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IMPROVED INTERFACE FOR LIQUID CHROMATOGRAPHY–ELECTRON-CAPTURE DETECTOR COUPLING. I

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SUMMARY

An improved design of an interface for the coupling of a liquid chromatograph and an electron-capture detector (ECD) is described and the dependence of extra-column band broadening on the dimensions of the capillary tube in this interface is studied. Use of the improved design enables the detection of high-boiling compounds which could not be handled with a previously available commercial interface. For several high-boiling halogenated bi- and terphenyls, and organochlorine pesticides, ECD detection limits (which often are in the low-picogram range) have been determined and compared with UV detection limits. The ECD calibration curves are linear over 3 orders of magnitude.

The optimized liquid chromatography–ECD system is applied to environmental analysis in the system silica–dry hexane. Finally, it is demonstrated that the addition of up to at least 6% of dioxane to the hexane used as mobile phase can be tolerated without unacceptable loss of performance.

INTRODUCTION

High-performance liquid chromatography (LC) has gained widespread popularity during the last decade. Detection systems in LC, however, have not yet reached the sensitivity level of gas chromatographic (GC) detectors. Currently, the UV absorption spectrophotometer is the most useful of LC detectors on account of its versatility and reliability and its relatively high sensitivity for a broad range of compounds. The use of fluorescence and electrochemical detectors (which possess a higher selectivity) is increasing as these detectors can detect *ca.* 1 pg of a favourable solute, whereas the UV absorption detector is limited to *ca.* 1 ng in a favourable situation. Until now, the use of typical GC detectors such as the electron-capture detector (ECD) in LC has been limited[1], mainly owing to the fact that such detectors cannot readily handle a relatively high flow of solvent. Also, the use of the ECD is virtually incompatible with that of polar mobile phases such as are necessary in reversed-phase LC, and even the addition of moderate amounts of an organic modifier to a non-polar mobile phase causes serious problems.

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In our laboratory, a commercially available LC–(Pye-Unicam) ECD combination has previously been shown[2] to give good sensitivity for compounds such as, *e.g.*, polychlorinated biphenyls (PCBs) and organochlorine pesticides using silica–dry hexane as the LC system. The total LC effluent of *ca.* 1 ml/min is evaporated in a coil situated in an oven kept at a temperature of 300–350°C and, next, allowed to enter the ECD. With the commercial equipment the evaporation of high-boiling compounds such as decabromobiphenyl and the tetradecachloroterphenyl isomers could, however, not be carried out efficiently even at the maximum operating temperature.

In this communication the improved performance of a redesigned LC–ECD interface will be demonstrated using a series of high-boiling halogenated aromatic compounds as test solutes. Detection limits obtained in LC–ECD will be compared with those in LC–UV and GC–ECD. Data on the band broadening caused by various types of interface, and on the dependence of ECD performance on the addition of polar modifiers to the non-polar mobile phase, will be shown.

MATERIALS AND METHODS

The LC system consisted of an Orlita (Giessen, G.F.R.) Model FE 034 sRC reciprocating pump, a Valco (Houston, TX, U.S.A.) six-port injection valve with a 25-μl loop, a 25 cm × 4.6 mm I.D. stainless-steel column prepacked with 5-μm LiChrosorb SI 100 silica (Brownlee, Santa Clara, CA, U.S.A.), a Pye-Unicam (Philips, Eindhoven, The Netherlands) Model LC 3 variable-wavelength UV detector and a Pye-Unicam ^{63}Ni ECD.

The effluent from the LC column passes through the UV detector and then, after splitting in a Valco tee piece, a suitable portion of the effluent (generally *ca.* 30–50 %) is directed to the ECD. With the commercial Pye-Unicam ECD unit, vaporization is achieved by passing the effluent into a stainless-steel transfer tube mounted in an oven which is maintained at a sufficiently high temperature. The increase in volume in this vaporization unit forces the vapour into and through the ECD, which is contained in the same oven. A purge gas stream of 30 ml/min oxygen-free nitrogen (which, in theory, is not necessary for reliable LC–ECD operation because of the high LC effluent gas flow-rate) is maintained primarily to prevent back diffusion of effluent vapour into the purge conduit. The effluent is finally collected as a liquid from a stainless-steel condenser tube mounted at the outlet of the ECD.

