Simultaneous Determination of Endocrine Disruptive Alkylphenolic Compounds as Dansyl Derivatives

Bunji UNO,[†] Yoshiaki KATO, and Shigeru MIWA

Gifu Pharmaceutical University, Mitahora-higashi, Gifu 502-8585, Japan

(Received February 25, 2002; Accepted April 8, 2002)

Introduction

Development of a method of conventional trace analyses for alkylphenolic compounds such as 4-octylphenol and 4-nonylphenol is currently required to estimate the potential estrogenic properties and the environmental quantity of these compounds. Most analytical schemes for the compounds employ GC-MS detection after transformation into trimethylsilyl ethers, acetates or trifluoroacetates combined with liquid-liquid extraction or solid-phase extraction (SPE) of the samples.¹⁻³ HPLC analyses with MS,³⁻⁵ electrochemical,⁶⁻⁸ UV-Vis^{8,9} and fluorescence detection¹⁰⁻¹² are also applied to determination of the alkylphenolic compounds in the environment. Although many different methods have been published, the environmental analysis of alkylphenols is still a challenging task when one seeks simple and easy treatments, high sensitivity, and simultaneous determination. Dansyl chloride is a fluorescent reagent frequently used for precolumn derivatization of aliphatic and aromatic amines and phenolic compounds.¹³⁻¹⁶ In this study we have developed a simple and highly sensitive assay method for simultaneous determination of endocrine disruptive alkylphenolic compounds as dansyl derivatives for fluorescent detection.

Experimental

Chemicals

The alkylphenolic compounds used here are illustrated in Fig. 1. They were of the best available grade from Nacalai Tesque and Tokyo Chemical Industry, and were used as received without further purification. Dansyl chloride was purchased from Tokyo Chemical Industry, and was used as received without further purification.

Preparation of the standards of dansyl phenols

The standards of dansyl phenols were synthesized with reference to the literature.¹⁷ Potassium hydroxide was added to mixtures of acetone solutions of dansyl chloride and the phenols. The reaction mixtures were stirred for 2 h at 50°C, and were then evaporated after drying over sodium sulfate. The oily products obtained were run on preparative TLC plates. The structure and purification of the derivatives were checked by means of TLC, UV spectra, elemental analyses, and MS.

HPLC apparatus and conditions

A Jasco HPLC system was used; it comprises a HPLC pump (880-PU) connected to a ternary gradient unit (880-02) and a 2-line degasser (880-51), UV-VIS spectrophotometric (875-UV) and fluorometric (FP-920) detectors with a Shimadzu chromatopac (C-6A), a column oven (860-CO) regulated at 40°C, a system controller (802-SC), and a Rheodyne 7125 syringe-loading sample injector with a 20 µL loop. The HPLC separations were performed on a Nacalai silica-based C₄ column (150 × 4.6 mm i.d.) with a mobile phase consisting of 0.1 mol/L acetate buffer (pH = 4.8) and CH₃OH at a flow rate of 1.0 mL min⁻¹.

SPE from environmental water

Tap water spiked with the alkylphenols was preconcentrated on a Waters Sep-pack® C₁₈ environmental cartridge at a flow rate of 5 mL min⁻¹ using a Shimadzu LC-7A pump. The phenols were eluted with 10 mL of organic solvents. Sample solutions were prepared as 1 mL of acetone solutions after evaporation of the eluates to dryness under reduced pressure.

Results and Discussion

Optimized conditions on dansylation of the phenols

A dansylation method in a mixture of water and acetone prepared in weak basicity is favorable with regard to the

- 1 R₁ = H, R₂ = C(CH₃)₃
- 2 R₁ = H, R₂ = CH(CH₃)C₂H₅
- 3 R₁ = C(CH₃)₃, R₂ = OCH₃
- 4 R₁ = H, R₂ = C(CH₃)(CH₃)C₂H₅
- 5 R₁ = H, R₂ = C(CH₃)(CH₃)CH₂C(CH₃)₃
- 6 R₁ = H, R₂ = C₉H₁₉
- 7 R₁ = H, R₂ = C(CH₃)₂-

Fig. 1 Structure of alkylphenolic compounds used here.

[†] To whom correspondence should be addressed.

E-mail: uno@gifu-pu.ac.jp

Note: This article was prepared as a camera ready copy.

