Journal of Chromatography, 317 (1984) 533–544
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 425

DETERMINATION OF PHENYLUREA PESTICIDES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV AND PHOTOCONDUCTIVITY DETECTORS IN SERIES

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SUMMARY

High-performance liquid chromatographic (HPLC) separations of eighteen phenylurea pesticides were investigated using both reversed-phase and normal-phase systems. A photoconductivity detector, which responds selectively to ionic products formed via postcolumn UV irradiation of photolabile analytes, was connected in tandem with a UV detector permitting serial dual detection of these compounds. The photoconductivity detector responded selectively to the thirteen halogen and sulfur containing compounds whereas the UV detector responses were of the same order of magnitude for all eighteen compounds at 250 nm. The HPLC–UV–photoconductivity detection system was successfully applied to the determination of chloroxuron in strawberries at the official tolerance level of 0.5 ppm. The tandem detectors combined with a choice of columns and chromatographic modes offers enhanced selectivity for the HPLC analysis of these pesticides as trace contaminants in complex samples.

INTRODUCTION

Various phenylurea compounds are approved for agricultural usage primarily as herbicides, though applications as an insecticide (diflubenzuron) and as a plant growth regulator (thiadiazuron) are also indicated[1]. Because of their thermal lability in direct gas chromatographic analysis, Lawrence[2] investigated the use of high-performance liquid chromatography (HPLC) with UV detection for six of these compounds as residues in foods. Applications of HPLC–UV to linuron in water and soil have also been reported[3,4].

Selectivity as well as sensitivity are desirable in the analysis of foods and other complex matrices for residues of chemical contaminants. A previous evaluation of a commercial HPLC photoconductivity detector indicated that this detector is adequately sensitive and selective for residue analysis of pesticides which photodegrade to form ionic products[5]. Photoconductivity detection (PCD) was subsequently applied successfully to the analysis of three chlorine- and sulfur-containing fungicides in fresh produce[6].

0021-9673/84/$03.00

Since many phenylurea pesticides contain either halogens or sulfur, the utility of HPLC–PCD for their analysis appeared practical. Good PCD sensitivity was previously observed for three chlorinated phenylurea herbicides[5]. Eighteen standard reference materials for these pesticides were found listed in the EPA index of available compounds[1] and were subsequently acquired. Since it could be predicted that some of these compounds would give very little response in PCD but that all would respond reasonably well to UV detection, a UV detector was connected in series ahead of the photoconductivity detector.

The first step in the present study was to investigate the HPLC separation of the compounds. While most of the HPLC–PCD work done in the authors' laboratory has been with reversed-phase systems, applications of PCD to pesticide analysis with normal-phase systems have been reported by other workers[7,8,9]. Thus, both reversed-phase and normal-phase chromatography were evaluated in this study. The relative sensitivity, and hence the selectivity, of PCD to the phenylurea compounds were subsequently determined.

EXPERIMENTAL

HPLC instrumentation

The instrumental system consisted of a Perkin-Elmer Series 3B reciprocating pump, a Waters WISP 710B autosampler, a Kratos Model 773 variable-wavelength UV detector and a Tracor Model 965 photoconductivity detector with a 254-nm mercury irradiation lamp. The photoconductivity detector flow splitter was adjusted to give an even split of the column effluent through the analytical and reference compartments of the detector. The UV detector was connected directly to the HPLC column and the photoconductivity detector was connected to the UV detector outlet with 0.009-in. I.D. stainless-steel tubing. The detectors were connected to separate Heath-Zenith Model SR-204 recorders, each set at 10 mV output and 0.1 in./min chart speed.

Columns and mobile phases

Columns used included (1) a 25 cm × 4.6 mm I.D. column packed with ODS-bonded, 6-μm spherical silica (Zorbax ODS; DuPont, Wilmington, DE, U.S.A.); (2) a 10 cm × 4.6 mm I.D. column packed with ODS-bonded, 3-μm spherical silica (Microsorb C_{18}; Rainin Instrument, Emeryville, CA, U.S.A.); and (3) a 25 cm × 4.6 mm I.D. column packed with cyano-bonded, 6-μm spherical silica (Zorbax CN; DuPont).

