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Analysis of pesticide residues in drinking water by planar chromatography

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Abstract

Planar chromatography is described in the field of water analysis. The principle of automated multiple development (AMD) technique is mentioned, the strategy of the whole procedure which became a German standard is demonstrated and separations of pesticide mixtures, as well as water samples containing pesticides are presented. The suitability of this method was proved for 283 pesticides and the corresponding ISO Standard has been applied for.

Keywords: Water analysis; Environmental analysis; Automated multiple development; Pesticides

1. Introduction

In order to assess the quality of ground, raw and drinking water including mineral water, a large number of water samples has to be analyzed. Thereby pesticides of a wide polarity range can be present in the water samples which can differ in matrix content. Normally not more than 4–5 pesticides occur simultaneously in one sample, but one does not know which. Thus, multiple methods in the ultratrace range suitable for high sample throughput are postulated. Planar chromatography, in this case, thin layer chromatography (TLC), is best suited for this purpose, because samples are determined simultaneously on one plate side by side. A special gradient development technique, the automated multiple development (AMD) technique, can be exploited to cover the wide polarity range. Furthermore the enormous flexibility of TLC is advantageous, e.g. a great variety of sorbents and/or solvents can be employed. Because all fractions remain stored on the plate, detection can be repeated at will, with the same or with changed parameters. Thus UV multi-wavelength detection can readily be confirmed by UV spectra and microchemical or enzymatic postchromatographic in situ reactions. Moreover, solvent consumption and variable costs in general are low and matrix contamination need not to be cared about because the TLC plate is disposable. Therefore sample preparation can often be simplified.

Pesticides in general [1,2] or compounds of triazines [3–7], organophosphates or carbamates [8–13], phenyl ureas [14–17], organochlorins [18,19], phenoxy carboxylic acids [20–22], dicarboximides [23,24] and anilins [25] can be separated by classical TLC (SN ~ 12). However, AMD [26–36] is better suited as a multimethod for any kind of pesticide and the zones are focused resulting in a higher peak capacity, that is separation number (SN>40).

In the following paragraphs the principle of the AMD technique is described, the strategy of the whole procedure which became a German Standard [37] is demonstrated and separations of pesticide mixtures, as well as water samples containing pesticides are presented.

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2. Principle of AMD

The concept of automated multiple development (AMD) has been derived by Burger [38] from an earlier technique, 'programmed multiple development' (PMD), introduced by Perry et al in 1973 [39]. The chromatogram is developed repeatedly in the same direction over increasing solvent migration distances. Thereby samples are concentrated into narrow bands in that molecules in the 'lower' part of a sample zone start their upward movement earlier than those in the upper part of the zone, each time the solvent front passes through that area. Usually the number of development runs is between 10 and 40. The solvent for each successive run normally differs from the one used before, so that a stepwise gradient can be obtained. Unlike in column liquid chromatography, an AMD gradient starts with the solvent having the strongest elution power and is varied towards decreasing solvent strength. The first development is performed using a polar solvent of very high elution power which transfers the components capable of migration from the starting zone as a focused zone over a very short distance. By gradually decreasing elution power while the running distance increments (typically 1 to 3 mm) increase from step to step, one fraction after the other will stop its migration and become stationary (Fig. 1) while the solvents of the successive steps pass over the area. The elution gradient is used to simulate a long separation distance and to increase the peak capacity of the HPTLC plate. Between developments, the solvent is completely removed from the developing chamber and the layer is dried under vacuum. At the end of each drying step the layer is conditioned with inert gas or a special conditioning vapour phase.

