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# ON-LINE TRACE ENRICHMENT ON A REVERSED-PHASE PRE-COLUMN FOR NORMAL-PHASE LIQUID CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION

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## SUMMARY

The trace enrichment of chlorophenols in aqueous samples has been achieved through a technique involving sorption on a liquid chromatographic (LC) pre-column packed with a styrene–divinylbenzene copolymer, drying of the pre-column with nitrogen and desorption. Analysis was carried out by normal-phase LC with on-line electron-capture detection. For the pre-concentration of 0.4 ml of standard solutions, recoveries of 70–110% were observed. Breakthrough volumes of di- and higher chlorinated phenols were over 40 ml, which allowed their detection down to low-ppt levels. The applicability of the method was tested on tap water, river water and urine samples.

## INTRODUCTION

In recent years, distinct progress has been made in the field of column liquid chromatography (LC) with on-line electron-capture detection (LC–ECD)[1]. Normal-phase LC (NPLC)–ECD is a straightforward technique which provides high sensitivity and selectivity and causes little operational problems. Several real applications have demonstrated the potential of NPLC–ECD[2,3]. The use of on-line reversed-phase LC (RPLC)–ECD creates more problems[4]. In principle, only narrow-bore LC, with flow-rates of about 20 μl/min, can be used and, although detection limits in mass units are about the same as with conventional-size NPLC–ECD, the limited injection volumes in narrow-bore LC cause the detection limits in concentration units to be distinctly higher in RPLC–ECD than in NPLC–ECD.

Conventional-size RPLC can be directly coupled with ECD only if a post-column extraction system is used, which is inserted between the LC column outlet and the evaporation interface inlet[5,6]. The noise levels are about ten times higher than in NPLC–ECD, but excellent selectivity is obtained, as has been demonstrated for the determination of pentachlorophenol in urine samples[6]. An on-line trace enrichment set-up was included in that application in order to improve the sensitivity further.

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An advantage of RPLC over NPLC is that aqueous samples can be directly injected. From our earlier experiments with an LC trace enrichment set-up coupled on-line with capillary gas chromatography[7], the idea was conceived that such a pre-column, based on a reversed-phase pre-concentration mechanism, could also be used in NPLC–ECD. Actually, in 1984 Boo and Krohn[8] developed an automated system for on-line trace enrichment on a reversed-phase pre-column with NPLC. They built a complicated system involving two pre-columns, and used a nitrogen purge and a vacuum system to dry the pre-columns before and after desorption. They used it for the determination of some organochlorine pesticides and polyaromatic hydrocarbons in standard solutions. They were able to pre-concentrate the analytes from at least 50 ml of water sample and obtained detection limits of below 1 ppb using UV detection. Trace-enrichment parameters were varied extensively in order to obtain optimal recoveries, but no further analytical data were given and only standard solutions were analysed. So far, no follow-up of this interesting and promising study has been published.

In this study, an LC pre-column packed with the reversed-phase sorbent PLRP-S (see below) was coupled on-line to an NPLC system. A large volume (0.4–50 ml) of an aqueous sample containing chlorophenols is passed through the pre-column. After a brief period of nitrogen flushing to remove the residual water from this column, the retained organic compounds are desorbed by the NPLC mobile phase, transferred to the separation column and analysed.

## EXPERIMENTAL

### *Chemicals*

Analytical-reagent grade toluene and HPLC-grade *n*-hexane (Baker, Deventer, The Netherlands) were purified by treatment with a dispersion of 45% sodium in paraffin (Fluka, Buchs, Switzerland) and subsequent distillation[9]. Analytical-reagent grade hydrochloric acid, glacial acetic acid, acetone and phosphoric acid and HPLC-grade water and methanol (Baker) were used as received.

All compounds used as test solutes were commercially available analytical-reagent or technical-grade products.

### *LC system*

NPLC was carried out using a 250 × 2.1 mm I.D. stainless-steel column laboratory-packed with 5-μm LiChrosorb Si-60 silica (Merck, Darmstadt, F.R.G.) with *n*-hexane–toluene–glacial acetic acid (80:19:1 or 75:24:1) as the eluent. The mobile phase was delivered by an Orlita (Giessen, F.R.G.) Model 034 sRC reciprocating pump at a flow-rate of 100–200 μl/min. The LC effluent was led to a Pye Unicam $^{63}$Ni constant-current electron-capture detector (Philips, Eindhoven, The Netherlands) via an evaporation interface as described earlier[10]. The interface and detector were kept at a temperature of 300°C. For optimal LC–ECD operation, a stream of oxygen-free nitrogen make-up gas was used at a flow-rate of 40 ml/min. The detector current was $1 \cdot 10^{-10}$ A. For integration and data handling a Nelson Analytical (Cupertino, CA, U.S.A.) Series 3000 Laboratory Data System was used in combination with a Commodore PC20 computer.

