Determination of Pesticides in Drinking Water by AMD A-28.7

Key words

Instrumental HPTLC – quantitative analysis – qualitative identification – trace analysis – densitometry (absorbance) – AMD – environmental analysis – pesticides:
Atrazin – Chlortoluron – Cyanazin – 2,4-D – Desethylatrazin – Metazachlor – Metobromuron – Metoxuron – Monuron – Pendimethalin – Phenmedipham – Propazin – Simazin – Terbutylazin – Vinclozolin

Scope

A method is described for identification and quantification of active ingredients of plant protecting agents in ground, drinking and mineral water. The analysis method has been accepted as German Standard Method DIN 38407, Part 11.

After extraction and enrichment of the pesticides from the water sample with solid phase extraction, the extract is chromatographed on silica gel by automated multiple development (AMD). Detection of the pesticides is performed by UV multi-wavelength scanning. In-situ UV spectra are taken for further confirmation of positive results.

Following the strategy described below, it is possible to distinguish practically all non-volatile water extractable active ingredients of plant protecting agents on the market. The suitability of this method was proved for more than 265 pesticides [5].

Advantages of performing this analysis by instrumental TLC

- Simultaneous screening for many pesticides
- Possibility to distinguish between all pesticides on the market
- No derivatization necessary.
- High sample throughput at low operating costs
- Determination limit in the range of 50 ng/L.

Reagents

All solvents used as mobile phases, conditioning liquids, extraction liquids, and for prewashing of the TLC plates must be free of nonvolatiles, especially UV absorbing residues. Only tested lots should be used for the analysis. (Reference R1)

For the experiments described in this procedure, the following solvents and reagents were used.

Solvent / reagent	Origin	#
Acetonitrile	Riedel de Häen	34851
Ammonia	Riedel de Häen	30501
Dichloromethane	American Burdick & Jackson (Distr. Fluka)	66747
Formic acid	Riedel de Häen	33015
Hydrochloric acid	Riedel de Häen	17932
Isopropanol	Riedel de Häen	34863
Methanol	American Burdick & Jackson (Distr. Fluka)	65544
n-Heptane	Riedel de Häen	34873
n-Hexane	American Burdick & Jackson (Distr. Fluka)	52763
tert-Butyl methyl ether	Riedel de Häen	65293

Today, special AMD grade solvents are available from Riedel de Häen.

Material for solid phase extraction

Bakerbond Octadecyl (C18), 40 µm (Baker 7025)
Glass cartridge (Baker 7328-03)
PTFE frits (Baker 7329-03)

Standards, all Pestanal (R) from Riedel de Häen

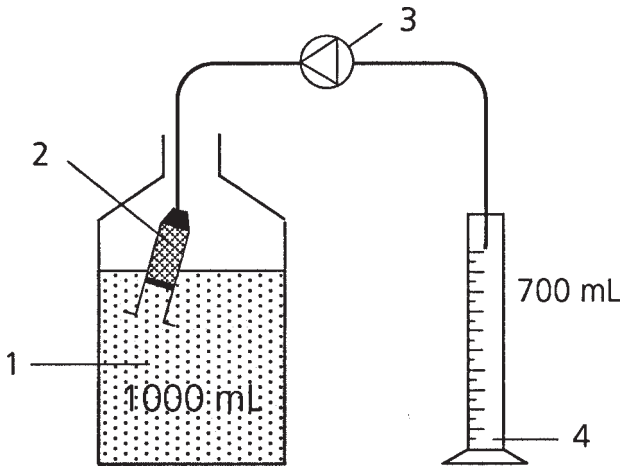
2,4-D	Metazachlor	Pendimethalin
Atrazine	Metobromuron	Propazin
Benzanilde	Metoxuron	Simazine
Chlortoluron	Monuron	Terbutylazine
Cyanazine	Phenmedipham	Vinclozolin
Desethylatrazine		

Other substances can be chosen as standards, if these have to be analyzed.

Sample preparation

Solid Phase Extraction (SPE) is carried out using glass cartridges equipped with PTFE frits and filled with of C18 material; use glass tubes and glass connections only. For enforcing the appropriate throughflow, arrange a peristaltic pump behind the outlet of the cartridge. A simple set-up is diagrammatically depicted in fig. 1.

- Condition the C-18 cartridge with 3 mL n-hexane, then 3 mL dichloromethane and finally 3 mL methanol.
- Acidify 1000 mL of the water sample with hydrochloric acid to pH 2.
- Connect the column entry to the 1000 mL water sample. Adjust a flow rate of 6 mL per minute.
- After exactly 700 mL has been percolated, dry the cartridge by blowing heated (35°C) nitrogen through for two hours; pass the nitrogen through activated carbon before it enters the cartridge.
- Elute the cartridge with 2 mL dichloromethane, then with 3 mL methanol.
- Evaporate the combined eluates to dryness in a stream of clean nitrogen at 35°C.
- Dissolve the residue in 250 µL acetonitrile - n-hexane 95:5 and add 280 ng Benzanilid as internal standard.
- For obtaining the blank solution, elute a conditioned and dried cartridge through which no water was percolated and treat the residue in the same way as described for the unknowns.



1 Water sample
2 C-18 cartridge
3 Peristaltic pump
4 Measuring cylinder

Fig. 1 Set-up for solid phase extraction

Identification/ calibration standards

For identification and calibration, the 15 standards are combined in three calibration mixtures. Each mixture contains 1 ng of each individual pesticide per µL acetonitrile.

