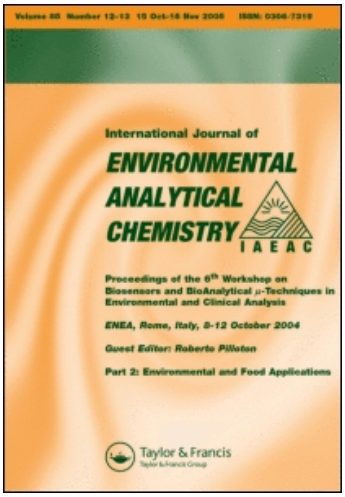

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The Use of Various Chromatographic Techniques for the Determination of Phenylurea Herbicides and Their Corresponding Anilines in Environmental Samples, II. Applications

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The Use of Various Chromatographic Techniques for the Determination of Phenylurea Herbicides and Their Corresponding Anilines in Environmental Samples,†‡

II. Applications

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In earlier work, various strategies have been developed for the trace-level determination of phenylurea herbicides and the anilines which are their main degradation products. They include catalytic hydrolysis of the phenylureas on silica, liquid chromatographic fractionation of complex mixtures of herbicides and anilines, derivatization of anilines and herbicides with electron-capture-sensitive reagents, and final analysis by means of capillary gas chromatography. In the present paper, the application of these principles to trace-level analysis of surface water, soil and crop samples is demonstrated.

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†This paper is part of the thesis of A. de Kok (present address: Food Inspection Service, Burgpoelwaard 6, Alkmaar, The Netherlands). For Part I, see ref. 5.

‡Part of this paper has been presented at the workshop "Handling of Environmental and Biological Samples in Chromatography", 24–25 November 1983, Lausanne (Switzerland).

INTRODUCTION

Among the present-day herbicides, nitrogen-containing compounds such as triazines, carbamates, phenylureas and anilides have a prominent place. The phenylureas, which are the object of our present study, are used as so-called pre- and post-emergence herbicides. Most ureas are strongly adsorbed by the soil, then absorbed by roots; some are taken up almost equally well by roots, shoots and leaves. Depending on e.g., herbicide structure, soil composition and weather conditions, the phenylureas show half-life periods of between several weeks and many months. Determination of herbicide residues in soil is important since knowledge about speed of degradation and, consequently, length of intervals between application of the herbicides, is required: delayed degradation of a herbicide used for one crop may well lead to damage of the next crop. Analysis in plant material is necessary since it will provide us with vital information about the mode of action of the compounds used. Residue analysis in surface water is primarily important within the framework of the generally occurring environmental pollution.

Trace-level determination of the phenylurea herbicides and their main—more toxic—degradation products, substituted anilines, unfortunately turns out to be particularly difficult. The main problems are as follows. Their rather polar nature and, often, their thermolability make the phenylureas unsuitable for direct gas chromatographic (GC) analysis. With the more polar anilines, neither direct GC nor direct column liquid chromatography (LC) can, as a rule, be recommended. Besides, the high polarity of the anilines creates problems during extraction (from e.g., soil) and clean-up procedures, with severe losses occurring due to irreversible adsorption. A further problem is the lack of detectors having a sensitivity comparable to that of the electron-capture detector (ECD) towards halogen-containing compounds. Finally, anilines are also widely used bulk chemicals and show up in the environment in industrial effluents and as degradation products of, e.g., other types of pesticides (carbamates, acylanilides) or aniline-based dyes. This, of course, hampers all analytical procedures—and there are several of these—involving conversion of the parent herbicides to their corresponding anilines with subsequent derivatization to products having suitable chromatographic and detection properties.

In previous papers,[1-5] a four-fold approach has been used to solve the above problems:

- heating of phenylureas on silica columns has been used instead of time-consuming reaction in aqueous solution to effect rapid hydrolysis of these herbicides;
- adsorption LC, with UV monitoring, has been studied for fractionation purposes, i.e., for the separation of groups of herbicides and/or anilines;
- derivation of the anilines with fluorinated acid anhydrides such as heptafluorobutyric anhydride (HFBA), with subsequent analysis by means of capillary GC-ECD has been optimized as a final separation and trace-level detection procedure;
- derivatization of the intact herbicides with HFBA has been combined with either GC-ECD or LC-ECD as an alternative method for phenylurea determination.

The combined methodology has been employed[5] to design various schemes of residue analysis for the phenylureas and corresponding anilines. In the present paper, the feasibility of the several approaches will be demonstrated for surface water, soil and crop samples. The names and structures of the phenylureas and anilines are given in Table I. For the sake of convenience, a summary of the various analytical procedures and schemes of analysis is included in the appropriate sections below.

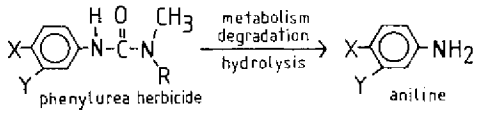
MATERIALS AND METHODS

Materials

The herbicides and 3-chloro-4-methoxyaniline were gifts from the Food Inspection Service (Amsterdam, The Netherlands) and Sandoz (Basle, Switzerland), respectively. The other anilines were purchased from Fluka (Buchs, Switzerland) and Aldrich (Beerse, Belgium) or prepared according to a published method.[5] All solvents were analytical-grade products from Baker (Deventer, The Netherlands) and were distilled before use. HFBA and silica (Kieselgel 60, reinst; 70–230 mesh) were obtained from Merck (Darmstadt, G.F.R.). Dimethylamine was purchased from Fluka.

TABLE I

Names and structure of phenylurea herbicides and anilines.



