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# CHROMATOGRAPHIC DETERMINATION OF PHENYLUREA HERBICIDES AND THEIR CORRESPONDING ANILINE DEGRADATION PRODUCTS IN ENVIRONMENTAL SAMPLES. I.*

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## SUMMARY

Several chromatographic procedures for the determination of fifteen phenylurea herbicides and their corresponding degradation products are reported. Methods of analysis for each separate class of compounds and multiresidue methods which allow selective determination of herbicides and anilines in each other's presence are discussed. The various procedures involve steps such as extraction, clean-up, catalytic hydrolysis of herbicides to anilines, liquid or gas chromatographic fractionation and/or separation, chemical derivatization and detection.

## INTRODUCTION

The extensive use of substituted phenylureas as selective herbicides[1] in agriculture has led to the elaboration of suitable analytical methods for determination of trace levels of these compounds in crops[2,3], soil[4,5] and surface water[6,7] samples. The general structure and names of the fifteen major phenylurea herbicides and their corresponding aniline degradation products are presented in Table I. It is of interest that in The Netherlands, in 1976, over 800 tons of phenylureas were applied over an area of about 6000 $km^2$. In the same year, in the U.S.A., the combined production of the well known phenylurea herbicides linuron and diuron amounted to *ca.* 4000 tons[8,9].

Direct determination of the phenylureas by either high-performance liquid (HPLC) or gas chromatography (GC) has often been reported in the literature (see, *e.g.*, ref. 10). However, a lack of sensitivity and selectivity in the case of HPLC, and thermal decomposition of several herbicides in GC, have caused many workers to use an indirect method of analysis, *i.e.*, hydrolysis of a herbicide to its aniline, and subsequent derivatization for sensitive and selective detection in GC with electron-

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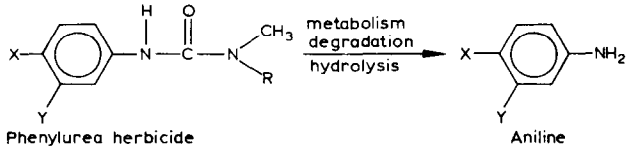
TABLE I

NAMES AND STRUCTURE OF PHENYLUREA HERBICIDES AND CORRESPONDING ANILINE DEGRADATION PRODUCTS




| *Herbicide (H)* | *Code* | *Substituents* | | | *Aniline (A)* |
|---|---|---|---|---|---|
| | | *X* | *Y* | *R* | |
| Fenuron | Fe | H | H | $CH_3$ | Aniline |
| Isoproturon | Ip | $(CH_3)_2CH$ | H | $CH_3$ | 4-$(CH_3)_2CH$- |
| Fluometuron | Fm | H | $CF_3$ | $CH_3$ | 3-$CF_3$- |
| Monuron | Mo | Cl | H | $CH_3$ | 4-Cl- |
| Chlortoluron | Ct | $CH_3$ | Cl | $CH_3$ | 3-Cl-4-$CH_3$- |
| Diuron | Di | Cl | Cl | $CH_3$ | 3,4-$Cl_2$- |
| Metoxuron | Mx | $OCH_3$ | Cl | $CH_3$ | 3-Cl-4-$OCH_3$- |
| Chloroxuron | Cx | 4-Cl-$C_6H_4O$ | H | $CH_3$ | 4-(4-Cl-$C_6H_4O$)- |
| Difenoxuron | Dx | 4-$CH_3O$-$C_6H_4O$ | H | $CH_3$ | 4-(4-$CH_3O$-$C_6H_4O$)- |
| Buturon | Bu | Cl | H | $CH(CH_3)C\equiv CH$ | 4-Cl- |
| Neburon | Nb | Cl | Cl | $C_4H_9$ | 3,4-$Cl_2$- |
| Monolinuron | Ml | Cl | H | $OCH_3$ | 4-Cl- |
| Linuron | Li | Cl | Cl | $OCH_3$ | 3,4-$Cl_2$- |
| Metobromuron | Mb | Br | H | $OCH_3$ | 4-Br- |
| Chlorbromuron | Cb | Br | Cl | $OCH_3$ | 3-Cl-4-Br |

capture detection. Quantitative conversion of phenylureas into their anilines may well take many hours[11–16]. A recent alternative which involves rapid catalytic hydrolysis of the herbicides on silica[17,18] has considerably enhanced the potential of all methods based on hydrolysis. Such procedures, however, do involve a lack of specificity. This may pose problems since anilines are widely used bulk chemicals and can be released into the environment via a number of ways, such as direct industrial disposal, (bio)degradation of phenylureas, phenylcarbamates, acyl anilides and dye products, reduction of nitrobenzene or combustion of polyurethanes.

In the present study we have attempted to overcome the specificity problem by combining HPLC, for suitable group separation of anilines and herbicides, with subsequent hydrolysis and derivatization, as required, and final analysis by means of capillary GC; in addition, the catalytic hydrolysis procedure has been optimized. Special attention has been devoted to the elaboration of a true multiresidue method for all fifteen phenylurea herbicides and their corresponding aniline degradation products.

## EXPERIMENTAL

### *Materials*

The herbicides were gifts from the Food Inspection Service (Amsterdam, The Netherlands); the anilines were purchased from Fluka (Buchs, Switzerland) or Al-

drich (Beerse, Belgium). 3-Chloro-4-methoxyaniline was a gift from Sandoz (Basle, Switzerland). 4-[4-Chlorophenoxy]- and 4-[4-methoxyphenoxy]aniline were prepared as described below.

Stock solutions of herbicides and anilines were made in toluene, ethyl acetate or acetone. For dilutions, hexane and dichloromethane were usually employed. All solvents were analytical grade products from Baker (Deventer, The Netherlands); they were distilled before use. Heptafluorobutyric anhydride (HFBA) from Merck (Darmstadt, F.R.G.) was used as received. For hydrolysis of herbicides, Kieselgel 60, for column chromatography (reinst, 70–230 mesh) from Merck was used. Dimethylamine was purchased from Fluka.

*Apparatus*

GC with packed columns (2 m × 2 mm I.D.) was carried out on a Model 419 gas chromatograph (Packard-Becker, Delft, The Netherlands) equipped with a $^{63}$Ni electron-capture detector. The glass columns were packed with 4% OV-210 or 4% free fatty acid phase (FFAP) on Chromosorb W AW (80–100 mesh). With OV-210 the column temperature was programmed from 110°C (2 min isothermal) to a final temperature of 230°C at a rate of 8°C min$^{-1}$; in the case of FFAP, the program was from 150 to 260°C at a rate of 4°C min$^{-1}$. With both phases the injector and detector temperatures were 230 and 300°C, respectively, and the flow-rate of the nitrogen used as carrier gas was 30 ml min$^{-1}$.