Normal-phase chromatography is usually employed in AMD as is the case in over 90% of all TLC/HPTLC applications. 'Universal gradient' is the term for a normal-phase AMD gradient that starts with a very polar solvent and is varied via a solvent of medium polarity, the 'basis' solvent, to a non-polar solvent. The central or 'basis' solvent, to a certain extent also the non-polar component, determine the chromatographic selectivity of the system. Either dichloromethane or an ether (usually diisopropyl ether or *tert.*-butyl methyl ether) is used as the basis solvent in over 80% of AMD applications. However, gradient elution on silica gel offers a wide variety of solvents which can be used for gradient composition. For gradient optimization, the densitogram of a chromatogram track can be superimposed with a matched scale diagram of the gradient. This permits easy identification of that segment of the gradient that has resolved which

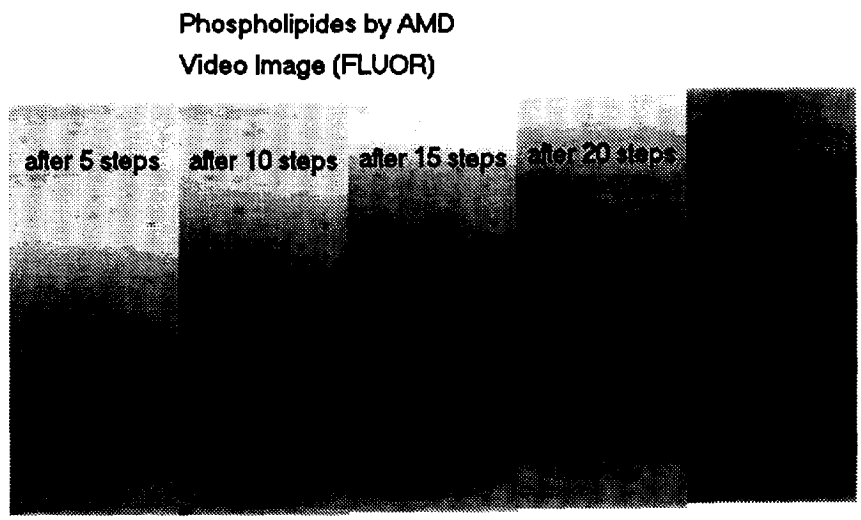


Fig. 1. Separation of phospholipids after various steps.

fractions. This way one can determine which part of the gradient needs to be modified in order to achieve the desired resolution.

3. Strategy

After sampling and pH-adjustment to pH of 2, the pesticides are extracted from the water by solid-phase extraction. First, the non-endcapped RP 18 material is rinsed and conditioned by *n*-hexane, dichloromethane, methanol and water of pH 2. One gram of the material is sufficient for 500 ml of water. Besides RP18 material also polystyrene divinylbenzene sorbent can be employed. After drying the cartridge in a stream of nitrogen the cartridge is rinsed with *n*-hexane and then the pesticides are eluted with dichloromethane and/or methanol. Usually aliquots of this extract can directly be applied to the TLC plate. To prevent interferences of a high humic acid content of the sample, it can be helpful to pass the dichloromethane extract through an Extrelut cartridge impregnated with aqueous buffer of pH 10, but of course acidic pesticides are lost [30].

Standard mixtures and samples are co-chromatographed on the same plate. That means for e.g., on one TLC plate the extracts of twelve water samples can be chromatographed in parallel with six different standard mixtures covering the wide range of common pesticides i.e. in total up to 60 different pesticides. Aliquots of the water extracts and the standard mixtures are chromatographed on silica gel using two gradients of different selectivity. The first separation is performed by a universal gradient, measured by UV multi-wavelength detection and, if required, measured again after in situ derivatization. This way, twelve samples are screened for up to 60 pesticides which gives twelve times 60, that is 720 analytical answers. The samples with positive results are chromatographed, together with suitable amounts of the relevant standards, with a second gradient of different selectivity. Again peaks in the unknowns are related to standards by position and multi-wavelength pattern. For further confirmation of positive results in situ UV spectra are taken from the unknown spot and the corresponding standard zone. Additional confirmation is possible by chemical or enzymatic derivatization reactions and, if required, by further spectroscopic evaluation. Using this strategy the statement can be confirmed stepwise from 'substance possibly present' to 'present almost for sure'. Quantification is done using suitable densitograms already recorded in the procedure.