Herbicides		Substituents			Aniline
Name	Code	X	Y	R	Code†
Fenuron	Fe	H	H	CH_3	1
Isoproturon	Ip	$(CH_3)_2CH$	H	CH_3	5
Fluometuron	Fm	H	CF_3	CH_3	2
Monuron	Mo	Cl	H	CH_3	4
Chlortoluron	Ct	CH_3	Cl	CH_3	7
Diuron	Di	Cl	Cl	CH_3	9
Metoxuron	Mx	OCH_3	Cl	CH_3	10
Chloroxuron	Cx	4-Cl-C_6H_4O	H	CH_3	13
Difenoxuron	Dx	4-CH_3O-C_6H_4O	H	CH_3	14
Buturon	Bu	Cl	H	$CH(CH_3)C{\equiv}CH$	4
Neburon	Nb	Cl	Cl	C_4H_9	9
Monolinuron	Ml	Cl	H	OCH_3	4
Linuron	Li	Cl	Cl	OCH_3	9
Metobromuron	Mb	Br	H	OCH_3	6
Chlorbromuron	Cb	Br	Cl	OCH_3	11
					3: 3-Cl
					8: 3,5-diCl
					12: 3,4,5-triCl

†3-Y-4-X-anilines corresponding with the parent phenylureas, excepting anilines No. 3, 8 and 12 which were used as (potential) internal standards.

Apparatus

Capillary GC was done on a Pye Unicam (Philips, Eindhoven, The Netherlands) Model GCV or a Packard–Becker (Delft, The Netherlands) Model 427 or 433 instrument equipped with a ^{63}Ni ECD or a nitrogen/phosphorus detector (NPD). A 25 m × 0.22 mm I.D. fused silica column wall coated with CP-Sil 5 was used, and 1-μl injections were made with a solid injector or via a septum injector (splitless, according to Grob). The standard temperature program was from 110°C (5 min isothermal) to 250°C with a rate of

6°C min^{-1}, and with injector and detector at 240 and 300°C, respectively. The gas flow-rates (in ml min^{-1}) were for the ECD: nitrogen carrier, 1 and purge gas, 30; for the NPD: hydrogen, 4, air, 50 and nitrogen make-up gas, 20.

The LC system consisted of an Orlita (Giessen, G.F.R.) Model AE 1044 or TW 1515 reciprocating pump, a Valco (Houston, TX, USA) six-port injection valve with a 100-μl loop, and a Pye Unicam (Philips) Model LC 3 variable-wavelength UV detector or a Pye Unicam (Philips) ^{63}Ni ECD connected with the LC column outlet via a home-made vaporization interface (75 cm × 0.25 mm I.D. stainless-steel capillary) kept at a temperature of 250–300°C. For construction and operating details of the LC-ECD system, one should consult Refs. 6 and 7. Separations were carried out on 25 cm × 4.6 mm I.D. stainless-steel columns packed with 5-μm LiChrosorb SI 60, 10-μm LiChrosorb NH_2 or 10-μm LiChrosorb RP-18 (Merck).

Methods

Hydrolysis[3,5] Silica is mixed with a solution of dimethylamine in hexane (1 mMole amine/g silica). After impregnation, the hexane is slowly evaporated at room temperature and, next, the DMA-silica is heated in an oven at 250°C for 3 days. The final product is allowed to cool down and stored in a stoppered flask at room temperature. Prior to use, 1 g of DMA-silica which is poured in a small glass column provided with a glass frit is activated at 165–200°C for 4 h. A sample solution in dichloromethane is applied to the top of the column, whch is then rinsed with the same solvent. The column is put into an oven heated at 165°C for 20 min for the hydrolysis to take place. After cooling, the anilines formed are eluted with 5 ml of ethyl acetate. A fresh column is used for each experiment

Derivatization[3–5] 5 ml of an aniline (herbicide)-containing solution in ethyl acetate (hexane) are mixed with 20 μl of HFBA in a centrifuge tube. After stoppering, the tube is shaken for 1 min on a Whirli mixer and then set aside for 5 min at room temperature (1 h at 60°C). Next, 3 ml of an aqueous 1 M NaOH solution (pH-8 buffer solution) are added and, after shaking and layer separation, the organic phase is transferred to another tube and dried over

anhydrous Na_2SO_4. The derivatives of the herbicides are stable for at least about 1 day; the aniline derivatives do not show noticeable breakdown even after several months. The procedure reported for the herbicides (conditions within brackets) can, of course, also be used for anilines.

Extraction (1) 10–20 ml *water* samples are adjusted to pH 10 and then extracted with 2×5 ml dichloromethane and, next, 1×5 ml hexane in a 25-ml glass-stoppered centrifuge tube by shaking on a Whirli mixer for 1 min. After separation of the layers, the organic phase is transferred to another tube. The combined organic phases are dried over anhydrous Na_2SO_4 and then concentrated to the appropriate volume.

2) To a 20-g *soil* sample in a glass-stoppered Erlenmayer flask approx. 100 ml of methanol are added, and care is taken that the soil sample is completely submerged in the solvent. The contents of the flask are shaken gently for 1 h on a shaking machine. After filtration over a Büchner funnel, the extraction procedure is repeated with 75 ml of methanol, and the soil on the Büchner funnel is finally rinsed with another 75 ml of methanol. The combined organic extracts are transferred to a round-bottomed flask and concentrated to a volume of 50 ml with a rotavapor at slightly elevated temperature (30–35°C). The concentrated methanol extract is centrifuged for 30 min at 5200 rpm and, next, transferred to a round-bottomed flask to which 10 ml of 1 M H_2SO_4 are added. After evaporation of the methanol, the remaining aqueous phase is transferred to a PTFE-stoppered glass tube and the round-bottomed flask is rinsed with 5 ml of demineralized water which are also transferred to the glass tube. After centrifugation, the pH is adjusted to 10 with 1 M NaOH, and extraction of the herbicides and/or anilines proceeds as reported above for the water samples.