Capillary GC was done on a Pye Unicam (Philips, The Netherlands) or a Packard-Becker Model 427 or 433 gas chromatograph equipped with a $^{63}$Ni electron-capture or a nitrogen–phosphorus detector. 1-$\mu$l Injections were made with a solid injector or via a septum injector (splitless, according to Grob). The stationary phases in the 25 m × 0.22 mm I.D. WCOT fused-silica columns were CP-Sil 5 and OV-25. The standard column temperature programs were from 110°C (5 min isothermal) to 250°C at a rate of 6°C min$^{-1}$ (CP-Sil 5) and from 130°C (5 min isothermal) to 240°C at a rate of 8°C min$^{-1}$ (OV-25). The injector and detector temperatures were 240 and 300°C, respectively. The nitrogen carrier and purge-gas flow-rates were 1 and 30 ml min$^{-1}$, respectively. The hydrogen and air flow-rates, for the nitrogen–phosphorus detector, were 4 and 50 ml min$^{-1}$, respectively; make-up gas ($N_2$) was supplied at 20 ml min$^{-1}$.

Gas chromatography–mass spectrometry (GC–MS) was performed on a Model 9500 gas chromatograph (Finnigan, Sunnyvale, CA, U.S.A.) connected with a Finnigan 3200 quadrupole mass spectrometer operated in the electron impact mode at 70 eV and an ion-source temperature of 230°C. The GC conditions were as given above; however, helium was used as carrier gas.

Thin-layer chromatography (TLC) was carried out in Hellendahl staining jars, using precoated silica gel $F_{254}$ (Merck) and apolar chamically bonded $KC_{18}$ thin-layer plates (Whatman, Springfield Mills, U.K.). Visualization was by inspection under UV (254 nm) light.

$^1$H NMR spectra were recorded with a Bruker WH 90 F.T. spectrometer (Bruker Physics, Karlsruhe, F.R.G.). $^1$H chemical shifts were measured with deuterochloroform as solvent.

The HPLC systems consisted of a Model AE 1044 or TW 1515 reciprocating pump (Orlita, Giessen, F.R.G.), a six-port injection valve with a 100-$\mu$l loop (Valco,

Houston, TX, U.S.A.) and a Pye-Unicam Model LC 3 or a Model PM2 DLC variable-wavelength UV detector (Zeiss, Oberkochen, F.R.G.).

Adsorption HPLC was carried out on 25 cm × 4.6 mm I.D. stainless-steel columns packed with 5-μm LiChrosorb Si 60 silica (Merck) or 10-μm LiChrosorb $NH_2$ or diol (Merck) and Polygosil 60-D10 CN (Macherey-Nagel, Düren, F.R.G.) chemically bonded phases. Mixtures of hexane with dioxane or dichloromethane were used as mobile phase. Reversed-phase HPLC was performed on a 25 cm × 4.6 mm I.D. stainless-steel column packed with 10-μm LiChrosorb RP-18 (Merck). Methanol–water mixtures were used as mobile phase. All experiments were done at ambient temperature.

For HPLC with electron-capture detection (ECD) a normal-phase HPLC system was connected with a Pye-Unicam $^{63}Ni$ electron-capture detector via an evaporation interface designed in our laboratory[19,20]. The column effluent was passed through a 75 cm × 0.25 mm capillary enclosed in an oven kept at temperatures between 250 and 300°C, was vaporized there and transported as vapour to the detector. A nitrogen stream of *ca.* 30 ml $min^{-1}$ was maintained for the detector.

*Methods*

*Hydrolysis.* For the hydrolysis step, a conventional grade of silica was used, either as such or after impregnation with dimethylamine. One gram of silica, activated at about 200°C for 4 h, was poured into a 15 cm × 3 mm I.D. glass column provided with a glass frit, and packed by gentle tapping. An appropriate amount of sample dissolved in dichloromethane was applied to the top of the column, which was then rinsed with the same solvent; care was taken that no more than 0.5 ml of solvent was eluted from the column. The column was placed into an oven heated at 165°C for 20 min for the hydrolysis to take place. After cooling, the anilines formed were eluted with 5 ml of ethyl acetate.

*Derivatization.* A 5-ml volume of a solution of the anilines dissolved in ethyl acetate was mixed with 20 μl of HFBA in a centrifuge tube. The tube was stoppered, shaken for 1 min on a Whirli mixer and then set aside for 5 min at room temperature. Next, 3 ml of an aqueous 1 *M* sodium hydroxide solution were added to destroy the excess of reagent and extract it, as the sodium salt of the acid formed, into the aqueous phase. After separation of the layers, the organic phase was transferred to another centrifuge tube, and dried over anhydrous $Na_2SO_4$.

For the derivatization of intact herbicides the above procedure can also be used with, however, the following modifications[10]: (1) a reaction time of 1 h at 60°C instead of 1 + 5 min at room temperature; (2) washing with a phosphate buffer (pH 8) instead of 1 *M* NaOH solution; (3) reaction in hexane rather than ethyl acetate. The modified procedure also allows the quantitative conversion of anilines and can, therefore, be used for mixtures of herbicides and anilines.

*Preparation of anilines from chloroxuron and difenoxuron.* A 20–40 mg amount of chloroxuron or difenoxuron was applied to a 2-g silica column which was then heated at 165°C for 30 min. The anilines formed and some by-products were eluted with 5 ml of ethyl acetate. After evaporation to 0.5 ml, the concentrated eluate was applied onto a preparative silica thin-layer plate (Kieselgel 60 $F_{254}$ plate; layer thickness 2 mm; Merck). After development with dichloromethane–ethanol (98:2) over a distance of about 15 cm, the aniline band ($hR_F$ = 70) was scraped off the plate,

ground in a mortar and then applied on the top of a 1-g silica column. Upon development with ethyl acetate the aniline was eluted as a light yellow zone of high purity. This zone was studied by means of HPLC-UV and, after derivatization, by GC–ECD.

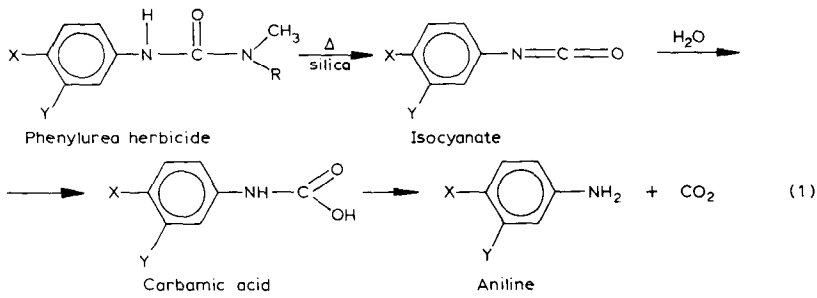
## RESULTS AND DISCUSSION

In the sections on hydrolysis, derivatization procedure and GC analysis below, information is presented which is supplementary to that published earlier[17].

### *Hydrolysis*

Due to the thermolability of phenylurea herbicides, their direct analysis by means of GC poses a problem. Degradation results in an often uncontrollable formation of isocyanates and/or anilines and prevents quantitative analysis. With special precautions, the GC determination of a limited number of phenylurea herbicides has been accomplished. A multiresidue method, however, requires a more convenient and reliable procedure for all relevant compounds. Derivatization of the NH moiety via silylation[21] or alkylation[22–25] permits GC analysis of the phenylureas. The required sensitivity for trace analysis is, however, only reached via derivatization with electrophilic reagents such as halogen-containing acid anhydrides, and subsequent GC–ECD analysis. According to the literature[26–28], acylation often causes problems if utilized for the parent herbicides. No such problems are encountered, however, with the corresponding aniline degradation products and this has prompted many workers to determine the phenylureas via an initial hydrolysis step, and subsequent derivatization of the anilines formed by means of acylation[29–31], and also bromination[2,15] or iodination[3,16].