Mobile phases consisted of methanol–water mixtures (reversed phase) and isooctane–methanol–2-propanol mixtures (normal phase). Methanol and water were pretreated by circulating through the ion-exchange resins supplied with the photoconductivity detector for at least 24 h prior to use; this was found to improve significantly the responsiveness of the photoconductivity detector. Acetonitrile–water was avoided for reversed-phase chromatography since the baseline stability of both the photoconductivity detector and the UV detector has previously been found[5] to deteriorate as a result of circulating this solvent through the resins. Circulation of the normal-phase eluent through the resins also increased baseline noise and drift and was, therefore, omitted. All organic solvents were from Burdick & Jackson

(Muskegon, MI, U.S.A.). Water was obtained from a Millipore (Bedford, MA, U.S.A.) Milli-Q system.

Standard solutions

Reference standards of all eighteen compounds were obtained from the U.S. Environmental Protection Agency (Research Triangle Park, NC, U.S.A.). Standard solutions were prepared in methanol for reversed-phase chromatography and in isooctane–methanol–2-propanol (90:5:5) for normal-phase chromatography.

Procedure

Separation of a mixture of the compounds was attempted on the ODS columns by reversed-phase chromatography, and on the CN column in both the reversed- and normal-phase modes. Peak identities were determined by comparing retention times with standards injected individually. PCD peak height responses obtained using the ODS columns were measured and compared. The HPLC–UV–PCD system in reversed-phase mode was applied to a fortified strawberry extract.

RESULTS AND DISCUSSION

Reversed-phase separation

ODS- and CN-bonded columns were selected for study on the basis of their differing polarities. The two ODS columns selected were compared largely as a matter of curiosity with respect to their different particle sizes and manufacturing processes, *i.e.*, the 3-μm particle column is reportedly end-capped with trimethylsilane to minimize possible exposure of silanol groups, whereas the 6-μm particle column is not.

Isocratic elution with methanol–water mixtures (50:50) gave separations of different quality on all three columns, but retention times of late-eluted compounds were excessive under these conditions. Gradients were therefore applied to achieve elution of all compounds within a reasonable time (<30 min) at a flow-rate of 1.0 ml/min. The PCD response decreases significantly with increased flow-rate due to decreased photolytic reaction time, and, thus, a compromise between speed, response, and baseline stability must be found[5].

Good separation of all eighteen compounds was achieved on the two ODS columns (Figs. 1 and 2). As predicted, the degree of separation was not significantly different, since the calculated theoretical plate count [based on the linuron peak with capacity factor (k') $\approx$ 5] was about the same ($N \approx 11{,}000$) for both columns. (The peaks are identified in Table I). The separation on the 10-cm, 3-μm particle column was improved as a result of raising the temperature to 35°C. A higher methanol content was required for elution of the compounds in a comparable volume from the 25-cm, 6-μm particle column. This was obviously due in part to the greater length and lower operating temperature of the latter, but was perhaps also due to a heavier stationary phase coating typically applied to Zorbax packings. The more notable difference in these columns is the shift in elution order for monuron (3), fluometuron (7), and siduron (14) which may be attributable to differences in manufacturing processes.

The compounds were less retained on the CN column (Fig. 3). A visible separation of only fourteen peaks was achieved on this column, which had a smaller