3) *Crop* samples were generally treated according to the method of Ambrus *et al.*,[8] which involves an acetone extraction in a Waring blender followed by an extraction into dichloromethane. The dried and concentrated (1 ml) extract is then applied to a silica column or used for direct derivatization. To avoid evaporation steps and/or the handling of large solvent volumes, in most cases only an aliquot of the acetone extract was used for the extraction into dichloromethane.

One should realize that the catalytic hydrolysis procedure at the

same time provides a (partial) clean-up of the sample extract. If no hydrolysis step is involved—i.e., in cases of direct derivatization with HFBA or determination of anilines—introduction of a clean-up step over a silica column is recommended (for details, see Ref. 8 and below).

RESULTS AND DISCUSSION

Various applications will be discussed which demonstrate the usefulness of the several approaches to the determination of phenylureas and their anilines presented before (Refs. 3–5; cf. INTRODUCTION). Here one should realize that the selection of the analytical procedure will always depend heavily on both the nature of the sample and the number and type of compounds to be determined.

Determination of anilines or herbicides

Anilines For the direct determination of anilines in surface water samples a suitable extraction procedure and, next, derivatization with HFBA and analysis by means of GC-ECD will usually suffice. Herbicides, as a rule, will not interfere even if they are converted into their direct HFB derivatives, because of efficient separation in capillary GC.

A detailed study has been made for the determination of 3,4-dichloroaniline in tap and several types of surface water. 10–100 ml water samples were extracted twice with 5–20 ml of dichloromethane and, finally, with 5–20 ml of hexane. For all types of water samples, over a wide concentration range—i.e., 0.01 ppb to 1 ppm—recoveries of 85–105% were invariably obtained, provided the aqueous phase was buffered to pH 10. Results obtained for an industrial discharge water from the Rotterdam harbour area are shown in Figure 1. The sample was found to contain 0.25 ppb of 3,4-dichloroaniline, while three further samples taken at different locations in the same area displayed aniline levels of below 0.05 ppb. In all cases, 3,5-dichloroaniline was used as internal standard. Depending on the degree and nature of the background pollution present in these and other types of samples, detection limits of 0.01–0.1 ppb were readily obtained.

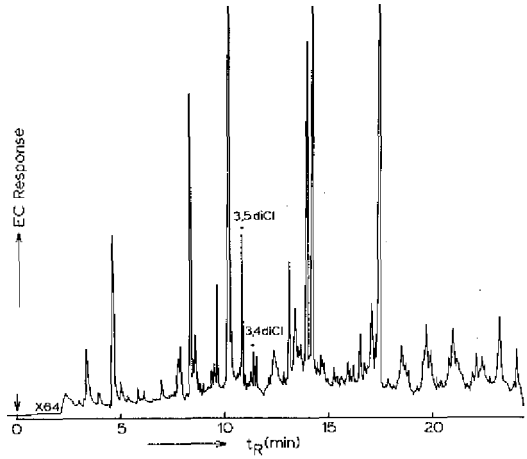


FIGURE 1 Capillary GC on CP-Sil 5 of industrial discharge water sample from the Rotterdam harbour area after extraction and derivatization with HFBA. The extract contained 0.25 ppb of 3,4-dichloroaniline, and 1.0 ppb of 3,5-dichloroaniline (added as internal standard). For details, see text.

Surface water samples from the Bosbaan, in a semi-rural area near Amsterdam, spiked with 1–10 ppb of each of 12 anilines, were used to compare the performance of the ECD and the NPD. When extracting 20-ml samples according to the above procedure, the detection limit for most anilines was found to be about 1 ppb with the NPD. In that case, the 4-ml extract volume obtained after derivatization had to be reduced to 0.2 ml before the final analysis. With the ECD, on the other hand, such an evaporation step was superfluous and, even so, distinctly higher signal-to-noise ratios were obtained as compared with GC-NPD (see Figure 2). One can conclude that for these aqueous samples, GC-ECD turns out to be 20–100 times more sensitive than is GC-NPD; besides, the NPD does not possess a higher selectivity than does the ECD. Analogous results were obtained with other aqueous and also with spiked soil samples.

Using 100-ml water samples, the ECD detection limit for the entire series of substituted anilines was found to be between 0.01 and 0.1 ppb, with average extraction recoveries in the 75–105% range.

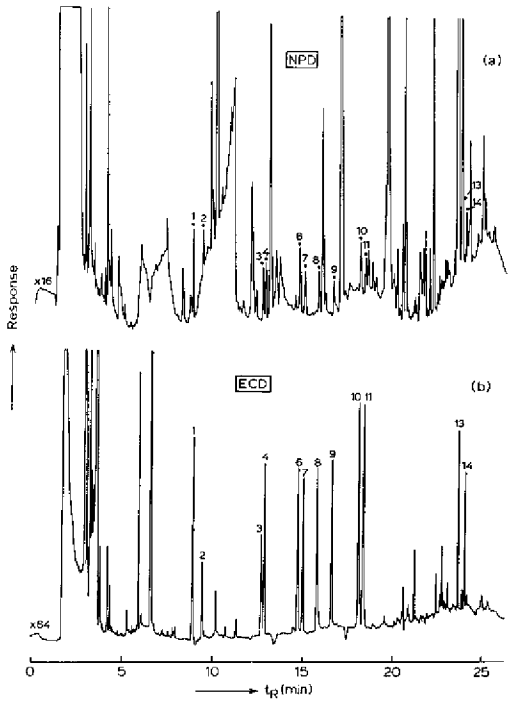


FIGURE 2 Comparison of capillary GC on CP-Sil 5 with (a) NPD, and (b) ECD detection for the extract of a surface water sample spiked with 1 ppb of 12 anilines, and derivatized with HFBA. For NPD: 4-ml extract concentrated by a factor of 20; for ECD: no concentration (injected amounts, 5 and 100 pg of each aniline for ECD and NPD, respectively). Column temperature program: 60–(6°C min^{-1})–180–(20°C min^{-1})→250°C. For aniline codes, see Table I.

