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PRE-COLUMN TECHNOLOGY IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF PHENYLUREA HERBICIDES IN WATER IN THE PRESENCE OF THEIR ANILINES*

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SUMMARY

Several metal-loaded phases were investigated for their use in the analysis of residues of phenylurea herbicides and their corresponding anilines. A special platinum phase packed in a short pre-column can act as an aniline filter for a group separation of the herbicides from the anilines. Coupling of such a Pt pre-column with a C₁₈ pre-column permits subsequent pre-concentration of the herbicides and on-line transfer to and separation on a reversed-phase analytical column. The feasibility of this method for the on-line automatable analysis of intact phenylurea herbicides in the presence of their corresponding anilines was demonstrated on polluted river water samples.

INTRODUCTION

Substituted phenylureas are well known and widely used herbicides with the general structure given in Fig. 1. Residue analysis of phenylureas in environmental samples is often based on gas (GC)^{1,2} or column liquid (LC)³⁻⁶ chromatographic

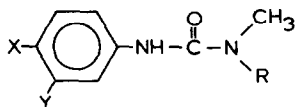


Fig. 1. Structures of the phenylurea herbicides and corresponding substituted anilines. X = H, Cl, Br, CH₃, OCH₃, (CH₃)₂CH-, 4-Cl-C₆H₄O-, or 4-CH₃O-C₆H₄O-; Y = H, CF₃ or Cl; R = CH₃, CH(CH₃)CCH-, -OCH₃ or C₄H₉. For detailed identification, see ref. 2.

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methods. A serious disadvantage of GC analysis is the thermolability of many of the phenylureas; consequently, prior to chromatography, steps such as hydrolysis and/or derivatization are required. LC methods are more attractive as they allow direct determination of the herbicides. In the literature, both normal- and reversed-phase separations with UV detection at 240–245 nm have been reported. With such methods, detection limits are typically about 10 ng. Consequently, for residue analysis a pre-concentration procedure is required that effects at least a 100-fold trace enrichment.

In the determination of the phenylureas, a special problem is the discrimination between these analytes and the substituted anilines which are their main (bio)degradation products. In addition, the latter are generally present in abundance as they are bulk chemicals as well as products of several industrial activities. Discrimination between a single herbicide and its corresponding aniline can easily be achieved by means of LC. Severe problems are encountered, however, when a relatively large number of phenylureas and anilines have to be separated from each other² and/or traces of the herbicides in the presence of larger amounts of anilines have to be determined. Recently, several combined LC–GC analysis schemes have been elaborated for the determination of 15 phenylureas and the 11 corresponding anilines⁷. These are, however, rather complicated and there still is a need for simpler procedures which, in the case of water samples, may well involve the use of pre-column technology.

In previous papers^{8–11} we have described the design and utilization of small pre-columns (length 2–5 mm, I.D. 2–4.6 mm) packed with sorbents such as C₁₈-bonded silica, carbon-based supports and divinylbenzene–styrene-type sorbents for the on-line pre-concentration of organic pollutants in aqueous samples. In this work, the same methodology was used for the trace-level pre-concentration and determination of the phenylurea herbicides. Also, an attempt was made to achieve herbicide/aniline group separation via insertion of a pre-column packed with a metal-loaded stationary phase. A suitably selected metal-loaded phase may be expected to provide the required selectivity as complexation of metal ions with primary amines (anilines) and substituted ureas is known to be distinctly different¹².

EXPERIMENTAL

Methods

Automated pre-concentration and LC analysis were carried out using a Kontron (Zürich, Switzerland) liquid chromatograph, consisting of two Model 410 pumps, a Model 200 programmer, an MCS 670 column-switching apparatus and a Uvikon LC 720 variable-wavelength UV detector set at 241 nm. Signals were recorded on a W + W 900 (Kontron) recorder and processed manually. The apparatus used is shown schematically in Fig. 2.

Separations were carried out using a 25 cm × 4.6 mm I.D. stainless-steel tube packed with CP-Spher C₁₈ (Chrompack, Middelburg, The Netherlands) and 12.5 cm × 4.0 mm I.D. stainless-steel columns home-packed with RP-8 and RP-18, 5 μm (Merck, Darmstadt, F.R.G.), and 2-amino-1-cyclopentene-1-dithiocarboxylic acid (ACDA) support; columns were slurry-packed with methanol at 35–50 MPa using isopropanol as a slurry liquid. Various methanol–water mixtures were used as the