Using extra thin layers, e.g. a layer of 100 μm thickness, the limit of determination of most pesticides is around 10 ng. If an extract equivalent to 250 ml of water sample is applied to the TLC plate for one chromatographic analysis, the limit of determination was found to be about 50 ng pesticide per liter for this method.

4. Separation of pesticides

A universal gradient used for the separation of pesticides is illustrated in Fig. 2. 16 pesticides of different substance classes are baseline-separated (Fig. 3).

The separation of a pesticide mixtures according to the example of a screening gradient in DIN 38407 part 11 [37] (Fig. 4) is pictured in the Fig. 5. The second gradient for confirmation of positive results is

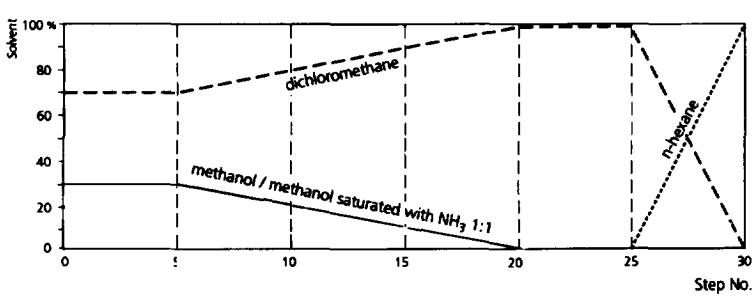


Fig. 2. 30 step universal gradient from methanol/dichloromethane to *n*-hexane using dichloromethane as 'basis' solvent [40].