Herbicides The feasibility of the catalytic hydrolysis procedure for the determination of 11 herbicides was examined for tap and surface water samples spiked at the 1–100 ppb level. Monuron and buturon, and neburon and diuron were not included since upon hydrolysis they are converted into 4-chloro- and 3,4-dichloroaniline, respectively, as are monolinuron and linuron—which is an inherent disadvantage of methods involving hydrolysis of the phenylurea herbicides.

20-ml samples (pH 10) were extracted with 2×5 ml dichloromethane and 1×5 ml hexane. The extraction recoveries

which were calculated relative to those for a standard mixture of herbicides that was hydrolyzed on a silica column in a parallel experiment, were in the 80–100% range for the 10- and 100-ppb level experiments, and still in the 75–90% range for the samples spiked with 1 ppb of herbicides (cf. Figure 3). The detection limits, calculated for 20-ml samples, were 0.2–0.5 ppb, but these can easily be improved 3–5-fold by increasing the sample volume. This is the more true since for the surface water analyzed by us, the main contribution to the background signal was found to be caused by impurities present in the DMA-impregnated silica. These are obviously eluted with ethyl acetate and, probably, derivatized with HFBA to yield ECD-sensitive compounds.

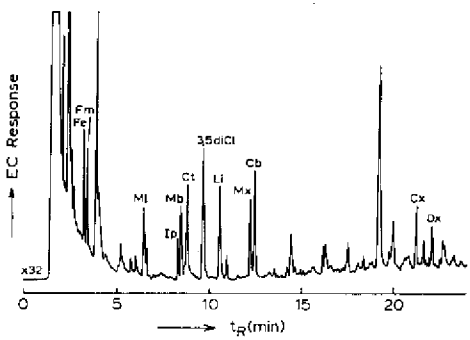


FIGURE 3 Capillary GC on CP-Sil 5 after extraction of a surface water sample spiked with 1 ppb of 11 phenylurea herbicides (for codes, see Table I), catalytic hydrolysis on silica and derivatization with HFBA. 3,5-Dichloroaniline was added as internal standard prior to derivatization. Injected amount corresponds with 5 pg of each herbicide.

In another study, potatoes were spiked with a mixture of 0.1 ppm of each of the herbicides monolinuron, metobromuron, linuron and metoxuron. After extraction with acetone, back-extraction into dichloromethane, catalytic hydrolysis and derivatization of the anilines eluted from the silica column, capillary GC yielded the chromatogram shown in Figure 4. The result demonstrates that the introduction of further clean-up steps is superfluous. The recoveries for the four herbicides were 65–115%. Further work with metoxuron-spiked potatoes gave similar recoveries down to the 10-ppb level.

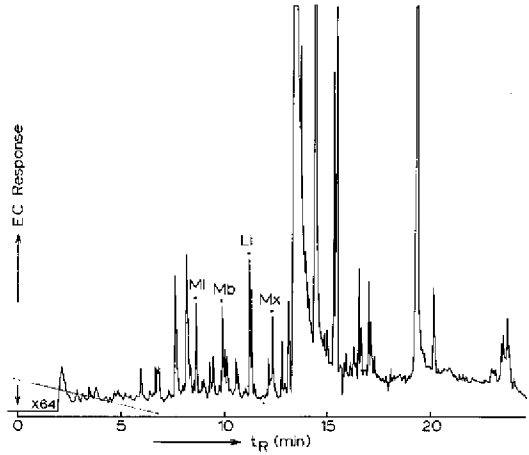


FIGURE 4 Capillary GC on CP-Sil 5 of HFB-amides obtained after extraction of a potato sample spiked with 0.1 ppm of monolinuron (Ml), metobromuron (Mb), linuron (Li) and metoxuron (Mx), catalytic hydrolysis on silica and derivatization with HFBA.

In certain cases, LC is an interesting alternative for the analysis of phenylurea herbicides. Figure 5 shows the reversed-phase LC chromatogram of an extract from a soil sample spiked with 11 herbicides at the 1-ppm level; detection was by UV absorption at 245 nm. A relatively clean chromatogram was obtained by extracting the soil sample with methanol, evaporating this solvent and displacing it by water, and extracting the herbicides from the aqueous phase with dichloromethane. The latter solvent was, in its turn, evaporated and the residue redissolved in the mobile phase solvent mixture. Total recoveries varied between 85 and 95%. Direct analysis of the methanol extract—which would have been highly compatible with the LC system used—turned out to be impossible. Many interfering peaks obscured most of the herbicides in the chromatogram.