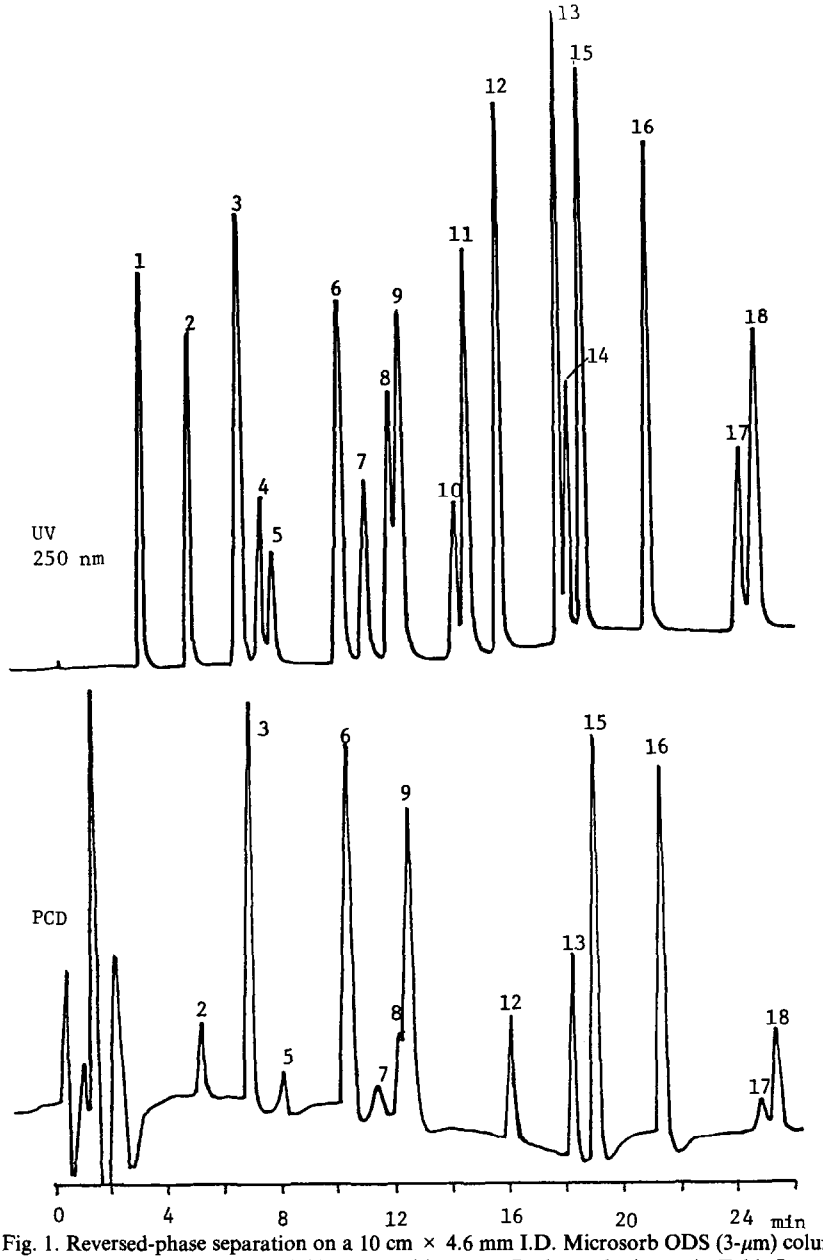

Fig. 1. Reversed-phase separation on a 10 cm × 4.6 mm I.D. Microsorb ODS (3-μm) column at 35°C by a concave gradient from 48 to 65% methanol in water. Peak numbering as in Table I.

calculated theoretical plate count (N ≈ 7300). A significant shift in peak elution order was observed, however, for at least five of the compounds.

