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Sensitive liquid chromatographic determination of alkyl-, nitro- and chlorophenols by precolumn derivatization with dansyl chloride, postcolumn photolysis and peroxyoxalate chemiluminescence detection

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ABSTRACT

A liquid chromatographic method is described for the determination of alkyl-, nitro- and chlorophenols at sub-ppb levels using a very sensitive and selective detection system. The phenols are labelled by two-phase dansylation. The deprotonated phenolic anions are extracted as tetrabutylammonium ion pairs into an organic phase in which dansyl chloride is dissolved. After derivatization, the excess of reagent is removed on an amino-bonded column; dansyl chloride reacts with the amino groups whereas the phenol derivatives are not retained. Chromatography is carried out with a methanol–water gradient followed by photolysis of the derivatives. The strongly quenching electronegative nitro- and chlorophenol groups are photochemically removed from the derivative and the products, dansyl hydroxide and dansyl methoxide, are sensitively detected by peroxyoxalate chemiluminescence. Chemical excitation is carried out by adding 2-nitrophenyl oxalate and hydrogen peroxide dissolved in acetonitrile to the column eluate. Detection limits of about 0.01–0.1 ng/ml have been achieved and the method has been applied to the determination of several phenolic compounds in surface water.

INTRODUCTION

Nowadays several substituted phenolic compounds with low (pentachlorophenol) to relatively high (phenol and nitrophenols) polarity have to be monitored at sub-ppb levels of environmental samples such as surface water [1]. Established methods, including liquid–liquid extraction and/or distillation followed by liquid chromatography (LC) with UV detection [2–4], lack both sensitivity and selectivity. Recently, other methods, including electrochemical detection [5], mass spectrometry [6], gas chromatography [7], micellar electrokinetic chromatography [8] and capillary zone electrophoresis [9], have been described. However, as electrochemical detection is still not widely accepted for routine analysis, mass spectrometry is expensive, gas chroma-

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tography is less suitable for polar analytes in aqueous samples and electrokinetic methods lack concentration sensitivity, LC methods are to be preferred.

A fast and simple derivatization reaction for phenolic compounds was developed by De Ruiter *et al.* [10]. The labelling procedure involves ion-pair extraction of the deprotonated phenol with a tetrabutylammonium counter ion to an organic phase in which the non-polar derivatization reagent (dansyl chloride) is dissolved. The sensitivity can be significantly improved by application of a postcolumn photochemical reaction [11]. The highly fluorescent dansyl hydroxide and/or methoxide is/are liberated from the strongly quenching electronegative chlorophenol moiety. Detection limits of *ca.* 200 pg for tri, tetra- and pentachlorophenols were obtained. For the determination of the complete range of phenols at the required level, a 100-fold preconcentration would be necessary. In that event the determination of the polar phenols will cause problems because their breakthrough volumes on hydrophobic precolumns often are too small for such a preconcentration step [12].

Peroxyoxalate chemiluminescence (CL) detection of dansyl derivatives has been shown to be one to two orders of magnitude more sensitive than fluorescence detection [13–15]. The aim of this study was to combine the selective two-phase dansylation and photolysis of substituted phenols with sensitive peroxyoxalate CL detection. Further, the applicability of the method for a wide range of phenols, including polychloro-, alkyl- and nitrophenols, was investigated for tap water and river water samples.

EXPERIMENTAL

Chemicals

High-performance liquid chromatographic (HPLC)-grade solvents and amino-bonded (type 7088-03) and C_{18}-bonded (type 7020-03) SPE columns were purchased from Baker (Deventer, The Netherlands). Bis(2-nitrophenyl) oxalate (2-NPO) was synthesized as described [16]. 5-Dimethylamino-1-sulphonyl chloride (dansyl chloride), 2,4-dichlorophenol (DCP) and tetrabutylammonium bromide (TBABr) were purchased from Aldrich (Brussels, Belgium), phenol (P) from Baker, 4-nitrophenol (4-NP) from Fluka (Buchs, Switzerland), 2-nitrophenol (2-NP) and 2,4,6-trichlorophenol (TCP) from Janssen (Beerse, Belgium) and 2-chlorophenol (2-CP), pentachlorophenol (PCP), 2,3-, 2,5-, 3,4- and 3,5-dimethylphenol and 2-isopropyl-5-methylphenol from Merck (Darmstadt, Germany). 2,4-Dimethylphenol (DMP), 3-methyl-4-chlorophenol (CMP), 2,4-dinitrophenol (DNP), 2,4-dinitro-6-methylphenol, 2,4-dinitro-6-*sec.*-butylphenol (dinoseb) and 2,4-dinitro-6-*tert.*-butylphenol (dinoterb) were received as a gift from H. van der Zouwen (Provinciaal Waterleidingbedrijf Overijssel, The Netherlands). Stock solutions of all phenols were prepared in methanol and kept at 4°C in the dark. All other chemicals were of analytical-reagent grade.

