Journal of Chromatography, 459 (1988) 139–149
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1477

RHODAMINE LABELLING REAGENT FOR THE DETERMINATION OF CHLOROPHENOLS BY LIQUID CHROMATOGRAPHY WITH PEROXY-OXALATE CHEMILUMINESCENCE DETECTION

P. J. M. KWAKMAN, J. G. J. MOL, D. A. KAMMINGA, R. W. FREI, U. A. Th. BRINKMAN and G. J. DE JONG*

Department of Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)

SUMMARY

Lissamine Rhodamine B sulphonyl chloride was applied as a pre-column labelling reagent for the peroxyoxalate chemiluminescence detection of phenolic compounds. The advantages of rhodamine labels are the high quantum yield, the long wavelength emission (>550 nm), which permits a considerable reduction of the chemiluminescence background, and the fact that electronegative heavy atom substituents do not quench the chemiluminescence.

By using a two-phase derivatization, the labelling procedure for chlorophenols can be made quantitative in 1 min at room temperature. The sensitivity of the method is limited by contaminants present even in the purified reagent and the formation of by-products during derivatization. For reversed-phase liquid chromatography, the oxalate and hydrogen peroxide were premixed in acetonitrile and added to the column effluent. For normal-phase liquid chromatography studies, the oxalate was dissolved in toluene–acetonitrile and pumped through a perhydrit reactor before its addition to the column effluent. The detection limits for several chlorophenols in both the reversed- and normal-phase systems are in the low picogram range.

INTRODUCTION

Peroxyoxalate chemiluminescence (CL) detection has been shown to be a highly sensitive detection principle for column liquid chromatography (LC)[1–7]. However, most analytes do not possess good chemiluminescence properties and derivatization is therefore necessary. Polycyclic aromatic compounds which exhibit good fluorescence properties, such as perylene derivatives[4,8], or small aromatic ring systems with electron-donating substituents, such as dimethylaminonaphthalene derivatives[1–3,6,7,9], are suitable labels for CL detection. Generally, the CL labels should have a low oxidation potential and a singlet excitation energy lower than about 105 kcal/mol, *i.e.*, an excitation wavelength higher than about 272 nm[10].

Sigvardson and Birks[5] investigated the inherent CL background emission and concluded that a weak emission occurs with a maximum at 440 nm. This background

0021-9673/88/$03.50

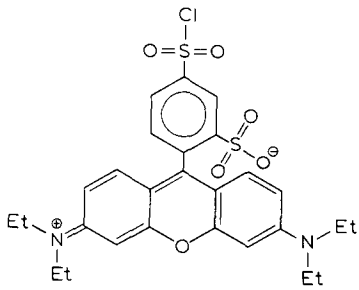
emission appears to be the main factor which limits the sensitivity of the method. Unfortunately, the major part of the background emission occurs in the wavelength region where most CL labels emit themselves. Therefore, it would be advantageous to apply labels with emission maxima at wavelengths of 550 nm or longer. In this case the CL background can be efficiently reduced by using a suitable filter. Recently, Metrione[11] described the application of Lissamine Rhodamine B sulphonyl chloride (laryl chloride) as a fluorescent label for the LC and thin-layer chromatographic (TLC) detection of amino acids. These rhodamine-type derivatives have an emission maximum at 595 nm and are well known for their excellent CL properties[4].

In this paper the application of laryl chloride as a pre-column labelling reagent for phenolic compounds in LC with peroxyoxalate CL detection is described. 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 is dissolved[12]. CL detection was not only coupled with reversed-phase LC, but also a CL detection system for normal-phase LC was developed. The latter system seemed promising because, in general, luminescence detection is favoured by the use of relatively non-polar media[13].

EXPERIMENTAL

Chemicals

HPLC-grade solvents were purchased from Baker (Deventer, The Netherlands). Bis (2,4,6-trichlorophenyl) oxalate (TCPO), bis(2-nitrophenyl) oxalate (2-NPO) and bis(2,4-dinitrophenyl)oxalate (DNPO) were synthesized as described in the literature[14]. Lissamine Rhodamine B sulphonyl chloride (laryl chloride, see Fig. 1) was purchased from Kodak (Weesp, The Netherlands). Tetrabutylammonium iodide and perhydrit tablets (hydrogen peroxide held on a urea support) were bought from Merck (Darmstadt, F.R.G.), 3,5-dichlorophenol, 2,4,6-trichlorophenol and 2,3,4,5-tetrachlorophenol from Aldrich (Beerse, Belgium) and pentachlorophenol from Merck. All other chemicals were of analytical-reagent grade.