Both LC and GC were used to good advantage as independent methods of analysis with a soil sample known to contain a herbicide contamination. Since, in soil, microbial degradation of phenylurea herbicides to anilines occurs, as a first step direct derivatization of the extract with HFBA was executed to identify the aniline. Capillary GC revealed the presence of 0.15 ppm of 3,4-

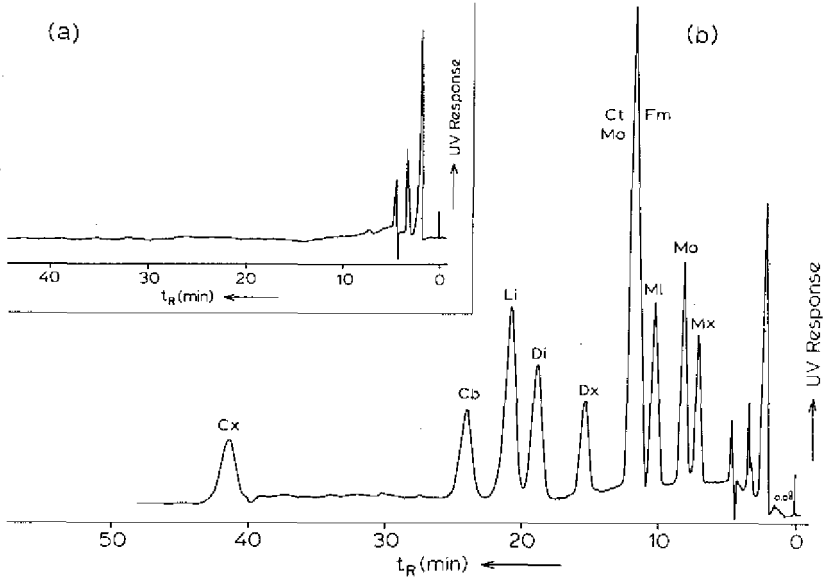


FIGURE 5 Reversed-phase LC of a soil sample extract: (a) blank soil, (b) soil spiked with 1 ppm of 11 phenylurea herbicides (for codes, see Table I). LC system: LiChrosorb RP-18/methanol-water (55:45); flow-rate, 1 ml min^{-1}; UV detection at 245 nm.

dichloroaniline. Next, an aliquot of the extract was hydrolyzed on silica, eluted with ethyl acetate and derivatized with HFBA. GC analysis showed the aniline level now to be much higher, indicating that part of the herbicide—either diuron, linuron or neburon (cf. Table I)—was still present in the soil sample. For unequivocal identification, an aliquot of the soil extract was injected onto a silica/dichloromethane-hexane-ethanol-triethylamine (90:10:0.45:0.01) LC system, retention time showing the unknown herbicide to be diuron. Its presence was further confirmed by collecting the diuron-containing fraction of the LC effluent and subjecting it to the complete hydrolysis procedure. From the very clean GC chromatogram, a diuron content of 0.40 ppm was calculated; this was in good agreement with the result of the GC measurement after direct hydrolysis on silica.

Determination of herbicides and anilines

Strategy The problem of separate versus joint determination of (parent) herbicides and anilines has been recognized in the literature, but it has not really been solved. All methods of analysis for herbicides based on an intermediate hydrolysis step will self-evidently produce "joint" data only. A short discussion of attempts to improve this situation is as follows.

An obvious method to separate herbicides and anilines would be via selective extraction. It is relatively easy to achieve complete extraction of the herbicides into a solvent such as dichloromethane. Earlier work[1] has, however, shown that it is virtually impossible to retain the anilines quantitatively in the aqueous phase. Even at pH values as low as 1 the more highly substituted anilines are partly extracted into dichloromethane. In other words, although separation involving, for example, aniline or 3-chloro-4-methoxyaniline can be nicely accomplished, liquid–liquid extraction cannot be recommended for a multiresidue method. For the rest, substitution of an apolar solvent such as hexane for dichloromethane will cause problems because of incomplete extraction of the herbicides.[9]

As an alternative, one can determine (1) the aniline content by derivatization with HFBA, and (2) the aniline + herbicide content via direct derivatization, subsequent hydrolysis and another derivatization step. Subtraction of the values found yields the herbicide content. Unfortunately, this procedure—which was successfully utilized in the example involving diuron quoted above—will lead to grossly inaccurate results if the aniline/herbicide ratio is very high.

Another possibility to discriminate between herbicides and anilines is via direct derivatization of both classes of compounds, using the modified procedure developed for herbicides, reported in the experimental section. This attempt is worthwhile because all HFB-herbicides and HFB-amides can be separated from each other in a single run in capillary GC (cf. Ref. 5). Still, the problem of determining 20–30 relatively closely related compounds in a complex matrix—with the pre-chromatographic derivatization with HFBA no doubt adding to the complexity of the final chromatogram—should not be underestimated. Generally speaking, direct derivatization of the complete sample will therefore be most valuable for mixtures

containing a restricted number of herbicides and anilines. A relevant example is included in the applications presented below.

For a real multiresidue method of the entire group of herbicides and anilines, the combined use of LC and GC appears to be the most relevant solution. In a recent paper,[5] LC is recommended for fractionation and purification purposes, with the combined potential of derivatization with HFBA and final analysis by capillary GC providing the required sensitivity and selectivity. Two schemes of analysis are briefly discussed below and their application to real samples is reported.

Scheme I (*Figure 6*) The herbicide/aniline-containing sample extract is fractionated in the LC system silica/dichloromethane-hexane-ethanol-triethylamine (90:10:0.45:0.01). The fractions are either

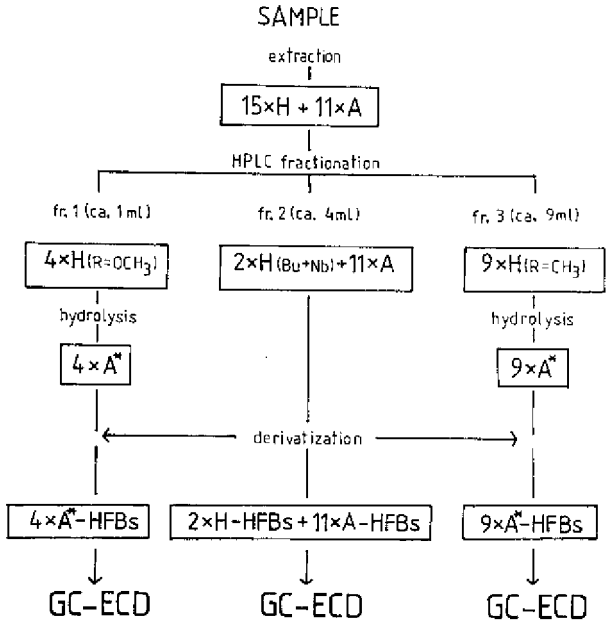


FIGURE 6 Analysis Scheme I for the selective determination of 15 phenylurea herbicides (H) and the 11 corresponding substituted anilines (A) based on LC fractionation as the first step. A*, anilines obtained after hydrolysis of herbicides; A-HFBs and H-HFBs, HFB derivatives of anilines and herbicides, respectively. For further details, see text.