Samples were directly introduced either with a laboratory-made micro-injection valve with a 0.5-μl internal injection loop or via a pre-column.

*Trace enrichment of chlorophenols from water*

Pre-concentration of chlorophenols from water samples was carried out by a valve switching system consisting of three laboratory-made six-port Rheodyne-type valves with Valco fittings. A Kipp (Delft, The Netherlands) 9208 LC pump was used for loading and flushing the pre-column with an aqueous 1 m*M* phosphoric acid–methanol (90:10) solution. The following pre-columns were tested: (1) 30 × 4.6 mm I.D., Brownlee (Santa Clara, CA, U.S.A.), packed with 10-μm Spheri ODS; (2) 20 × 4.6 mm I.D. and (3) 2 × 2 mm I.D., laboratory-made[11]; (4) 4 × 2.2 mm I.D., Chrompack (Middelburg, The Netherlands); and (5) 4 × 1 mm I.D., laboratory-made micro-pre-column[7]. The original screens of the Chrompack pre-column were replaced with screens with a pore size of 5 μm and pre-columns 2–5 were laboratory packed with 10-μm Hypersil ODS (Shandon, Runcorn, U.K.). As will be explained below, for the final measurements the 4 × 2.2 mm I.D. Chrompack pre-column laboratory packed with 20-μm 1000-Å PLRP-S (Polymer Labs., Shropshire, U.K.) styrene–divinylbenzene polymer material was used. The general set-up of the reversed-phase trace enrichment system coupled on-line with normal-phase LC–ECD is shown in Fig. 1.

In order to activate the polymer material in the pre-column prior to the trace enrichment step, the 0.4-ml injection loop (V1) was filled with methanol. By turning valves V1, V2 and V3 to the appropriate positions (as shown in Fig. 1), the methanol was passed through the pre-column at a flow rate of 0.3 ml/min (pump 1). This was followed by 2–5 ml of carrier stream [1 m*M* phosphoric acid–methanol (90:10)] to remove the excess of methanol. After turning valve V1, the 0.4-ml loop was then filled with the sample and, by turning valve V1 again, the sample was passed through the pre-column. After rinsing the pre-column with an extra 2.6 ml of carrier stream, valve V2 was turned to allow nitrogen (0.5 bar inlet pressure) to pass through the pre-column and remove the residual water from the packing material. Passage of nitrogen was continued until no further water condensation was observed in the PTFE waste tubing connected to the pre-column valve; this took about 5 min. For safety, a drying time of 7 min was always used. Next, valve V3 was turned to desorb

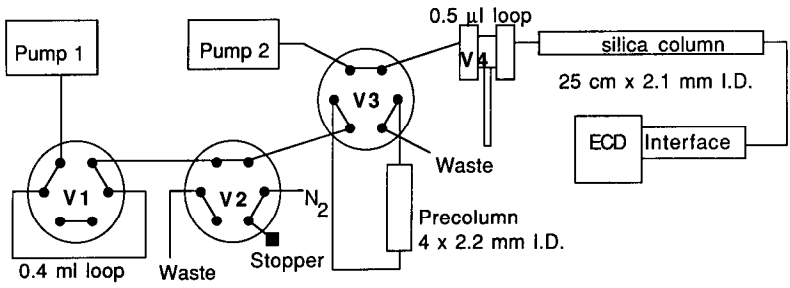


Fig. 1. Scheme of the LC system with four valves (V1, V2, V3, V4) for trace enrichment and sample introduction. The loading step of a sample on to the pre-column is shown. After turning V2 the pre-column will be dried, and after turning V3 the sorbed compounds will be desorbed and transferred to the LC column. Valve V4 is for direct injection on to the separation column. Pump 1 delivers 1 m*M* phosphoric acid–methanol (90:10) as carrier stream and pump 2 delivers the LC eluent (*n*-hexane–toluene–glacial acetic acid). Pump 1 was also used in a system without valve V1 for delivering large sample volumes.

the retained compounds with the mobile phase, *n*-hexane–toluene–acetic acid, from the PLRP-S packing material directly on to the separation column.