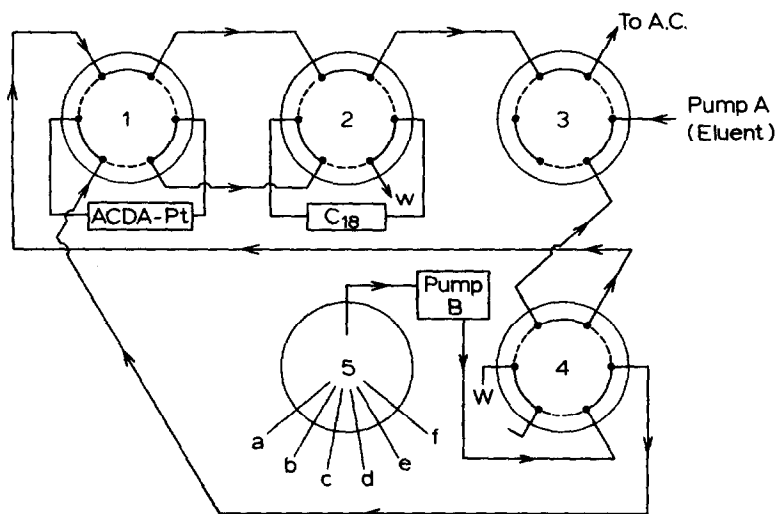


Fig. 2. Switching-valve configuration for the on-line removal of anilines and pre-concentration of phenylurea herbicides prior to LC analysis. 1, 2, 3, 4 = High-pressure switching valves; 5 = low-pressure six-port selector valve (a = water; b = sample 1; c = acetonitrile; d = water; e = sample 2; f = acetonitrile); ACDA-Pt and C_{18} , pre-columns (11×2 mm I.D., packed with the indicated material); A.C. = analytical column; A and B = HPLC pumps; W = waste.

mobile phase. Stainless-steel pre-columns (11×2 mm I.D.) designed as described earlier⁸ were slurry-packed with a syringe using a dense slurry of the packing material in methanol.

River water samples were filtered over a paper filter followed by a $0.8\text{-}\mu\text{m}$ cellulose acetate filter (Sartorius, Göttingen, F.R.G.).

Materials

Methanol, acetonitrile and hydrochloric acid were of analytical-reagent grade and were purchased from Baker (Deventer, The Netherlands). LC-quality water was prepared by purifying demineralized water in a Milli Q filtration system (Millipore, Bedford, MA, U.S.A.).

The herbicides were gifts from the Food Inspection Service (Amsterdam, The Netherlands). 3- and 4-chloroaniline, 4-bromoaniline and 3,4-dichloroaniline were purchased from Fluka (Buchs, Switzerland), and 3-chloro-4-methoxyaniline from Aldrich (Beerse, Belgium); 4-chlorophenoxy- and 4-(4-methoxyphenoxy)aniline were prepared from the corresponding herbicides via catalysed hydrolysis on silica⁷. Stock solutions of the herbicides and anilines were prepared in methanol; chloroxuron and difenoxuron were, however, first dissolved in toluene and then diluted with methanol. Stock solutions were stored at -20°C . Aqueous solutions were stored at -4°C in the dark. Aqueous solutions of the herbicides were stable for several weeks; with isoproturon, however, appreciable breakdown occurred within a few days. Aqueous solutions of the anilines had to be prepared fresh each week.

A $7\text{-}\mu\text{m}$ silica-based chelating ion exchanger containing an ACDA functional group, without and with added metal ions, was the stationary phase material used for herbicide/aniline separations. The structure is given in Fig. 3. The synthesis of

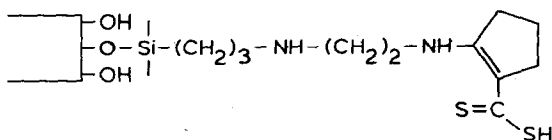


Fig. 3. Structure of the ACDA-Pt phase.

the ACDA phase has been reported earlier¹³. In this study, three different batches of ACDA were used; these were all prepared from the same batch (No. 15-B.5, $S_{BET} = 426 \text{ m}^2 \text{ g}^{-1}$) of 6.65- μm LiChrosorb Si 60 silica (Merck, Darmstadt, F.R.G.).

Metal loading was performed by dissolving an appropriate amount of metal salt in a small volume of 0.01 *M* hydrochloric acid, adding the corresponding amount of ACDA-silica and shaking it for at least 2 h on a shaking machine [example: 1.517 g of $\text{Na}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ (2.7 mmol) were dissolved in 25 ml of 0.01 *M* hydrochloric acid and 3 g of ACDA-silica were added].

d.c. argon emission spectrometer investigations of the supernatant liquid showed that there was no difference whether the shaking time was either 2 or 15 h. From these investigations the capacity of the Pt-ACDA-silica was determined to 0.65 mmol g^{-1} of Pt(IV), which is in good agreement with earlier reported values¹³. The results of the elemental analysis of three different Pt-ACDA phases were C 9.6%, N 2.3%, S 3.2%, Pt 9.2%.