*Reaction conditions.* Hydrolysis of the phenylureas typically has to be carried out in a strongly basic or acidic medium in a reflux system for 1–10 h. In TLC[32–34], the herbicides can be detected via heating of the silica thin-layer plate containing the sample spot, derivatization (with, *e.g.*, dansyl chloride or fluorescamine) on the plate and measurement of the fluorescence intensity of the derivatives. The products formed upon heating of the thin-layer plate have been identified as substituted anilines; that is, they are identical with the products formed in classical hydrolysis experiments. A distinct advantage of hydrolysis on the thin-layer plate is its rather high speed (about 30 min at 160–180°C). This suggests a catalytic function of the silanol groups during the hydrolysis process when the phenylureas are in the adsorbed state:



The formation of isocyanates has often been demonstrated[35] by GC and, in the present work, by GC–MS of the residue remaining at the origin during TLC of the anilines from chloroxuron and difenoxuron (*cf.*, above and ref. 18).

*Optimization.* From our earlier work[17] it is known that a procedure similar to the above for TLC can be used with small silica columns. Optimum conditions for all herbicides then are as follows: up to 1 ml of sample in, *e.g.*, dichloromethane, is applied to 1 g of activated silica contained in a small glass column, rinsed with 0.5 ml of hexane, heated at 165°C for 15–30 min and eluted with 3–5 ml of ethyl acetate. This ensures 80–100% recovery of the anilines formed from all herbicides. Prolonged heating of the silica column causes only small losses of the anilines (0–20% after 1 h), which contrasts sharply with the very rapid loss of anilines during evaporation of a solution in, *e.g.*, hexane or dichloromethane, under a stream of nitrogen at room temperature.

Further study revealed that samples and standards should be dissolved in the same solvent prior to their application to the silica column. Remarkable differences in GC response (after hydrolysis and derivatization) were observed between, for example, toluene and dichloromethane. Probably, this can be explained by the different times required to evaporate different solvents in the oven; as a consequence, the actual hydrolysis time also is different. As an additional safety measure, we recommend application of about equal volumes of sample and standard solution, even though one may expect a rather minor effect from volume differences when using the highly volatile dichloromethane.

All the above experiments were performed by applying 1–10 μg samples onto the silica column. When, however, smaller samples of 10–500 ng were applied, distinctly lower recoveries were obtained, and the reproducibility deteriorated. For obvious reasons, attention was first focused on the elution problem. Elution of anilines applied on a silica column activated at 200°C was found not to be a problem; with 5 ml of ethyl acetate, recoveries of 80–100% were obtained irrespective of the analyte amount. Insertion of a 20-min heating time, at 165°C, between application of the anilines and their elution, however, drastically changed the situation. With high aniline amounts (5 μg), recoveries still were 80–99% with a rel. S.D. of 5–20% ($n = 6$). When, however, 100 ng of each aniline were applied, recoveries decreased to 15–80%, and the rel. S.D. increased to 10–30% ($n = 6$).

During initial attempts to improve elution of the anilines via the use of silica impregnated with NaOH or KOH —which, indeed, met with some success— it was observed that with both non-impregnated and impregnated silica somewhat higher recoveries were obtained for anilines formed on-column via hydrolysis of the parent herbicides than with anilines which were applied as such. Since, during hydrolysis of the phenylureas, an amine, $RHNCH_3$ (*cf.*, eqn. 1), is formed which in all probability is adsorbed very strongly by the silica and, thus, may successfully compete with the anilines for the active surface adsorption sites and promote desorption of anilines, several amine-impregnated silicas were tested. The adsorbent was treated with 1 mmol of dimethylurea, dimethylamine (DMA), triethylamine or dibutylamine per g of silica. DMA-treated silica gave the most promising results, with recoveries for the aniline elution step at the 10–100 ng analyte level being fairly high (70–100%) and closely similar to recoveries obtained for the full, *i.e.*, hydrolysis plus elution, procedure (65–95%).

*DMA-impregnated silica.* The loading of silica was varied between 0.1 and 10 mmol of DMA per g of silica. Aniline recovery increased over the 0.1–1 mmol range; too high loadings had, however, an adverse effect on aniline recovery. In all probability, a large excess of DMA interferes with the catalytic hydrolysis of the phenylureas on the free silanol groups. Also, at high loading, the trace-level impurities present in DMA and other such amines were found to cause serious disturbances in the final gas chromatogram. Loading of silica with DMA was more effective from solutions in hexane as compared with ethyl acetate, and final solvent evaporation should be carried out gently, *viz.*, with a rotavapor. The final activation and purification of the impregnated silica by prolonged heating to remove the excess of DMA and volatile impurities was also found to be important. After heating, the DMA-impregnated silica has a yellowish colour, the intensity of which is a fair measure of the amount of amine loaded per g of silica.

The complete procedure is as follows. An appropriate amount of silica is mixed with a solution of DMA in hexane containing 1 mmole of DMA per g of silica. If necessary, additional hexane is added to create a homogeneous suspension and, thereby, promote an even loading. The round-bottomed flask containing the suspension is connected to a rotavapor and the hexane is slowly evaporated at room temperature. Next, the DMA-impregnated silica is placed in an oven and heated at 250°C for 3 days. The final product is allowed to cool and stored in a stoppered erlenmeyer flask at room temperature; prior to use, it is activated at 165°C for several hours. A freshly packed column is used for each experiment.

Using DMA-impregnated silica, the hydrolysis of the phenylurea herbicides and subsequent elution of the aniline degradation products was studied. As can be

TABLE II

PERCENTAGE RECOVERIES OF ANILINES AFTER HYDROLYSIS OF PHENYLUREA HERBICIDES ON DMA-IMPREGNATED SILICA

The analytes were applied in 0.5 ml of dichloromethane, and washed into the column with 1–1.5 ml of the same solvent. For further experimental conditions, see the text.