Column liquid chromatography

The LC mobile phase flow-rate of 0.5 ml/min was delivered by a Gilson (Villiers-le-Bel, France) gradient system consisting of a Model 305 pump (A), a Model 302 pump (B), a Model 805 manometric module and a Model 811B dynamic mixer.

Pump A delivered methanol–100 m*M* imidazole buffer (pH 7.0) (97.5:2.5, v/v) and pump B methanol–2.5 m*M* imidazole buffer (pH 7.0) (2.5:97.5, v/v). The gradient programme was as follows: 75% A from 0 to 9.5 min, 85% A from 10 to 14.5 min, 95% A from 15 to 19.5 min, 100% A from 20 to 40 min and 100% to 75% A from 40 to 41 min. For every new run the column was allowed to equilibrate for at least 20 min at 75% A. A Rheodyne six-port valve with a 100-μl loop was used for introduction of samples onto a 200 × 3.1 mm I.D. column packed with 3-μm LiChrosorb RP-18 (Merck) by a standard slurry technique.

Detection system

After chromatography, the dansyl derivatives were irradiated in a photochemical reactor designed by Scholten *et al.* [17]. It was equipped with a Philips (Eindhoven, Netherlands) Model 93110E mercury lamp (90 W, 0.9 A). The reaction coil was a 130 × 0.3 mm I.D. × 1/16 in. O.D. polytetrafluorethylene (PTFE) capillary (helix diameter 72 mm), which was placed concentrically around the lamp. The reactor was cooled by a fan. Hydrogen peroxide and 2-NPO dissolved in acetonitrile at final concentrations of 50 and 5 m*M*, respectively, were mixed just before use and added to the column effluent (after photolysis) with a pulseless Isco (Lincoln, NE, USA) μLC-500 syringe pump. The mobile phase (0.5 ml/min) and the reagent stream (0.3 ml/min) were mixed with a standard Valco T-piece immediately before the detector. An ATTO (Tokyo, Japan) AC 2220 CL detector (operated at 700 V) equipped with a 65-μl spiral flow cell and a 470-nm cut-off filter was used for detection. Fluorescence detection was carried out with a Perkin-Elmer (Beaconsfield, U.K.) LS-2 fluorescence detector (λ_{exc} = 340 nm, λ_{em} = 470 nm).

Derivatization of phenolic compounds with dansyl chloride

Determination of phenolic compounds in the concentration range 10–1000 ng/ml. To 500 μl of an aqueous phenol-containing solution, adjusted to pH 12 with 1 *M* sodium hydroxide solution, 100 μl of an aqueous solution of TBABr (30 mg/ml, pH 12) and 600 μl of a solution of 0.1 mg/ml dansyl chloride in dichloromethane were added in a standard reagent tube (100 × 9 mm I.D.). The tube was capped with aluminium foil to avoid evaporation of dichloromethane and vortex mixed vigorously for 2 min. A 500-μl volume of the organic phase was slowly brought onto an amino-bonded SPE column (previously washed with dichloromethane and dried with nitrogen or air) and for 10 min the excess of dansyl chloride was allowed to react with the amino groups. The dansyl derivatives were eluted from the SPE column with 3 ml of dichloromethane, gently evaporated to dryness and the residue was dissolved in 500 μl of methanol–water (50:50, v/v). Finally, 100 μl were injected onto the reversed-phase column.

Determination of 2- and 4-nitrophenol, phenol and 2-chlorophenol in water samples at 0.1–10 ng/ml levels. A 2.0-ml volume of an aqueous phenol-containing solution, 300 μl of the TBABr solution and 800 μl of the dansyl chloride solution were treated as described above. A 500-μl volume of the organic phase was brought on to the amino-bonded SPE column and the same procedure was followed.

Determination of 2,4-dimethyl-, 3-methyl-4-chloro, 2,4-dichloro-, 2,4,6-trichloro- and pentachlorophenol in water samples at 0.1–10 ng/ml levels. A 3.0-ml volume of an aqueous acidified (pH 3.0) phenol-containing solution was preconcentrated on a

C_{18}-bonded SPE precolumn, desorbed with 3 ml of dichloromethane and evaporated to dryness under a stream of nitrogen. To the residue 100 μl of the TBABr solution, 500 μl of water (pH 12) and 600 μl of the dansyl chloride solution were added. The above procedure was then followed.