From the N/S ratio, it can be calculated that about 60% of the available aminoethylaminopropyl spacer has reacted with ACDA. Taking the S value (sulphur) as an indication of the amount of the bonded ACDA the surface concentration can be calculated with a mean molecular weight of the bonded species to be about 1.4 $\mu\text{mol m}^{-2}$ of ACDA. The amount of Pt corresponds to 0.52 mmol g^{-1} of Pt, which is lower than the amount determined by sorption studies. A possible explanation is that in uptake studies usually the equilibrium concentration is measured. This means that both coordinated and physisorbed metal ions are determined. By washing the silica and using it in chromatography, the physisorbed metal ions are then washed out.

RESULTS AND DISCUSSION

Chromatography

Reversed-phase chromatography. Separations of phenylurea herbicides on C_2 - and C_{18} -bonded phases have been described in the literature⁴⁻⁶. The resolving powers of three reversed-phase materials differing in chain length are compared in Table I. The eluent for the C_8 - and C_{18} -type column was methanol-water (60:40) and for the C_2 -type column methanol-water (15:85). The best results were obtained with the C_{18} column, on which 14 out of the 15 herbicides could be resolved. Monolinuron and buturon showed coinciding peaks but can well be separated on a C_2 column. On the C_{18} column resolution from the corresponding anilines was also tested and could not be achieved; neither could the anilines be resolved from each other.

Ligand-exchange chromatography. Portions of a batch of ACDA were loaded with different metal ions that are known to form complexes with amines^{12,14,15} and both the loaded and unloaded phases were slurry packed into analytical columns.

TABLE I
RETENTION OF SOME PHENYLUREA HERBICIDES AND CORRESPONDING ANILINES ON DIFFERENT REVERSED-PHASE SYSTEMS

Herbicide	Abbreviation	Capacity factor*		Retention time (min)*
		<i>C</i> ₁₈ column (250 × 4.6 mm I.D.) eluent 0.6**	<i>C</i> ₈ column ⁷ (250 × 4.6 mm I.D.) eluent 0.6**	<i>C</i> ₂ column ⁵ (500 × 3.5 mm I.D.); eluent 0.15**
Fenuron	Fe	1.0	—	—
Metoxuron	Mx	1.7	1.6	4.2
3-Cl-4-OCH ₃ -aniline	1	1.1	—	—
Monuron	Mo	2.8	2.7	4.6
Monolinuron	MI	4.2	3.4	4.9
Buturon	Bu	4.2	—	6.1
4-Cl-aniline	2	1.7	—	—
Chlortoluron	Ct	4.6	3.9	6.3
3-Cl-4-CH ₃ -aniline	5	3.3	—	—
Metobromuron	Mb	5.1	3.9	5.7
4-Br-aniline	3	1.9	—	—
Fluometuron	Fm	2.1	—	5.8
3-CF ₃ -aniline	4	—	—	—
Isoproturon	Ip	5.6	—	8.0
4-CH(CH ₃) ₂ -aniline	8	5.1	—	—
Diuron	Di	6.3	4.4	8.1
Linuron	Li	9.3	6.0	11.4
Neburon	Ne	19.0	—	6.2
3,4-Cl ₂ -aniline	6	3.5	—	—
Chlorbromuron	Cb	10.5	6.9	12.8
3-Cl-4-Br-aniline	7	4.3	—	—
Chloroxuron	Cx	15.0	8.8	33.0
Difenoxuron	Dx	17.0	4.1	17.0

* —, Not determined.

** Volume fraction of methanol in methanol-water.

The retention behaviours of some phenylurea herbicides and the corresponding anilines on the different columns were compared. Methanol-water mixtures of different compositions were used as eluents. The results are presented in Table II. The retention of the herbicides and anilines on ACDA and the ACDA-metal-loaded phases increases with decreasing methanol content, indicating a reversed-phase type solute-sorbent interaction. Retention of the herbicides is much less on the ACDA phase than on *C*₁₈. Even when a higher water content is used in the eluent, neither the bare ACDA phase nor the metal-loaded phases exert an appreciable resolving power towards the phenylurea herbicides.

The addition of metal ions does not greatly influence the retention of the herbicides, the only exception being Ag(I), which causes a 1.25–1.50-fold increase in *k'* when methanol-water (40:60) is used as the eluent. The increase in retention of the anilines on addition of Ag(I) is even 3–5-fold with this eluent. With methanol-water (60:40) the addition of Ag(I) to the ACDA stationary phase causes an up to 6-fold increase in retention of the anilines. Unfortunately, it does not lead to the large

TABLE II
CAPACITY FACTORS OF SOME PHENYLUREA HERBICIDES AND CORRESPONDING ANILINES ON ACDA AND ACDA LOADED WITH DIFFERENT METAL IONS

Column: 125 × 4 mm I.D.