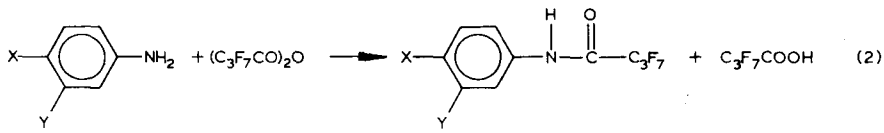
| *Herbicide* | *% recovery for amount (μg) of herbicide applied* | | | | |
|---|---|---|---|---|---|
| | *5* | *2* | *1* | *0.2* | *0.02* |
| Fenuron | 87 | 92 | 94 | 87 | 91 |
| Isoproturon | | 102 | | 99 | 93 |
| Fluometuron | 75 | 84 | 85 | 80 | 81 |
| Monuron | | | | | 86 |
| Chlortoluron | 86 | 99 | 126 | 94 | 94 |
| Diuron | | | | | 85 |
| Metoxuron | 87 | 100 | 91 | 101 | 77 |
| Chloroxuron | 100 | 122 | 121 | 120 | 69 |
| Difenoxuron | 91 | 112 | 101 | 111 | 60 |
| Buturon | | | | 102 | 103 |
| Neburon | | | | 100 | 99 |
| Monolinuron | 65 | 74 | 72 | 70 | 63 |
| Linuron | 112 | 128 | 123 | 125 | 112 |
| Metobromuron | 79 | 90 | 90 | 88 | 78 |
| Chlorbromuron | 100 | 114 | 105 | 116 | 98 |

seen in Table II, good recoveries of at least 75%, and typically 80–110%, were obtained in all but one case (monolinuron, 65–75%) down to 200 ng of each analyte. Even at the rather low level of 20 ng of herbicide, acceptable results were obtained for all fifteen compounds with recoveries of 60–110% in a series of duplicate experiments. Because of small variations in day-to-day conditions and/or batch-to-batch variations of the DMA-treated silica, in work with real samples a few hydrolysis experiments of standard mixtures at, *e.g.*, the 2-μg and 200- and 20-ng levels are strongly recommended. If such precautions are taken, the catalytic hydrolysis procedure is reliable for trace analysis.

Finally we note that substitution of aluminium oxide for silica did not improve the results. In contrast, even with high amounts (10 μg) of each herbicide, recoveries upon elution with 5 ml of ethyl acetate never exceeded 50%, and with metoxuron, chloroxuron and difenoxuron no aniline was recovered.

*Derivatization*

*Anilines.* Derivatization of anilines with perfluoro agents such as HFBA was described by Bradway and Shafik[29] several years ago and, recently, studied in more detail by our group[17,18]. Further comments on the optimization of the reaction, which proceeds according to




and is generally carried out in ethyl acetate, are as follows.

Provided the reaction mixture is initially shaken for 1 min on a Whirli mixer, the reaction time of 1 h recommended earlier[29] can be reduced to a mere 5 min. The reaction goes to completion (>99%) at room temperature without the addition of a catalyst. Besides ethyl acetate, solvents such as hexane and dichloromethane can also be used. With the latter two solvents, care should be taken as regards extraction with 1 *M* NaOH, because the aniline derivatives tend to stay in the aqueous phase. To solve this problem ethyl acetate has to be added so that the ratio of ethyl acetate to the second solvent is at least 3/1. Alternatively, use of a phosphate buffer (pH 8) instead of 1 *M* NaOH results in complete extraction even when the organic phase is pure hexane or dichloromethane. We have also observed that the use of a relatively large volume as compared to that of Bradway and Shafik has no adverse effect on the speed of reaction. It is not necessary, therefore, to concentrate large volumes of aniline-containing solutions prior to derivatization, which prevents the loss of volatile anilines during evaporation. Since ethyl acetate is also suitable for the elution of anilines from the silica column used for hydrolysis, derivatization can immediately follow elution.

The reproducibility of the derivatization reaction was tested with capillary GC–ECD. At the 100-ng level (absolute amount derivatized) a rel. S.D. of between 1.6 and 4.5% ($n = 6$ for each aniline) was obtained for the complete series of anilines.

As regards to mass spectrometry of the HFB-amides, the reader is referred to

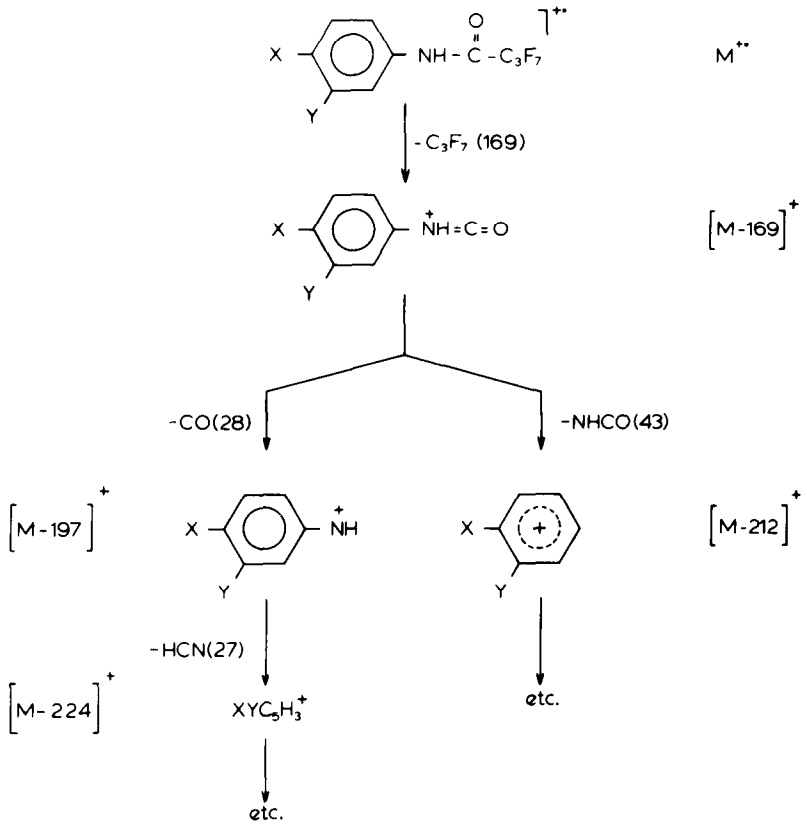
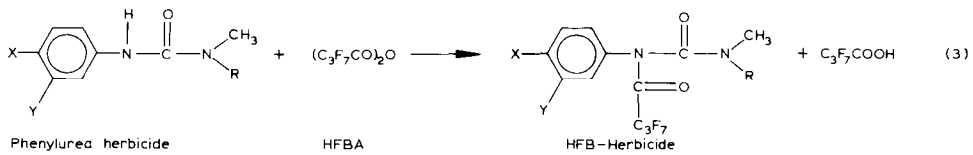



Fig. 1. Schematic representation of the general fragmentation pattern of the HFB derivatives of substituted anilines.

the discussion in ref. 17. The information contained therein is summarized in Fig. 1.

*Intact herbicides.* Recently, the few reports[26–28,36] on direct derivatization of phenylureas with reagents such as HFBA according to




have been augmented with a more detailed study[10]. The experimental procedure outlined in that paper has been adopted here (see *Methods*) and will not be discussed further. The following points should, however, be appreciated. Compared with the anilines, herbicides react more sluggishly with HFBA. Consequently, reaction takes more (*ca.* 60 *vs.* 5 min) time at a higher (60 *vs.* 20°C) temperature in hexane rather than in ethyl acetate as solvent, and removal of excess of HFBA should be carried out more gently (pH 8 buffer *vs.* 1 *M* NaOH). Finally, the HFB-herbicides are dis-

tinctly less stable than the HFB-amides (6–16 h *vs.* at least several weeks at room temperature) and, again contrary to the HFB-amides, the HFB-herbicides can be hydrolyzed to the corresponding anilines when they are applied to a silica or DMA-impregnated silica column and subjected to the catalytic hydrolysis conditions for the parent herbicides, *i.e.*, 20 min heating at 165°C. This fact will be seen to play a rôle in one of the schemes used for discrimination between anilines and herbicides to be outlined below.