The signal from the ECD is amplified by a Pye-Unicam constant-current electron-capture amplifier. The detector (standing) current can be varied between $1 \cdot 10^{-10}$ and $50 \cdot 10^{-10}$ A. The ECD and UV chromatograms were recorded simultaneously by means of a Kipp (Delft, The Netherlands) BD 41 dual-pen recorder.

For optimal performance of the LC–ECD system, high-quality solvents are required[2]. HPLC-grade *n*-hexane from Baker (Deventer, The Netherlands) was used as mobile phase in LC. ECD-active contaminants were exhaustively eliminated from this solvent by refluxing it for 2 h over a dispersion of 45 % sodium in paraffin (Fluka, Buchs, Switzerland) and subsequent distillation. Dioxane (Aldrich, Beerse, Belgium), used as polar modifier, was treated in the same way. Waste hexane and dioxane were re-used after refluxing and distillation according to the same procedure.

All compounds used as test solutes were commercially available analytical-grade products (see ref. 3 for suppliers).

RESULTS AND DISCUSSION

Interface design

In a previous paper[2] we have discussed the potential of the LC–(Pye-Unicam) ECD system. Provided dry solvents are used which contain a low level of electron-capturing impurities, excellent sensitivity is observed for compounds such as halomethanes, PCBs, polychlorinated naphthalenes and organochlorine pesticides. However, even if using the maximum allowed (interface) oven temperature of 350°C, no useful results are obtained for compounds such as decabromobiphenyl or highly chlorinated terphenyls: peaks either tail badly or do not show up at all. Our primary aim was therefore to improve the design of the commercial interface so as to create more efficient heat transfer, thereby opening up the possibility of making even the high-boiling compounds mentioned amenable to LC–ECD analysis.

The commercial interface consists of a coiled capillary transfer tube of 150 cm × 0.8 mm I.D., which is merely enveloped by a stainless-steel tube. In the new design (see Fig. 1), the capillary is coiled around a grooved cylindrical stainless-steel block so that it fits snugly in the grooves in this block. In addition, a stainless-steel tube is slipped over the block, thereby causing the capillary to make contact on all sides with the heated block plus tube.

Using the modified design the contribution of capillaries of varying dimension to band broadening was studied. In these experiments, the UV detector and the ECD were connected in series without in-between splitting; the oven temperature was

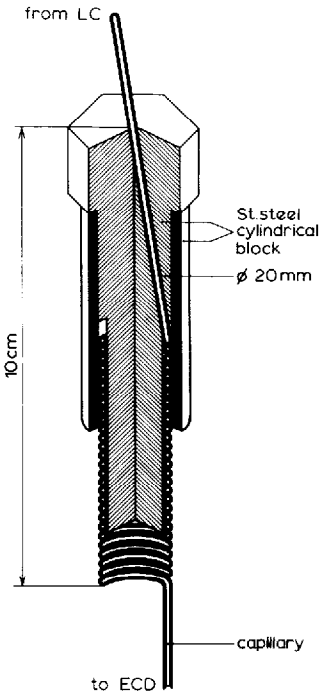

Fig. 1. Cross-sectional view of the modified interface for LC–ECD. For further details see text.

invariably kept at 300°C. Six test solutes were selected, *viz.* 2,4,4′-trichlorobiphenyl (TCB) (which was included as a relatively low-boiling reference compound) and decachlorobiphenyl (DCB), decabromobiphenyl (DBB) and the three isomeric tetradecachloroterphenyls (TDCTs), which exemplified high-boiling halogenated aromatics. The peak widths of the UV and ECD signals were recorded as their standard deviation, σ. The band broadening due to the interface plus ECD, σ_{ECD}, was calculated from the relationship

$$\sigma^2_{total} = \sigma^2_{UV} + \sigma^2_{ECD} \quad (1)$$

where σ_{total} and σ_{UV} are the standard deviations read from the ECD and UV chromatograms, respectively. The results are summarized in Table I. The relative standard deviation for the σ_{ECD} data typically was *ca.* 3% ($n = 5$).

TABLE I

COMPARISON OF BAND BROADENING (σ_{ECD}) CAUSED BY VARIOUS LC–ECD INTERFACE DESIGNS

LC system: silica–dry hexane. Flow-rate: 0.88 ml/min; oven temperature, 300°C. k' = Capacity ratio.

