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Determination of Residues of Substituted Phenylurea Herbicides in Grain, Soil and River Water by Use of Liquid Chromatography

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A rapid and sensitive procedure is described for the determination of trace amounts of eight substituted phenylurea herbicides. High-performance liquid chromatography is used, with an ultraviolet spectrophotometric detector. The herbicides are extracted from grain and soil samples with methanol and from water samples with dichloromethane. They are chromatographed on microparticulate silica bonded with octadecyltrichlorosilane using a mixture of methanol, water and ammonia as mobile phase.

Keywords: *Herbicide residues determination*; *phenylurea herbicides*; *high-performance liquid chromatography*

Substituted phenylurea compounds (urons) are widely used as selective herbicides in agriculture[1] and consequently can give rise to residues in crops. In addition, some herbicide can be absorbed by the soil and can be leached out into river water. Methods for the determination of residues of phenylurea herbicides have been based on gas - liquid chromatographic or colorimetric procedures.[2] Gas - liquid chromatography of phenylurea herbicides is difficult because of their ease of thermal decomposition. Procedures have been reported in which careful control of conditions allows these compounds to be chromatographed intact.[3,4] Alternatively, the urons can be hydrolysed to the corresponding substituted anilines; these compounds are then determined by either gas - liquid chromatography, directly[5] or as derivatives,[6] or colorimetrically[7] after coupling with a suitable chromophore. Methods based on hydrolysis lack specificity and involve lengthy procedures.

These disadvantages can be overcome by using liquid chromatography. Following the earlier work of Kirkland[8] on phenylurea herbicides, Sidwell and Ruzicka[9] applied liquid chromatography to the identification and determination of active ingredient contents of phenylurea herbicide formulations. Smith and Lord[10] have used liquid chromatography for the determination of chlortoluron residues in soil, but diuron and monuron interfered in their chromatographic system. It was thought that the technique of Sidwell and Ruzicka could form the basis of a method for routine monitoring. By modification of the mobile phase a technique has been devised for the direct determination of residues of chlorbromuron, chlortoluron, chloroxuron, diuron, linuron, metobromuron, monolinuron and monuron in grain, soil and river water.

Experimental

Apparatus

Liquid chromatograph. A Waters Associates constant-volume solvent-delivery system, Model 6000, was used. A variable-wavelength ultraviolet monitor (Cecil Instruments, Model CE 212), fitted with a 10-μl flow cell and set at 240 nm was used as a detector.

Preparation of column. A stainless-steel column tube, 300 × 4.6 mm i.d., was washed with chloroform and methanol and polished on the inner surface. One end was fitted with a $\frac{1}{4}$ × $\frac{1}{16}$ in Crawford Patent column end fitting and the other was coupled to a 400-mm pre-column reservoir through a $\frac{1}{4}$ × $\frac{1}{4}$ in Swagelock union. A slurry of 5-μm Spherisorb ODS (Phase Separations Ltd.) was packed into the column by releasing 5 000 lb in^{-2} of solvent (acetone) pressure. The column was prepared for stop-flow injection by removing the top few milli-

metres of packing and inserting a disc of stainless-steel fine-mesh gauze, of 8-μm nominal porosity, a plug of silanised glass-wool and a top plug of porous PTFE.

Sample injection. A Varian Associates stop-flow injector was used and samples were injected on to the stainless-steel fine-mesh gauze fitted on top of the packing. A needle guide was incorporated in the injector to ensure that samples were introduced on to the centre of the top of the column.

Rotary evaporator. This is used with a water-bath that can be maintained at 55 °C.

Reagents

All reagents should be of analytical-reagent grade unless otherwise specified.

Dichloromethane. Laboratory-reagent grade.

Methanol.

Methanol. Spectrograde (Fisons).

Mobile phase. Prepare a solution of 60% methanol in water and add 0.6% *V*/*V* of ammonia solution (sp. gr. 0.88).

Sodium sulphate, anhydrous, granular.

Uron standard solutions. Prepare a standard solution in Spectrograde methanol containing 0.25 mg l^{-1} of phenylurea herbicide; dilute as necessary.