Normal-phase separation

Solvents that can be used in mobile phases for HPLC with PCD are necessarily

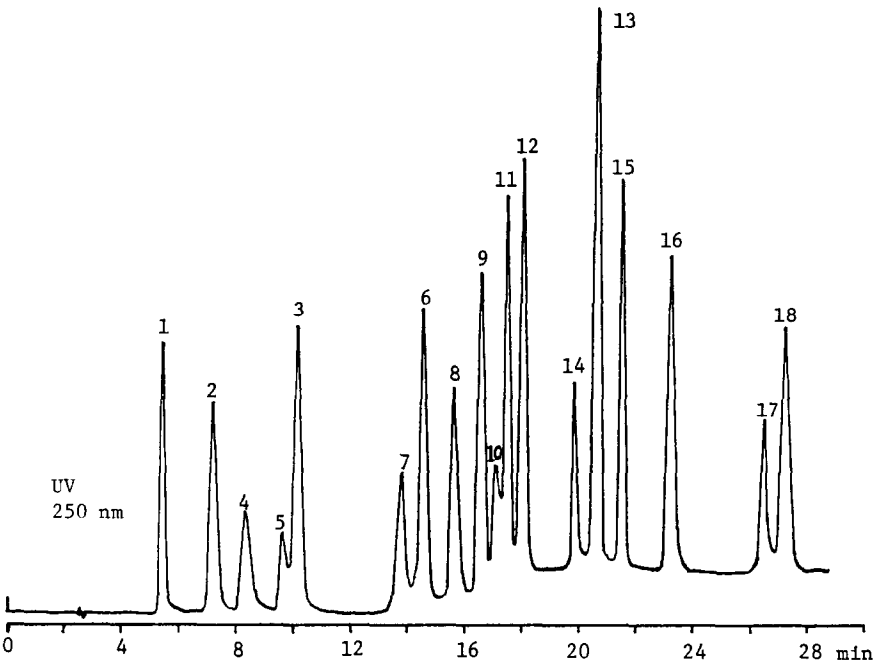

Fig. 2. Reversed-phase separation on a 25 cm × 4.6 mm I.D. Zorbax ODS (6-μm) column at 25°C by a concave gradient from 60 to 75% methanol in water. Peak numbering as in Table I.

TABLE I

RELATIVE PCD PEAK HEIGHT RESPONSES ON MICROSORB ODS COLUMN

100 ng of each compound injected.

Peak No.	*Pesticide*	*Halogen or sulfur bonds present*	*Relative response (Chlorobromuron = 100)*
1	Fenuron	None	0
2	Metoxuron	C–Cl	18
3	Monuron	C–Cl	93
4	Karbitulate	None	0
5	Thiadiazuron	C–S–N	10
6	Monolinuron	C–Cl	85
7	Fluometuron	C–F_3	8
8	Chlortoluron	C–Cl	19
9	Metobromuron	C–Br	73
10	Difenoxuron	None	0
11	Isoproturon	None	0
12	Diuron	C–Cl (2)	29
13	Linuron	C–Cl (2)	48
14	Siduron	None	0
15	Chlorbromuron	C–Cl, C–Br	100
16	Chloroxuron	C–Cl	86
17	Diflubenzuron	C–Cl, C–F (2)	7
18	Neburon	C–Cl (2)	25

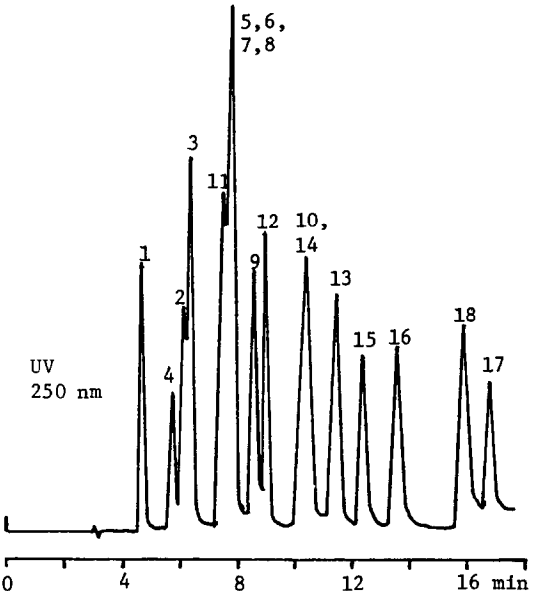

Fig. 3. Reversed-phase separation on a 25 cm × 4.6 mm I.D. Zorbax CN (6-μm) column at 25°C by a concave gradient from 55 to 65% methanol in water. Peak numbering as in Table I.

limited by their susceptibility to photoionization, *e.g.*, halogenated solvents are not recommended. Mobile phases which have been used for HPLC–PCD applications in the normal-phase chromatographic mode include isooctane[6,10] and cyclohexane[8,9] as primary solvents with methanol and 2-propanol added as polar modifiers in all cases. 2-Propanol is used to effect miscibility of methanol with the non-polar solvents. Isooctane–methanol–2-propanol was therefore used in the present study.

The Zorbax CN column is described by the manufacturer as a versatile column, which can be used for normal- as well as reversed-phase chromatography, *i.e.*, when used with relatively non-polar solvents, its separation properties are similar for many polar compounds to non-bonded silica. A good separation of thirteen peaks for the eighteen compounds was achieved in 28 min on this column by isocratic elution with isooctane–methanol–2-propanol (90:5:5) (Fig. 4). The elution order was shifted considerably and several of the peaks which had been eluted early in the reversed-phase systems appeared late in the normal-phase chromatogram and *vice versa*.