Analysis of water samples

Amsterdam tap water spiked with a number of selected phenols was either adjusted to pH 12 with 1 *M* sodium hydroxide solution for direct derivatization of the phenols or adjusted to pH 3 with 1 *M* nitric acid for preconcentration on a C_{18} precolumn. River water (Rhine) was filtered through a Millipore (Etten-Leur, Netherlands) 2-μm membrane filter prior to preconcentration and/or derivatization.

RESULTS AND DISCUSSION

Derivatization

Removal of excess of reagent. De Ruiter *et al.* [10] developed a rapid method for the dansylation of phenolic steroids using a two-phase system and phase-transfer catalysis. The deprotonated phenols are extracted as an ion pair with a tetrabutylammonium salt into an organic phase in which the apolar reagent (dansyl chloride) is dissolved. The reaction between the phenolic anion and dansyl chloride is very fast (*ca.* min at room temperature). The only disadvantage of the two-phase derivatization is that the excess of reagent remains in the same layer as the derivatized phenol. In normal-phase LC with fluorescence detection this is seldom a problem as dansyl chloride does not fluoresce. In reversed-phase LC, however, the excess of reagent will present a real problem as it will be partly converted into strongly fluorescent products.

Under the experimental conditions used in this study, the reagent appeared as a broad band with a retention close to that of the dansyl derivative of 2-CP. It was therefore necessary to remove the excess of reagent before injection into the LC system. In the literature the excess of reagent is often converted into a polar derivative by adding a small amount of a concentrated solution of a polar amine, *e.g.*, taurine [18] or glycine [19], after the derivatization of the analytes. This approach did not work here because a polar amine (or phenol) does not dissolve in the organic layer in which dansyl chloride is dissolved. Slightly more apolar amines such as ethylamine do remove the excess of reagent, but cause a broad tailing band in the early part of the chromatogram which interferes with the early-eluting dansylated phenol derivatives. Moreover, a large reagent band eluting from the column at t_0 causes a photomultiplier overload. This is especially inconvenient when using the ATTO AC-2220 CL detector, which has no overload safety.

De Ruiter *et al.* [11] described the used of amino-bonded TLC plates for the preparative dansylation of chlorophenols. The reagent dansyl chloride reacts with the amino groups and remains on the TLC plate with an R_F value of zero. This approach was slightly modified here by using amino-bonded SPE columns instead of TLC plates. After the normal two-phase dansylation procedure, 500 μl of the organic layer are brought onto a dry amino column where the excess of dansyl chloride will react with the amino groups. The volume should not exceed 500 μl in order to prevent breakthrough. The reaction time of the unreacted dansyl chloride with the amino

groups on the column should be at least 10 min at room temperature before elution of the dansyl derivatives from the amino column is carried out. Elution is typically performed with 3 ml of dichloromethane; the recovery of all dansylated phenols was 98–100%. For trace determinations (0.1–1 ng/ml) each analysis should be carried out with a new amino SPE column previously washed with dichloromethane and dried with nitrogen or air. The amino columns should preferably be dry because unreacted dansyl chloride is sometimes washed from a wet column. For analysis at higher levels (10–1000 ng/ml) it is possible to use the amino columns two or three times with intermediate washing with dichloromethane and drying. The chromatograms resulting after the removal of the excess of dansyl chloride were sufficiently clean even for the detection of dansyl derivatives of relatively polar phenols such as P and 2-CP. Injections of 100 μl were possible without excessive band broadening by using methanol–water (50:50, v/v) for injection. An example of a chromatogram with nine phenols derivatized according to the procedure described above is shown in Fig. 1.

Influence of the pH in the two-phase reaction. Using an aqueous phase with a pH of 10–11, irreproducible results were obtained for P and 2,4-DMP. Changing the pH