*GC analysis*

The HFB derivatives of the anilines are much more suitable for GC than are the parent compounds: they are thermally very stable, less prone to adsorption on glass walls or support material and display good separation characteristics. In a previous paper[17], GC of a limited number of HFB-amides on OV-210 and FFAP as stationary phases was shown to give good results, while separation on OV-101 was unsatisfactory; retention times are presented in Table III. It has recently been shown[10] that the peak shape of HFB-herbicides in packed-column GC is even better than that of the HFB-amides. Further attention was, however, solely devoted to capillary GC, since the superior separation efficiency of this technique will no doubt be required in the analysis of complex real samples.

Retention times for the HFB-amides on fused-silica columns wall-coated with CP-Sil 5 and OV-25 are also given in Table III. Both columns display good separation characteristics, but CP-Sil 5 was preferred for further work because it shows less bleeding. One should note the several differences in elution order between the two columns, which may be helpful when trying to confirm the identity of a particular aniline derivative in residue analysis. A comparison of the retention data, on CP-Sil

TABLE III

GC RETENTION TIMES OF HFB DERIVATIVES OF SUBSTITUTED ANILINES ON PACKED (OV-210 AND FFAP) AND CAPILLARY (OV-25 AND CP-Sil 5) COLUMNS

For column temperature programs, see *Apparatus* section.

| *HFB-amide of aniline** | | $t_R$ *(min) on* | | | |
|---|---|---|---|---|---|
| | | *4% OV-210* | *4% FFAP* | *OV-25* | *CP-Sil 5* |
| (1) | Aniline | 3.9 | 3.3 | 5.20 | 3.45 |
| (2) | 3-$CF_3$ | 5.0 | 4.3 | 4.55 | 3.60 |
| (3) | 3-Cl | 6.6 | 8.0 | 8.00 | 5.75 |
| (4) | 4-Cl | 6.6 | 8.0 | 8.35 | 5.90 |
| (5) | Iso-$C_3H_7$ | 6.9 | – | 8.75 | 7.40 |
| (6) | 4-Br | 7.9 | 10.4 | 10.45 | 7.60 |
| (7) | 3-Cl-4-$CH_3$ | 7.9 | 8.9 | 9.80 | 7.90 |
| (8) | 3,5-$Cl_2$ | 8.4 | 13.8 | 10.35 | 8.70 |
| (9) | 3,4-$Cl_2$ | 9.3 | 14.0 | 11.80 | 9.50 |
| (10) | 3-Cl-4-$OCH_3$ | 10.7 | 15.1 | 14.10 | 11.15 |
| (11) | 3-Cl-4-Br | 10.3 | 16.8 | 13.80 | 11.30 |
| (12) | 3,4,5-$Cl_3$ | 11.2 | – | 14.50 | 12.80 |
| (13) | 4-(4-Cl-$C_6H_4O$) | 15.7 | 24.5 | 22.45 | 19.85 |
| (14) | 4-(4-$CH_3O$-$C_6H_4O$) | 16.2 | 25.6 | 24.10 | 20.70 |

* Anilines 3, 8 and 12 included as internal standards.

5, for the HFB-amides and HFB-herbicides (*cf.*, ref. 10) shows excellent resolution for each pair and efficient separation even for a mixture of some 25 herbicides and anilines. Typical chromatograms for standard mixtures of the HFB-amides on a packed and a capillary column are shown in Fig. 2.

Under optimized conditions, the HFB-amides have detection limits of 0.2–0.3 pg in capillary GC; no remarkable response differences occur between them. Response differences do exist with the HFB-herbicides which display[10] detection limits of between 0.4 and 2.0 pg. For both types of derivatives, good linearity ($R$ = 0.999) is obtained for injected amounts of up to 250–500 pg.

If the phenylurea herbicides are determined via hydrolysis to the anilines and subsequent derivatization with HFBA, the detection limits are, of course, higher than with the anilines themselves, because of (1) the herbicide/aniline molecular weight ratio, and (2) losses during hydrolysis. In practice, the detection limits are increased about 2- to 3-fold.

A nitrogen-sensitive detector has been used in some of our studies. The relative sensitivity of this detector and ECD was evaluated for the entire group of HFB-amides. Taking the HFB-amides as model compounds is slightly unfair, since derivatization with HFBA has deliberately been selected because of the GC–ECD analysis. On the other hand, one should keep in mind that determination of the underivatized compounds, whether anilines or herbicides, will almost invariably present serious problems such as peak tailing and/or thermal instability. With the nitrogen–phosphorus detector, which satisfactorily met the manufacturer's specifications, the absolute detection limits of *ca.* 10 pg were a factor of 20–50 higher than those for ECD. Within the series of HFB-amides the mutual response differences did not exceed 20%.

An additional serious drawback of the nitrogen–phosphorus detector was the poor reproducibility of the response after replacement of the rubidium bead, due to

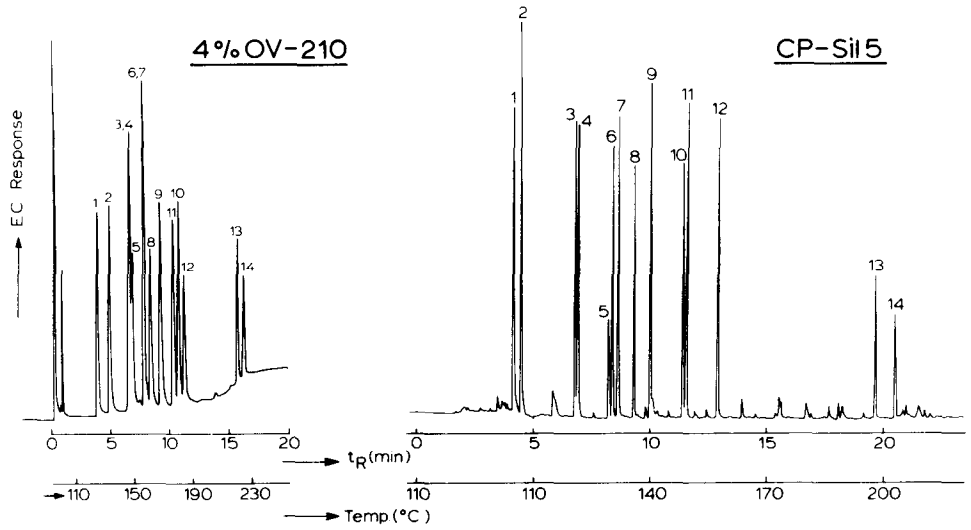



Fig. 2. Packed-column (4% OV-210; left) and capillary (CP-Sil 5; right) GC of the HFB derivatives of substituted anilines. For peak numbers see Table III.