Solute	k'	σ_{UV} *(sec)*	σ_{ECD} *(sec) for capillary dimensions (mm)*·***			
			1500 × 0.8	*300 × 0.5*	*750 × 0.5*	*750 × 0.25*
DCB	0.26	3.55	4.30	3.65	2.80	2.80
m-TDCT	0.45	4.15	–	4.50	3.40	2.90
p-TDCT	0.55	4.45	–	5.40	3.85	3.40
o-TDCT	0.75	5.15	–	5.10	3.95	3.70
TCB	1.05	5.70	3.90	3.40	2.65	2.60
DBB	1.20	6.35	–	6.50	5.30	4.60

* 1500 × 0.8 mm: Pye-Unicam; with the other designs, *ca.* 100 mm of transfer capillary protrudes from the heating block at each end (*cf.* Fig. 1).

** – = Not detectable.

The data in Table I demonstrate that the commercial 150 cm × 0.8 mm I.D. interface* is clearly inferior to the other designs. This is evident from (1) the high σ_{ECD} values for TCB and DCB and (2) the complete absence of peaks due to the other test solutes. As regards the latter aspect, the inefficient heat transfer obviously prevents vaporization of these test solutes despite the relatively long residence time in the interface. Replacing the commercial interface by a redesigned one of much smaller dimensions (75 cm × 0.5 mm I.D.) considerably improves the performance: all test solutes now show up, and band broadening is much reduced. Still better results are obtained with the smaller-bore 75 cm × 0.25 mm I.D. transfer capillary, the further improvement being especially manifest in the case of the high-boiling TDCTs and DBB. The data for the 30 cm × 0.5 mm I.D. capillary cannot simply be compared with those of the 75-cm versions since, because of the small length (only 10 cm

* These are the dimensions of the commercially sold unit. They differ from those quoted in an early paper on the Pye-Unicam design (40 cm × 0.25 mm, ref. 4).

inside block; *cf.* footnote to Table I) a straight instead of a coiled transfer capillary had to be used. In this case, efficient heating was achieved by mounting the capillary in a straight channel of appropriate diameter which had been drilled in the stainless-steel heating block. The short-transfer capillary also shows much better performance than does the commercial design; evaporation apparently is no problem. Band broadening is, however, significantly larger than with the 75-cm capillaries. This may well be due to less efficient radial mixing in the straight as compared with the coiled capillaries.

Fig. 2 will serve as an illustration of the above. Here, for five of the test solutes, an LC–UV chromatogram is compared with LC–ECD chromatograms obtained with the Pye-Unicam and the 75 cm × 0.25 mm I.D. interface. The improved performance of the redesigned interface in the case of DCB and, much more so, the TDCT isomers is evident.

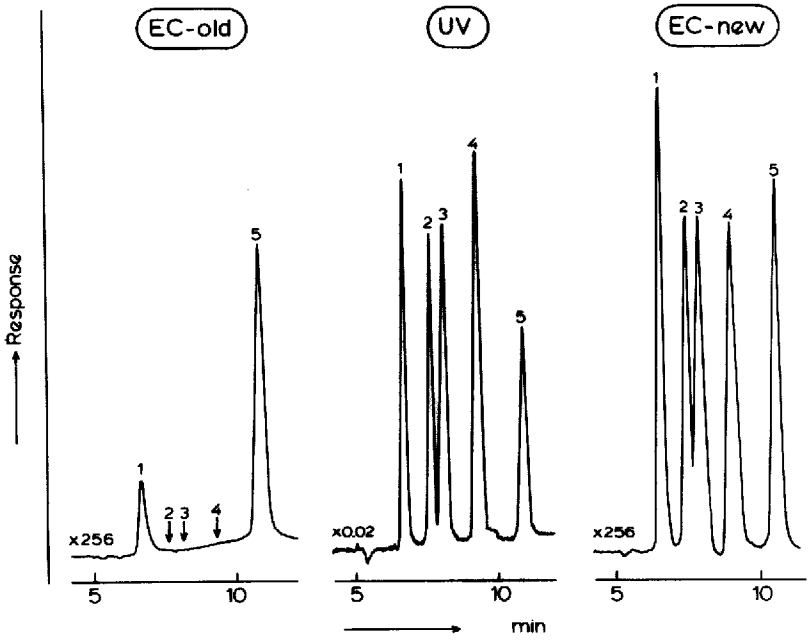

Fig. 2. Comparison of LC–UV chromatogram and LC–ECD chromatograms obtained with the Pye-Unicam (EC-old) and the 75 cm × 0.25 mm I.D. redesigned (EC-new) interface. Peak: 1 = DCB; 2 = *m*-TDCT; 3 = *p*-TDCT; 4 = *o*-TDCT; 5 = TCB. LC system: silica–dry hexane; flow-rate: 0.88 (UV) and 0.33 (ECD) ml/min. UV detection at 215 nm. Detector current, $3 \cdot 10^{-10}$ A. Oven temperature, 300°C.