Detector responses

For the purpose of investigating the chromatographic separations, 10-μl injections, containing *ca.* 100 ng of each pesticide, were made in each case. The UV spectra of the eighteen phenylurea pesticides, which have the common basic structure $C_6H_5NHCON\lessgtr$, all have a maximum between 245 and 250 nm. The UV detector was therefore set at 250 nm. At this wavelength, the peak height responses obtained were within the same order of magnitude for all eighteen compounds.

A PCD chromatogram of the standard mixture is shown in Fig. 1 to illustrate the relative responses of the eighteen pesticides in the photoconductivity detector. Some peak resolution is lost in the photoconductivity detector due to bandbroad-

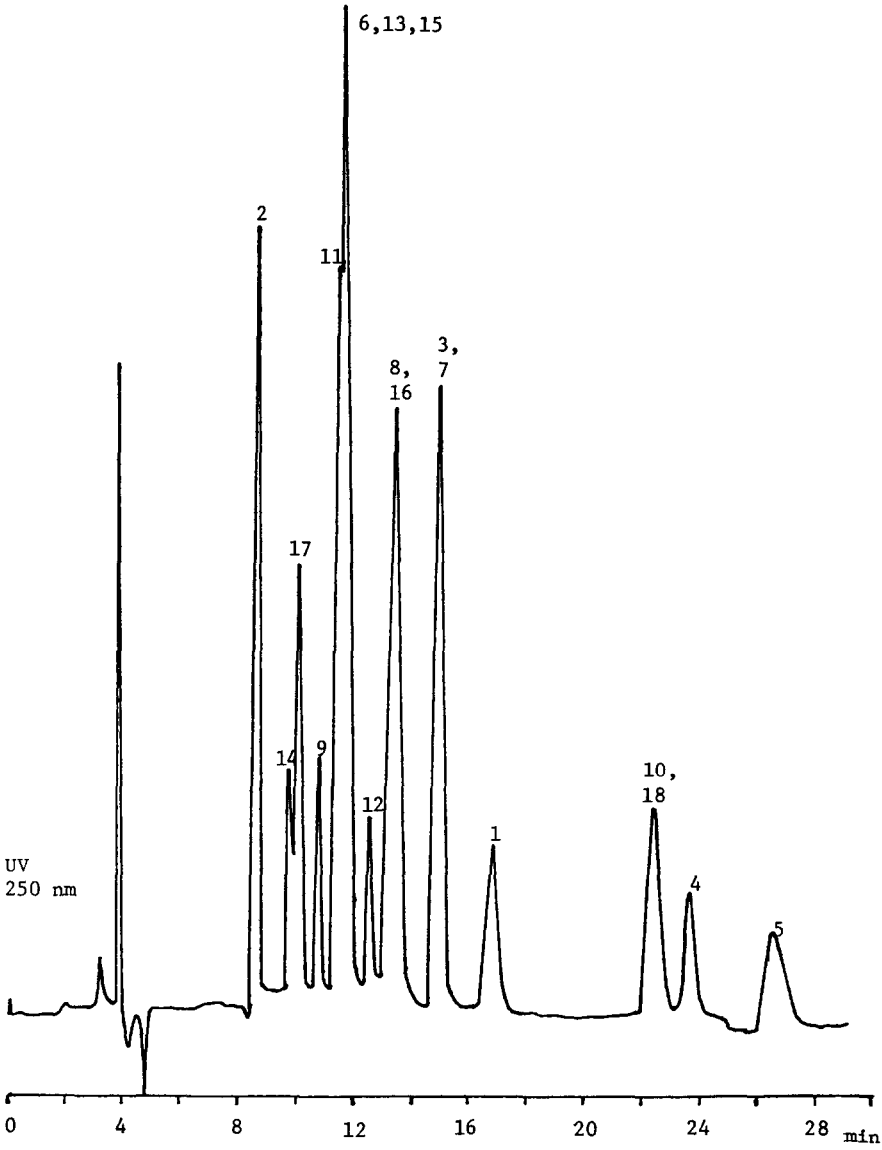

Fig. 4. Normal-phase separation on a 25 cm × 4.6 mm I.D. Zorbax CN (6-μm) column at 25°C using isocratic elution with isooctane–methanol–2-propanol (90:5:5). Peak numbering as in Table I.

