psoralen production in plants infected with Ecc, this effect was examined in greater detail and quantified by high-performance liquid chromatography. Moreover, the study was extended to determine the accumulation of psoralens in celery parts other than diseased tissues and in liquid media inoculated with Ecc. A parallel study on the effect of elicitor preparations from Ecc on cell suspension cultures of celery is under way, and the results will be reported separately.

MATERIALS AND METHODS

Plant Material. Mature celery plants of the variety Elne were purchased from growers, placed in plastic bags, and kept cool during transport to the laboratory. Plants were used no later than 3 h after harvesting. Celery

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