TABLE IV

HPLC RETENTION TIMES OF PHENYLUREA HERBICIDES

Column: LiChrosorb RP-18. Eluent: methanol–water (60:40); flow-rate, 1 ml $min^{-1}$.

| *Herbicide* | $t_R$ *(min)* | *Herbicide* | $t_R$ *(min)* | *Herbicide* | $t_R$ *(min)* |
|---|---|---|---|---|---|
| Fenuron | 3.7 | Fluometuron | 6.8 | Diuron | 9.1 |
| Metoxuron | 4.3 | Chlortoluron | 7.6 | Linuron | 12.0 |
| Monuron | 5.4 | Metobromuron | 7.6 | Chlorbromuron | 13.4 |
| Monolinuron | 6.7 | Difenoxuron | 8.7 | Chloroxuron | 18.6 |
| Buturon | 6.7 | Isoproturon | 8.7 | Neburon | 26.6 |

difficulties in its adjustment. This was observed for the HFB-amides but not for the test compound, azobenzene. Occasionally, the late-eluting HFB-amides of chlorophenoxy- and methoxyphenoxy-aniline were not detected at all, while all others were. This problem could only be remedied after time-consuming readjustment of the bead.

*HPLC analysis*

The potential of various HPLC systems for the fractionation and separation of mixtures of herbicides and anilines was studied in some detail. Detection was generally by means of UV absorption at 245 nm, which provides good sensitivity for all analytes studied.

*Reversed-phase (RP) system.* Preliminary TLC work on $C_{18}$-bonded silica (Whatman $KC_{18}$) with methanol–water mixtures indicated that successful separations of herbicides and anilines cannot be expected in RP-HPLC. In addition, substituted anilines are known often to yield tailing peaks, due to silanol-group inter-

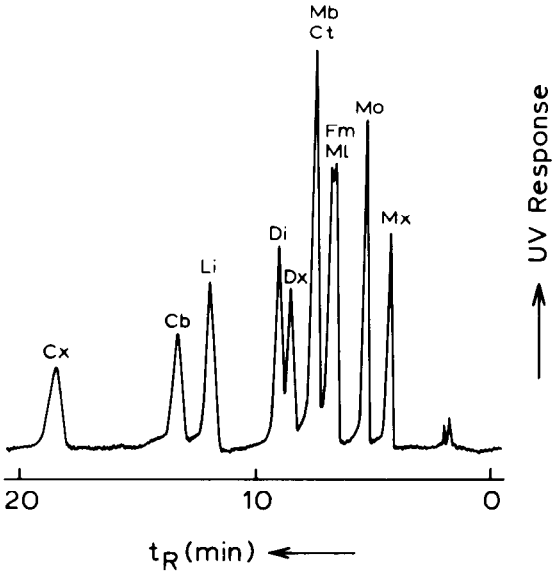



Fig. 3. RP-HPLC of eleven phenylurea herbicides (for codes see Table I) on LiChrosorb RP-18 with methanol–water (60:40) as eluent; UV detection at 245 nm.

action and/or pH effects. Research was therefore limited to the analysis of herbicides on a LiChrosorb $C_{18}$-bonded phase, with methanol–water mixtures as mobile phase. As is seen from Table IV, an efficient separation can be obtained with methanol–water (60:40). A typical chromatogram of a mixture of eleven herbicides is shown in Fig. 3. Although the selectivity and sensitivity of the RP-HPLC-UV system is not adequate for real trace analysis, it is interesting to note that direct injection of 100 µl of a spiked water sample yields detection limits of about 50 ppb. Preconcentration of 10 ml of a herbicide-containing water sample on a short (2–5 × 4.6 mm I.D.; *cf.*, refs. 37 and 38) precolumn packed with a $C_{18}$-bonded phase will effect a decrease of this detection limit by about two orders of magnitude. Such preconcentration has been shown to be possible for all herbicides, except for fenuron (1–3 ml).

*Normal-phase (NP) system.* Generally speaking, RP-HPLC systems have important and well known advantages over NP-HPLC systems. In the present study, however, NP-HPLC combines the advantages of superior group separation (see next paragraph) and good compatibility of the non-aqueous mobile phase with the subsequent hydrolysis, derivatization and GC–ECD and/or HPLC–ECD procedures.

The selection of NP-HPLC rather than RP-HPLC for aniline/herbicide fractionation was based on TLC on silica with dichloromethane (with or without a second solvent). A typical example is shown in Fig. 4. This suggested an approach along one of the following routes:

(I) The aniline–herbicide mixture is separated into three fractions: (1) the rapidly eluted anilines; (2) the slowly eluted anilines plus rapidly eluted herbicides and (3) the slowly eluted herbicides*. Since the ultimate determination of the herbicides proceeds via hydrolysis to the aniline and derivatization, the main problem is to prevent elution of any one herbicide in a fraction containing the corresponding aniline.

(II) The aniline–herbicide mixture is first derivatized to form HFB-amides and HFB-herbicides, respectively, and, next, hydrolyzed on silica. The HFB-amides remain unchanged during this step; the HFB-herbicides, however, are converted into the corresponding anilines. Complete group separation of anilines (as HFB-amides) and herbicides (as anilines) should now pose no problem.

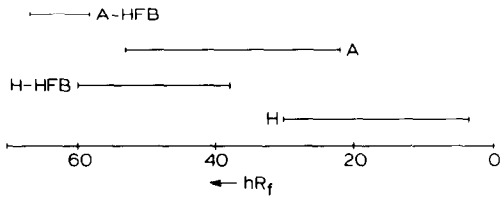



Fig. 4. Schematic presentation of the TLC behaviour of phenylurea herbicides (H), the corresponding aniline degradation products (A) and their HFB derivatives in the normal-phase HPLC system silica/dichloromethane–methanol (99:1).

* Since the order of elution of the herbicides and anilines was found to be dependent on the actual chromatographic conditions used (also see text below), at this stage the analytes contained in each fraction cannot be specified.

*Scheme I.* In preliminary HPLC studies, several chemically bonded (amino, nitrile and diol) phases were studied for their usefulness in herbicide/aniline fractionation. Dichloromethane and dioxane, with or without a certain percentage of hexane, were used as mobile phase, dioxane being tested because of its compatibility with HPLC–ECD. No acceptable results were obtained with any of the chemically bonded phases. Two main problems were encountered: (1) co-elution of some of the rapidly eluted herbicides from the second fraction with the anilines from the first fraction, which would give rise to discrimination problems; (2) broad and/or considerably tailing peaks for several substituted anilines. Silica was, therefore, the stationary phase selected for all further work.

In the system silica/hexane–dioxane, co-elution of part of the herbicides from the second fraction with the anilines present in the first fraction was invariably observed; the peak shapes, however, were excellent. Initial results with silica/dichloromethane–hexane (dried on molecular sieve) were according to expectations based on TLC results. The activity of the silica column, however, steadily increased and, as a result, severe peak tailing and/or increased retention times were observed with some of the anilines and late-eluted herbicides. These deleterious effects could be partially counteracted by adding a small amount of triethylamine (TEA) to the mobile phase. The total fractionation time remained, however, unacceptably high. This had the added disadvantage of too large effluent volumes being collected for the next (derivatization) step.