ening, which occurs in the reaction and detection chambers. Only the thirteen compounds containing halogens or sulfur gave any response in the photoconductivity detector at the 100-ng level (Table I). As previously observed[5], brominated compounds typically respond well presumably due to lower bromide-bond strength. The responses of fluorinated compounds were relatively small, which corresponds to higher fluoride-bond strength. The response of thiadiazuron, which contains no halogens, can most likely be attributed to the sulfur bond. Bond strengths are also affected by

molecular configuration (*i.e.*, positions of the reactive groups in the molecule relative to electron-withdrawing substituents, etc.) and relative responses of different compounds are therefore difficult to predict.

Using the methanol–water mobile phase at a flow-rate of 1.0 ml/min, it was observed that the photoconductivity detector gave a 50% of full-scale recorder deflection (f.s.d.) for low-ng levels of strongly photoionized compounds (setting of range 10, attenuation × 5) (Table II). The amounts of these compounds required to obtain the same response in the UV detector were of the same order of magnitude at a sensitivity setting of 0.005 a.u.f.s. At these sensitivity settings, both detectors began to show slight (< 1%) baseline noise. A greater than 100-fold increase in concentration of isoproturon was required for an equivalent PCD response. Nitrogen compounds which do not contain an N–O bond do not give a strong PCD response, and none of the compounds studied herein contain this bond. Thus, the sensitivity of the photoconductivity detector for the halogen- and sulfur-containing phenylurea pesticides is 2 to 3 orders of magnitude higher than that for compounds lacking these groups.

The photoconductivity detector is very sensitive to solvent and pressure changes, and therefore the baseline is affected rather drastically by gradient elutions. As the chromatogram in Fig. 1 indicates, the photoconductivity detector baseline is somewhat unstable at a sensitivity setting of range 10, attenuation × 50. At the higher sensitivites generally used for residue analysis (*e.g.*, range 10, attenuation × 5), the use of gradient elution with the photoconductivity detector is not considered very practical.

The use of normal-phase chromatography with the photoconductivity detector is complicated by the generally low polarity of the mobile phases used in this mode. The low polarity of these solvents presumably creates poor ion mobility and hence poor charge transfer in the conductivity cell; this results in short, broad, tailing peaks. This problem was observed in our earlier work[5] with the photoconductivity detector when it was found that saturating the isooctane–methanol–2-propanol mixture with water (*ca.* 0.15%) improved the peak shape significantly. The PCD chromatogram obtained with the normal-phase solvent [isooctane–methanol–2-propanol (90:5:5)] in contrast to the UV detector chromatogram in Fig. 4 was an unresolved mass of broad, tailing peaks (not shown). In the recently reported HPLC–PCD applications in which normal-phase chromatography on silica gel was used[8,9], *ca.* 1% acetic acid

TABLE II

COMPARISON OF AMOUNTS PRODUCING 50% FULL-SCALE DEFLECTION IN UV AND PHOTOCONDUCTIVITY DETECTORS

Zorbax CN column in reversed-phase mode.

Pesticide	*Amount equivalent to 50% f.s.d. (ng)*	
	UV (250 nm, 0.005 a.u.f.s.)	*PCD (range 10, attenuation × 5)*
Monolinuron	14	7
Thiadiazuron	53	36
Isoproturon	44	5500

was added to the mobile phases for the purpose of reducing the tailing on silica gel[8]. We, therefore, proceeded to add 1% acetic acid to our mobile phase and compared the UV and PCD responses to linuron before and after this addition (Fig. 5). The photoconductivity detector peak shape improved dramatically, whereas the UV detector peak shape remained the same. Thus, we concluded, as before[5], that the abnormal photoconductivity detector peak shape in relatively non-polar mobile phases is a result of the conductivity cell response rather than chromatography. The reduced tailing observed in the aforementioned report[8] may, in fact, be largely attributable to an improved PCD response after the polarity of the mobile phase is increased with acetic acid. We found that for this purpose, adding acetic acid was more effective than adding water due in part to the fact that acetic acid is far more miscible than water with the mobile phase and thus the concentration can be increased. One disadvantage of adding acetic acid is that photoconductivity detector background noise