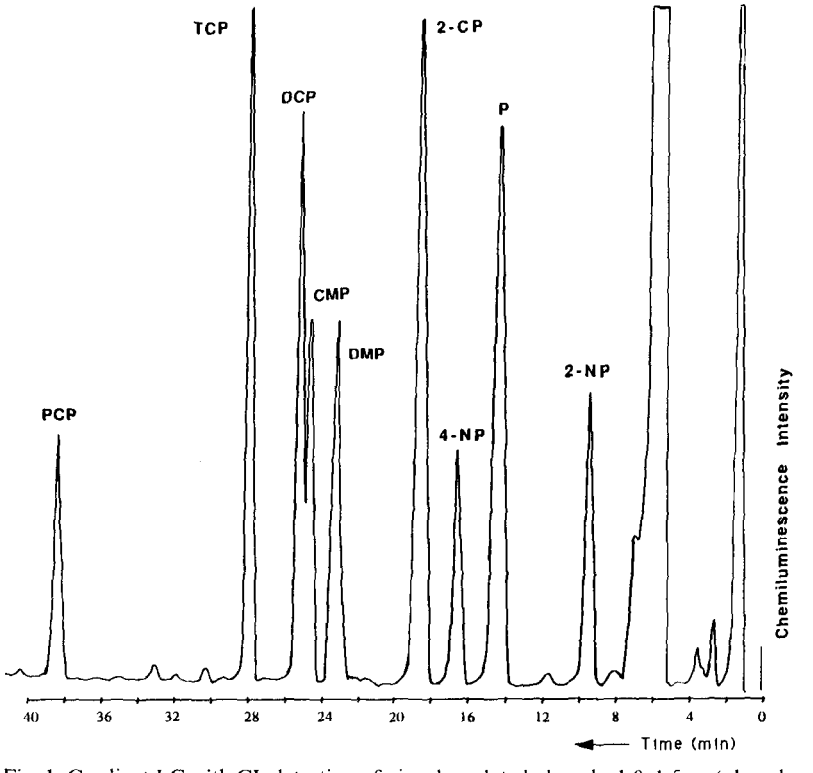

Fig. 1. Gradient LC with CL detection of nine dansylated phenols; 1.0–1.5 ng (phenol and chlorophenols) and 5.0 ng (mononitrophenols) injected. Sensitivity, 700 V; attenuation, 50. 2-NP = 2-nitrophenol; P = phenol; 4-NP = 4-nitrophenol; 2-CP = 2-chlorophenol; DMP = 2,4-dimethylphenol; CMP = 3-methyl-4-chlorophenol; DCP = 2,4-dichlorophenol; TCP = 2,4,6-trichlorophenol; PCP = pentachlorophenol. For derivatization and gradient elution programme, see Experimental.

to 12 improved the results for both phenols. Apparently, the pH of the aqueous phase should be at least one unit above the pK_a values of the phenolic compounds under investigation. Unfortunately, the higher chlorinated phenols containing three or more chlorine atoms are very unstable in pH 12 solutions. Their pK_a values are, admittedly, much lower than 12, but this pH is necessary for P and 2,4-DMP. For example, after 1 h at room temperature in a pH 12 solution, the derivatization yield for PCP had decreased by 10–30%, whereas a *ca.* 90% loss was found after 4 h.

Volume ratio in the two-phase reaction. Normally, 500 μl of an aqueous sample, 100 μl of a 30 mg/ml solution of TBABr and 600 μl of a 0.1 mg/ml solution of dansyl chloride in dichloromethane were vortex mixed for 2 min, and 500 μl of the organic layer were brought onto an amino SPE column for removal of the excess of reagent. In this way a sensitivity of *ca.* 0.1–0.4 ng/ml can be achieved. To obtain detection limits lower than 0.1 ng/ml, another procedure was necessary, *e.g.*, preconcentration on a short C_{18} precolumn. For apolar chlorophenols this is a reliable approach; polar phenols (2-NP, 4-NP and P), however, break through almost immediately. An alternative is to increase the aqueous phase volume in the two-phase reaction from 500 to 2000 μl, the TBABr solution volume from 100 to 300 μl and the dansyl chloride solution volume from 500 to 800 μl. For the relatively polar phenols (2- and 4-NP, P and 2-CP) this approach worked well and a gain in sensitivity by a factor of about four was obtained. However, for the more apolar compounds such as DMP, CMP and DCP (see Fig. 1), large interfering peaks appeared in the chromatogram. Therefore, all apolar phenols (DMP, DCP, CMP, TCP and PCP) were preconcentrated from 3.0 ml of an acidified aqueous sample on a C_{18} precolumn, as described [20]. The C_{18} precolumn was desorbed with 3 ml of dichloromethane and the eluate was evaporated under a stream of nitrogen. To the residue 600 μl of the dansyl chloride solution in dichloromethane, 500 μl of water (pH 12) and 100 ml of the TBABr solution were added and the normal derivatization procedure was followed. It is an advantage that the higher chlorinated phenols are in contact with the pH 12 solution for only a relatively short time.