After the desorption was complete (after 0.5 min), valve V2 was turned to the drying position so that nitrogen could pass through the pre-column again to remove the remaining solvent from the pre-column. A drying time of 5 min was sufficient at this stage. By turning valve V2 to the sorption position the system was returned to its initial setting where methanol can be passed through the pre-column to activate the packing material. In the analysis of real samples, the activation with methanol was preceded by a 0.4-ml flush with acetone for cleaning purposes.

For the pre-concentration of large sample volumes of tap water and filtered Amstel river water, the solvent reservoir of the Kipp pump (pump 1) was filled with the sample after acidifying it with phosphoric acid to pH 2 and adding up to 10% of methanol.

*Analysis of urine samples*

A 1-ml volume of methanol was added to 9 ml of urine in a reagent tube and hydrochloric acid was added to adjust the pH to 1.5. After stoppering the reagent tube, hydrolysis was carried out by placing the tube in a water-bath at 100°C for 1 h[12]. After homogenization on a Vortex mixer, the 0.4-ml injection loop was filled with the sample solution.

## RESULTS AND DISCUSSION

*Optimization of the pre-column system*

For the direct analysis of aqueous samples via NPLC, it is important that all water is completely removed from the sample or the pre-column in order to avoid changes in the mobile phase composition, as retention in NPLC is very sensitive to small changes in water content. Boo and Krohn[8] found that the design and dimensions of the enrichment column influence the effective removal of the water from this column. Of their pre-columns, the smallest (10 × 4.6 mm I.D.) packed with a 5-$\mu$m Hypersil ODS $C_{18}$ bonded phase appeared to be the most suitable. They did not observe any problems caused by the pressure drop between the pre-column and the top of the NPLC column after switching the evacuated enrichment column to the desorption position, although the volume of the pre-column (about 170 $\mu$l) and, therefore, the pressure drop was large. Still, reduction of the pre-column volume will be advantageous in order to reduce the pressure drop and the volumetric band broadening, and to speed up and simplify the drying of the pre-column. On the other hand, one should realize that this will also reduce the breakthrough volume of the analytes of interest.

The five pre-columns specified under Experimental were tested. It was found that the two large (20 and 30 cm long) pre-columns were difficult to dry using only a nitrogen stream (no vacuum) and ambient temperature. Heating these pre-columns in a water-bath at 40–50°C reduced the drying time; however, after desorption a large unretained peak often showed up in the NPLC–ECD chromatogram. The results obtained with the 2 × 2 mm I.D. laboratory-made pre-column were not satisfactory because the frits tended to become clogged (when analysing biological samples). The results with the 4 × 2.2 mm I.D. Chrompack and 4 × 1 mm I.D. laboratory-made

pre-column, both equipped with metal screens instead of frits, were good; drying of the pre-column for 5 min at ambient temperature with a nitrogen purge only was found to be sufficient.

In this study, the 4 × 2.2 mm I.D. pre-column (with a volume of about 15 μl) was further used because this pre-column is commercially available and the packing can be easily exchanged. To compensate for the relatively small volume of this pre-column, the styrene–divinylbenzene PLRP-S copolymer sorbent was used as the packing material instead of a $C_{18}$-bonded silica. It is well known that for, *e.g.*, dichlorophenols at least 10-fold higher breakthrough volumes are obtained on such polymer material than with a $C_{18}$ sorbent[13]. An additional advantage of the polymer over $C_{18}$-bonded silica is its higher chemical stability; carrier streams and/or LC solvents of pH 1–13 can be used. In RPLC, the use of the polymer sorbent for the pre-concentration of hydrophobic compounds is often hampered because of difficulties in the desorption step, *e.g.*, low recoveries and/or extra band broadening. In this study, the desorption was carried out with non-polar solvents and did not give any problems. The pressure drop between the pre-column and the top of the analytical column occurring on switching valve V3 (see Fig. 1) to the desorption position did not cause a noticeable reduction in the lifetime of the analytical column.

Various di-, tri-, tetra- and pentachlorophenol(s) (DCP, TCP, TeCP and PCP, respectively) were used as test compounds. The optimization of the NPLC–ECD separation of those chlorophenols has been studied before; in order to obtain symmetrical peak shapes for the slightly acidic compounds, about 1% of glacial acetic acid has to be added to the *n*-hexane–toluene mixture used as the eluent[2].