Procedure

Grain

Grind a 50-g sample and transfer it into a 500-ml flat-bottomed flask. Add 100 ml of methanol and shake on a wrist-action shaker for 1 h. Filter the resulting slurry through a Whatman No. 1 filter-paper using reduced pressure. Wash the flat-bottomed flask with 50 ml of methanol and add the washings to the filter-funnel, leave for 3–5 min, then apply the reduced pressure. Repeat the washing procedure with a further 50 ml of methanol. Combine the extract and washings and then remove the methanol using a rotary evaporator with a water-bath at 55 °C. Dissolve the residue in dichloromethane, using a total volume of 50 ml, and pass the dichloromethane extracts through a column of anhydrous sodium sulphate (50 g). Wash the sodium sulphate with 50 ml of dichloromethane, combine the extract and washings and evaporate to dryness at 55 °C in a rotary evaporator. Cool the flask and add 5.0 ml of methanol; swirl the flask to dissolve the residue and filter the solution through a Whatman No. 42 filter-paper. Using a flow-rate of the mobile phase of 0.6 ml min^{-1}, inject 5 μl of the sample solution into the liquid chromatograph. Calculate the uron content of the sample by comparing the peak height obtained with those obtained from 5-μl injections of standard solutions.

Soil

Air-dry a sample of soil and transfer 50 g into a 500-ml flat-bottomed flask. Extract and filter the sample using the method described under *Grain*. Remove the methanol by using a rotary evaporator with a water-bath at 55 °C. Cool the flask and add 5.0 ml of methanol, swirl to dissolve the residue and filter the solution through a Whatman No. 42 filter-paper. Using a flow-rate of 0.6 ml min^{-1}, inject 5 μl of extract into the liquid chromatograph and determine the uron content using the procedure described under *Grain*.

Water

Add 100 ml of dichloromethane to 1 l of river water in a 2-l separating funnel and shake for 30 s. Run off the dichloromethane and repeat the extraction twice, using 50-ml portions of dichloromethane. Dry the extracts by passing them through a column of anhydrous sodium sulphate (50 g) and wash the column with 50 ml of dichloromethane. Combine the extracts and washings and remove the dichloromethane in a rotary evaporator with a water-bath at 55 °C. Cool the flask and add 5.0 ml of methanol, swirl the flask to dissolve the residue and inject 5 μl of the solution into the chromatograph. Determine the uron content of the sample using the procedure described under *Grain*.

Results and Discussion

Methanol was chosen as the extraction solvent for wheat and soil because Khan *et al.*[4] found it to be the best solvent for extraction of urons from soil. Variable recoveries were obtained

from wheat samples when a Soxhlet extraction apparatus was used and this effect was attributed to breakdown of some of the uron herbicides by prolonged heating with methanol.[11] However, satisfactory recoveries were achieved consistently by using a wrist-action shaker. An extraction time of 1 h was chosen as this time has been reported as the optimum for soil.[4] Care was taken during the evaporation of solutions of urons in methanol to ensure that the temperature did not rise above 55 °C as it has been reported that at higher temperatures degradation occurs.[10]

TABLE I

RECOVERY OF URONS FROM FORTIFIED SAMPLES

Five determinations were carried out on each sample. Results given are percentage recoveries.