Mixture 1	Mixture 2	Mixture 3
Desethylatrazine	Metoxuron	2,4-D
Simazine	Monuron	Atrazine
Cyanazine	Chlortoluron	Metazachlor
Terbutylazine	Propazin	Pendimethalin
Vinclozolin	Phenmedipham	Benzanilid (int. Standard)
	Metobromuron	

Strategy

Extracts of the unknowns, together with all three identification/ calibration standards, are chromatographed on two plates with two different AMD gradients (gradient A and B). Usually, gradient A is considered as a screening run, gradient B as confirmatory with regard to qualitative identification. However, either chromatogram confirms the result of the other. Quantification need to be done with only one of the two, i.e. where separation from extraneous peaks is best. This means, if several pesticides are found in the unknowns, quantification of the individuals can be taken from different plates.

Chromatogram layer and sample application

(R2 and R3 refer to recommendations in appendix 2)

Precoated HPTLC plates silica gel MERCK 60 F 254, 100 µm, 20 x 10 cm.

Immerse the plates in isopropanol for at least one hour. Then dry at 120°C for 1 hour.

Protect the prewashed plates suitably against contamination from the atmosphere during cooling, storage before use (R2), and sample application (R3).

Apply samples bandwise with a Linomat, preferably with a Linomat IV-Y: bands 7 mm long, 3 mm apart, distance from lower edge 8 mm, 25 mm from the side = 16 samples per plate; delivery speed 10 s/µL.

With a Linomat IV-Y, adjust the Y-drive at 6 mm so that rectangular sample zones 7 x 6 mm are obtained. If you have no Linomat IV-Y, apply with a regular Linomat each sample in three parallel bands, 2 mm apart in Y-direction.

Recommended application pattern for quantitative analysis:

B1	S1	S2	S3	U1	S4	S5	S6	B2	S7	S8	S9	U2	..-..
Blank	Mixture 1			Sample	Mixture 2			Bla-nk	Mixture 3			Sample	..-..

Apply 20, 50 and 100 µL of each standard mixture corresponding to 20, 50 and 100 ng per pesticide and 100 µL of the unknowns (corresponding to 250 mL of the original water sample) and 100 µL of the blank on two plates.

For processing large numbers of samples it is recommended to use only the 100 µL amounts of the identification/ calibration mixtures in the first (screening) run. On the second plate, which is used for confirmation and quantification, a complete set of three calibration standards of the mixture(s) corresponding to the suspected positives is run.

Chromatography

Chromatographic development is performed in the AMD System using two different universal gradients.

Gradient A is most often used in a screening function. It is based on dichloromethane as the central solvent governing the selectivity. Gradient A is diagrammatically depicted in fig. 2.

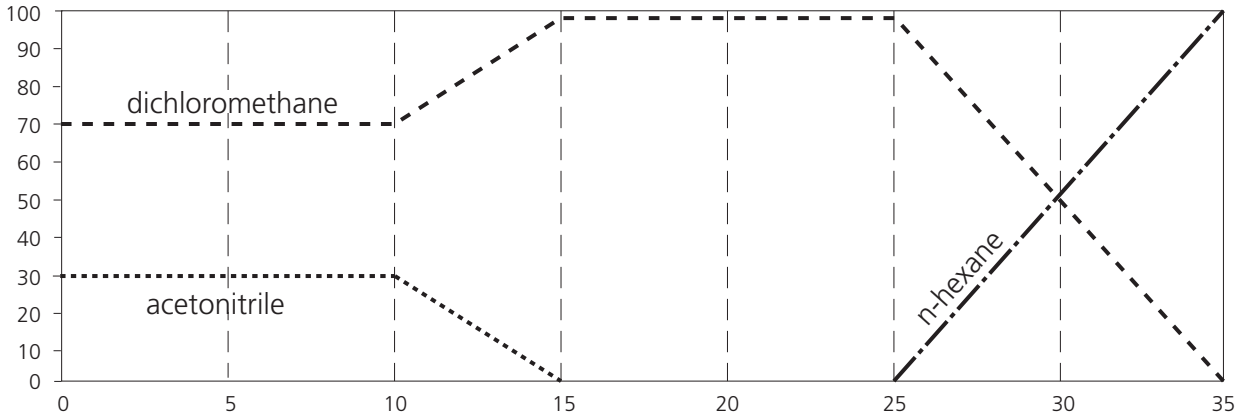


Fig. 2 AMD gradient A; operational details are given in appendix 2.

Development starts with 10 short isocratic runs with acetonitrile - dichloromethane 30:70 over 10 mm, thus extracting the active ingredients from the sample application area (away from humic acids). During steps 16 through 28 the mobile phase is acidified with small amounts of formic acid. By interaction with the weakly alkaline buffered layer, this establishes a pH gradient from weakly alkaline to acidic conditions. This is important for proper chromatography of all types of pesticides in the presence of humic acids. These are prevented from moving away from the starting area under the prevailing conditions.

The second plate is developed with AMD gradient B, which is most often used in a confirmatory function. This gradient is based on *tert*-butyl methyl ether and exhibits a distinctly different selectivity compared to the dichloromethane based gradient A.