directly reacted with HFBA (Fraction 2; all anilines plus the herbicides buturon and neburon) or applied to a silica column for rapid hydrolysis (20 min at 165°C) with subsequent ethyl acetate elution of the anilines formed and derivatization with HFBA (Fraction 1, R=OCH_3 herbicides; Fraction 3, R=CH_3 herbicides). Final analysis of all fractions is by capillary GC-ECD.

Analysis according to this scheme has been tested using surface water samples spiked with 0.1–100 ppb of each of the herbicides and anilines under investigation. Sample size at the 0.1–1 ppb level typically was 100 ml; extraction and all further steps were carried out as described in the appropriate sections above.

For a sample spiked at the 1-ppb level the capillary GC chromatograms are shown in Figure 7. 3-Chloro-, 3,5-dichloro- and 3,4,5-trichloroaniline were used as internal standards which were added immediately prior to derivatization. The chromatograms of Figure 7 correspond with the three fractions obtained after LC fractionation, hydrolysis of the herbicides and derivatization of the anilines with HFBA. The composition of these fractions is seen to agree fully with the expectations based on the scheme shown in Figure 6. For the rest, the chromatograms adequately illustrate the inherent selectivity and sensitivity of the method; the detection limit for most of the test compounds is about 0.1 ppb. Recoveries were in the same range as in the separate determination of either herbicides or anilines, viz., 60–100, and 65–105%, respectively with a rel. S.D. of 5–18% ($n=5$) for the entire class of herbicides and anilines.

Scheme 1A (*Figure 8*) Recently, it has been shown (Ref. 4; cf. Methods section) that direct derivatization of the phenylureas with HFBA can be carried out with over 95% yield for all 15 compounds tested. Such direct derivatization can be included in Scheme I and, then, will reduce the time of analysis. In the modified scheme, Fraction 1—which may now include neburon and buturon—and Fraction 3 can be derivatized without prior hydrolysis, and analyzed by LC-ECD or GC-ECD. For Fraction 2, the situation remains unchanged. If final analysis is by GC-ECD only, Fractions 1 and 3 can even be combined since all HFB-herbicides are resolved in capillary GC (whereas they are not in LC-ECD!). One should realize, however, that GC analysis of Fraction 3 will prevent one from determining chloroxuron and difenoxuron: their HFB derivatives only show up at room temperature, i.e., in LC analysis.[5]

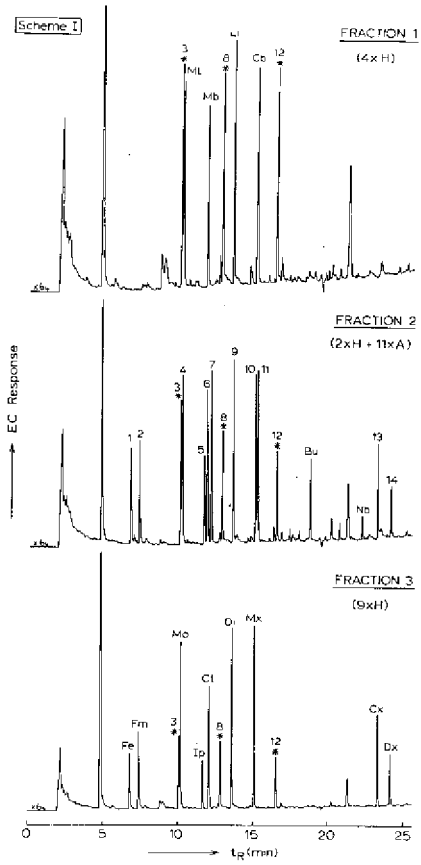


FIGURE 7 Capillary gas chromatograms, on CP-Sil 5, obtained after extraction of a 100-ml surface water sample spiked with 1 ppb of 11 anilines and 15 phenylurea herbicides and subjection to Analysis Scheme I. For aniline and herbicide codes, see Table I; for further details, see text.

Examples of the use of LC-ECD and/or direct derivatization of phenylureas with HFBA for the analysis of surface water, crop and soil samples have recently been published.[4, 7] An illustrative example, viz. the determination of the Fraction-3 herbicides in a surface water sample spiked at the 1-ppb level is shown in Figure 9.

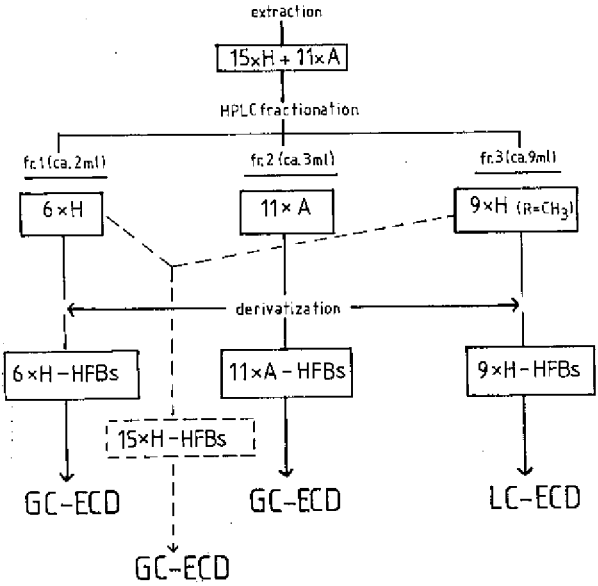


FIGURE 8 Analysis Scheme IA. For further details, see Scheme I and text.