	Wheat									
	Fortified at 5 mg kg^{-1}		Fortified at 2 mg kg^{-1}		Fortified at 0.5 mg kg^{-1}		Soil, fortified at 2 mg kg^{-1}		Water, fortified at 0.1 mg kg^{-1}	
Uron	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Chlorbromuron	91.0–95.5	92.5	87.5–93.0	91.0	84.5–92.5	88.0	98.0–101.0	99.5	98.0–100.0	99.0
Chlortoluron	89.5–94.0	92.0	87.0–92.5	89.5	85.5–94.0	89.0	100.0–103.0	101.5	97.0–100.5	99.0
Chloroxuron	87.5–91.5	89.5	87.0–91.0	88.5	86.5–94.0	89.5	97.0–103.0	99.0	94.5–100.5	97.0
Diuron	89.0–94.5	91.5	88.0–90.5	89.0	87.0–92.5	89.0	98.0–100.5	99.5	97.5–102.0	100.0
Linuron	90.5–94.5	92.5	86.5–93.5	89.0	85.5–96.0	91.5	94.0–100.5	97.5	96.0–100.5	98.5
Metobromuron	90.5–95.5	92.0	89.5–93.5	91.5	84.0–93.0	86.5	96.5–101.0	99.5	96.5–100.5	99.0
Monolinuron	90.0–95.5	92.5	89.0–94.0	92.5	87.0–95.5	90.0	100.5–103.0	102.0	97.5–100.0	98.5
Monuron	87.0–94.5	90.0	91.0–97.0	94.5	89.0–95.5	92.5	99.0–102.0	100.5	98.0–100.5	99.5

The eluting agent originally used for liquid chromatography was 60% methanol in water, as recommended by Sidwell and Ruzicka.[9] This solvent gave adequate resolution of all eight urons but a clean-up procedure, based on that of Onley and Yip,[12] was required in order to remove interfering co-extractives. Even with the clean-up, sample extracts of wheat injected on to the chromatograph showed an interfering co-extractive peak that had the same retention

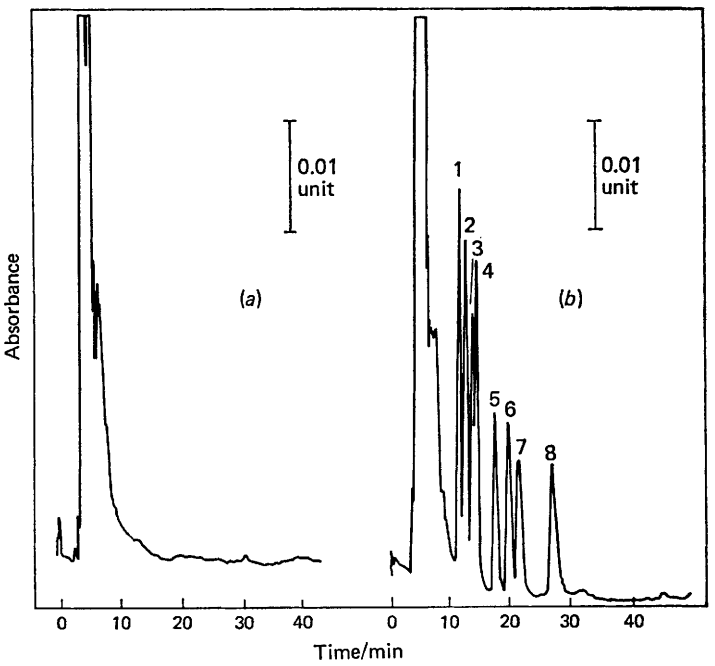

Fig. 1. Typical chromatograms obtained from 5-μl injections of wheat extracts: (*a*) unfortified; and (*b*) fortified with uron herbicides at 2 mg kg^{-1}. 1, Monuron; 2, monolinuron; 3, metobromuron; 4, chlortoluron; 5, diuron; 6, linuron; 7, chlorbromuron; and 8, chloroxuron.

time as chloroxuron. It was then found that the incorporation of a small amount of ammonia in the eluting agent solved the problem of co-extractive interference by moving the interfering peaks to a smaller retention volume while leaving the resolution of the urons unaltered. This