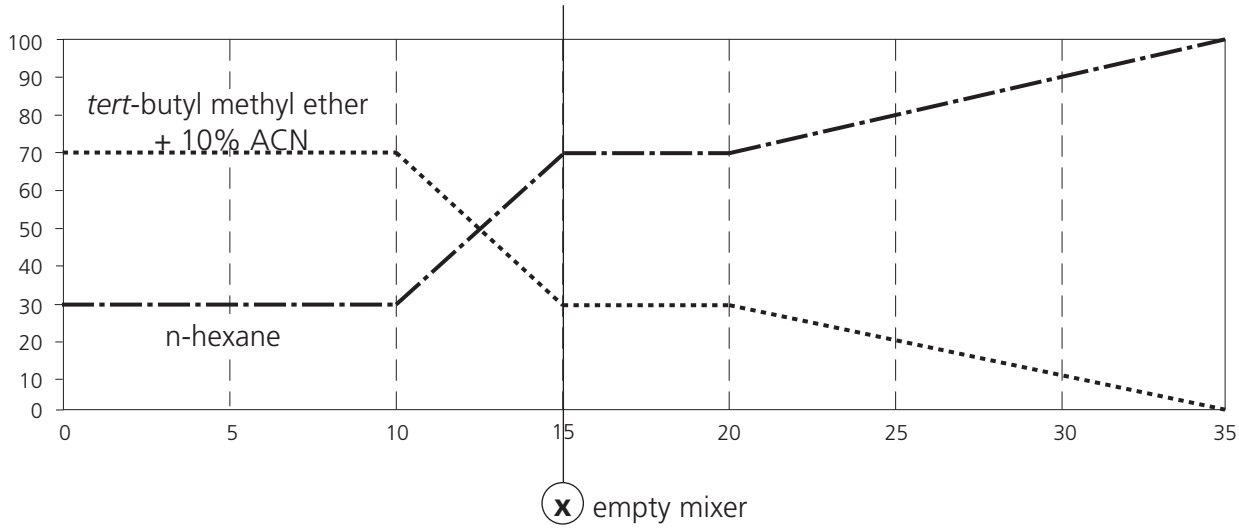


Fig. 3 AMD gradient B; operational details are given in appendix 2.

Similar to gradient A, this gradient starts with 10 isocratic steps with *tert*-butyl methyl ether containing 10% acetonitrile - hexane 70:30 slightly alkaline, for fixing humic acids in the starting area and extracting the active ingredients. Formation and function of the pH-gradient too is similar to that in gradient A.

Densitometric evaluation

With CAMAG TLC Scanner II with Labdata System and CATS evaluation software. Multi-wavelength scanning by absorbance at 190, 200, 220, 240, 260, 280, 300 nm with deuterium lamp, monochromator bandwidth 10 nm, slit dimensions 0.2 x 3 mm, scanning speed 4 mm/sec, sens 0, span 7.

☛ In order to obtain reliable results at wavelengths 190 and 200 nm, it is strongly recommended to flush the monochromator with nitrogen (about 0.5 L/min).
The substances are evaluated with linear regression via peakheight at their optimum wavelength. Benzanilide is used as internal standard for the correction of the sample volume applied to the TLC plate.

Results

Reference appendix 1 for details.

Fig. 4 through 9 show the distinctly different separation of the three calibration mixtures in gradient A and B. Not only the distance of migration for the same pesticide is different in both gradients, even the sequenz of elution is often changed.

Combining the results from both gradients is the only way to distinguish between the several hundreds of pesticides on the market.

Conformable positive results obtained with both gradients have proved over more than ten years of experience, to be almost as secure as GC/MS.

Fig. 10 and 11 show the chromatograms of a fortified sample of drinking water in gradients A and B respectively. Component 1 in gradient A (Fig. 10) has the same distance of migration as 2,4-D, but the behaviour of this component in UV multi-detection differs from 2,4-D: the peakheight at 240 nm is relatively too high. In the chromatogram of the same sample in gradient B (Fig. 11) component 1 is UV pure 2,4-D and the interfering component X (1a) is separated from 2,4-D more than half the length of the chromatogram. All other positive results from Fig. 10 are confirmed in Fig. 11 qualitatively and quantitatively.

All concentrations of pesticides stated in the legends of the chromatograms of the samples are uncorrected for recovery.

Further confirmation of positive results is possible by taking in-situ UV spectra of standards and components in the unknown.

For this purpose some conditions are essential:

1. The amount of substance in sample and calibration spot should be at least in the same order of magnitude; the smaller the difference of masses the better.
2. Reference spectra should be measured for each individual UV spectrum as close as possible to the substance spot, to minimize matrix influences.
3. Because of possible matrix interference, taking the spectra of the unknown and the identification standard from the same plate, is preferable over comparison using the spectrum library.