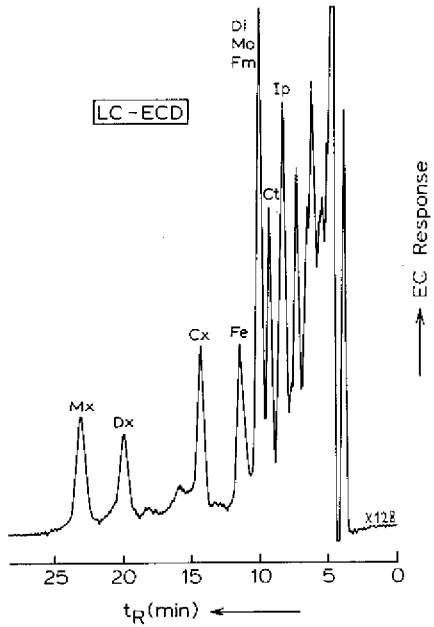


FIGURE 9 LC-ECD of HFB derivatives of 9 phenylurea herbicides (for codes, see Table I) obtained after extraction of a surface water sample (spiked at the 1-ppb level), subjection to Analysis Scheme IA and derivatization of Fraction 3 (containing the R=CH_3 herbicides) with HFBA. LC system: LiChrosorb NH_2/hexane-dioxane (90:10); flow-rate, 1 ml min^{-1}; 0.5 ml min^{-1} directed to ECD. EC detector current, 1×10^{-10} A.

Scheme II (*Figure 10*) The herbicide/aniline-containing sample extract is firstly reacted with HFBA to yield HFB-herbicides and HFB-amides, respectively. After 20-min heating at 165°C on a silica column—which effects hydrolysis of the HFB-herbicides to anilines, while the HFB-amides remain intact—LC fractionation occurs in the same system as used above. The second fraction which contains the anilines is derivatized with HFBA to yield HFB-amides. Analysis of this fraction and of the first fraction (which requires no further treatment) is by capillary GC-ECD. Utilization of Scheme II for the

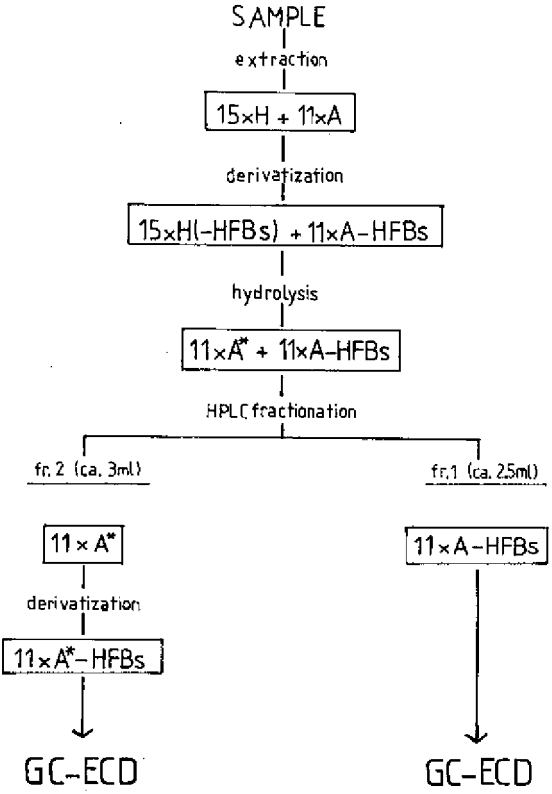


FIGURE 10 Analysis Scheme II for the selective determination of 15 phenylurea herbicides (H) and the 11 corresponding substituted anilines (A) based on derivatization as the first step. A*, anilines obtained after hydrolysis of herbicides; A-HFBs and H-HFBs, HFB derivatives of anilines and herbicides, respectively. For further details, see text.

analysis of surface water samples gave results fully comparable to those presented above. For example, at a spiking level of 5 ppb, the recoveries for herbicides and anilines were 50–100%, and 70–95%, respectively. Chromatograms obtained in the case of a soil sample spiked with 1 ppm of each of the model compounds are shown in Figure 11. Selectivity and sensitivity again are fully satisfactory; it should be noted, however, that because of extraction problems with the soil type studied—which had a high organic matter content—

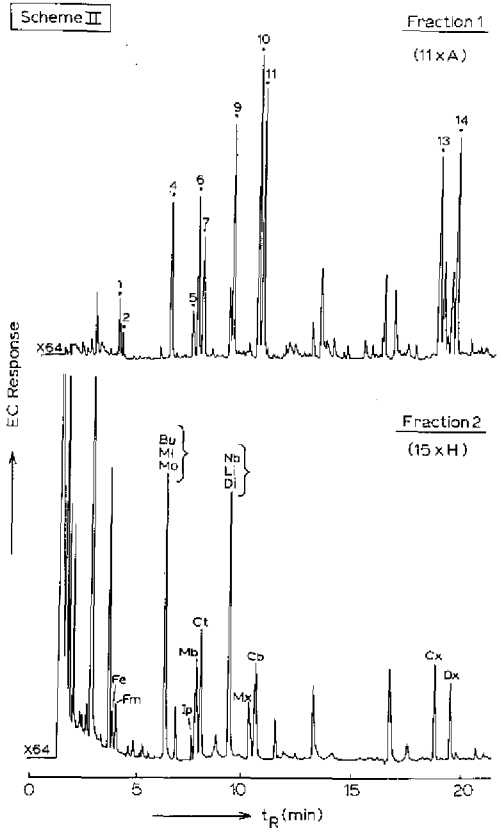


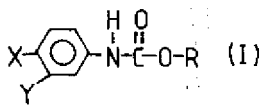
FIGURE 11 Capillary gas chromatograms, on CP-Sil 5, obtained after extraction of a 20-g soil sample spiked with 1 ppm of 11 anilines and 15 phenylurea herbicides and subjection to Analysis Scheme II. For aniline and herbicide codes, see Table I; for further details, see text.