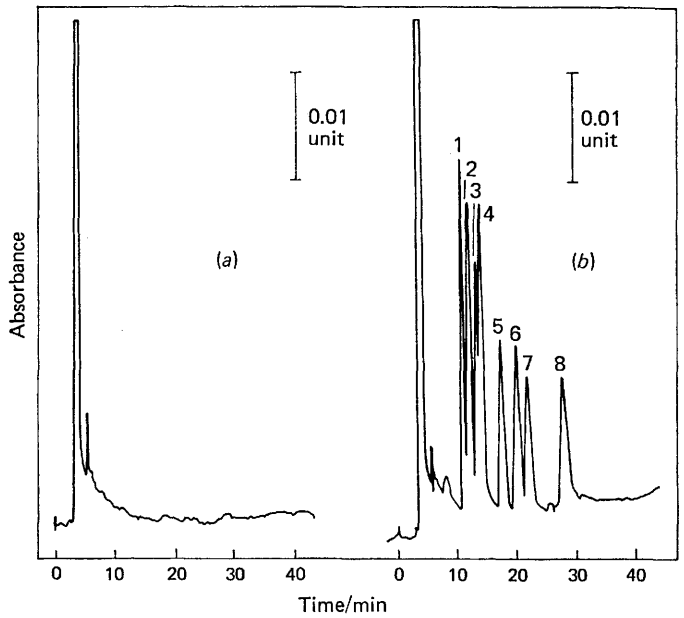

Fig. 2. Typical chromatograms obtained from 5-μl injections of soil extracts: (*a*) unfortified; and (*b*) fortified with uron herbicides at 2 mg kg^{-1}. 1, Monuron; 2, monolinuron; 3, metobromuron; 4, chlortoluron; 5, diuron; 6, linuron; 7, chlorbromuron; and 8, chloroxuron.

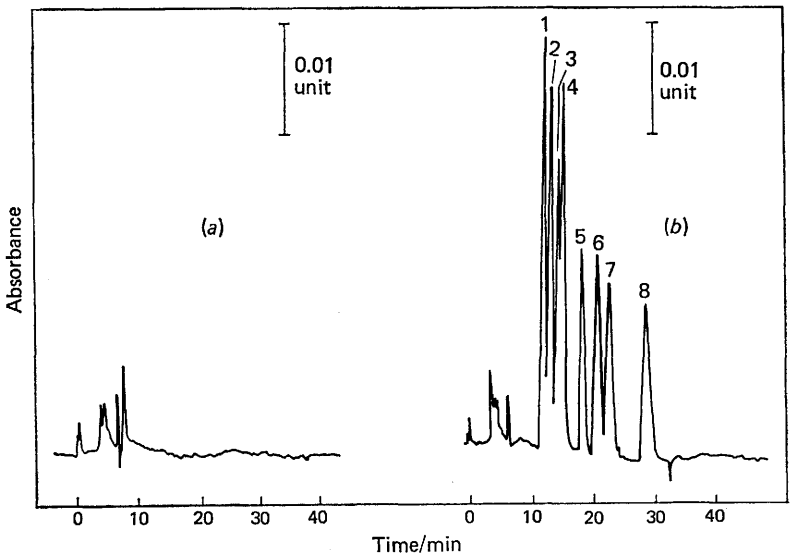

Fig. 3. Typical chromatograms obtained from 5-μl injections of river water extracts: (*a*) unfortified; and (*b*) fortified with uron herbicides at 0.1 mg kg^{-1}. 1, Monuron; 2, monolinuron; 3, metobromuron; 4, chlortoluron; 5, diuron; 6, linuron; 7, chlorbromuron; and 8, chloroxuron.

modification was subsequently applied to samples that had not been subjected to a clean-up procedure and it was found that satisfactory chromatographic traces could be obtained for all of the substrates, and thus the method could be simplified by incorporation of ammonia into the eluting agent. However, as the wheat extracts are oily, it is advisable to flush the column periodically with Spectrograde methanol to wash off any oils not eluted by the mobile phase.

The recoveries obtained for urons from samples of wheat, soil and water are shown in Table I. Samples of wheat and soil were fortified by adding known volumes of solutions containing (*a*) monuron, metobromuron, diuron and chlorbromuron or (*b*) monolinuron, chlortoluron, linuron and chloroxuron. The lower recoveries from wheat may be attributable to residual oil, which remains after the evaporation of the dichloromethane, increasing the volume of the solvent as this oil is miscible with methanol.

Typical chromatograms, obtained from extracts of wheat, soil and river water, are shown in Figs. 1–3. The lower limits of detection were estimated to be 0.2 p.p.m for wheat, 0.2 p.p.m. for soil and 0.01 p.p.m. for river water; below these levels both co-extractives and signal noise interfere.

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