Appendix 1 - typical results

Chromatograms of identification/calibration mixtures

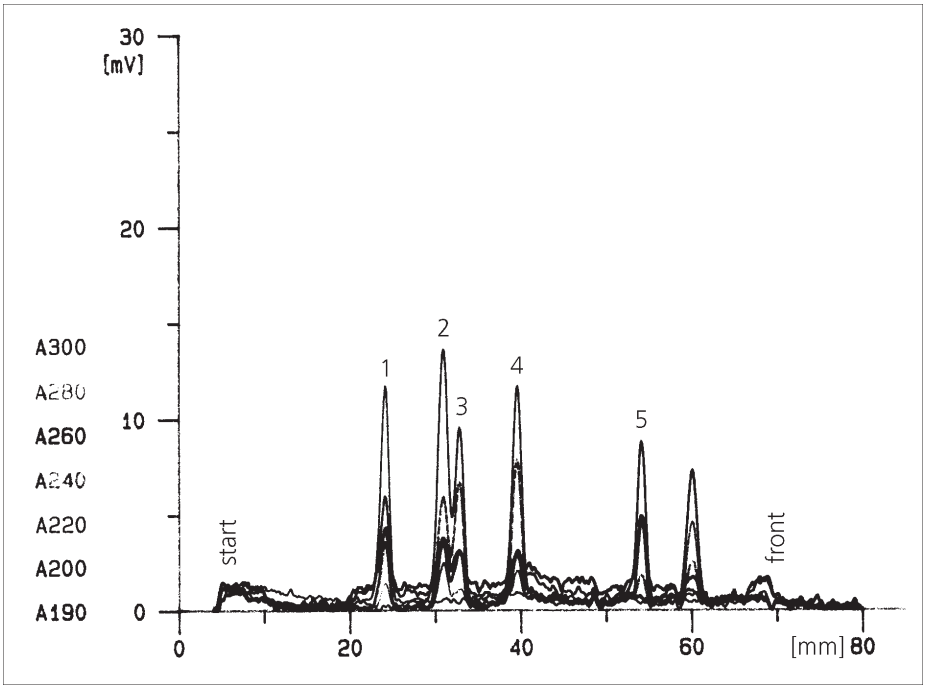


Fig. 4: Calibration mixture 1, gradient A, 50 ng each component; 1 = Desethylatrazine, 2 = Simazine, 3 = Cyanazine, 4 = Terbutylazine, 5 = Vinclozolin

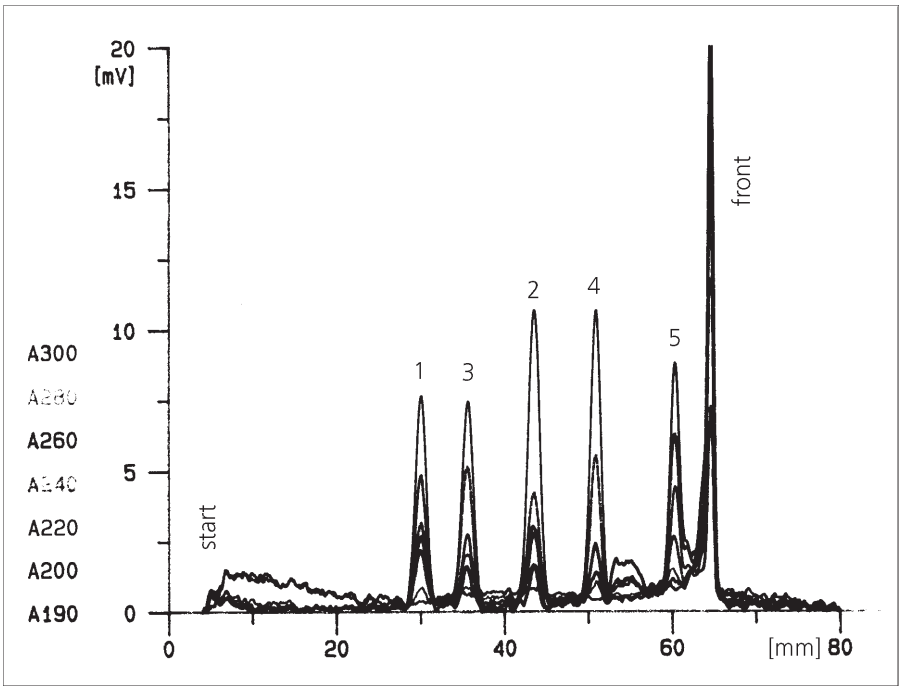


Fig. 5: Calibration mixture 1, gradient B, 50 ng each component; 1 = Desethylatrazine, 2 = Simazine, 3 = Cyanazine, 4 = Terbutylazine, 5 = Vinclozolin