Analyte	Capacity factor		ACDA loaded with metal ions										
	ACDA	(A)*	Ag(I) (A)	Hg(II) (A)	Cd(II) (A)	Cu(II) (A)	Pd(II) (B)	ACDA (C)	Ag(I) (C)	Hg(II) (C)	Cd(II) (C)	Cu(II) (C)	Pt(IV) (C)
Metoxuron	1.3	1.9	1.9	1.1	1.5	1.1	1.4	0.6	0.7	0.5	0.6	0.3	0.7
3-Cl-4-OCH ₃ -aniline	—	—	—	—	—	—	∞	—	—	—	—	—	∞
Monuron	1.2	1.8	1.8	1.4	1.5	1.1	—	0.6	0.6	0.5	0.5	0.3	0.6
Monolinuron	1.2	1.6	1.6	1.1	1.5	1.1	—	0.3	0.5	0.4	0.5	0.3	0.5
Buturon	1.6	1.7	1.7	—	2.1	1.8	—	0.7	—	0.7	0.6	0.4	0.7
4-Cl-aniline	1.1	4.4	4.4	0.7	1.2	0.9	∞	0.6	2.9	0.5	0.5	0.5	∞
Chlortoluron	1.6	2.5	2.5	1.3	1.8	1.4	1.6	0.6	0.7	0.5	0.6	0.4	0.7
3-Cl-4-CH ₃ -aniline	1.4	6.7	6.7	1.2	1.5	1.5	∞	0.6	3.6	0.5	0.5	0.5	∞
Metobromuron	1.6	2.6	2.6	1.4	2.0	1.5	—	0.7	0.7	0.5	0.6	0.4	0.6
4-Br-aniline	—	—	—	—	—	—	∞	0.8	4.8	0.7	0.7	0.7	∞
Diuron	2.2	3.1	3.1	1.8	2.6	2.2	2.8	0.7	0.8	0.7	0.7	0.5	0.3
Linuron	2.3	2.8	2.8	1.8	2.6	2.1	2.4	0.7	0.7	0.7	0.7	0.4	0.7
Neburon	3.3	4.4	4.4	2.6	4.2	3.5	—	0.9	0.8	0.8	0.7	0.5	0.9
3,4-Cl ₂ -aniline	2.4	7.8	7.8	2.3	2.8	2.5	∞	0.9	3.0	0.9	0.9	0.7	∞
Chlorbromuron	—	—	—	—	—	—	—	—	—	—	—	—	—
3-Cl-4-Br-aniline	3.1	11.2	11.2	—	—	—	∞	1.2	3.9	1.1	1.1	0.9	∞

* Eluent: methanol-water, (A) 40:60, (B) 70:30, (C) 60:40.

differences in selectivity towards the herbicides and anilines that are required for group separation. The selectivity of ACDA-Ag towards anilines could, however, be used for the determination of anilines in the absence of phenylurea herbicides. A chromatogram of six substituted anilines separated on ACDA-Ag is given as an example in Fig. 4.

The addition of Cd(II), Cu(II) and Hg(II) does not cause a significant interaction with the anilines in aqueous mobile phases, contrary to earlier reports where, e.g., Cd(II)-loaded phases have been applied successfully to the normal-phase separation of substituted anilines¹⁴. The influence of the addition of Pd(II) and Pt(IV) on group selectivity is much more pronounced. The anilines investigated are infinitely retarded in the system used, whereas no influence of Pd(II) and Pt(IV) on the retention of the urea herbicides is observed. Complexation of phenylurea herbicides with metal ions can apparently only take place through interaction with the aromatic ring, as the nitrogen atoms are sterically and electronically shielded. Of the metal ions investigated, only Ag(I) is well known for its affinity towards aromatic systems^{15,16}, which explains the increased retention of the herbicides on ACDA-Ag¹⁵.

In principle, all investigated metal ions are able to form strong complexes with anilines, by complexation with the NH₂ group. Ag(I) can form complexes with both nitrogen and the aromatic ring. Pd(II) and -IV and Pt(II) and -IV are known to form extremely strong and kinetically stable complexes with amines^{16,17}. For the latter reason they are not employed in the ligand-exchange chromatography of amines. As our results indicate, however, they are suitable for irreversible sorption of anilines and could therefore be useful as selective filters for the removal of anilines from herbicide solutions. The applicability of this concept is further developed in this study. As the Pd(II) and Pt(IV) phases behaved very similarly in our experiments, we arbitrarily chose ACDA-Pt(IV) to continue our studies.

Retention of herbicides and anilines on an ACDA-Pt pre-column

As we indicated in the Introduction, in order to obtain adequate sensitivity for herbicide residue analysis by LC with UV detection, at least a 100-fold concentration of the samples is required. This means that with on-line pre-concentration, several millilitres have to be sampled and hence the same volume should pass the ACDA-Pt pre-column without anilines breaking through.