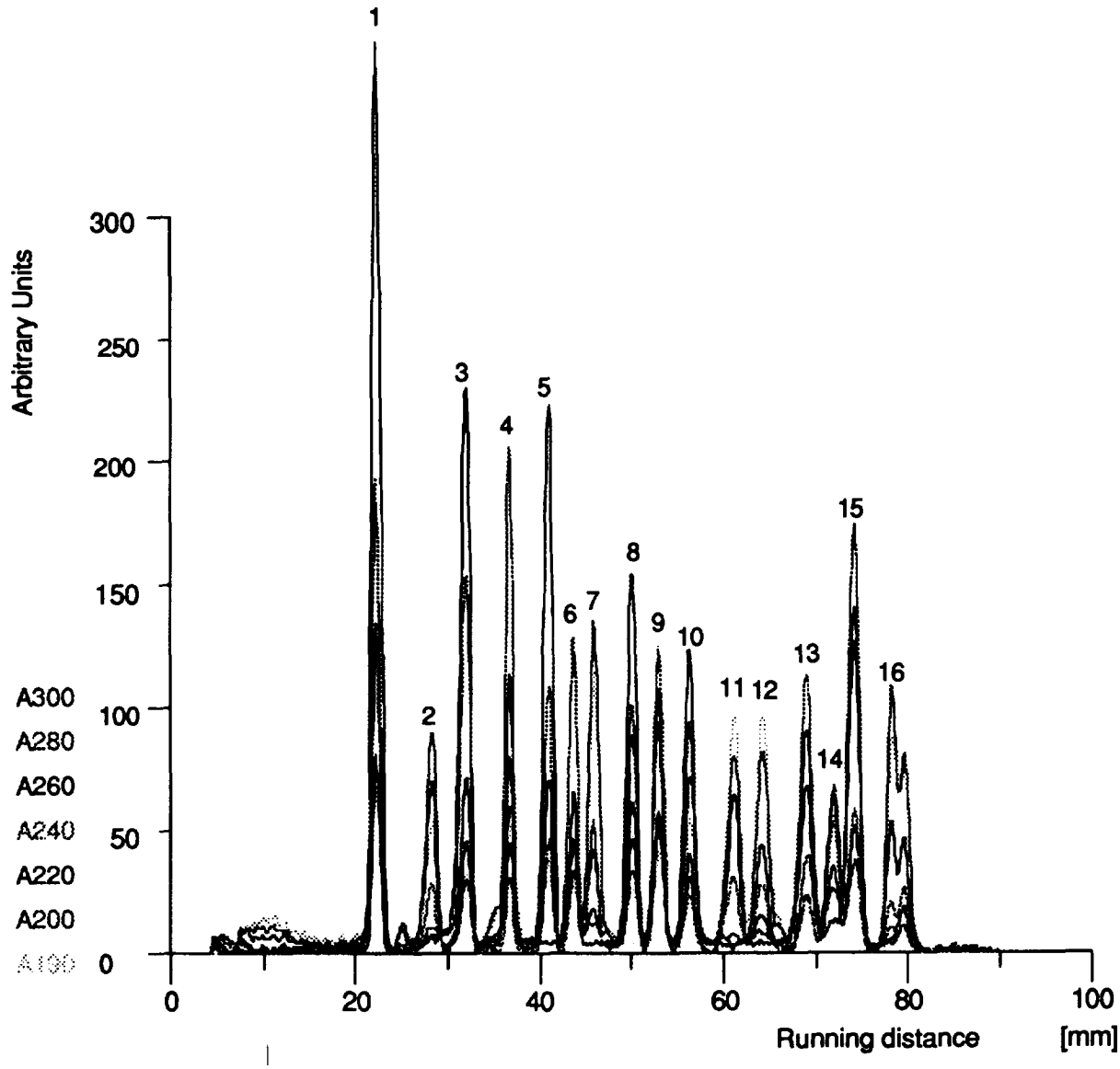


Fig. 3. Multi-wavelength scan of various pesticides (100 ng each) at seven different wavelengths from 190 to 300 nm obtained from the gradient pictured in Fig. 2. (1=naphtalin-1-sulfonic acid, 2=procloraz, 3=triazoxid, 4=ethidimuron, 5=simazine, 6=bromazil, 7=carbofuran, 8=metribuzin, 9=azinphos methyl, 10=coumaphos, 11=prosulfocarb, 12=dichlofuanid, 13=parathion, 14=fenthion, 15=dinoseb, 16=prothiofos) [40].

illustrated in Fig. 6. The corresponding separation of a pesticide mixture is shown in Fig. 7. The isocratic steps 1 to 10 (Figs. 4 and 6) at the beginning are advisable to ensure that no compounds remain stationary at the line of sample application, because active components could be entrapped. Thereby the starting zone is focused to a sharpened band by ten fold development to the same short running distance. Both gradients start with an alkaline solvent mixture and end with an acidic solvent gradient. In this way it is possible to move or fix particular compound classes, such as acids or amines. The running

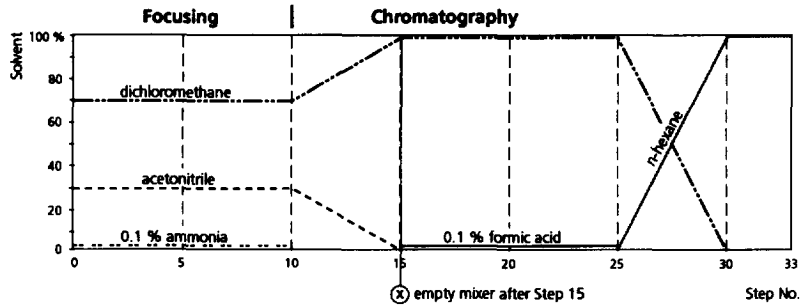


Fig. 4. Gradient example for screening in DIN 38407 part 11.