Photochemical conversion of dansylated phenols

As has been described above, photochemical conversion of dansyl derivatives into more strongly fluorescent products can be used conveniently in a postcolumn LC system [11]. Photochemical conversion is important, because dansylated chlorophenols containing two or more chlorine atoms exhibit little native fluorescence, especially when the chlorine atoms are in the *ortho* position to the phenolic OH group. After LC separation the dansylated phenols are irradiated by a mercury lamp, and two main products are formed: the non-fluorescent chlorophenol and the highly fluorescent dansyl hydroxide and/or dansyl methoxide group.

When analysing a mixture of dansylated chloro- and nitrophenols (present at the same molar concentration level) by means of LC and postcolumn photolysis, surprisingly we observed that the peak heights of the mononitrophenols were about four times lower than those of the chlorophenols, and the dinitrophenols (DNP, DNMP, dinoseb and dinoterb) gave no peak at all. This aspect was investigated further by injecting relatively high concentrations (*ca.* 1 μg/ml) onto an LC column, with subsequent photolysis and UV monitoring, isolating the irradiated peaks and reinjecting a fraction of these peaks in the same system. In principle, each peak should

contain the following analytes: dansyl hydroxide and/or methoxide and, if still present, the non-decomposed dansylated phenol. The photochemical conversion can be calculated by comparing the peak height of the dansylated phenol peak in the first chromatogram with the peak in the same position after the second analysis. For several chlorophenols a 90–95% yield was observed, as against 30–40% for the mononitrophenols. For all dialkylphenols tested (2,3-, 2,4-, 2,5-, 3,4- and 3,5-dimethylphenol and 2-isopropyl-5-methylphenol), a photochemical conversion of 100% was found. This means that dansylated chloro- and dialkylphenols are very efficiently decomposed after irradiation for only 5 s, whereas in the same time the dansylated mononitrophenols are converted into fluorescent products to the extent of only 30–40%. Changing the irradiation time in the PTFE coil from 5 to 15 s did not improve the results. In fact, the peak heights of the dansylated mononitrophenols remained about the same, while all dansylated chlorophenol peak heights were 30–50% lower, probably owing to photochemical breakdown. The use of a more transparent quartz coil instead of a PTFE coil also did not improve the photochemical conversion of the nitrophenols, the peak-height ratio of chlorophenols to nitrophenols remaining about the same. As a quartz coil is more expensive and fragile than a PTFE capillary, all further studies were carried out with the standard PTFE capillary.

At a pH of 8, ion-pair extraction of dinitrophenols into chloroform or dichloromethane could easily be carried out, but no reaction with either dansyl chloride or laryl chloride was observed. It seems likely that the negative charge of the phenolate anion is strongly delocalized by the nitro groups. The nucleophilic character of the phenolate anion is reduced and, consequently, the reactivity. In other words, the present method is not suitable for dinitro-substituted phenolic compounds.

Chemiluminescence detection

Peroxyoxalate CL detection is usually combined with acetonitrile–water mobile phases. In that case, the oxalate, 2-NPO and hydrogen peroxide dissolved in acetonitrile can be added to the mobile phase without mixing problems. In this study, methanol was necessary as mobile phase modifier, because in postcolumn photochemistry acetonitrile is not compatible with a PTFE coil. Radicals are formed and rapid leakage of the PTFE coil will occur. Using the quartz coil described above, acetonitrile could be compared with methanol as mobile phase modifier. The signal-to-noise ratio for a dansylated phenol in acetonitrile–water mixtures was three times better than in methanol–water, but not all the peaks could be separated with an acetonitrile–water gradient; therefore, methanol–water was used.

When combining gradient elution with peroxyoxalate CL detection, care must be taken that both solvents contain an equal amount of buffer [21]. The mobile phase will then continuously contain the same concentration of imidazole buffer, an important parameter in CL detection. Furthermore solvents should be filtered and degassed very carefully, because air bubbles and microprecipitates in the mixing chamber will cause baseline disturbances when performing a gradient at trace-level sensitivity.

The imidazole buffer was varied both in concentration (1 and 10 m*M*) and pH (6 and 7.5), two of the most critical parameters in peroxyoxalate CL detection in LC systems [22,23]. With dansylated phenol as the test compound, the imidazole concentration gave a maximum in the CL signal-to-noise ratio at about 2.5 m*M*, and the pH at 7.0.

The oxalate (2-NPO, 5 m*M*) and hydrogen peroxide (50 m*M* in acetonitrile) were used as described in an earlier paper [24]. The addition of the 2-NPO and hydrogen peroxide mixture in acetonitrile (at 0.3 ml/min) to the methanol–water column eluate (0.5 ml/min) never led to any mixing or precipitation problems.