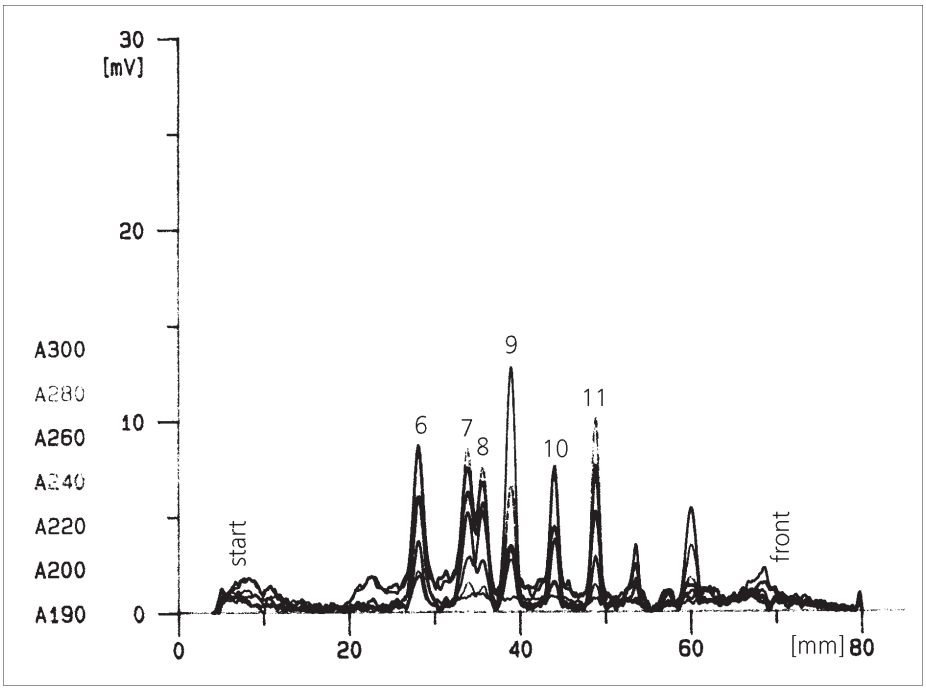


Fig. 6: Calibration mixture 2, gradient A, 50 ng each component; 6 = Metoxuron, 7 = Monuron, 8 = Chlortoluron, 9 = Propazine, 10 = Phenmedipham, 11 = Metobromuron

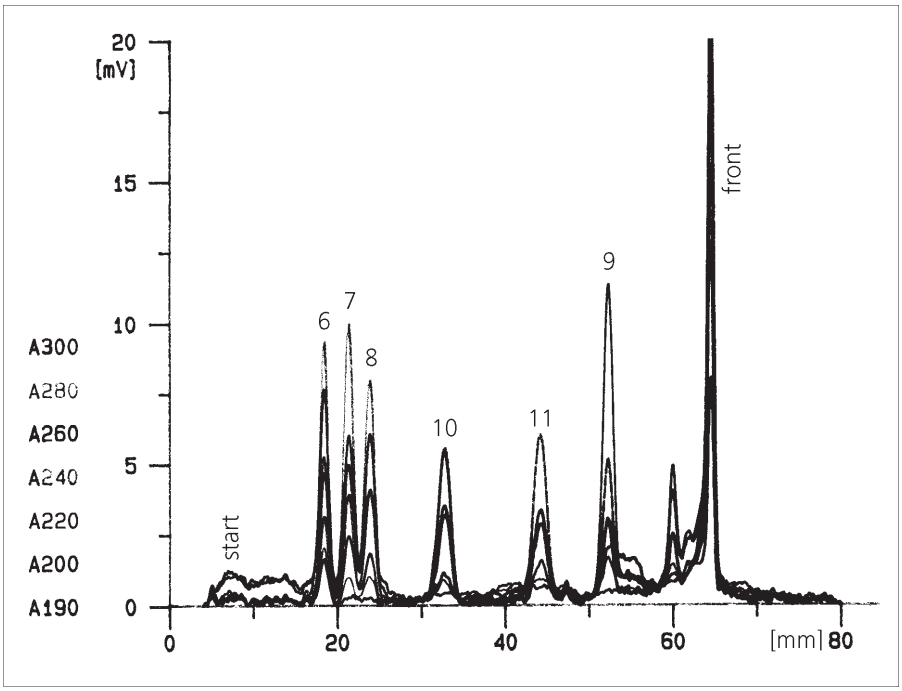


Fig. 7: Calibration mixture 2, gradient A, 50 ng each component; 6 = Metoxuron, 7 = Monuron, 8 = Chlortoluron, 9 = Propazine, 10 = Phenmedipham, 11 = Metobromuron

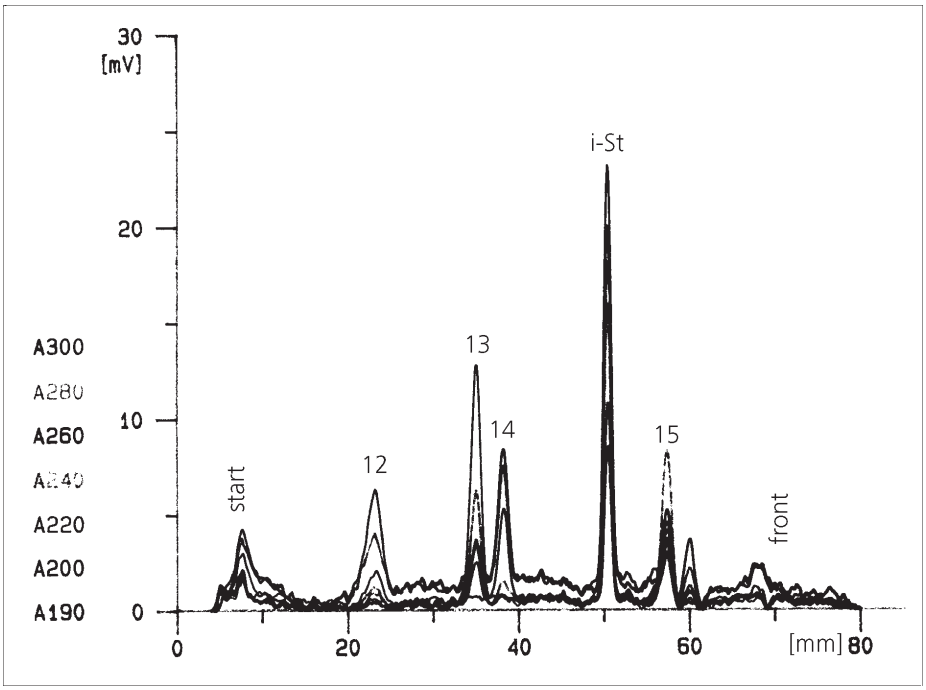


Fig. 8: Calibration mixture 3, gradient A, 50 ng each component; 12 = 2,4-D, 13 = Atrazine, 14 = Metazachlor, i-St = Benzanilid, 15 = Pendimethalin