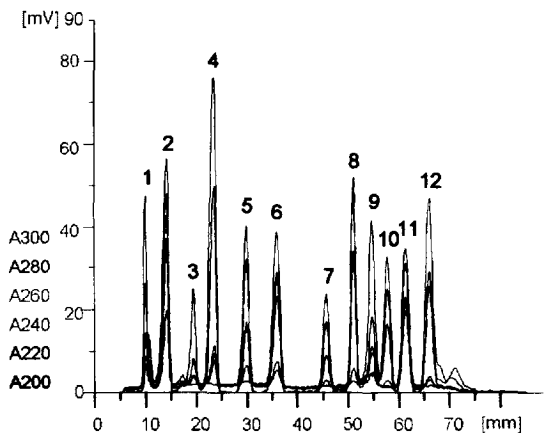


Fig. 5. Multi-wavelength scan of a pesticide mixture separated according to the gradient in Fig. 4. (1=hydroxyatrazin, 2=formetanat, 3=triadimenol, 4=metalaxyl, 5=isoproturon, 6=diuron, 7=dimethylaminosulphanilide, 8=methidathion, 9=2,4-D-isobutyl ester, 10=endrin, 11=ethalfluralin, 12=2,2-*bis*-(4-chloro-phenyl)-1,1-dichloroethane).

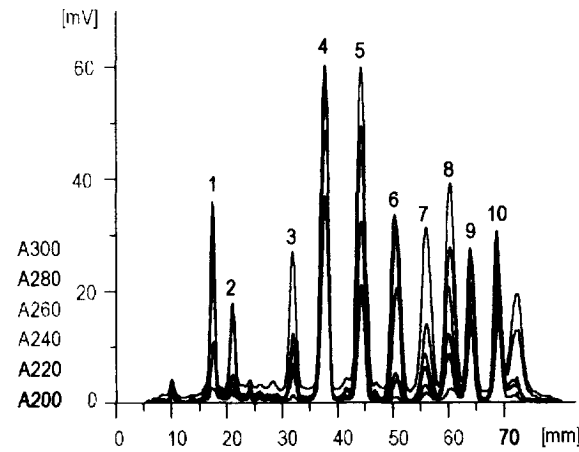


Fig. 7. Multi-wavelength scan of a pesticide mixture separated according to the gradient in Fig. 6. (1=oxamyl, 2=triadimenol, 3=2,4-DB, 4=azinphos-ethyl, 5=coumaphos, 6=chlorbufam, 7=dichlorprop-methyl, 8=2,4,5- TP-methyl ester, 9=fluchloralin, 10=profluralin).

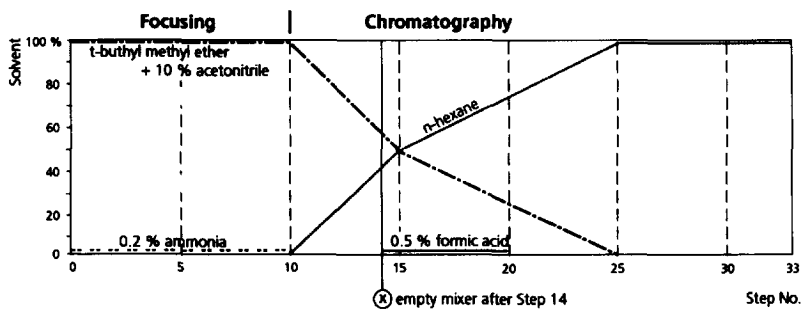


Fig. 6. Gradient example for confirmation of positive results in DIN 38407 part 11.