The ATTO AC 2220 CL detector is one of the few commercially available CL detectors for LC. Its photomultiplier tube is operated at a standard voltage of 1000 V. Unfortunately, in our system the noise was too large for us to be able to use the whole range of attenuation settings on the ATTO AC 2220. Therefore, our electronic workshop changed the photomultiplier power supply in such a way that it could be manually adjusted between 600 and 1000 V. Throughout this study a voltage of 700 V was applied. At this photomultiplier voltage the complete range of attenuation settings could be used.

Comparison of fluorescence with CL detection

The two detection modes were compared by injecting a mixture of four dansylated phenols. Fluorescence detection was carried out with an Perkin-Elmer LS-2 fluorescence detector equipped with a xenon lamp (excitation wavelength 340 nm, 470 nm, time constant 1 s) and CL detection was performed as described under Experi-

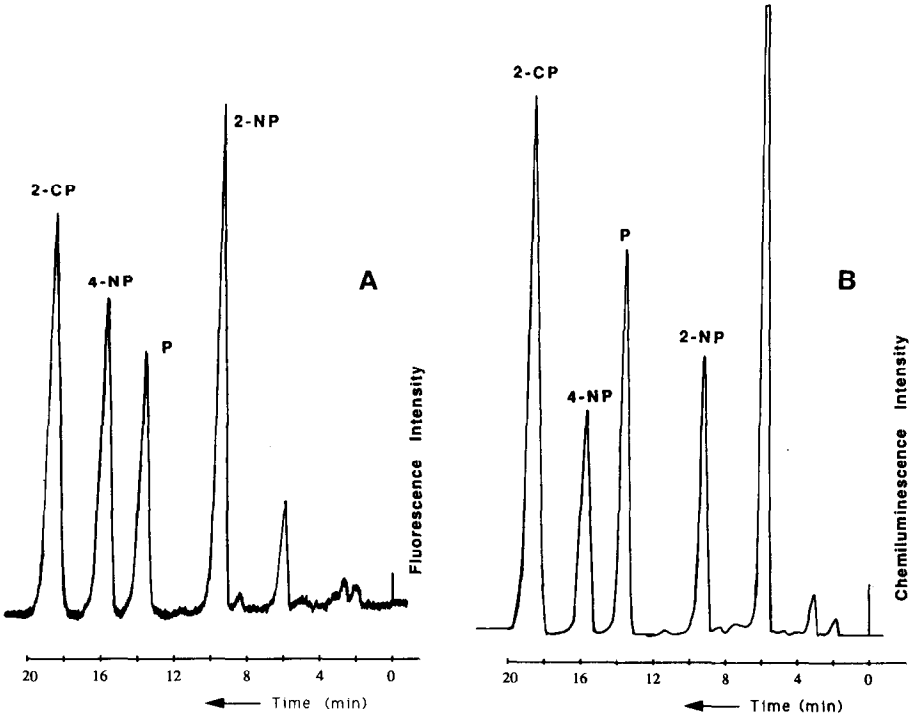

Fig. 2. (a) LC with fluorescence detection of a mixture of four dansylated phenols (see Fig. 1); 4 ng (phenol and 2-chlorophenol) and 20 ng (mononitrophenols) injected. Detector, Perkin-Elmer LS-2 (λ_{exc} = 340 nm, λ_{em} = 470 nm; response time, 2 s); mobile phase, A–B (75:25) flow-rate, 0.5 ml/min. For derivatization and further details, see Experimental. (B) LC with CL detection of the same dansylated phenols as in (A). Detector, ATTO AC-2220; attenuation , 200; noise is obtained at attenuation 2 (100 times more sensitive); mobile phase A–B (75:25); flow-rate, 0.5 ml/min. For derivatization and further details, see Experimental.

mental. As can be seen from Fig. 2, the signal-to-noise ratio with CL detection is about two orders of magnitude better than that with fluorescence detection. Another interesting point is that the peak-height ratio of nitrophenols to chlorophenols is slightly higher with fluorescence detection. This may be due to the excitation lamp irradiating the dansyl derivatives during analysis.

Linearity and detection limits

The linearity of the complete system (derivatization, photolysis and CL detection) was measured for a mixture of four dansylated phenols, 2- and 4-NP, P and 2-CP, in the range 5–500 ng/ml. In all instances the linearity was good ($n = 9$, $r = 0.996$–0.998).

The limits of detection for normal derivatizations (500 μl of aqueous phenolic solution) were 0.4 and 0.05 ng/ml for phenol and 2-chlorophenol, respectively and 0.4 ng/ml for 2-NP and 4-NP. For the higher chlorinated phenols slightly higher limits of detection were obtained than for 2-CP, ranging from 0.1 ng/ml for 2,4-DCP to 0.2 ng/ml for PCP. When using a larger aqueous phase volume or preconcentration via a precolumn, the limits of detection were improved accordingly (see Table I). In some instances they were influenced by the appearance of small peaks due to the formation of derivatization side-products (especially with P and DMP), but in almost all instances the required detection limit of 0.1 ng/ml was achieved.