Lissamine Rhodamine B Sulphonylchloride

Fig. 1. Structure of Lissamine Rhodamine B sulphonyl chloride. Et = C_2H_5.

Column liquid chromatography

In the reversed-phase system the mobile phase was delivered by a Gilson Model 302 pump equipped with a Gilson Model 308 manometric module (Gilson, Villiers-le Bel, France). A Valco injection valve with a 25-μl loop was used for the introduction of samples on to a 150 × 3.1 mm I.D. analytical column packed by a slurry technique with 5-μm LiChrosorb RP-18 (Merck). Reversed-phase LC was carried out with acetonitrile–imidazole nitrate buffer (10 m*M* pH 7.0) (75:25, v/v) at a flow-rate of 0.5 ml/min as the eluent. In the normal-phase system, a Gilson Model 302 pump was used to deliver the LC mobile phase. A Valco injection valve with a 14-μl loop was used for injection on to a 250 × 3.1 mm I.D. 5-μm LiChrosorb Si 60 (Merck) silica column. Chromatography was carried out using a mobile phase of toluene–acetonitrile–methanol (60:40:5, v/v/v) containing 5 m*M* imidazole.

Detection systems

The reversed-phase system is shown schematically in Fig. 2a. Hydrogen peroxide and 2-NPO dissolved in acetonitrile at final concentrations of 50 and 10 m*M*, respectively, were mixed just before use and added to the column effluent with a

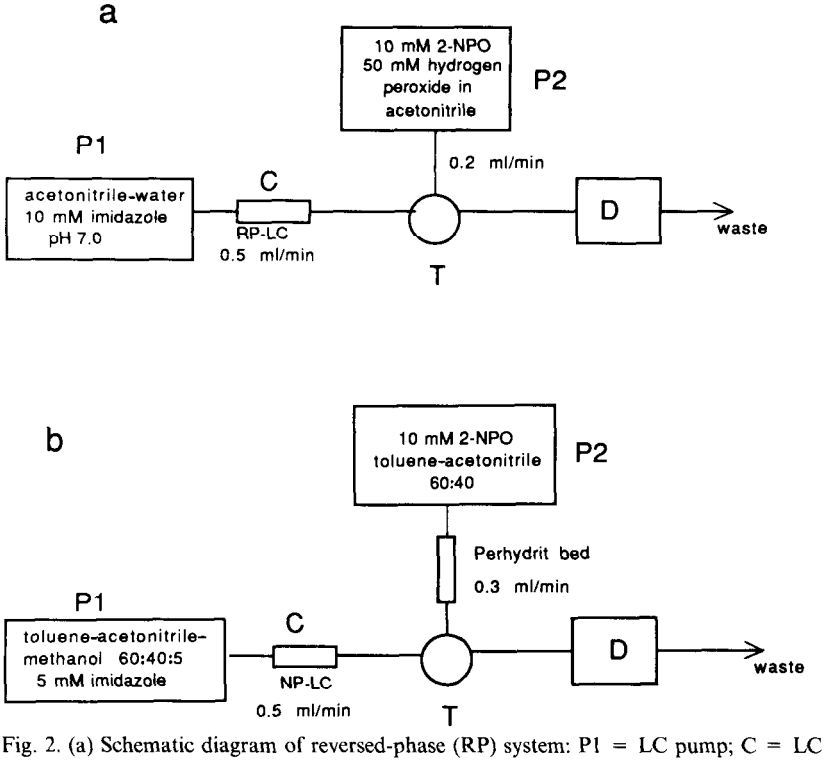


Fig. 2. (a) Schematic diagram of reversed-phase (RP) system: P1 = LC pump; C = LC column; P2 = pulseless syringe pump; T = tee-piece; D = Kratos detector. (b) Schematic diagram of normal-phase (NP) system: P1 = LC pump; C = LC column; P2 = pulseless syringe pump with perhydrit bed in-line; T = tee-piece; D = photomultiplier housing with 80-μl flow cell. For chromatographic details, see Experimental.

pulseless Isco (Lincoln, NE, U.S.A.) μLC-500 syringe pump. The mobile phase (0.5 ml/min) and the reagent (0.2–0.4 ml/min) were mixed using a standard Valco T-piece immediately in front of the detector. A Kratos (Ramsey, NJ, U.S.A.) FS 970 fluorescence detector equipped with a 2π steradian mirror, with the lamp turned off, a laboratory-made 50-μl flow cell[15] and a 580-nm emission cut-off filter were used for detection.