As is to be expected, an incidental advantage of the efficient heat transfer of the new interface is that relatively low oven temperatures can be used to achieve maximum ECD response. To quote an example, for DCB 250°C now suffices as against 350°C with the Pye-Unicam interface. This implies that in many cases the ECD (which is placed in the same oven as is the interface) will not have to be held at its maximum temperature any more.

Detection and detection limits

For a series of organochlorine pesticides and other highly halogenated

TABLE II

MINIMUM DETECTABLE AMOUNTS (MDA) OF A NUMBER OF HALOGENATED AROMATIC COMPOUNDS VIA UV AND ELECTRON-CAPTURE DETECTION

LC system, see Table I; UV detection at 205 nm (pesticides) or λ_{max}; detection limits at signal-to-noise ratio of 3:1.

Solute	*k'*	*MDA (pg)*	
		UV	*EC*
Decachlorobiphenyl	0.26	100	5
Octachloronaphthalene	0.27	200	5
m-Tetradecachloroterphenyl	0.45	200	10
p-Tetradecachloroterphenyl	0.55	270	10
o-Tetradecachloroterphenyl	0.75	280	10
2,4,4'-Trichlorobiphenyl	1.05	460	10
2,4,5,2',4',5'-Hexabromobiphenyl*	1.10	540	10
Decabromobiphenyl	1.20	510	20
Organochlorine pesticides**			
Group I	0.14–3.35	600–4500	8–30
Group II	3.70–28.5	>1000	20–100

* Major constituent of FireMaster BP-6.

** Group I: HCB, Aldrin, *p,p'*-DDE, Heptachlor, *o,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD and *p,p'*-DDD. Group II: α-HCH, γ-HCH, heptachlorepoxide, β-endosulfan, endrin and dieldrin.

aromatic compounds of environmental interest, minimum detectable amounts obtained with UV and electron-capture detection are given in Table II. UV detection limits were determined at or near the wavelength of maximum absorption of each individual compound to allow a fair comparison of the relative sensitivities of UV and ECD. For the compounds mentioned in Table II, ECD detection limits all are in the low-picogram range, *viz.* 5–100 pg. As an illustration, in Fig. 3 LC–ECD chromatograms of a duplicate injection of 8 pg of DCB are shown. The noise level amounts to *ca.* 1% of full scale deflection at attenuation ×16 (detector current, $3 \cdot 10^{-10}$ A). For DCB, good linearity was obtained over three orders of magnitude, *viz.* in the 10 pg–10 ng range; the repeatability of the ECD signal for six replicate injections of 80 pg DCB was 1.5%.

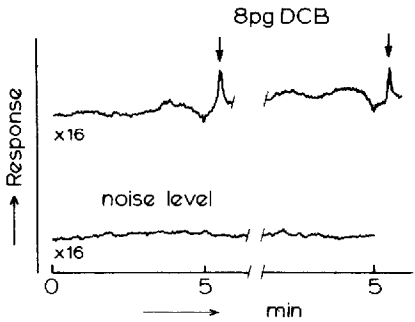

Fig. 3. LC–ECD chromatograms of a duplicate injection of 8 pg DCB. LC conditions as in Fig. 2.

For strongly UV-absorbing compounds, such as DCB, hexachlorobenzene and pesticides of the DDT group, gain factors of 20–50 are achieved on going from UV to electron-capture detection. The gain in sensitivity is between 2 and 4 orders of magnitude for the weakly UV-absorbing non-aromatic pesticides such as, *e.g.*, aldrin, the hexachlorocyclohexane (HCH) isomers, endrin and dieldrin. As regards the comparison of sensitivity of electron-capture detection in LC and GC, detection limits of LC–ECD typically are 20–100-fold higher than are those in GC–ECD. Since, however, injection volumes in LC and GC are *ca.* 100 and 1–5 μl, respectively, LC–ECD and GC–ECD can be said to possess comparable sensitivity. These conclusions, and the above results, clearly demonstrate the potential of the LC–ECD system for reliable and sensitive quantitative analysis.