Tap and river water samples

Tap and river water were spiked with phenols at the 0.5–2.5 ng/ml level. For the four early-eluting phenols (2- and 4-NP, P and 2-CP), the water was adjusted to pH 12 with 1 *M* sodium hydroxide solution and 2000 μl were derivatized as described under Experimental. For the five late-eluting phenols (DMP, CMP, DCP, TCP and PCP) the water was acidified to pH 3 and 3.0 ml were preconcentrated on a C_{18} precolumn. A larger preconcentration volume, *e.g.*, 10–20 ml, is still below the breakthrough volumes of these relatively apolar phenols and will probably result in lower

TABLE I

LIMITS OF DETECTION IN ng/ml (SIGNAL-TO-NOISE RATIO = 3) FOR SEVERAL PHENOLIC COMPOUNDS

Dansylated derivative of	Aqueous sample volume[a]		
	500 μl	2.0 ml	3.0 ml
2-Nitrophenol	0.4	0.1	
4-Nitrophenol	0.4	0.1	
Phenol	0.4	0.1	
2-Chlorophenol	0.05	0.01	
2,4-Dimethylphenol	0.5		0.1
3-Methyl-4-chlorophenol	0.1		0.02
2,4-Dichlorophenol	0.1		0.02
2,4,6-Trichlorophenol	0.1		0.03
Pentachlorophenol	0.2		0.04

[a] For procedures, see Experimental.