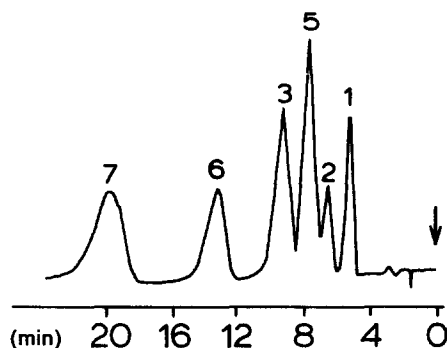


Fig. 4. Separation of (1) 3-chloro-4-methoxy-, (2) 4-chloro-, (5) 3-chloro-4-methyl-, (3) 4-bromo-, (6) 3,4-dichloro- and (7) 3-chloro-4-bromoaniline. Column: 250 × 4 mm I.D., ACDA-Ag. Eluent: methanol-water (30:70). Flow-rate: 1 ml min⁻¹. Volume injected: 20 μl. Detection: UV, 243 nm.

In order to determine the maximum volume of water that can be fed through an ACDA-Pt pre-column, breakthrough curves of the very polar 4-chloroaniline were recorded. The pre-column dimensions were 11×2.0 mm I.D. and the flow-rate was varied between 0.5 and 2.0 ml min⁻¹. Breakthrough was measured for a solution containing 0.3 µg of 4-chloroaniline per millilitre of water. At a flow-rate of 2 ml min⁻¹ breakthrough took place after 2 ml, while the breakthrough volume was 10 ml at a flow-rate of 0.5 ml min⁻¹. This large difference is caused by the slow mass transfer due to the apparently slow complexation kinetics.

As to repeatability of this filtering procedure on this type of pre-column, 10 ml of a solution containing 8–15 ng ml⁻¹ of aniline were repeatedly injected and up to five successive injections can be made before breakthrough of aniline, probably due to overloading, is noticed. As it is desirable for the ACDA-Pt pre-column to perform a complete group separation between phenylurea herbicides and the corresponding anilines, the retention properties of the ACDA-Pt pre-column towards herbicides in aqueous solution was also investigated. Chloroxuron, a late-eluting compound in reversed-phase LC (see Table I) was used as the test compound. The breakthrough curve of a 0.15 µg ml⁻¹ solution of this solute was recorded. Breakthrough was found to take place within 1 ml.

Desorption of anilines from ACDA-Pt

As the breakthrough volume of the early eluting 4-chloroaniline on a short ACDA-Pt pre-column is low (10 ml, see above) and also its sorption capacity is limited, the utility of an on-line ACDA-Pt filter column is fully dependent on the possibility of fast, on-line regeneration of the filter. Therefore, the desorption of the anilines from ACDA-Pt was studied. These experiments were carried out with 45×4.6 mm I.D. columns, slurry-packed with ACDA-Pt, on which 1-ml aliquots of an aqueous solution of 4-chloro-, 3,4-dichloro- and 3-chloro-4-bromoaniline were pre-concentrated. The elutotropic properties of different eluents were investigated. Neither methanolic-aqueous mixtures nor pure methanol could be used to desorb the anilines from the column. As the retention of anilines on ACDA-Pt is due to the formation of (very strong) complexes with the stationary phase, solvents with higher elutotropic strength than methanol in reversed-phase systems, such as dioxane and THF, were not successful either.

An exception was acetonitrile, which is able to form complexes with Pt¹⁸ and can therefore act as a displacer of the anilines. Other possible displacers such as non UV-absorbing organic amines (*e.g.*, aminoethane) dissolved in an aqueous mobile phase and dilute hydrochloric acid were also investigated without success.

A 5% solution of diaminoethane caused an immediate loss of Pt and was therefore not further investigated. A solution of hydrochloric acid (pH 2) was able to remove the most polar anilines (4-chloro- and 3,4-dichloroaniline) but the capacity factors were very high and the peaks broad and tailing. Addition of 60% and 80% of methanol did not improve the situation substantially, although the peak shape was better with the latter eluents. With pure acetonitrile all anilines were immediately desorbed from the ACDA-Pt column (capacity factor $k' = 0.8$). Acetonitrile-water (10:90 and 30:70) and acetonitrile-methanol-water (10:50:40 and 15:50:40) mixtures were able to elute all three anilines as broad and tailing peaks, but contrary to the situation with pure acetonitrile, the desorption was far from complete (recovery less

than 10%). Acidification of acetonitrile-water mixtures did not improve the situation as protonation of acetonitrile hinders Pt complexation.

As acetonitrile acts as a displacer it can be expected that it will block the complexing sites of the stationary phase and thus prevent further interaction. It was indeed found that a 36- μ l pre-column, flushed with 1 ml of acetonitrile, had completely lost its retention properties towards anilines. Only thorough flushing with approximately 23 ml (700 column volumes) of water could restore the initial properties of the material. Nevertheless, as these operations can be carried out on-line the desorption and regeneration step can potentially be automated.