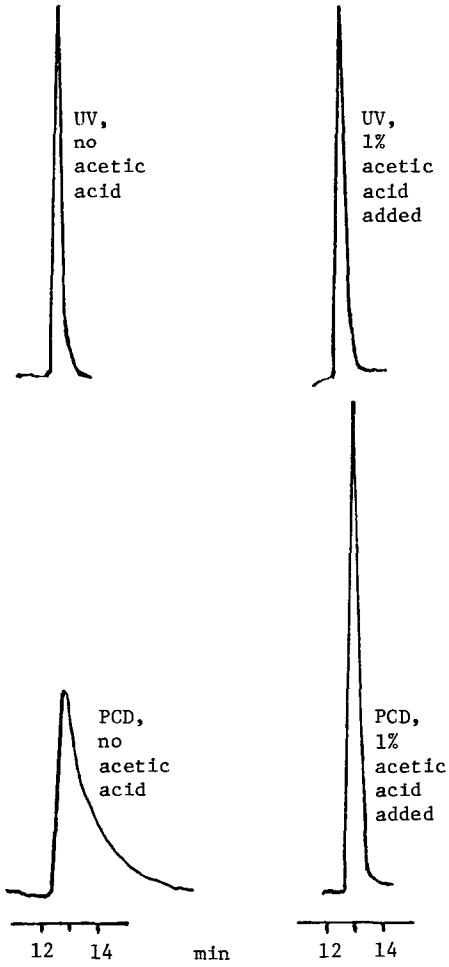

Fig. 5. Effect of adding acetic acid to isooctane–methanol–2-propanol (90:5:5) normal-phase eluent on responses of detectors (20 ng linuron injected in each case).

is also increased, and this ultimately diminishes the signal-to-noise output of the photoconductivity detector in the normal-phase mode. The detection limits with the acidified normal-phase solvent were roughly comparable to those obtained in the reversed-phase mode.

From the standpoint of signal-to-noise ratio, a better approach to modifying normal-phase solvents is to increase the polar alcohol content. By substantially increasing the alcohol content to *ca.* 50%, the photoconductivity detector could be operated at maximum sensitivity (range 1, attenuation × 1) with normal shaped peak responses and very little baseline noise (< 1%). As a result, another ten-fold or larger reduction in detection limit appeared possible (*e.g.*, 50% f.s.d. for *ca.* 0.5 ng monuron). However, column retention and resolution of the compounds are vir-