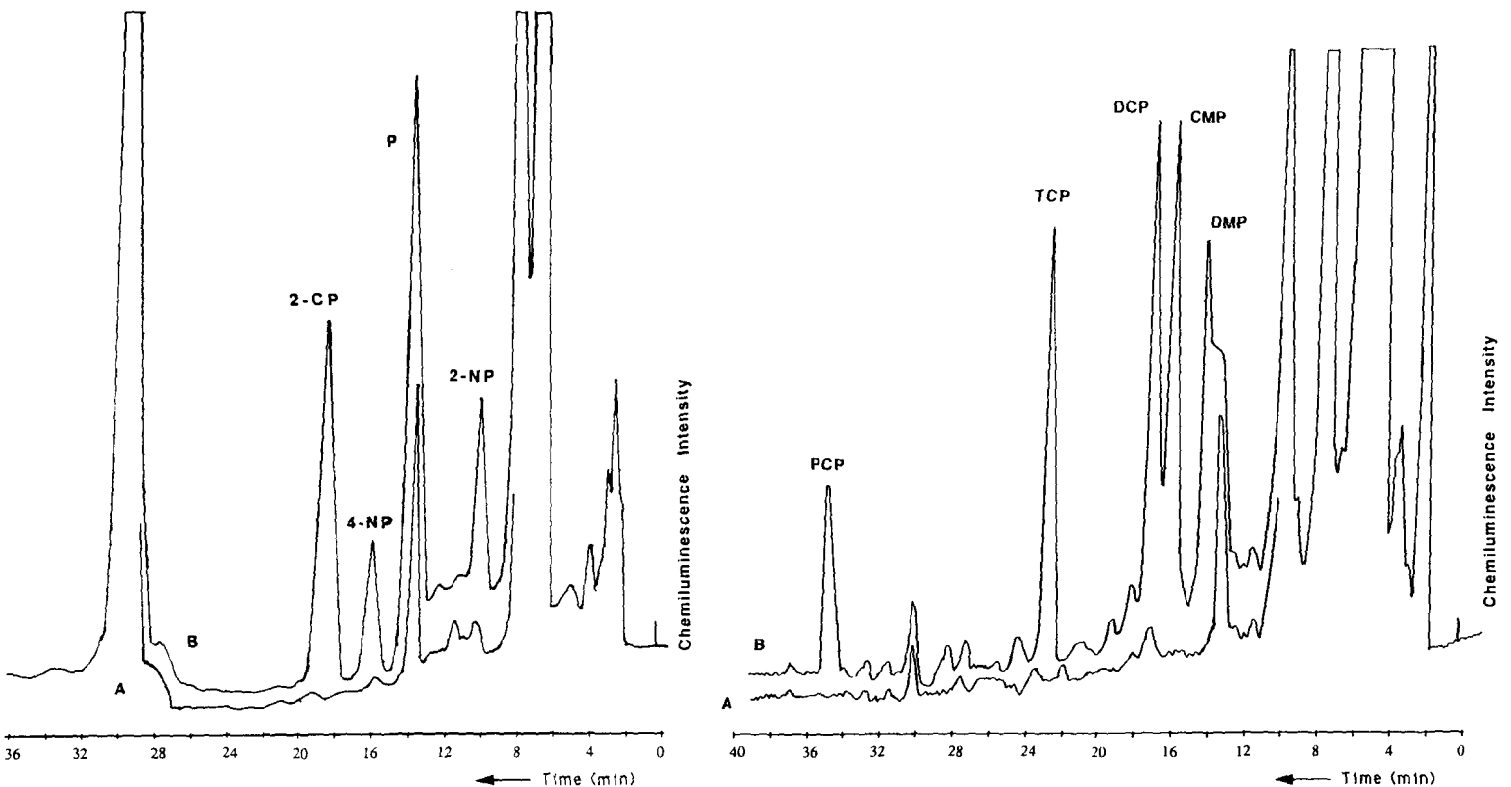

Fig. 3. LC with CL detection of Rhine water, (A) unspiked and (B) spiked with 0.5 ppb each of phenol and 2-chlorophenol and 2.0 ppb of mononitrophenols. The peak coeluting with dansylated phenol in the chromatogram of unspiked Rhine water is caused by derivatization side-products. Attenuation, 2; time constant, 5 s; mobile phase, A – B (75:25); flow-rate, 0.5 ml/min. For derivatization and further details, see Eperimental.

Fig. 4. Gradient LC with CL detection of Rhine water, (A) unspiked and (B) spiked with 0.8 ppb of phenols. Attenuation, 5; time constant, 2 s. Gradient: 0–6 min, 85% A (15% B); 6–20 min, 85–95% A (15–5% B); 20–35 min, 95% A (5% B); 35-36 min, 95–85% A (5–15% B); flow-rate, 0.5 ml/min. For derivatization and further details, see Experimental.

concentration detection limits. After desorption with dichloromethane and gentle evaporation of the solvent with a stream of nitrogen, the derivatization was carried out as described under Experimental. Fig. 3 shows a chromatogram of a Rhine water sample (spiked with 2- and 4-NP, P and 2-CP) and demonstrates the high sensitivy and selectivity of the detection system. Fig. 4 shows a gradient elution chromatogram of river Rhine water spiked with five apolar phenolic compounds (DMP, CMP, DCP, TCP and PCP).

CONCLUSION

A rapid and relatively simple method for the sensitive and selective determination of phenolic compounds is described. Only 2–3-ml aqueous samples are used and laborious liquid–liquid extraction becomes superfluous. The two-phase dansylation procedure described earlier for tri-, tetra- and pentachlorophenols by means of LC with fluorescence detection can be extended to relatively polar phenols such as phenol, mononitrophenols and 2-chlorophenol. However, derivatization is not successful for dinitrophenols in the two-phase ion-pair extraction dansylation. This may be ascribed to the strongly electron-withdrawing nitro groups which cause a strong decrease in electron density on the phenolic oxygen, leading to a very low reactivity. A major improvement of the derivatization scheme is the removal of the excess of dansyl chloride by an amino SPE column, which results in much cleaner chromatograms. In principle, this approach is amenable to the removal of other reagents suitable for primary amines, such as 9-fluorenyl methylchloroformate (FMOC), fluorescein isothiocyanate (FITC), 2,4-dinitrofluorobenzene (DNFB), 4-dimethylamino-4′-azobenzenesulphonyl chloride (dabsyl chloride) and lissamine rhodamine B sulphonyl chloride (laryl chloride). It may even be extended to reagents for other functional groups such as thiols, aldehydes, alcohols and carboxylic acids if suitable cartridges are available.

The postcolumn photochemical reaction for dansylated phenols leads to the formation of the fluorescent dansyl hydroxide and/or methoxide [5]. It should be emphasized that the system described above shows a nearly universal response for all chloro- and alkylphenols if molar concentrations are used. However, the photochemical conversion of dansylated mononitriphenols is about four times lower than that for dansylated chlorophenols and the detection limits are accordingly higher. The postcolumn photochemical reaction can easily be combined with peroxyoxalate CL detection, yielding detection limits which are about 100 times lower than with fluorescence detection. Linearity is good for all phenols from 5 to 500 ng/ml and the detection limits are at sub-ppb levels.

The applicability of the present system for the trace analysis of other phenolic compounds, *e.g.*, drugs and their metabolites, will be investigated in the near future.

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