Pre-concentration of phenylurea herbicides from aqueous solution on C₁₈

Following the aniline removal step the phenylurea herbicides are trapped and pre-concentrated on small (11 \times 2 mm I.D.) pre-columns packed with C₁₈ material. In order to obtain acceptable detection limits (ppb level) using LC with UV detection (detection limit *ca.* 10 ng) a total sample volume of at least about 10 ml is required. Breakthrough curves of two polar, early eluting herbicides, fenuron and metoxuron, were measured. Fenuron broke through after 1.5 ml but the breakthrough volume of metoxuron was 36 ml. Metoxuron and all the less polar, later eluting herbicides are therefore expected to be trapped with 100% recovery from 10-ml water samples.

Aliquots of 10 ml of aqueous solutions of fenuron, metobromuron and linuron, all with concentrations between 2 and 100 ng ml⁻¹, were pre-concentrated and subsequently eluted on-line in a C₁₈/methanol-water LC system. Calibration graphs were recorded and linear responses ($R = 0.9995$ – 0.9998) were found for metobromuron and linuron ($n = 6$). The correlation coefficient for fenuron was slightly less good (0.9987) but, taking into account that breakthrough occurs, this result is not surprising and still acceptable.

Analysis of phenylurea herbicides in dilute mixtures of herbicides and anilines

Aliquots of 10 ml of an aqueous solution containing *ca.* 10 ng ml⁻¹ of each of twelve urea herbicides (see Table III) and *ca.* 5 ng ml⁻¹ of each of seven anilines (see Table I) were subsequently fed through an 11 \times 2 mm I.D. ACDA-Pt pre-column and a 11 \times 2 mm I.D. C₁₈ pre-column, at a flow-rate of 0.5 ml min⁻¹. After sampling, both pre-columns were switched on-line in a chromatographic system with a C₁₈ analytical column using methanol-water (60:40) as the eluent at a flow-rate of 0.85 ml min⁻¹. The apparatus used for these experiments is shown schematically in Fig. 2. In order to remove all the herbicides completely, the ACDA-Pt pre-column has to be flushed with a maximum of 200 μ l of eluent and the C₁₈ pre-column with at least 1.7 ml. A smaller desorption volume (<1.7 ml) causes a loss of the most retarded solute, difenoxuron. Flushing the ACDA-Pt pre-column with a larger portion of eluent (>200 μ l) is not allowed, as it causes desorption of the earliest eluting aniline.

Chromatograms of 10-ml samples of phenylurea herbicides concentrated on a C₁₈ pre-column and with and without anilines filtered out by an ACDA-Pt pre-column are shown in Fig. 5.

The complete program for automated sample handling is given in the Appendix.

Recoveries of the herbicides after filtration and pre-concentration were deter-

TABLE III

RECOVERY OF PHENYLUREA HERBICIDES PRE-CONCENTRATED FROM 10-ml WATER SAMPLES ON AN ACDA-Pt AND C₁₈ PRE-COLUMN COMBINATION

Herbicide	Abbreviation	Concentration in HPLC grade water (ng ml ⁻¹)	Recovery (%)	
			HPLC-grade water (vs. loop injection)	River water (vs. HPLC-grade water)*
Fenuron	Fe	8.8	45	—
Metoxuron	Mx	11.7	92	98
Monuron	Mo	8.4	98	102
Fluometuron	Fm	9.2	92	103
Monolinuron	Ml	8.0	96	100
Buturon	Bu	9.1	0	—
Chlortoluron	Ct	12.2	93	96
Metobromuron	Mb	10.6	93	—
Isoproturon	Ip	10.8	95	—
Difenoxuron	Dx	12.4	90	—
Diuron	Di	9.8	91	98
Linuron	Li	10.7	90	100

* —, Not measured.