recoveries are fairly low, i.e., 25–70% for the herbicides, and only 5–30% for the anilines. The 30–95% losses contrast with 5–40% losses observed when methanol was spiked at the same 1 ppm level and subjected to the complete analytical procedure.

It should be added that low recoveries of anilines from soils with a high organic matter content (as used by us) are a recurrent problem in the literature.[10–12] A major part of the anilines seems to be held very tightly by the quinoidal subunits of soil humus.[13–16]. Alkaline hydrolysis[17] or, even better, the Bleidner technique[18] is rather efficient but, of course, cannot be used in herbicide/aniline discrimination work. In that case, conventional extraction (herbicides + "free" anilines) and, next, alkaline hydrolysis under reflux ("bound" anilines) is a promising alternative. The extracts can be combined to be subjected to one of the schemes of analysis.

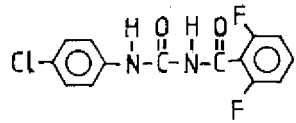
Determination of related classes of compounds

Preliminary work has shown that the principle of catalytic hydrolysis can be extended to (classes of) compounds related to the phenylureas, such as the N-phenylcarbamates (I) and the well-known insecticide diflubenzuron (II), which also generate substituted anilines upon hydrolysis.



(I)

N-Phenylcarbamates



(II)

Diflubenzuron

For the four carbamates tested—carbetamide (aniline), chlorpropham (3-chloroaniline), phenmedipham (3-methylaniline) and propham (aniline), where the compounds within brackets are the anilines formed upon hydrolysis—hydrolysis at the conventional temperature of 165°C took somewhat longer than with the phenylureas, viz., 30–40 min. For diflubenzuron, the optimum time was even 60 min. The hydrolysis efficiency was over 95% for the carbamates, and around 80% for diflubenzuron.

Two examples are shown in Figure 12. One relates to the determination of chlorpropham in a soil sample (found, 1.7 μg/g dry

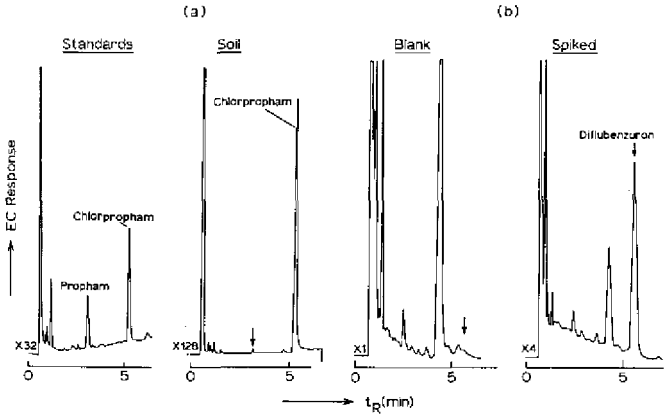


FIGURE 12 Packed column (4% OV-210 on Chromosorb W AW, 80–100 mesh) GC of (a) chlorpropham and, probably, pyrazon (see arrow) in soil, and (b) blank soil and diflubenzuron-spiked (0.25 ppm) soil. Analysis after extraction, catalytic hydrolysis on silica and derivatization with HFBA. For GC conditions, see Ref. 5.

soil) with the small aniline peak probably being caused by the fact that the soil had also been sprayed with the herbicide pyrazon which can form aniline upon hydrolysis. The other example deals with the analysis of a soil sample without and with added (0.25 ppm) diflubenzuron. The corresponding HFB-amide clearly shows up in the spiked sample, and is completely absent from the blank.

CONCLUSION

Several methods of sample pretreatment and chromatographic analysis have been tested for a variety of samples, and good sensitivity and selectivity have generally been observed. Herbicides and anilines can typically be detected at the 0.01–0.1 ppb level in surface water, and at the 10-ppb level in soil and crops. Repeatability is satisfactory but, for soil samples, recovery still needs further optimization.

For multiresidue analysis of complex mixtures of both herbicides and anilines the proposed schemes of analysis, which are based on the combined use of LC and GC, can certainly be recommended. In a specific case, the selection of the proper alternative will largely be

determined by the nature of the problem at hand. Scheme I, for example, has the advantage of being highly selective and discriminates between herbicides which yield the same aniline upon hydrolysis. If such discrimination is not of paramount importance, Scheme II can be recommended since the procedure involves the analysis of two instead of three fractions and is relatively fast. Scheme IA is rather similar to Scheme I, and its choice will mainly be determined by considerations regarding the separation efficiency of LC versus GC, and the possible presence of difenoxuron or chloroxuron.

Finally, the principle of catalytic hydrolysis on silica has been extended to other types of pesticides which generate anilines upon hydrolysis, e.g., the insecticide diflubenzuron and four N-phenylcarbamates. The procedure shows good efficiency and requires much shorter reaction times than does the classical procedure of heating strongly acidic or basic sample solutions at high temperature.[19–22]

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