The favourable noise level achieved in this study is partly due to a basic improvement in the design of the condenser tube installed at the detector exit. With the commercial coiled, and non-insulated, condenser tube, solvent droplets form immediately at the detector exit. This causes back-pressure variations and, consequently, a rather high noise level. This problem was eliminated by using a straight instead of the coiled condenser tube and, in addition, insulating its first part to prevent premature condensation. For the rest, in part of the present work a conventional ECD for GC (Series GCV, Pye-Unicam) was used instead of the Pye-Unicam ECD for LC. In such a case, the above problems self-evidently do not occur, the solvent vapours simply being evacuated with a suitable home vacuum line. The analytical results obtained with both types of ECD were fully comparable.

Finally, one should realise that in the above series of experiments no attempt has been made to optimize the flow directed to the ECD. Preliminary data indicate that flow-rates to the ECD of 0.3–0.4 ml/min (as used in the present work), instead of 0.8–1.0 ml/min, considerably (4–8 ×) improve signal-to-noise ratios and, thus, detection. A similar observation has been made by Krull and co-workers[5,6]. More systematic studies are, however, required to assess definitely the beneficial effects of low flow-rates and, consequently, the potential of microbore LC–ECD.

Use of polar modifiers

For efficient separations in adsorption LC, the use of solvent mixtures more polar than pure hexane is often required. Chamberlain and Marlow[7] have studied the compatibility of various polar modifiers dissolved in hexane with the use of an LC–ECD system. On the basis of their results (dioxane, benzene > tetrahydrofuran > propan-2-ol, methanol > acetonitrile) we selected dioxane for a short study on the influence of mobile-phase composition on LC–ECD performance.

Addition of 0, 0.3–3 and 6% dioxane to the hexane used as mobile phase, directing 0.4 ml/min to the ECD, resulted in a *ca.* 1% noise level at attenuator settings of ×16, ×32 and ×64, respectively (detector current, $1 \cdot 10^{-10}$ A). When 10% dioxane was mixed with hexane, the increase in background signal required the use of zero suppression of the recorder to record ECD chromatograms, and even then only the highest attenuation settings could be used. Thus the LC–ECD system only functions reliably with dioxane percentages of up to *ca.* 6%*. Under these conditions

* Recently, it has been found that with dioxane from Baker (Deventer, The Netherlands) an addition of 10–15% dioxane to hexane can be tolerated.

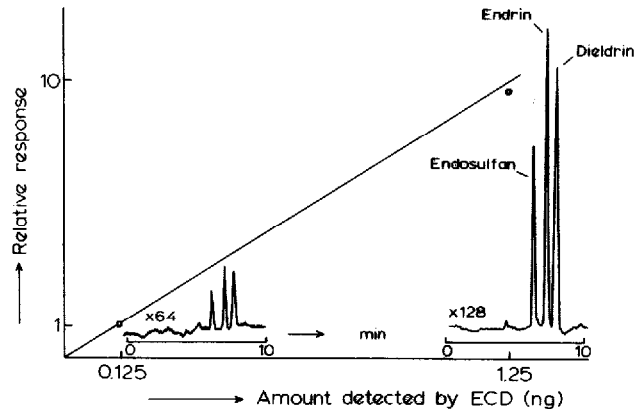

Fig. 4. LC–ECD chromatograms and comparison of electron-capture (EC) responses of 0.25 and 2.5 ng injections of the pesticides endosulfan, endrin and dieldrin. LC system: silica–hexane–dioxane (97:3); flow-rate: 0.8 ml/min; 0.4 ml/min directed to ECD. Detector current, $1 \cdot 10^{-10}$ A. Oven temperature, 300°C.

linearity of calibration curves for some selected pesticides was observed over a concentration range of at least 2 orders of magnitude; the repeatability was also satisfactory (relative S.D., 1.5%; $n = 5$). Typical chromatograms are shown in Fig. 4.

Environmental analysis

So far, the number of applications of LC–ECD has been rather limited, the most impressive examples being the milk pesticide monitor of Dolphin *et al.*[8], and the determination of nitro aromatics in post-blast explosion residues by Krull and co-workers[5,6]. We have further studied the use of LC–ECD in environmental analysis.