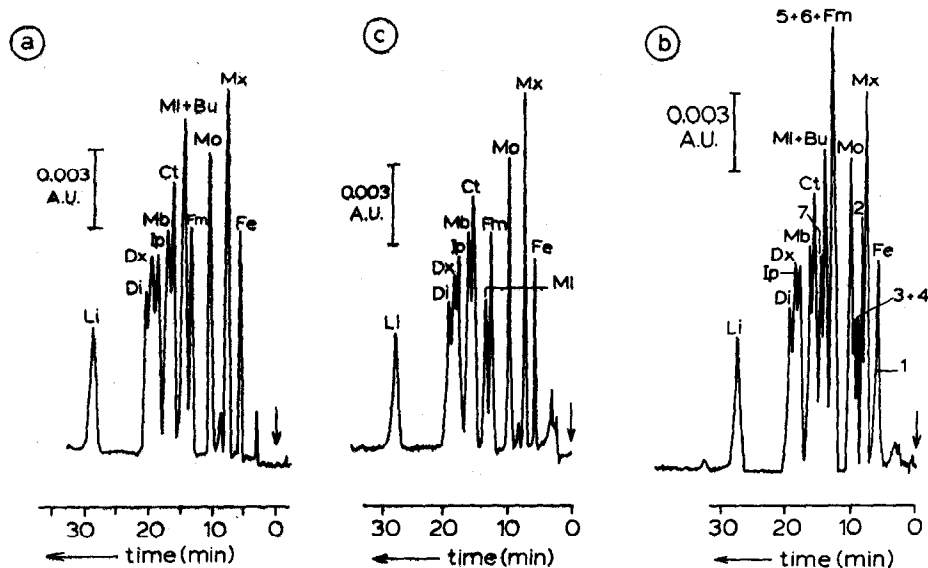


Fig. 5. Comparison of the results of one-line pre-concentration of 10 ml of an aqueous solution of herbicides with and without anilines on a C₁₈ pre-column with and without insertion of an ACDA-Pt filter. (a) Concentration of twelve herbicides on C₁₈; (b) concentration of twelve herbicides and seven anilines on C₁₈; (c) selective removal of seven anilines from solution b on ACDA-Pt, followed by pre-concentration of twelve herbicides on C₁₈. Apparatus: see text and Fig. 2. Flow-rate during sampling: 0.5 ml min⁻¹. For identification of the solutes, see Table I. Analytical column: 250 × 4.6 mm I.D., 8-μm CP-Spher C₁₈. Eluent: methanol-water (60:40). Flow-rate: 0.85 ml min⁻¹.

mined by comparing the peak heights of the analytes with those from loop injections and of samples that had only been concentrated on C_{18} . The recovery of fenuron is only 45% as this herbicide is not fully retained on the pre-columns (see above). The complete loss of buturon was surprising but this herbicide was found to be fully retained on ACDA-Pt. Buturon contains an alkyne group, which is well known for its ability to complex with Pt (see, *e.g.*, ref. 10).

All other herbicides were determined with over 90% recovery. As the peak shape is slightly influenced by insertion of the ACDA-Pt filter column, the recoveries would probably have been even better if peak areas had been compared instead of peak heights.

The above experiment could be repeated at least five times without cleaning of the ACDA-Pt pre-column before breakthrough of anilines became noticeable. In

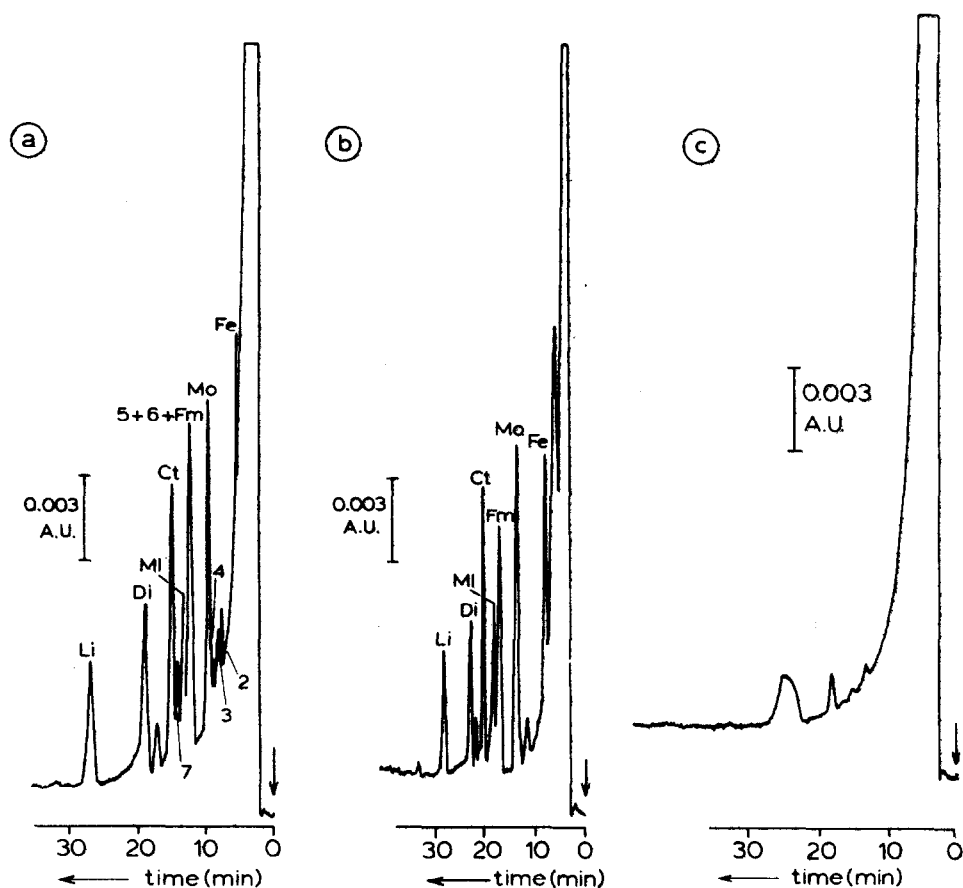


Fig. 6. Pre-concentration of 10 ml of a river water sample, spiked with seven herbicides and seven anilines. (a) Pre-concentration on a C_{18} pre-column; (b) as (a), but with insertion of an ACDA-Pt pre-column upstream from the C_{18} pre-column; (c) river water blank pre-concentrated on a C_{18} pre-column. Further conditions: see Fig. 5.

all further experiments, however, the ACDA-Pt pre-columns were flushed with 1 ml of acetonitrile to remove the anilines and subsequently with approximately 23 ml of water to remove the acetonitrile and re-equilibrate the pre-column. On introduction of these flushing steps, the separation pattern reproducibility remained excellent for several days.