The set-up for the normal-phase system is shown schematically in Fig. 2b. A laboratory-made pulseless syringe pump delivered a 10 m*M* solution of oxalate (TCPO or 2-NPO) in toluene–acetonitrile (60:40, v/v). This solution was pumped through a 100 × 4.6 mm I.D. perhydrit column (containing *ca.* 1.7 g of perhydrit) and added to the LC column effluent. The flow-rates of the mobile phase and the reagent stream were 0.5 and 0.3 ml/min, respectively. A laboratory-made photomultiplier housing with an Oriel 7070 power supply[16] and a laboratory-made 80-μl quartz flow cell with a 570-nm cut-off filter were used. The Model RCA 1P-28 photomultiplier tube was typically operated at 750 V.

Labelling of chlorophenols with laryl chloride

To 500 μl of a solution of chlorophenols in a 0.02 *M* carbonate buffer (pH 9.0), 100 μl of 0.2 *M* tetrabutylammonium iodide in acetone–water (1:1, v/v) were added, then 500 μl of a 0.5 mg/ml solution laryl chloride in hexane–toluene–acetonitrile (4:3:1, v/v/v) were added and the two-phase mixture was whirl-mixed for 60 s. For trace-level analysis the 0.5 mg/ml laryl chloride solution was diluted 10-fold. Next, 50 μl of a concentrated (*ca.* 2 *M*) propylamine solution in water were added to remove the unreacted laryl chloride and the solution was mixed for a further 60 s, then 400 μl of the organic layer were evaporated to dryness and the residue was dissolved in 500 μl of mobile phase.

RESULTS AND DISCUSSION

Derivatization conditions

In the literature[17], the derivatization of phenolic compounds with dansyl chloride is usually carried out in acetone for about 30 min at elevated temperatures with, *e.g.*, solid potassium carbonate as a base. Recently, De Ruiter *et al.*[12] have developed a fast two-phase derivatization for the selective dansylation of chlorophenols. The chlorophenols, in their nucleophilic anionic form, are extracted as a tetrabutylammonium ion-pair from an aqueous solution into an organic phase containing the fluorescent label. This procedure allows a rapid derivatization and an easy separation of the analyte of interest from the intensely fluorescent hydrolysis product, which remains in the aqueous phase. In principle this system works for every apolar label able to react with extracted nucleophiles.

By making minor adjustments to the procedure in ref. 12, it was possible to apply laryl chloride as a highly chemiluminescent label for chlorophenols. Dissolving laryl chloride in dichloromethane or chloroform gave rise to very large interfering peaks; therefore, toluene–hexane mixtures were used instead. In addition, a small percentage of acetonitrile had to be added to improve the solubility of laryl chloride; an organic phase of hexane–toluene–acetonitrile (4:3:1) appeared to be the best choice. The derivatization time was optimized by varying the mixing time prior to the

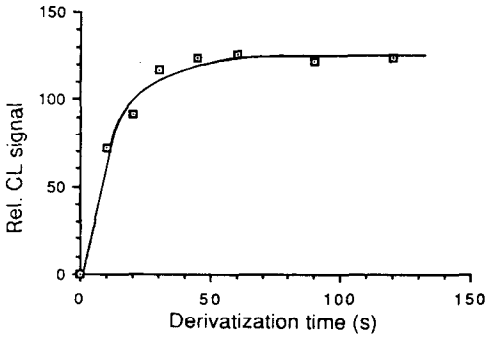


Fig. 3. Dependence of laryl derivative formation on reaction time. The derivatization time was varied by changing the addition time of the concentrated aqueous propylamine solution for removal of the excess of laryl chloride.

addition of the propylamine solution. After about 60 s of whirl-mixing at room temperature no further gain in signal was obtained (see Fig. 3).