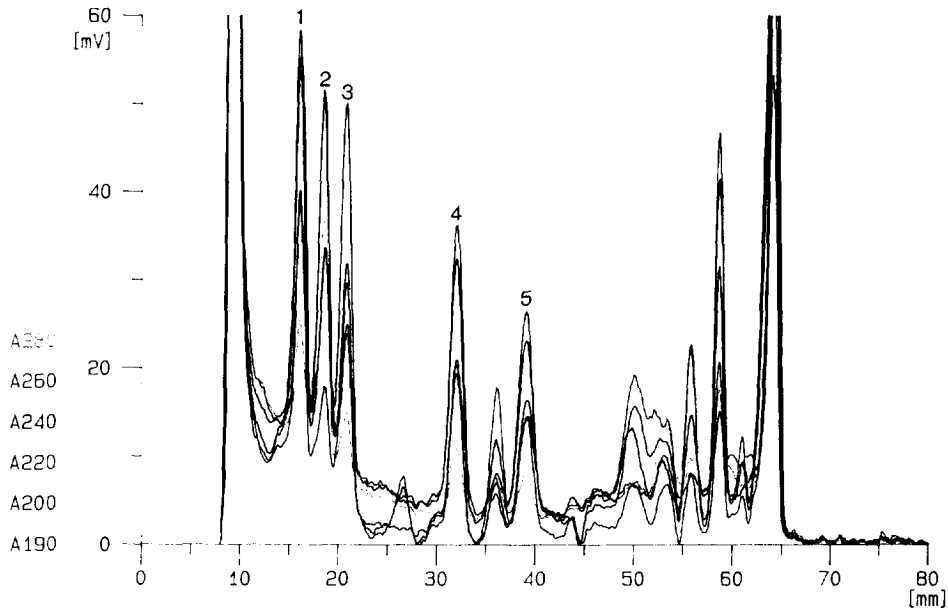


Fig. 8. Multi-wavelength scan of a fortified drinking water sample (1=metoxuron, 2=monuron, 3=chlortoluron, 4=neburon, 5=linuron).

distance increments for these gradients were 3 mm. As further example the chromatogram of a fortified drinking water sample is pictured in Fig. 8.

A shortened 20 step gradient just for the monitoring of diuron, 2,4-D, mecoprop, MCPA, simazine and atrazine is illustrated in Fig. 9. A drinking water sample fortified with some of those pesticides is presented in Fig. 10. Using 1 mm running distance increments the gradient takes about 90 min, for e.g. twelve samples on the plate.

For very complex mixtures, coupling of HPLC (reversed-phase system) with TLC/AMD (normal-phase system) is sucessfully employed [40,41]. As shown in Fig. 11, an HPLC fraction can easily be applied onto a TLC plate by a modified automatic TLC sampler and chromatographed on silica gel.

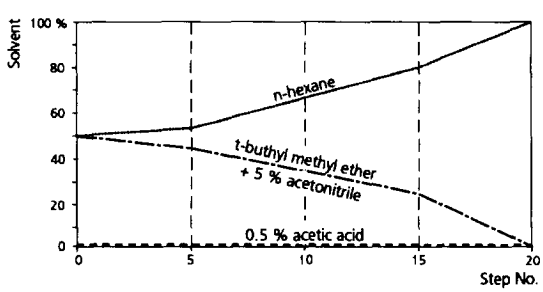


Fig. 9. Optimized shortened gradient for the determination of diuron, 2,4-D, mecoprop, MCPA, simazine and atrazine.

5. Conclusion

The AMD technique is a method that brings the advantages of stepwise gradient development into the field of thin-layer chromatography, leading to an enormous increase in the selectivity of the chromatographic separation. It should be noted that AMD is the only chromatography technique that offers reproducible gradient elution on normal-phase silica gel. AMD is very efficient for screening as well as for confirmation of positive results. Thus, this technique for identification and quantification of active ingredients of plant protecting agents in ground, raw, drinking and mineral water has been accepted as a German Standard [37]. The corresponding ISO Standard [42] has been applied for. By the way, the suitability of this method was proved for 283 pesticides [35,43].