In our previous paper it was shown that patterns of technical mixtures of PCBs can easily be recognized by means of LC–ECD. In Fig. 5, this is demonstrated for an industrial sewage-sludge extract. The peak pattern of the technical mixture Aroclor 1260 is clearly recognized from the LC–ECD, but not from the LC–UV chromatogram. In the latter case, the many UV-absorbing interfering compounds present in the heavily polluted sample completely obscure the PCB pattern. Using the three major peaks in the LC–ECD chromatogram, the PCB level in the sample was calculated to be 3.5 ppm, which value is within 15% of that calculated via capillary GC–ECD (4.0 ppm).

The detection of a number of organochlorine pesticides in a sewage-sludge extract is shown in Fig. 6. In this case, the advantage of LC–ECD over LC UV mainly is the gain in sensitivity, which is especially apparent with aldrin and hexachlorobenzene.

Another interesting application is the analysis of a sample of Kanemi rice oil which caused the notorious Yusho disease in Japan (Fig. 7). Next to the PCBs, another band is seen to elute, which is much more clearly visible via EC as compared to UV detection (note the detector settings in Fig. 7). This broad band can be attributed to polychlorinated quaterphenyls (PCQs) which have recently been shown to be present in large amounts in the said rice oil (see, *e.g.*, refs. 9 and 10). Whereas GC on non-polar phases involves long retention times even at high oven temperatures, LC–ECD of the PCBs plus PCQs can be accomplished at room temperature within 20

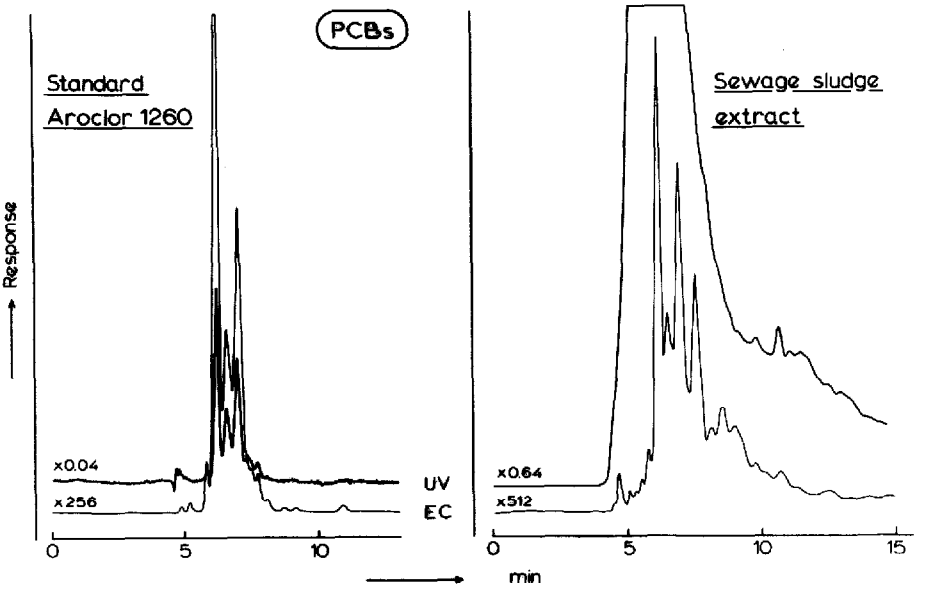

Fig. 5. LC–UV and LC–ECD chromatograms of a standard PCB mixture (Aroclor 1260) and a sewage-sludge extract. UV detection at 205 nm. For LC–ECD conditions see Fig. 2.

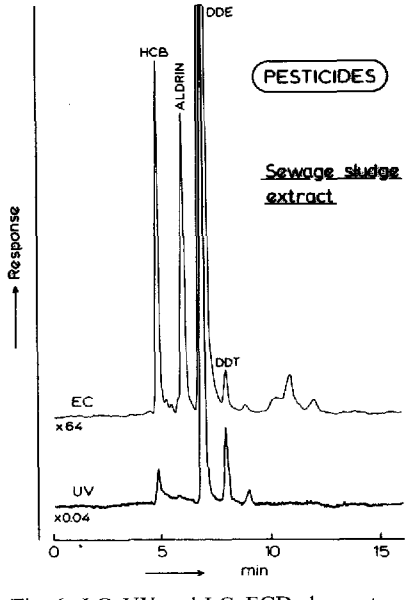

Fig. 6. LC–UV and LC–ECD chromatograms of a sewage-sludge extract. UV detection at 205 nm. For conditions see Fig. 2.