Characteristics of laryl chloride as a CL label

The main advantage of rhodamine-type compounds is the high CL quantum yield[4] combined with its red emission wavelength (λ_{em} = 595 nm), which allows the use of an emission filter with a cut-off above 550 nm and, hence, a reduction of the CL background. When using the filter, a signal-to-noise (S/N) gain by a factor of 10 was obtained in the reversed-phase system and by a factor of 5 in the normal-phase system. Also, ultrapure and expensive solvents are no longer required, because the presence of impurities emitting above 550 nm is unlikely. In the reversed-phase system, which uses acetonitrile and water as solvents, it was sufficient to distil the LC column effluent only once (as its acetonitrile–water azeotrope) to be able to re-use it as the LC mobile phase with the same noise level as before.

A further advantage of the laryl chloride label is that, in principle, the CL (and the fluorescence) quantum yield will be the same for every chlorophenol derivative. With dansylated chlorophenols the number and position of the chlorine atoms strongly influence the fluorescence quantum yield; for example, chlorine atoms in the positions *ortho* to the hydroxyl group act as strong quenchers, and derivatives with four or five chlorine atoms hardly fluoresce at all[18]. With laryl chloride, the luminescence characteristics will be mainly determined by the heterocyclic ring system and not by the side ringe which is used for derivatization. Therefore, the CL quantum yield of a pentachlorophenol derivative will be equal to that of, *e.g.*, a disubstituted phenol derivative. In other words, a single calibration graph for each LC system (in peak area units) should, in principle, be sufficient for all chlorophenols. This was confirmed experimentally in both reversed-phase and normal-phase LC by measuring the peak areas for the laryl derivatives of 3,5-dichloro-, 2,4,6-trichloro-, 2,3,4,5-tetrachloro- and pentachlorophenol. For all laryl derivatives the ratio of peak area to moles of chlorophenol was the same, within experimental error.

A serious disadvantage of the present label is its impurity and the formation of by-products during derivatization. For purification of the reagent simple approaches

such as recrystallization and filtration were applied without any real success. Preparative LC on a 200 × 3.1 mm I.D. cyano-bonded column with acetonitrile as the mobile phase led to only a minor improvement in the reagent purity. Finally, it was found that 10-fold dilution of the laryl chloride solution dramatically reduced the interfering peaks. This was partly caused by the simple fact of diluting the impurities present in the reagent and partly by slower by-product formation. The amount of the (unknown) by-products formed not only increases with increasing time of derivatization, but obviously also with increasing laryl chloride concentration. As a result, for trace levels (1–400 ppb*) of chlorophenols, the use of a diluted laryl chloride solution is recommended; for concentrations of over 400 ppb the undiluted laryl chloride solution (see Experimental) should be used.

Chemiluminescence detection

Reversed-phase LC. In order to elute the apolar laryl derivatives of the chlorophenols with acceptable peak shape and retention, the mobile phase acetonitrile–water (75:25, v/v) was used. Usually the addition of a solution of TCPO in ethyl acetate to an aqueous mobile phase causes mixing and precipitation problems. These problems were avoided by dissolving 2-NPO (10 m*M*) and hydrogen peroxide (50 m*M*) in acetonitrile. 2-NPO was chosen because of its high CL intensity, slow decay of signal intensity, good solubility in acetonitrile and high stability[4].

The rate of the CL reaction mainly depends on the concentration of imidazole in the mobile phase[7]. This concentration was varied from 25 to 2.5 m*M* at a pH of 7.0 (see Fig. 4). The best S/N ratio was obtained with 10 m*M* imidazole and this concentration was maintained throughout all reversed-phase LC experiments. The 2-NPO concentration in the reagent stream was varied between 1 and 20 m*M* (see Fig. 5). The CL signal first increases rapidly with increasing 2-NPO concentration, but between 10 and 20 m*M* the increase is nearly negligible. In order to prevent possible precipitation of 20 m*M* 2-NPO its addition to the aqueous mobile phase, 10 m*M* was chosen for all further work. The hydrogen peroxide concentration was varied between 500 and 25