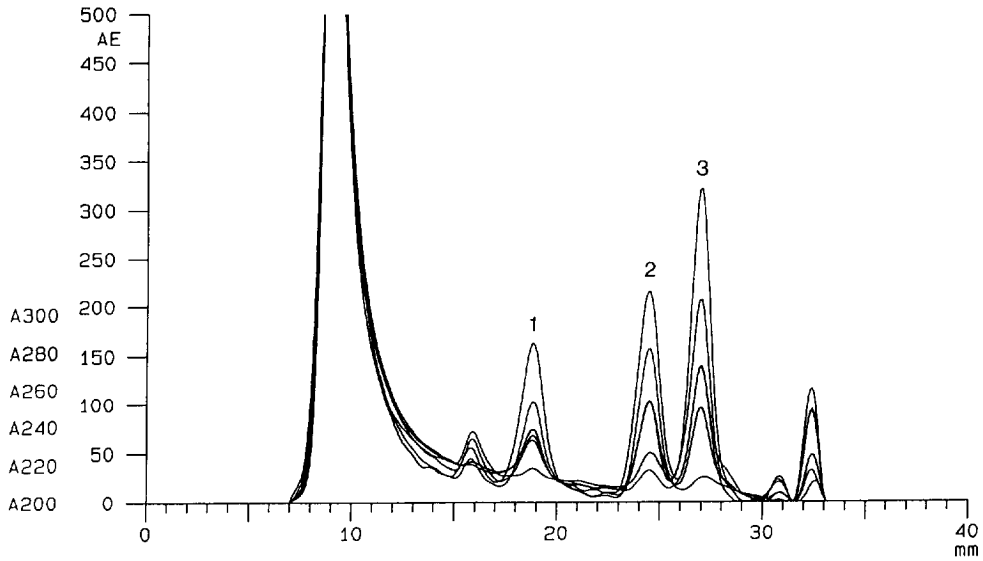
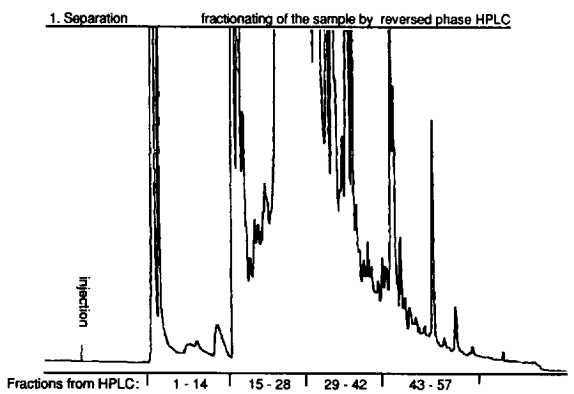


Fig. 10. Multi-wavelength scan of a fortified drinking water sample (1=2,4-D, 2=mecoprop, 3=simazine).



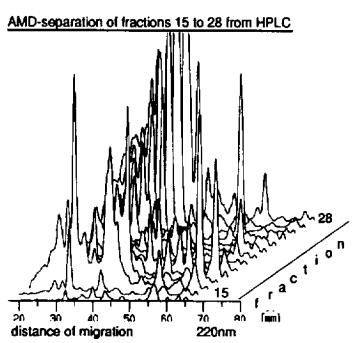


Fig. 11. Separation of a complex mixture by HPLC/AMD coupling [40].

References

[1] DFG, Dünnschicht-Chromatographie in der Rückstandsanalytik von Pflanzenschutzmitteln und deren Metaboliten, VCH, Weinheim, 1987.
[2] H.-E. Hauck, M. Mack, S. Reuke and H. Herbert, J. Planar Chromatogr., 2 (1989) 268.
[3] G.L. Biagi, A.M. Barbaro, A. Sapone and M. Recanatini, J. Chromatogr., 625 (1992) 392.
[4] R.M. Johnson, F. Halaweish and J.J. Fuhrmann, J. Liq. Chromatogr., 15 (1992) 2941.
[5] W. Ehlert, Staatsexamenarbeit, FR 12.5, University of Saarland, Saarbrücken, 1991.
[6] H. Jork and B. Roth, J. Chromatogr. 144 (1977) 39.
[7] L.M. Kostadinovic, S.T. Pavkov and M.B. Vojinovic, Arh. Farm., 41 (1991) 173.
[8] H.S. Rathore and S. Sharma, J. Liq. Chromatogr., 15 (1992) 1703.
[9] P.E.F. Zoun and T.J. Spierenburg, J. Chromatogr., 462 (1989) 448.
[10] O.V. Vashkevich and E.S. Gankina, J. Planar Chromatogr. 3 (1990) 354.
[11] W. Funk, V. Glück, B. Schuch and G. Donnevert, J. Planar Chromatogr., 2 (1989) 28.
[12] H. Ackermann, J. Chromatogr., 36 (1968) 309.
[13] C. Weins, Dissertation, FR. 12.5, University of Saarland, Saarbrücken, 1996.
[14] J. Sherma and L. Joel, J. Liq. Chromatogr., 6 (1983) 1183.
[15] A. Lawerenz, H. Goralczyk and H. Hermenau, Acta Hydrochim. Hydrobiol., 2 (1986) 121.
[16] W. Gries and H. Jork, J. Planar Chromatogr., 2 (1989) 290.
[17] V.D. Chmil and Zh. Anal. Khim., 40 (1985) 500.
[18] M.F. Yong, T.S.A. Hor and H.K. Loe, Bull. Singapore Natl. Inst. Chem., 18 (1990) 101.