Application to spiked river water

Water from the river Amstel (Amsterdam, The Netherlands) was spiked with 5–10 ng ml⁻¹ of each of seven herbicides and seven corresponding anilines. Aliquots of 10 ml were subsequently fed to an ACDA-Pt and a C₁₈ pre-column as described above. Recoveries of the herbicides were determined by comparison of the signals with those of a reference solution in HPLC-grade water, treated in the same manner (see Table III). The results for the river water samples are at least as good as those of the reference solutions. The seven anilines were completely removed from the solution by the ACDA-Pt filter column (compare Fig. 6a and b), even though this column obviously serves as an efficient filter for the removal of many other (polar) matrix constituents (*cf.*, Fig. 6b and c). After each run, the ACDA-Pt pre-column should be washed with 1 ml of acetonitrile to remove all sorbed compounds.

The combination of an ACDA-Pt filter column and a C₁₈ pre-column prior to reversed-phase LC with UV detection permits the simultaneous detection of fourteen phenylurea herbicides listed in Table I (with the exception of buturon) in environmental water samples at the ppb level, without interference from anilines.

Attempts to use a guard column to protect the ACDA-Pt filter from very dirty samples were not successful: small (30 μl) guard columns packed with C₂, silica and alumina removed 100, 30 and 15%, respectively, of the herbicides from 10-ml water samples while the bulk of the matrix was not removed.

CONCLUSIONS

The applicability of a platinum(IV)-loaded stationary phase, ACDA-Pt, for the removal of at least 0.1 ppm of anilines from aqueous solutions containing trace amounts of phenylurea herbicides is feasible. The combination of an ACDA-Pt filter and a C₁₈ pre-column permits fully on-line and therefore automatable sample clean-up and pre-concentration of river water samples for the determination of urea herbicides by reversed-phase LC with UV detection. The small platinum columns are regenerated by washing with acetonitrile and can then be used repeatedly when a final water washing step is used for regeneration of the ACDA-Pt pre-column.

Future studies will be directed towards the quantitation of trace amounts of anilines in environmental samples by means of pre-concentration on metal-loaded phases and suitable chromatography. Coupling of these pre-column procedures with selective detection modes such as photochemically sensitized fluorescence is also being investigated.

APPENDIX

Overall procedure for the automated pre-concentration and desorption/analysis of herbicides (1 cycle)

Pre-concentration.

<i>Time (min)</i>	<i>Event</i>	<i>Action</i>
0	Aux 1 dur 1.5 Aux 2 dur 0.5 Flow 1.5 ml min ⁻¹	Filling capillaries with water
0.5	Flow 1 ml ⁻¹	Flushing C ₁₈ pre-column with 1 ml of water
1.5	Aux 2 dur 3.5	Flushing ACDA-Pt pre-column with 1 ml of water
2.5	Aux 4 dur 2	Changing from water to sample (to waste)
4.5	Flow 2 ml min ⁻¹ Aux 1 dur 0.5 Flow 1.5 ml min ⁻¹ Flow 0.5 ml min ⁻¹	Filling capillaries with sample Pre-concentration of 10.0 ml sample
25.0	Flow 0	
25.1	End	

Desorption and analysis. Flow-rate, 0.85 ml min⁻¹; eluent, methanol-water (60:40).

<i>Time (min)</i>	<i>Event</i>	<i>Action</i>
0	Aux 1 dur 0.15	Desorption of ACDA-Pt
0.1	Aux 2 dur 2 Aux 3 dur 40	Desorption of C ₁₈ Eluent over analytical column only
2.4	Aux 4 dur 2 Aux 6 dur 0.01 Flow 2 ml min ⁻¹	Changing from sample to CH ₃ CN
4.4	Aux 1 dur 0.5 Aux 2 dur 19 Flow 1.5 ml min ⁻¹	Filling capillaries with CH ₃ CN
4.9	Flow 1 ml min ⁻¹	Desorption of ACDA-Pt with 1 ml of CH ₃ CN
5.9	Aux 4 dur 2 Aux 6 dur 0.01 Flow 2 ml min ⁻¹	Changing from CH ₃ CN to water
7.9	Aux 1 dur 0.5 Flow 1.5 ml min ⁻¹	Filling capillaries with water
23.4	Flow 0	Flushing pre-column with 22.5 ml of water
23.5	End	

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