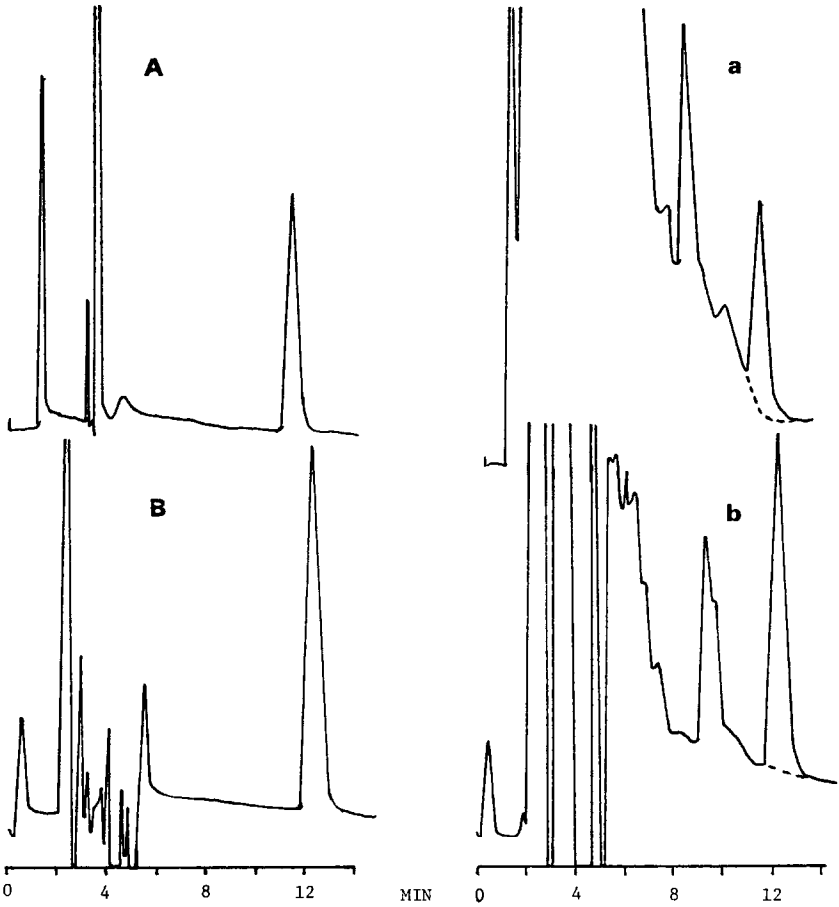

Fig. 6. HPLC–UV–PCD determination of chloroxuron in crude strawberry extract, fortified at 0.44 ppm. Zorbax CN column at 25°C used with methanol–water (55:45) at 1 ml/min. UV (A) and PCD (B) responses, respectively, to 12.5 ng chloroxuron standard in 10 μl methanol; UV (a) and PCD (b) responses, respectively, to 10-μl extract equivalent to 26 mg sample in methanol. UV detector at 245 nm and 0.01 a.u.f.s.; photoconductivity detector at range 10, attenuation × 5. Dashed lines indicate responses due to non-fortified extract.

tually destroyed with increasing alcohol content which makes this approach impractical.

Although not employed in this study, a 214-nm zinc irradiation lamp is also supplied with the photoconductivity detector. The intensity of this lamp is less than the 254-nm lamp but further selectivity in response can be achieved in some cases by substituting this lamp in the reaction chamber[5].

Application to sample extract

Tolerances for phenylurea pesticides have been established for certain commodities, *e.g.*, chloroxuron is permitted in fresh strawberries up to 0.5 ppm. A crude extract of fresh strawberries, which had been prepared previously for pesticide residue screening by the method of Luke *et al.*[10], was fortified at 0.4 ppm with standard chloroxuron, and 10 μl was chromatographed on the CN column with a methanol–water (55:45) mobile phase (Fig. 6). Lawrence[2] used the multi-residue method of Luke *et al.*[10], followed by a Florisil column clean-up, to achieve HPLC–UV quantitation of urea herbicides in foods with recoveries of $\geqslant 80\%$. The chromatograms in Fig. 6 were obtained without Florisil clean-up of the extract. (The extract was filtered through a 0.2-μm porosity membrane prior to injection). Though more sample background was present in the UV than in the photoconductivity detector chromatogram, quantitation of chloroxuron was possible with both detectors in this case. Interferences from sample co-extractives will vary with different commodities, and quantitation of phenylurea pesticides in crude extracts may not be possible in many cases without further clean-up of the extracts. In any case, additional clean-up is a good practice for multiple sample analysis, since column deterioration and/or detector contamination will likely occur more rapidly with crude extracts.

CONCLUSIONS

HPLC with a choice of columns and mobile phases offers stable and selective chromatographic separation of eighteen phenylurea pesticides. Good sensitivity to all of these compounds is provided by state-of-the-art liquid chromatography–UV detection at 250 nm, but the selectivity of the UV response is less than desirable for trace analysis in complex samples. The photoconductivity detector is only sensitive to those compounds which undergo photolysis to form strong ions, thus offering selective detection for thirteen of the 18 compounds studied which contain halogen or sulfur bonds. Tandem operation of the photoconductivity detector and UV detector with a choice of columns and chromatographic modes (*i.e.*, reversed phase or normal phase) significantly enhances the utility of HPLC for the analysis of these pesticides as trace contaminants in plant extracts and other complex matrices relative to the use of a single detector and chromatographic system. In addition to the obvious requirements that the mobile phases used with PCD must be transparent to UV light and must not undergo photoionization, it is apparent that they must also be sufficiently polar to facilitate free ion formation and efficient charge transfer in the photoconductivity process.

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