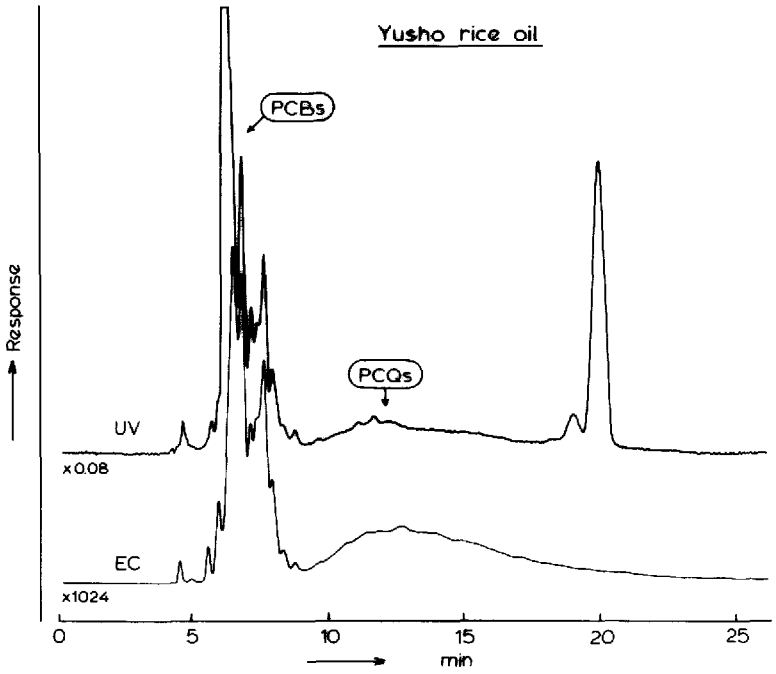

Fig. 7. LC–UV and LC–ECD chromatograms of a "Yusho" rice oil sample. UV detection at 205 nm. For LC–ECD conditions see Fig. 2.

min. The rather bad resolution in the PCQ peak profile is (both according to the literature[9,11] and our own experience) a problem common to LC and GC. Several authors[10–13] have, therefore, quantitated PCQs via perchlorination to the isomeric octadecachloroquaterphenyls. For these compounds, determination via LC–ECD will, to all probability, be highly suitable.

CONCLUSION

The construction of a redesigned interface and an improved condenser unit for use in on-line LC–ECD offers the possibility of detecting high-boiling compounds such as the fully halogenated biphenyls and terphenyls, and even polychlorinated quaterphenyls, without undue extra-column band broadening and/or baseline noise. Detection limits in the low (5–100) picogram range are obtained for these types of compounds as well as for organochlorine pesticides. The linear dynamic range amounts to 2–3 orders of magnitude. The successful application of LC–ECD to the analysis of environmental samples containing PCBs and pesticides reveals the superior sensitivity and selectivity of electron-capture compared to UV detection.

Mobile phases containing up to 6% dioxane (in hexane) are compatible with LC–ECD without undue loss of sensitivity of the ECD, or of linearity. An important means of using the full potential of adsorption LC–ECD will be the conversion of polar compounds into less polar derivatives, which are amenable to efficient elution within the range of mobile-phase compositions tolerated by the ECD. By selecting

suitable electron-capture reagents, derivatization may simultaneously serve as a means of increasing the sensitivity of compounds towards the ECD.

Other possibilities include the use of completely different phase combinations [CN or $N(CH_3)_2$ bonded phase–toluene; ref. 6], and of microbore LC columns with flow-rates of typically 50–100 μl/min. The latter alternative, amongst other things (*cf.* above) will allow the detector to work again under flow conditions comparable to those in GC–ECD. In such a case, the purge gas will regain its original function and can, then, hopefully be employed to optimize the performance of the LC–ECD system. Lastly, one should remark that the use of LC–ECD for systems employing really polar mobile phases can be expected only if an efficient wire- or belt-transport system is constructed or, alternatively, if an extraction detector is installed between the outlet of the LC column and the ECD inlet in order to extract the solute of interest from the polar LC effluent into a solvent compatible with the ECD.

Work along some of the lines indicated here is currently in progress in our laboratory[14].

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