[19] P.P. Singh and R.P. Chawla, Int. J. Environ. Anal. Chem., 36 (1989) 17.
[20] U. Siegel, M. Schmitt, H. Jork, J. Planar Chromatogr., 2 (1989) 304.
[21] M. Schmitt, Dissertation, FR. 12.5, University of Saarland, Saarbrücken 1995.
[22] M.A. Satter, J. Chromatogr., 209 (1981) 329.
[23] H. Cyroll, Dissertation, FR. 12.5, University of Saarland, Saarbrücken 1995.
[24] I. Wassmuth-Wagner and H. Jork, J. Planar Chromatogr., 2 (1989) 297.
[25] H. Pugge, Dissertation, FR. 12.5, University of Saarland, Saarbrücken, 1995.
[26] G. Lodi, A. Betti, E. Menziani, V. Brandolini and B. Tosi, J. Planar Chromatogr., 4 (1991) 106.
[27] G. Lodi, A. Betti, Y.D. Kahie and A.M. Mahamed, J. Chromatogr., 545 (1991) 214.
[28] K. Burger, Pflanzenschutz-Nachrichten, Bayer 41 (1988) 173.
[29] K. Burger, J. Köhler and H. Jork, J. Planar Chromatogr. 3 (1990) 504.
[30] K. Burger, in H.-J. Stan (Editor), Analysis of Pesticides in Ground and Surface Water II, Series Chemistry of Plant Protection, Vol. 12, Springer-Verlag, Berlin, Heidelberg, 1995, p. 181.
[31] U. De la Vigne and D.E. Jaenchen, J. Planar Chromatogr., 3 (1990) 6.
[32] E. Zietz and I. Ricker, J. Planar Chromatogr., 2 (1989) 262.
[33] H. Jork, G. Keller and U. Kocher, J. Planar Chromatogr., 5 (1992) 246.
[34] U. De la Vigne, D.E. Jaenchen and W.H. Weber, J. Chromatogr., 553 (1991) 489.
[35] S. Butz, H.-J. Stan, Anal. Chem., 67 (1995) 620.
[36] G. Morlock, Dissertation, FR. 12.5, University of Saarland, Saarbrücken 1995.
[37] German standard DIN 38 407 part 11, Beuth Verlag, Berlin, 1994.
[38] K. Burger, Fresenius Z. Anal. Chem., 318 (1984) 228.
[39] J.A. Perry, K.W. Haag and L.J. Glunz, J. Chrom. Sci., 11 (1973) 447.
[40] Private communication with K. Burger, Bayer, Dormagen, 1996.
[41] Private communication with M. Zeller, Ciba-Geigy, Basel, 1996.
[42] ISO/TC 147/SC 2/WG 37, ISO document CD 11370, 1993.
[43] S. Butz, in H.-J., Stan (Editor), Analysis of Pesticides in Ground and Surface Water II, Series Chemistry of Plant Protection, Vol. 12, Springer-Verlag, Berlin, Heidelberg, 1995, p. 197.