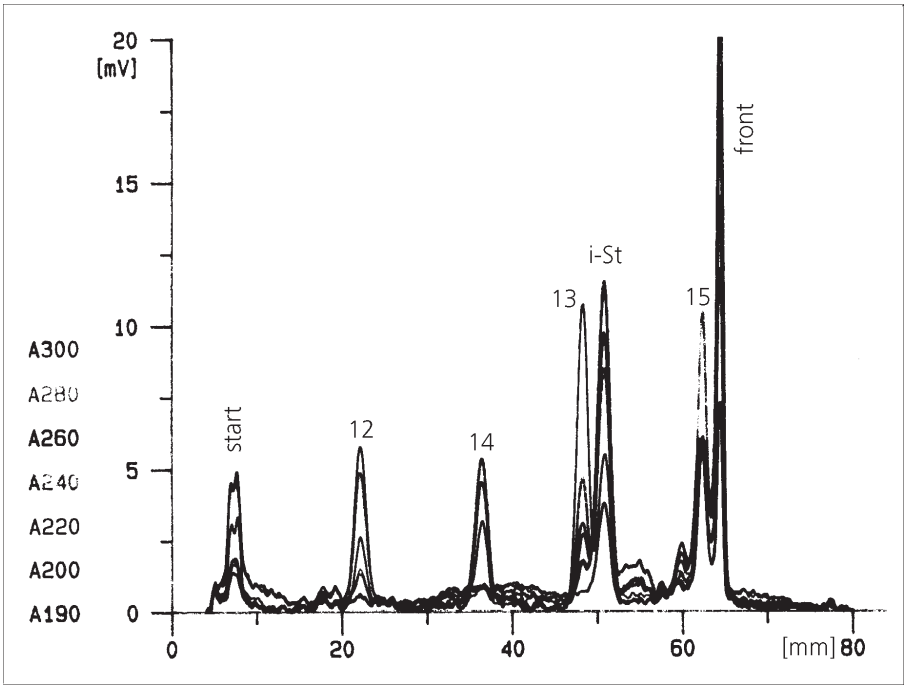


Fig. 9: Calibration mixture 3, gradient B, 50 ng each component; 12 = 2,4-D, 13 = Atrazine, 14 = Metazachlor, i-St = Benzanilid, 15 = Pendimethalin

Chromatograms of a Water Sample

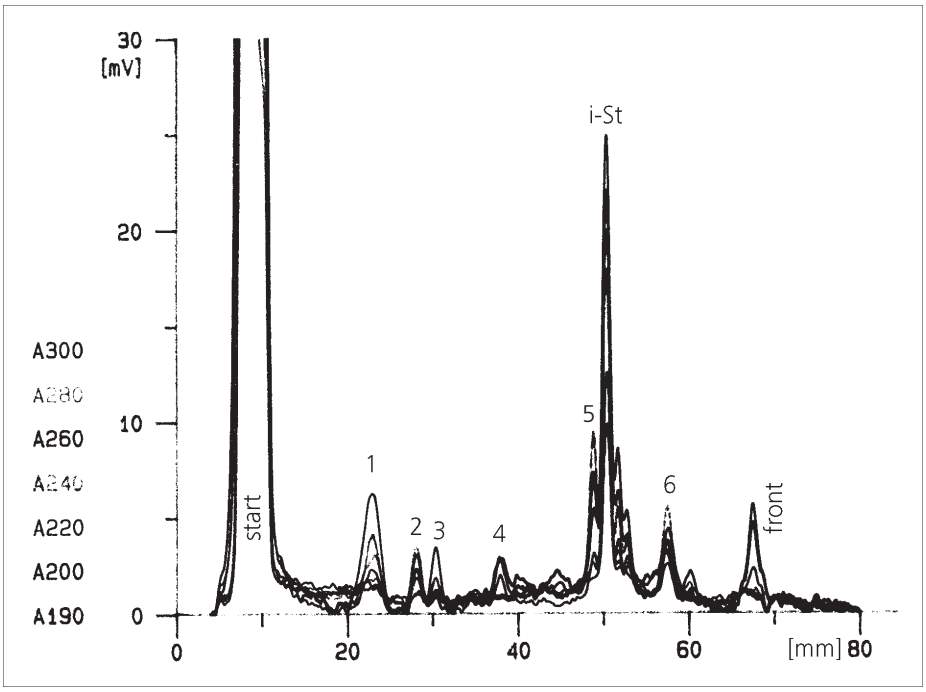


Fig. 10: Fortified water sample, gradient A; 1 = 2,4-D and interfering component X, 2 = Metoxuron (0,08 µg/L), 3 = Simazine (0,05 µg/L), 4 = Metazachlor (0,06 µg/L), 5 = Metobromuron (0,18 µg/L), i-St = Benzanilid, 6 = Pendimethalin (0,11 µg/L)