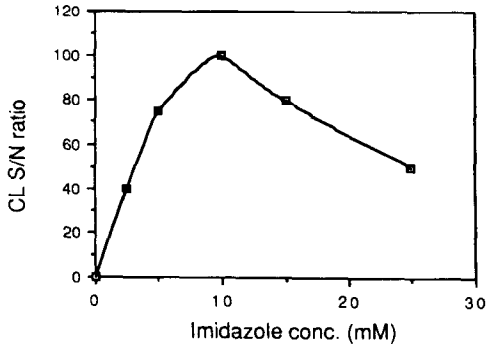


Fig. 4. Effect of imidazole concentration in the mobile phase on CL S/N ratio (10 m*M* 2-NPO; 50 m*M* hydrogen peroxide; 1.7 ng of laryl-2,4,6-trichlorophenol). For other conditions, see Experimental.

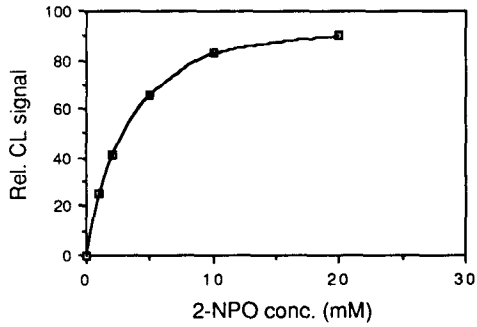


Fig. 5. Influence of oxalate concentration in the reagent stream on CL intensity (50 m*M* hydrogen peroxide; 10 m*M* imidazole; 1.7 ng of laryl-2,4,6-trichlorophenol). For other conditions, see Experimental.

* Throughout the article the American billion (10^9) is meant.

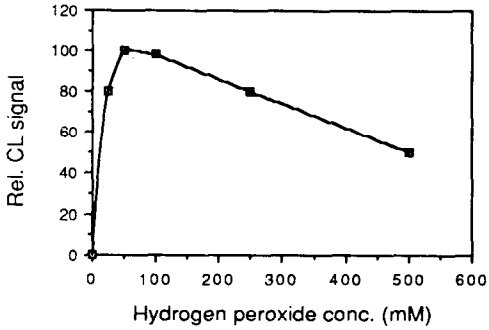


Fig. 6. Effect of hydrogen peroxide concentration in the reagent stream on CL intensity (10 m*M* 2-NPO; 10 m*M* imidazole; 1.7 ng of laryl-2,4,6-trichlorophenol). For other conditions, see Experimental.

m*M*, and an optimum was found at 50 m*M* (see Fig. 6). The rate of the CL reaction at high hydrogen peroxide concentrations seemed to be too fast, which resulted in the loss of a major part of the CL signal prior to the flow cell[15]. In addition, the mixture of 2-NPO and hydrogen peroxide turned out to be less stable at higher hydrogen peroxide (and hence water) concentrations. The solution of 10 m*M* 2-NPO and 50 m*M* hydrogen peroxide in acetonitrile was stable for at least 4 h at room temperature. It could even be stored overnight at −20°C with less than 1% loss in CL intensity, as measured by the injection of a standard rhodamine solution. Varying the flow-rate of the reagent mixture from 0.5 to 0.2 ml/min did not reduce the S/N ratio; 0.2 ml/min was preferred, because the 50-ml syringe pump volume then needed refilling only once every 4 h.

Normal-phase LC. In normal-phase solvents such as hexane and chloroform the fluorescence quantum yield is often higher than under reversed-phase conditions. In the present system, with a fairly high percentage of acetonitrile a small percentage of methanol was also necessary in order to elute the laryl derivatives with acceptable capacity factors and peak shape. Consequently, the polarity of the mobile phase was not as low as required for a real gain in CL quantum yield and, as is reported below, the limits of detection turned out to be essentially the same in both the reversed- and normal-phase systems.

Recently, Nozaki *et al.*[19] described a normal-phase LC system with peroxyoxalate CL detection, using separate pumps for the addition of the hydrogen peroxide and oxalate solutions. An aqueous hydrogen peroxide solution was added to methanol containing an acetate buffer; that is, the CL detection was still carried out under semi-aqueous conditions. In the non-aqueous system described in this paper, it sufficed to use only one reagent pump, *viz.*, by inserting the perhydrit column already described in an earlier paper[9]. A hydrogen peroxide concentration of about 13 m*M* was obtained by pumping a solution of 2-NPO or TCPO [10 m*M* in toluene–acetonitrile (60:40, v/v)] through a 100 × 4.6 mm I.D. perhydrit column; the oxalate concentration had been optimized by varying it between 2.5 and 20 m*M*. DNPO could not be used because of the rapid hydrolysis of this ester in the perhydrit column, as shown by the appearance of an intense yellow colour. Changing the toluene–acetonitrile ratio from 60:40 to 40:60 was expected to increase the hydrogen peroxide

concentration in the reagent stream. However, no increase in signal was found; therefore, it can be concluded that the concentration of hydrogen peroxide was already optimal at 40% acetonitrile.