Trace enrichment of organic compounds from water samples using pure water as the carrier stream occasionally results in low recoveries[14]. This can be the result of either adsorption of the analytes on the plastic parts of, *e.g.*, the valve system or

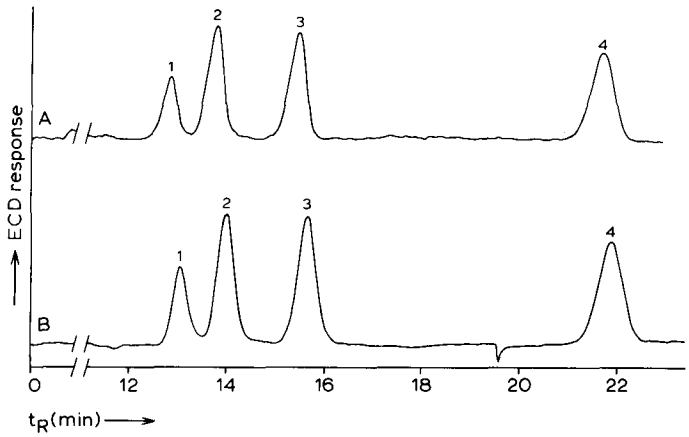


Fig. 2. LC–ECD chromatograms for a mixture of four chlorophenols (A) injected via a 0.5-μl loop and (B) after 0.4-ml trace enrichment. Conditions: 250 × 2.1 mm I.D. column packed with 5-μm LiChrosorb Si-60 silica; eluent, *n*-hexane–toluene–glacial acetic acid (79:20:1); flow-rate, 200 μl/min. Amount of sample for chromatogram A: (1) 17 ng 2,5-DCP; (2) 2,4 ng PCP; (3) 2.0 ng 2,3,5-TCP; (4) 2.3 ng 2,3,4,5-TeCP. Amount of sample for chromatogram B: (1) 22 ng 2,5-DCP; (2) 3.2 ng PCP; (3) 2.6 ng 2,3,5-TCP; (4) 3.1 ng 2,3,4,5-TeCP. ECD: detector current, $1 \cdot 10^{-10}$ A; attenuation, × 128.

the fact that the passage of large amounts of pure water through the pre-column changes the nature of the surface of the packing material. In order to avoid these problems, 10% methanol was added to both the carrier stream and the aqueous samples. In Fig. 2B, a typical NPLC chromatogram is shown of a 0.4-ml aqueous sample containing four chlorophenols (2.6–22 ng each) after their on-line enrichment. The chromatogram in Fig. 2A represents a sample containing 2–17 ng of each chlorophenol injected directly on to the NPLC column. The recoveries were determined by comparison of the peak areas in the two chromatograms; they varied from 70 to 110%. Fig. 2 also shows that use of the trace enrichment set-up leads to a shift in retention time of about 0.2 min.

The extra-column band broadening due to the trace-enrichment system was about 15–20 $s^2$. This resulted in an extra time variance of, *e.g.*, 15% for PCP ($k' = 2.8$) using a column with 10 000 plates. The rather high time variance is partly caused by the insertion of the micro injection valve V4 (see Fig. 1), which is necessary for the easy determination of the recovery and for calibration purposes.

Detection limits for the higher chlorinated phenols are in the range 40–100 pg (signal-to-noise ratio 3:1). This implies that for the pre-concentration of a 0.4-ml sample, the minimum detectable concentrations are 0.1–0.25 ppb. Repeated trace-enrichment experiments at concentrations of 5 ppb showed relative standard deviations (R.S.D.) of 7–20% ($n = 5$) for both peak-height and peak-area measurements. Considering the complexity of the system and the low concentrations involved, this is a satisfactory result.

*Water analysis*

The widespread use of chlorophenols has led to their presence in water, ranging from sub-ppb levels in river water to ppm levels in waste water[15,16].