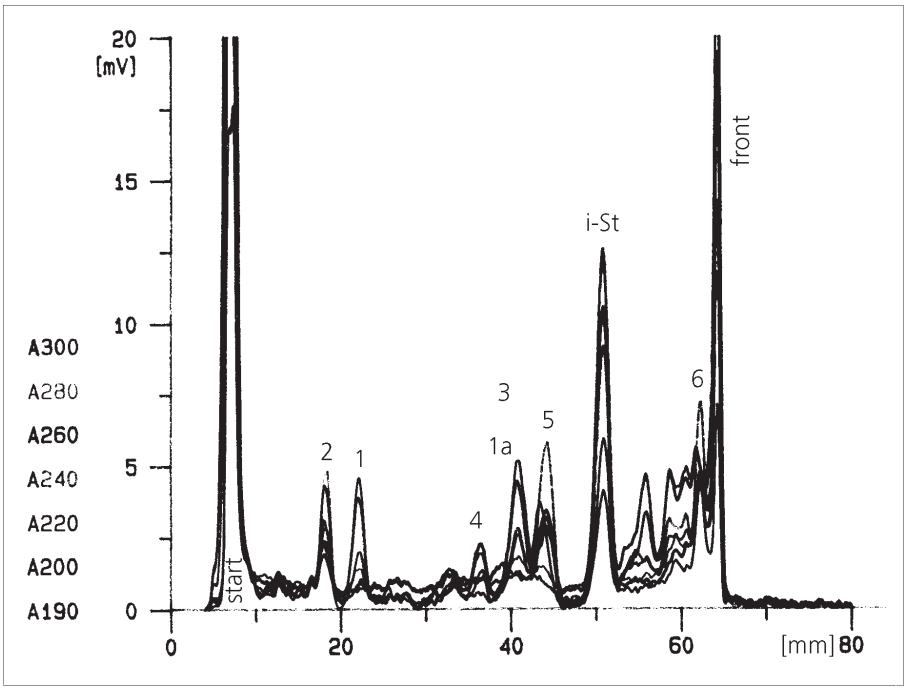


Fig. 11: Fortified water sample, gradient B; 1 = 2,4-D (0,13 µg/L), 1a = interfering component X from Fig 10, 2 = Metoxuron (0,08 µg/L), 3 = Simazine (0,06 µg/L), 4 = Metazachlor (0,06 µg/L), 5 = Metobromuron (0,18 µg/L), i-St = Benzanilid, 6 = Pendimethalin (0,10 µg/L)

Spectra

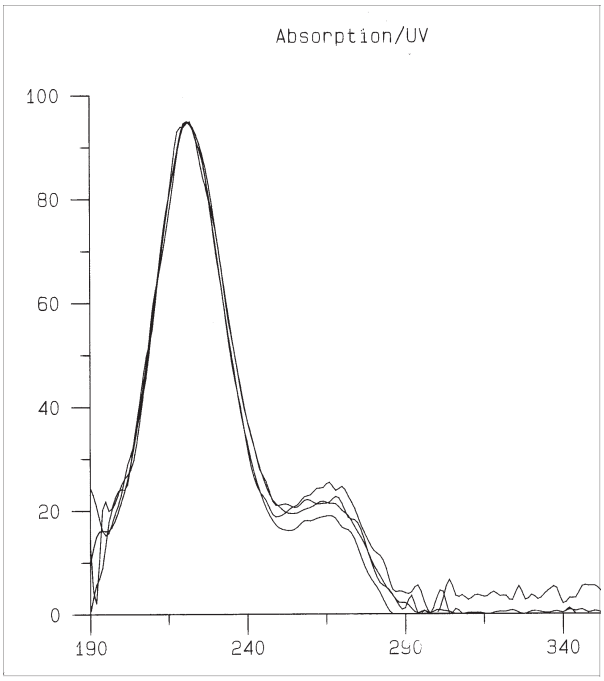


Fig. 12: In situ UV-spectra of 20, 50 and 100 ng Atrazine plotted together with the spectrum of the component in the sample, corresponding to 0.08 µg Atrazine per liter. All spectra are in an acceptable accordance.

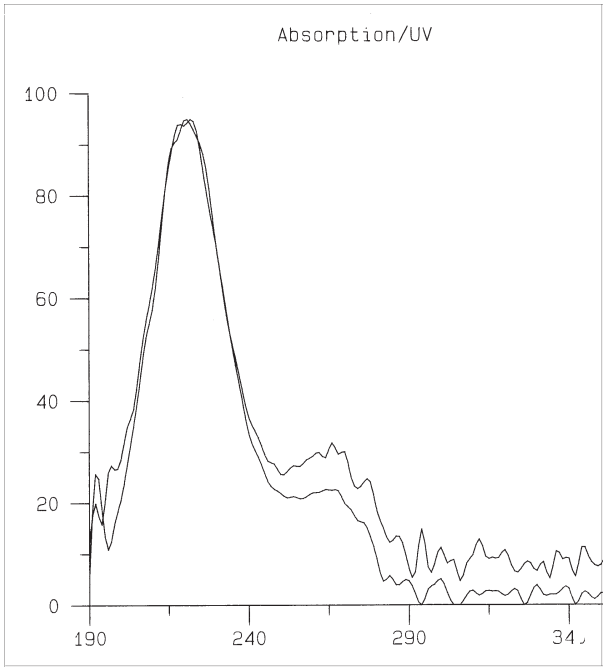


Fig. 13: In situ UV-spectrum of component 3 in fig. 8, corresponding to 0.05 µg Simazin per liter, plotted together with the spectrum of 20 ng Simazin (calibration substance). The accordance is satisfying.