The hydrogen peroxide concentration of 13 m*M* was calculated by running parallel experiments with a known amount of aqueous hydrogen peroxide in the reagent solution. This method of adding hydrogen peroxide could not be used routinely, because of the frequent formation of precipitates in the capillary inserted after the T-piece. The concentration of 13 m*M* hydrogen peroxide is in sharp contrast with the 600 m*M* concentration utilized in the normal-phase system described by Nozaki *et al.*[19] A direct comparison between both normal-phase systems cannot be made, because the systems are essentially different regarding, *e.g.*, the mobile phase modifiers, the amount of water in the solvent stream passing the flow-cell and the type of catalyst.

As with the reversed-phase system, the rate of the CL reaction is influenced most by the imidazole concentration in the mobile phase. Varying this concentration between 20 and 2.5 m*M* showed 5 m*M* imidazole to be the optimal concentration. Under these conditions the rate of the CL reaction is in the same range (less than about 30 s) as in the reversed-phase system.

Analytical data

In both the reversed- and normal-phase systems the linearity was measured by diluting a solution of derivatized 2,4,6-trichlorophenol in the range 5–500 ppb of trichlorophenol. The systems showed excellent linearity over more than two orders of magnitude (normal-phase system, $n = 9$, $r = 0.9998$; reversed-phase system, $n = 9$, $r = 0.9995$). The limits of detection (S/N = 3) were 3–4 pg (15–20 fmol) in both LC systems.

The performance of the laboratory-made photomultiplier housing was checked by comparing this detection system with the Kratos FS 970 detector with a 2π steradian mirror. Surprisingly, the S/N ratio was the same for both detection systems. This indicates that expensive fluorimeters are not really required for CL measurements.

The linearity of the derivatization reaction was tested by varying the chlorophenol concentration from 500 to 5 ppb. Initially, detection in the low-ppb range was limited by the appearance of large interfering peaks in the chromatogram. Use of the 0.05 mg/ml laryl chloride solution (see above) considerably reduced these interferences without influencing the linearity (normal-phase system, $N = 7$, $r = 0.9993$; reversed-phase system, $n = 7$, $r = 0.9991$).

A normal-phase chromatogram of 40 pg of the derivatized 2,4,6-trichlorophenol (obtained by 100-fold dilution of the derivative) is shown in Fig. 7a and the result of a direct derivatization of a 5-ppb trichlorophenol solution (66-pg loop injection) in Fig. 7b. A reversed-phase chromatogram of 44 pg of the derivatized 2,4,6-trichlorophenol (obtained by 50-fold dilution of the derivative) is shown in Fig. 8a and a direct derivatization of a 9-ppb solution (225-pg loop injection) in Fig. 8b. It is obvious that the chromatograms in Figs. 7b and 8b contain more interfering peaks than those in Figs. 7a and 8a. In practice, the detection limits of the various chlorophenols are about three times higher than those obtained when diluting a relatively concentrated stock solution, *i.e.*, they are about 10 pg (50 fmol).