In order to investigate the suitability of the present set-up for the determination of low-ppt (parts per $10^{12}$) levels of chlorophenols, it is necessary to load 10–40 ml of water on the pre-column. For testing, the water samples were spiked with 2,3,6-TCP, PCP, 2,3,5-TCP and 2,3,4-TCP. The breakthrough volumes for these compounds were found to be at least 40 ml. Because for 2.1 mm I.D. analytical columns injection volumes of 5–10 $\mu$l are commonly used, this implies that enrichment factors of at least about 4000 are obtainable. The recoveries for trace enrichment from 10–40-ml samples varied from 40 to 60%, the recovery for 2,3,4-TCP being the lowest. The substantial losses were probably due to adsorption in the PTFE supply tubing of the LC pump. In Fig. 3 three traces are shown, obtained after the pre-concentration of 40 ml of tap water, *viz.*, for a non-spiked, a 15–45 ppt and a 60–180 ppt spiked sample. In the blank tap water no peaks were observed. At both spiking levels the same recoveries for the chlorophenols were found. From the chromatograms for the spiked samples it can be seen that detection limits of about 10 ppt can be obtained for tri- and higher chlorinated phenols. The repeatability of the system was tested for tap water spiked with 60–180 ppt of the chlorophenols. The R.S.D. was 7–11% ($n = 5$). For cleaning of the pre-column after desorption, a wash step with acetone was introduced. This improved the repeatability of analysis considerably, especially with complex samples such as urine (see below).

In an Amstel river water sample clearly more ECD-sensitive compounds were present. Several peaks were recorded with the same peak height as 10–100 ppt of

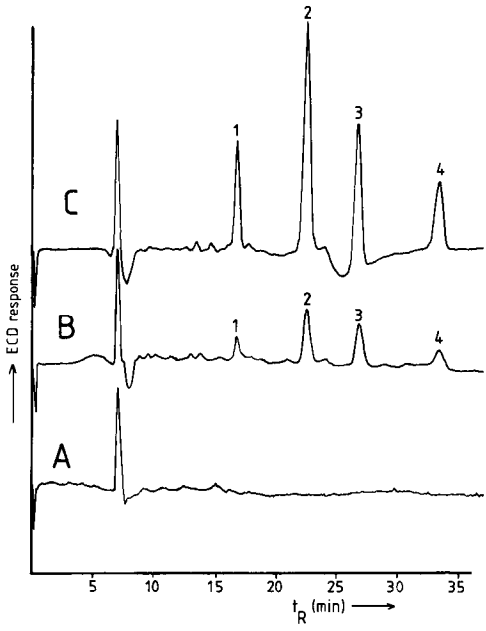


Fig. 3. LC–ECD chromatogram of 40 ml of tap water after trace enrichment. (A) Non-spiked, (B) spiked with 15–45 ppt and (C) spiked with 60–180 ppt of (1) 2,3,6-TCP, (2) PCP, (3) 2,3,5-TCP and (4) 2,3,4-TCP. Conditions: 250 × 2.1 mm I.D. column packed with 5-μm LiChrosorb Si-60 silica; eluent, *n*-hexane–toluene–glacial acetic acid (79:20:1); flow-rate, 100 μl/min. ECD: detector current, $1 \cdot 10^{-10}$ A; attenuation, × 64.

PCP. A peak having the retention time of PCP was also observed; it corresponded to a concentration of about 70 ppt of PCP. For positive proof of the presence of this compound, confirmation by other analytical techniques is required. De Ruiter *et al.*[17], who used fluorescence detection of dansyl hydroxide split off by post-column UV irradiation from dansyl derivatives of the phenolic compounds, found a PCP concentration of about 80 ppt in Amstel river water. Comparison of the two systems for the determination of chlorophenols in aqueous samples revealed about a 5-fold higher sensitivity and better selectivity (*e.g.* no large unretained peak) with our system; however, the repeatability seems to be better for the system developed by the Ruiter *et al.* [R.S.D. of 2.4% ($n$ = 5) for PCP at a spiking level of 1 ppb].

*Urine samples*

In the previous examples, the high sensitivity of the system was demonstrated. In practice, however, the trace analysis of complex samples is often hampered by a lack of selectivity. Therefore, as an alternative example, several hydrolysed urine samples were analysed. Fig. 4 shows the chromatograms of a spiked and a non-spiked urine sample. In the blank trace several peaks were observed with the same peak height as given by 5–20 ppb of PCP. With those peaks (1–6) the repeatability of the analysis of urine samples was measured and found to be acceptable with an R.S.D. of 4–10% ($n$ = 6).