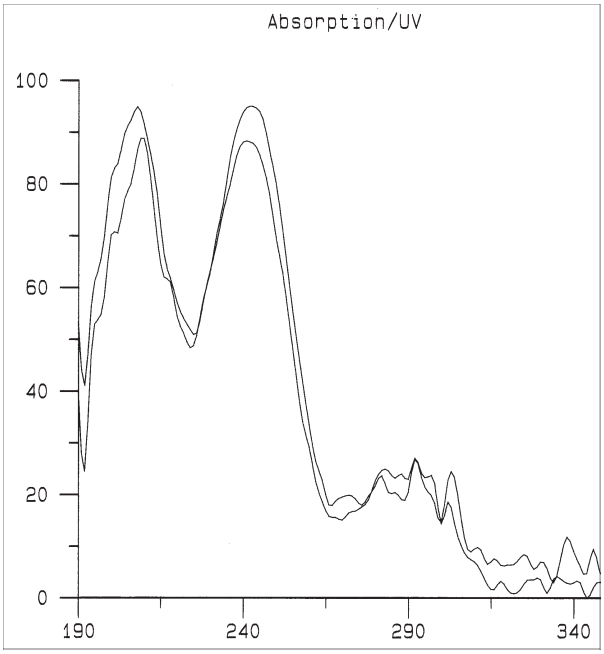


Fig. 14: In situ UV-spectrum of peak 2 in fig. 9, corresponding to 0.08 µg Metoxuron per liter, plotted together with the UV spectrum of 50 ng Metoxuron (calibration substance). In the range of low absorbance (> 260 nm), the limits of the method can be seen.

Recovery

The recoveries for pesticides with positive results in the samples of the confirmatory test are stated below. A drinking water sample was fortified with 0.2 µg of the individual pesticide, extracted and analyzed. The average of all recoveries was 80,1 % ± 13,0 %.

Recovery of the Pesticides (% compared to the spiked concentration)

Pesticide	**Recoveries observed**						**Mean recoveries**	**cv (%)**
Metoxuron	102	81	92	79	79	74	84.5	12.4
2,4-D	72	73	72	99	91	72	79.8	15.1
Metazachlor	88	71	79	68	97	88	81.8	13.6
Simazine	84	76	90	78	92	78	83.0	8.2
Metobromuron	96	77	94	76	87	70	83.3	12.7
Pendimethalin	73	82	62	65	81	96	76.5	16.4
Desethylatrazine	74	63	80	70	78	67	72.0	9.1
Atrazine	88	66	80	65	100	82	80.2	16.6

Appendix 2 - Operational data, recommendations, practical hints

Table 1: Program of running times per step - gradients A and B

step	min	step	min	step	min	step	min	step	min	step	min
1-10	0.8	11	1.0	16	2.9	21	6.0	26	9.6	31	13.9
		12	1.3	17	3.4	22	6.6	27	10.5	32	14.8
		13	1.6	18	4.0	23	7.3	28	11.2	33	15.6
		14	2.0	19	4.6	24	8.1	29	12.1	34	16.6
		15	2.5	20	5.3	25	8.7	30	13.0	35	17.5

Table 2: Program of AMD gradient A

Step	1-10	11-15	16-20	21-25	26-28	29-35
Bottle no.	1	2	3	4	5	6
Acetonitrile	30					
Dichloromethane	70	100	100	100	50	
n-Hexane					50	100
HCOOH conc.			0.1	0.1	0.1	
Wash bottle: empty	10					
Drying time (min)*	1.5	1.5	1.5	1.5	1.5	1.5

)* The (very short) drying time of 1.5 minutes requires that vacuum pump, all vacuum connections and the valves are in perfect condition. Selecting a drying time of 3.0 minutes would make the AMD procedure less critical, however, extends the total time by 2 hours.

Table 3: Program of AMD gradient B

Step	1-10	11-15	16-20	21-25	26-28	29-35
Bottle no.	1	2	3	4	5	6
Acetonitrile	70	30	30	20	10	
Dichloromethane	30	70	70	80	90	100
n-Hexane			0.1	0.3	0.3	
HCOOH conc.	0.1					
Wash bottle: empty		15				
Drying time (min)*	1.5	1.5	1.5	1.5	1.5	1.5

R1 Recommended method for testing solvents

- 10 mL of a lot of each solvent, blow down to dryness in a stream of nitrogen.
- Take up residues separately in 100 µL of solvent and apply aliquots corresponding to 5 mL of the original solvent to a TLC plate.
- Chromatograph the TLC plate by AMD gradient A.
- Scan the chromatogram plate by multi-wavelength scanning as described.
- The scan of the evaporation residue of a suitable solvent should not contain a significant peak besides the chromatographic blind.

R2 Storage of plates after drying

If this kind of trace analysis has to be carried out in a heavily contaminated environment, place prewashed plates in a desiccator, which you evacuate, Fill up the desiccator with nitrogen which you pass though activated carbon. Add nitrogen to pressure equilibration when the plates are cooled down to room temperature.

Before you remove a plate for use, cover its layer with a clean glass plate for protection.

R3 Sample application in a contaminated environment

If this kind of trace analysis has to be carried out in a heavily contaminated environment, sample application and possibly also AMD chromatography under a clean bench should be considered. Alternatively, sample application with Linomat IV or Linomat IV-Y can be done with the Inert Gas Blanket option.

Literature

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2 H. Jork, J. Köhler and U. Kocher: Application of AMD to the Determination of Crop Protection Agents in Drinking Water; Part 2: Limitations; Journal of Planar Chromatography **5** (1992), 246-250

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