The above problem was only solved when it was observed that the elution times, and even elution order, changed drastically upon replacing dichloromethane from Merck or Janssen Pharmaceutica by dichloromethane from Baker. This differ-

TABLE V

HPLC RETENTION TIMES OF PHENYLUREA HERBICIDES AND CORRESPONDING ANILINES (A) AND HFB-AMIDES (A-HFBs)

Column: LiChrosorb silica Si 60. Eluent: dichloromethane–hexane–ethanol–TEA (90:10:0.45:0.01); flow-rate, 1 ml $min^{-1}$.

| *Code* | *Herbicide* | $t_R$ *(min)* | *No.* | *Aniline* | $t_R$ *(min)* | |
|---|---|---|---|---|---|---|
| | | | | | *A* | *A-HFB* |
| Ml | Monolinuron | 4.9 | 5 | iso-$C_3H_7$ | 7.0 | 4.5 |
| Mb | Metobromuron | 4.9 | 13 | 4-(4-Cl-$C_6H_4O$) | 7.1 | 4.8 |
| Li | Linuron | 4.9 | 7 | 3-Cl-4-$CH_3$ | 7.3 | 5.0 |
| Cb | Chlorbromuron | 4.9 | 2 | 3-$CF_3$ | 7.4 | 5.7 |
| Bu | Buturon | 5.9 | 1 | Aniline | 7.6 | 5.0 |
| Nb | Neburon | 6.4 | 14 | 4-(4-$CH_3O$-$C_6H_4O$) | 7.7 | 4.8 |
| Ip | Isoproturon | 9.6 | 4 | 4-Cl | 8.0 | 5.4 |
| Cx | Chloroxuron | 10.3 | 6 | 4-Br | 8.1 | 5.4 |
| Dx | Difenoxuron | 11.1 | 9 | 3,4-$Cl_2$ | 8.3 | 5.9 |
| Fe | Fenuron | 12.2 | 11 | 3-Cl-4-Br | 8.4 | 5.9 |
| Ct | Chlortoluron | 12.2 | 10 | 3-Cl-4-$OCH_3$ | 9.1 | 6.0 |
| Fm | Fluometuron | 13.1 | | | | |
| Mo | Monuron | 14.0 | | | | |
| Di | Diuron | 15.6 | | | | |
| Mx | Metoxuron | 16.2 | | | | |

ence in behaviour was shown to be due to the different alcohol contents of the various brands of dichloromethane. The addition of a few tenths of a per cent of ethanol —which will act as a strong modifier— to dichloromethane from Baker resulted in separations which were completely comparable to those obtained when using the Merck- or Janssen-type dichloromethane (the former, according to its label, contains about 0.3% of ethanol).

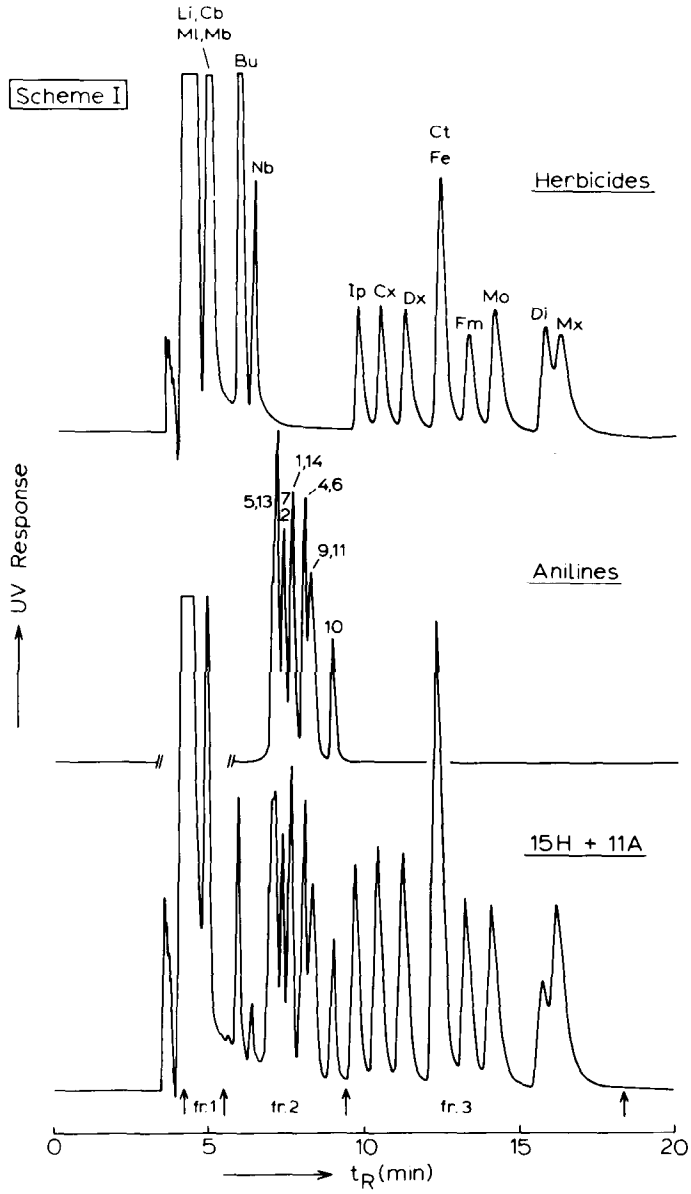



Fig. 5. NP-HPLC on silica of fifteen phenylurea herbicides (H), the eleven corresponding aniline degradation products (A) and their mixture, showing fractionation according to analysis scheme I. For mobile phase, codes and further details see Table V and text.

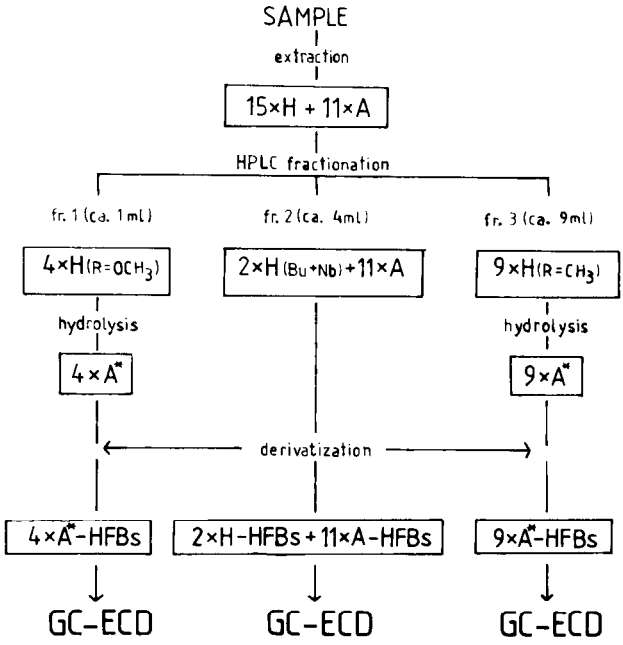



Fig. 6. Analysis scheme I for the selective determination of fifteen phenylurea herbicides (H) and the eleven corresponding substituted aniline degradation products (A) based on HPLC fractionation as the first step. A* = Anilines obtained after hydrolysis of herbicides; A-HFBs and H-HFBs = HFB derivatives of anilines and herbicides, respectively. For further details see text.