As an application, Fig. 9 shows the reversed-phase LC analysis of Amstel river

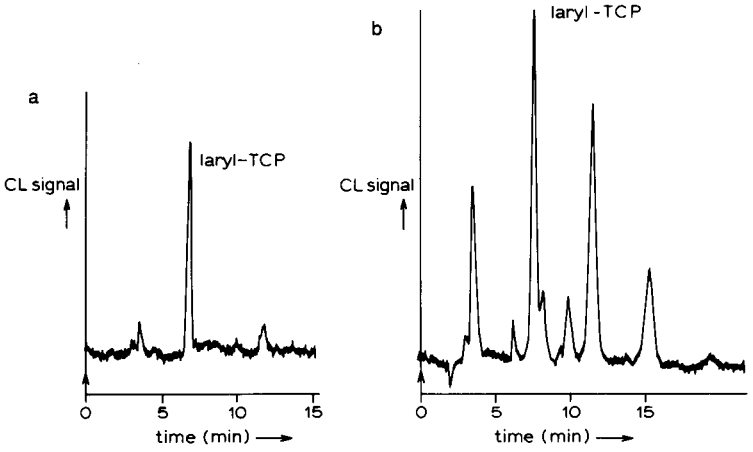


Fig. 7. (a) Normal-phase chromatogram of 40 pg of the laryl derivative of 2,4,6-trichlorophenol (laryl-TCP), obtained by 100-fold dilution of a relatively concentrated stock solution. (b) Normal-phase chromatogram of 66 pg of laryl-TCP, obtained by direct derivatization of a 5-ppb chlorophenol solution. For derivatization and LC conditions, see Experimental.

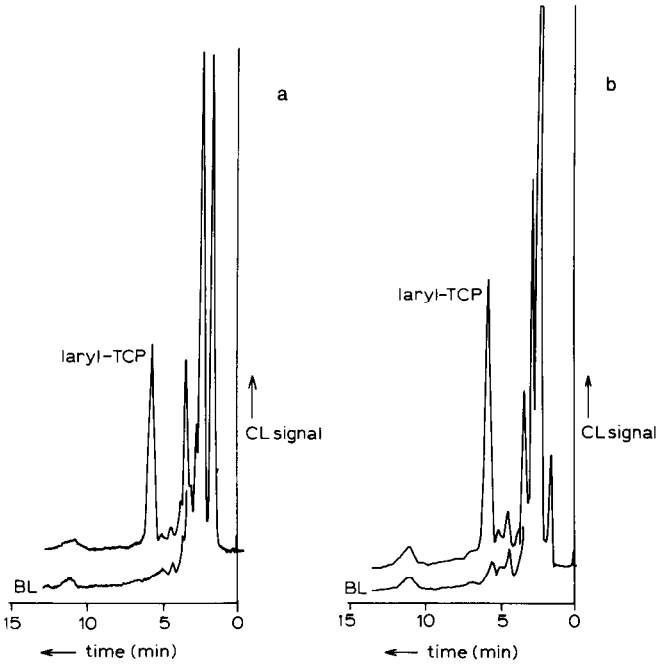


Fig. 8. (a) Reversed-phase chromatogram of 44 pg of laryl-TCP, obtained by 50-fold dilution of a relatively concentrated stock solution. The sensitivity of the detector is about three times higher than that in Fig. 8b. (b) Reversed-phase chromatogram of 225 pg of laryl-TCP, obtained by direct derivatization of a 9-ppb chlorophenol solution. Blanks (BL) are shown as reference. For derivatization and LC conditions, see Experimental.

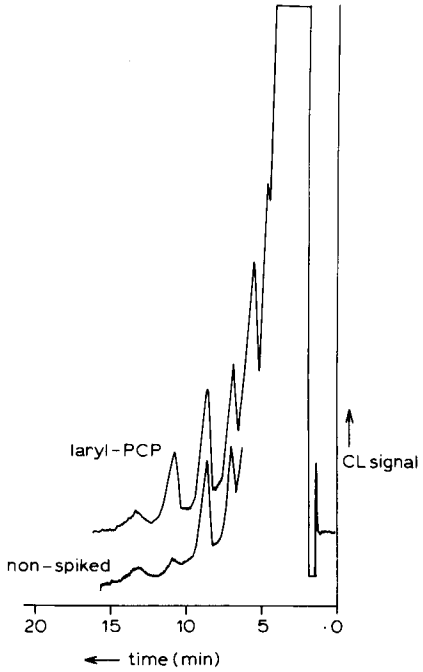


Fig. 9. Reversed-phase chromatogram of Amstel river water without and with 0.8 ppb pentachlorophenol spike; 200-μl loop injection (derivative indicated as laryl-PCP). For derivatization and LC conditions, see Experimental.

water spiked with 0.8 ppb of pentachlorophenol. The derivatization was carried out directly on the sample without any preconcentration or clean-up step(s). Peak compression occurring on the top of the analytical column allowed the use of a 200-μl injection volume (10% acetonitrile in water), which yielded an 8-fold higher sensitivity than with our conventional 25-μl loop injections. Under these conditions, the detection limit of pentachlorophenol in Amstel water is about 0.2 ppb when using only a 0.5-ml sample. If necessary, the result can be further improved by combining preconcentration with clean-up, *e.g.*, on a C_{18} precolumn.