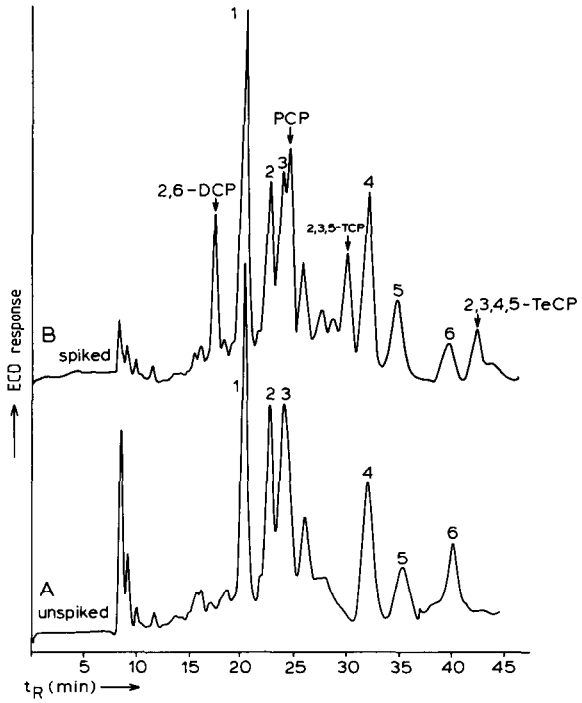


Fig. 4. LC–ECD chromatograms of 0.4 ml urine after trace enrichment. (A) Non-spiked; (B) spiked with 100 ppb of 2,6-DCP and 10 ppb each of PCP, 2,3,5-TCP and 2,3,4,5-TeCP. Conditions: 250 × 2.1 mm I.D. column packed with 5-$\mu$m LiChrosorb Si-60 silica; eluent, *n*-hexane–toluene–glacial acetic acid (74:25:1); flow-rate, 100 $\mu$l/min. ECD: detector current, $1 \cdot 10^{-10}$ A; attenuation, ×64.

The acetone used for cleaning of the pre-column after desorption had a dark colour after flushing the pre-column. Measurement of an aqueous standard after a urine sample revealed that memory effects were very low (< 0.5%). In other words, the wash step with acetone is efficient.

In Fig. 4B the trace obtained after spiking the urine sample with 100 ppb of 2,6-DCP and 10 ppb each of PCP, 2,3,5-TCP and 2,3,4,5-TeCP is shown. The recoveries of the chlorophenols at the 10 ppb level varied between 60 and 80%. It can be seen that in order to determine PCP concentrations below 10 ppb better resolution between peak 3 and PCP is necessary; this can probably be achieved via a reduction in the band broadening of the pre-column system by removing valve V4 and/or by using the miniaturized pre-column No. 5 (see Experimental). Although the 0.4-ml pre-concentration volume for the urine sample is far below the breakthrough volumes of the chlorophenols, pre-concentration from larger sample volumes presumably will not reduce the detection limits below about 1 ppb because of the large number of interfering ECD-sensitive compounds trapped on the pre-column. The use of a highly sensitive detector as an electron-capture detector, which ensures the pre-concentration from relatively small volumes to be sufficient, will have a good effect on the LC column lifetime. However, during the column usage the retention of the chlorophen-

ols tended to increase. In the present system, at least ten urine samples could be analysed before band broadening and clogging problems made it necessary to exchange the pre-column.

A comparison of the present system with the results obtained with a conventional-size RPLC–extraction module–ECD system[6] reveals a 10-fold higher sensitivity for standards and a significantly higher chromatographic selectivity for the present NPLC system; for non-spiked urine samples, one large tailing peak was obtained with the extraction system, whereas with the present system at least six peaks can be distinguished (see Fig. 4A).

## CONCLUSIONS

Trace enrichment on a reversed-phase pre-column for subsequent on-line analysis by normal-phase LC–ECD has been achieved. Small pre-columns with a volume of 5–15 $\mu$l and equipped with screens were found to be the most suitable. Packing the pre-column with styrene–divinylbenzene PLRP-S polymer resulted in breakthrough volumes for di- and higher chlorinated phenols of over 40 ml. After loading the sample on to the pre-column, a drying time of 5 min with a stream of nitrogen was sufficient. The results show that the method is applicable to the determination of chlorophenols in tap water and urine down to low-ppt and low-ppb levels, respectively. The lowest detectable concentrations which can be obtained depend on the complexity of the sample matrix and the selectivity and separation efficiency of the LC system. The use of the pre-column set-up did not lead to higher noise levels with the ECD and/or reduced lifetime of the LC column.

The use of RPLC pre-columns for NPLC is also possible using more common LC detectors. The easy handling of aqueous samples, the trace enrichment factors obtainable and the automation potential of the system are very promising.

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