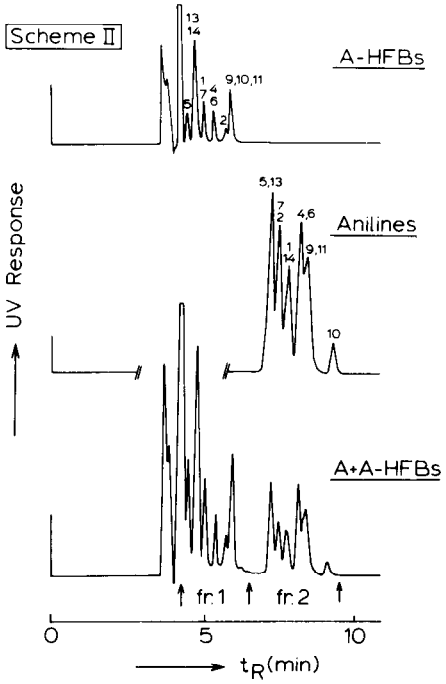



Fig. 7. NP-HPLC on silica of eleven anilines (A), the corresponding HFB derivatives (A-HFBs) and their mixture, showing fractionation according to analysis scheme II. For mobile phase, codes and further details see Table V and text.

Since increased ethanol concentration had a more pronounced effect on herbicide as compared to aniline retention, an optimum was selected so as to have the six early eluted herbicides [with R = $OCH_3$, $C_4H_9$ or $CH(CH_3)C \equiv CH$) elute ahead of all anilines, without losing the separation between the late-eluted anilines and the remaining nine herbicides (with R = $CH_3$). The best results were obtained with dichloromethane–hexane (90:10) containing 0.01% of TEA and 0.45% of ethanol as mobile phase. Fraction 1 now contains the four herbicides having R = $OCH_3$, fraction 2 neburon and buturon and all eleven anilines and fraction 3 the nine herbicides with R = $CH_3$; the pertinent retention times are recorded in Table V. A chromatogram of a mixture of all fifteen herbicides and the corresponding eleven anilines is shown in Fig. 5. The total analysis time is less than 20 min.

It is obvious that the final result is to be preferred to the initially intended fractionation. All anilines are now contained in the same fraction and can be immediately derivatized. Neburon and buturon which are also present in the fraction are easily converted into their HFB derivatives. Hydrolysis is, therefore, superfluous and there is no interference with the anilines of these herbicides. The herbicides which yield the same aniline upon hydrolysis, *viz.*, monolinuron, buturon and monuron (4-chloroaniline), and linuron, neburon and diuron (3,4-dichloroaniline), are nicely

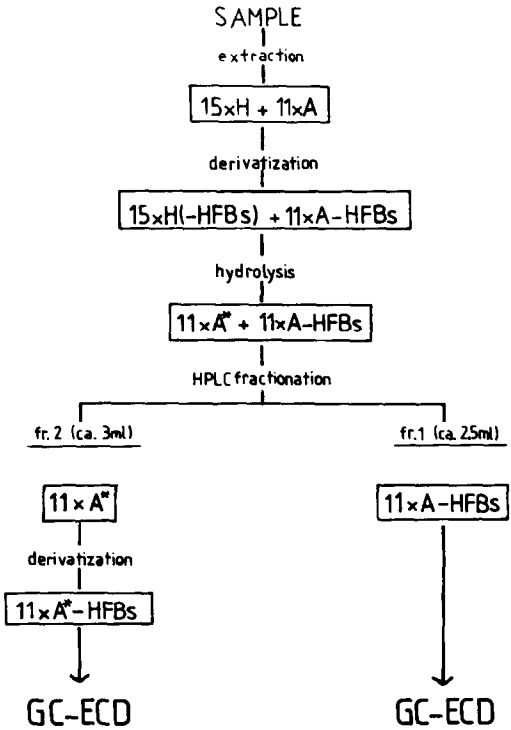



Fig. 8. Analysis scheme II for the selective determination of fifteen phenylurea herbicides (H) and the eleven corresponding substituted aniline degradation products (A) based on derivatization as the first step. A* = Anilines obtained after hydrolysis of herbicides; A-HFBs and H-HFBs = HFB derivatives of anilines and herbicides, respectively. For further details see text.

distributed over fractions 1, 2 and 3, respectively. The complete analysis scheme I is outlined in Fig. 6.

*Scheme II.* As an alternative to the above procedure, derivatization and subsequent hydrolysis of the complete herbicide–aniline mixture was suggested; in this case the final products are anilines and HFB-amides. Group separation, on silica, of these classes of compounds was shown to be rather simple in TLC (*cf.*, Fig. 4). Successful HPLC fractionation was indeed easily achieved, *e.g.*, with hexane–dioxane (90:10) or (85:15) and also with dichloromethane–hexane–ethanol–TEA (90:10:0.45:0.01). With the latter mobile phase, fractionation took only 10 min (see Fig. 7) and was distinctly more rapid than with the dioxane-containing mixtures or than fractionation in Scheme I. This mobile phase is therefore recommended for all further work, the more so since it can be used in both schemes; all relevant retention times are included in Table V. The complete analysis scheme II is outlined in Fig. 8.

Scheme II has the advantage over Scheme I of a more simple fractionation procedure, and final GC–ECD of only two fractions. That is, the total analysis time is considerably shorter. Scheme I, on the other hand, possesses a greater selectivity since herbicides yielding identical anilines end up in different fractions, whereas in Scheme II all herbicides are contained in the same fraction. Obviously, the ultimate choice will depend on the problem at hand.

## CONCLUSIONS

The determination of phenylurea herbicides and/or their aniline metabolites via various chromatographic procedures has been studied. For the determination of the separate classes of compounds, both reversed- and normal-phase HPLC and packed-column and capillary GC can be used successfully. For a real multiresidue method, the use of normal-phase HPLC (for fractionation purposes) combined with capillary GC–ECD (for ultimate separation and detection) seems to be required. In GC, the electron-capture detector is recommended since it is two orders of magnitude more sensitive than, and equally as selective as, the nitrogen–phosphorus detector.

Catalytic hydrolysis of the herbicides on a short silica column promotes their rapid conversion (20 min at 165°C) into the corresponding anilines, which are then derivatized with HFBA for sensitive GC–ECD detection. Impregnation of the silica with DMA (1 mmol/g) is recommended for accurate trace level analysis, *i.e.*, for ng amounts of herbicides.

The derivatization of anilines with HFBA has been optimized with respect to the reaction time and medium. Separation of the entire class of fourteen HFB-amides can be achieved on a CP-Sil 5 fused-silica column, with detection limits (for ECD) of about 0.2 pg.

The above procedures and other techniques, such as direct derivatization of the parent herbicides with HFBA[10] and the use of normal-phase HPLC–ECD[19,20], can be combined to give complete schemes of analysis for the determination of mixtures of herbicides and/or anilines. In the present work, two such schemes have been outlined (*cf.*, Figs. 6 and 8). In a subsequent paper[39], application of these schemes and other relevant procedures to the analysis of water, soil and crop samples will be discussed.

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