CONCLUSIONS

Laryl chloride can be utilized as a sensitive labelling reagent for the selective two-phase derivatization of chlorophenols with subsequent CL detection. Detection limits in both the reversed- and normal-phase modes are in the low picogram range.

The main advantages of the rhodamine-type labels are the possibility of filtering out part of the CL background and the fact that electronegative heavy-atom substituents do not quench the CL signal. With laryl chloride, the presence of impurities and the formation of by-products still limit its applicability and other types of rhodamines are therefore currently being studied.

Although peroxyoxalate CL detection in normal-phase LC systems is, in principle, a promising approach, the results of this work do not show any gain in sensitivity

compared with reversed-phase systems. This is probably due to the high polarity of the label, which is caused by the presence of the second sulphonate group, which necessitates the addition of relatively large amounts of polar modifiers to the mobile phase. In other words, the gain in CL quantum yield should be more pronounced when working with less polar labels, which will allow LC elution with, *e.g.*, hexane–chloroform mixtures (provided that optimal hydrogen peroxide concentrations can be reached). Future research will deal with this aspect.

ACKNOWLEDGEMENTS

The authors are grateful to J. H. M. van den Berg and N. G. F. M. Lammers of Duphar B.V. and C. de Ruiter for stimulating discussions. This work was supported by Duphar B.V. and the Dutch Foundation of Technical Sciences (S.T.W.) under grant No. 700-349-1301.

REFERENCES

1 K. Kobayashi and K. Imai, *Anal. Chem.*, 52 (1980) 424.
2 K. Miyaguchi, K. Honda and K. Imai, *J. Chromatogr.*, 303 (1984) 173.
3 K. Imai, Y. Matsunaga, Y. Tsukamoto and A. Nishitani, *J. Chromatogr.*, 400 (1987) 169.
4 K. Honda, K. Miyaguchi and K. Imai, *Anal. Chim. Acta*, 177 (1985) 104 and 111.
5 K. W. Sigvardson and J. W. Birks, *Anal. Chem.*, 55 (1983) 432.
6 T. Koziol, M. L. Grayeski and R. Weinberger, *J. Chromatogr.*, 317 (1984) 355.
7 G. J. de Jong, N. Lammers, F. J. Spruit, C. Dewaele and M. Verzele, *Anal. Chem.*, 59 (1987) 1458.
8 B. Mann and M. L. Grayeski, *J. Chromatogr.*, 386 (1987) 149.
9 P. J. M. Kwakman, U. A. Th. Brinkman, R. W. Frei, G. J. de Jong, F. J. Spruit, N. G. F. M. Lammers and J. H. M. van den Berg, *Chromatographia*, 24 (1987) 395.
10 C. L. R. Catherall, T. F. Palmer and R. B. Cundall, *J. Chem. Soc., Faraday Trans. 2*, 80 (1984) 823.
11 R. M. Metrione, *J. Chromatogr.*, 363 (1986) 337.
12 C. de Ruiter, R. R. Otten, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr.*, 436 (1988) 429.
13 M. Zander, *Fluorimetrie*, Springer, Berlin, 1981, Ch. 3.
14 A. G. Mohan and N. J. Turro, *J. Chem. Educ.*, 51 (1974) 528.
15 G. J. de Jong, N. Lammers, F. J. Spruit, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 353 (1986) 249.
16 G. Gübitz, P. van Zoonen, C. Gooijer, N. H. Velthorst and R. W. Frei, *Anal. Chem.*, 57 (1985) 2071.
17 L. A. Sternson, in R. W. Frei and J. F. Lawrence (Editors), *Chemical Derivatization in Analytical Chemistry*, Plenum Press, New York 1981.
18 C. de Ruiter, J. F. Bohle, G. J. de Jong, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 60 (1988) 666.
19 O. Nozaki, Y. Ohba and K. Imai, *Anal. Chim. Acta*, 205 (1988) 255.