Table 1 Derivatization yields, RSD values of the proposed method and recoveries from tap water

Compound	Yield, % ^a	RSD, % ^b	Recovery, % ^c
1	98.4	3.0	98.0
2	93.3	2.4	85.3
3	35.3	2.6	92.5
4	95.2	2.2	83.2
5	92.3	2.1	83.1
6	94.7	2.3	78.0
7	93.2 ^d	3.0	81.1

a. The dansylation yields for the phenolic compounds with dansyl chloride.

b. The RSD values were obtained from repeated 10 times experiments.

c. The yields for recoveries of the alkylphenolic compounds spiked in tap water with SPE. See the text for details.

d. The value corresponds to formation of the didansylated derivative.

competitive rates between the labeling reaction and the hydrolysis of dansyl chloride and solubility of samples.¹³ The dansyl phenolic compounds are usually obtained in aqueous acetone solutions saturated with sodium carbonate with long reaction times at relatively high temperatures. In order for researchers to avoid the hard and time-consuming conditions the dansylation procedures have been improved as solid-phase, anion-exchange catalytic and two-phase dansylation methods.^{14–16} Indeed, preliminary experiments using sodium carbonate did not give the dansyl derivatives of 1–7 with high yields. We investigated a dansylation procedure in an organic solvent combined with a base. The reaction mixture was prepared by addition of a 1.0 mL aliquot of 0.1 mol/L dansyl chloride in the organic solvent to a mixture of 1.0 mL of sample and base solutions of the solvent. Use of acetone and KOH gave the highest and most stationary yield of the dansyl phenols. Figure 2 shows the dependence of concentrations of KOH ([KOH]) as a reagent for pH control on the reaction yields. The [KOH] sensitively affects the dansylation yield. At [KOH] less than 0.01 mol/L the dansylation did not proceed smoothly. Hydrolysis of dansyl chloride prevented the derivatization reaction from proceeding with the high yield at higher [KOH] than 0.04 mol/L. On the other hand, the reaction time was not critical for the yields, except for some variation within 3 min after preparation of the sample solutions. The conditions were therefore defined as [KOH] = 0.02 mol/L and the reaction time of 5 min. The optimal conditions allow the dansylated phenols to be derived with the reaction yields more than 92% except for 3, as listed in

Fig. 2 Dependence of the peak heights on concentrations of the KOH acetone solution added to the reaction mixture.

Fig. 3 Elution profile of the dansyl derivatives of the alkylphenolic compounds. The number denotes the compounds shown in Fig. 1. The peak 7 corresponds to the didansylated derivative. The separation was performed on a C4 column with the gradient mobile phase consisting of acetate buffer (pH = 4.8) and CH₃OH. The gradients are as follows: 30% (CH₃OH) to 70% for 0 min to 10 min, 70% to 20% for 10 min to 80 min.

Table 1. The low yield for 3 seems to be due to steric hindrance caused by the bulky *tert*-C₄H₉ group at the ortho position. The reaction mixtures left for more than 1 h gave the chromatograms involving some decrease in the peak for the derivatives and the appearance of a few peaks arising from decomposition of the derivatives in the presence of KOH.

HPLC analyses of the phenolic dansyl derivatives

The dansyl derivatives of the phenols showed the absorption maximum at 350 nm, and the fluorescent maximum at 510 nm (ex. 340 nm). The fluorescent intensity was stronger in CH₃OH than in CH₃CN. The detectors were therefore set at 350 nm for UV detection and 510 nm (ex. 340 nm) for fluorescent detection.

For development of the simultaneous determination for the alkylphenolic compounds, efficient chromatographic separation is required. Some preliminary investigations showed that an ODS column did not allow the dansyl derivatives of the alkylphenolic compounds to be eluted within 60 min even when CH₃CN and CH₃OH were used as a mobile phase. A silica column modified by the butyl group (C₄-column) was thus used for the analytical purposes. The gradients of the acetate buffer (pH = 4.8) and methanol gave the well-resolved chromatogram of the dansyl derivatives, despite unfavorable separation by isocratic mobile phases. Figure 3 shows the typical chromatogram of the dansyl derivatives of 1–7.

Reproducibility, calibration and detection limits

The RSD values of reproducibility for the fluorescent detection involving the dansylation procedure are 2.1–3.0%, as listed in Table 1. A 10-μL volume of each dansyl standard mixture of the phenols 1–7 was injected into the HPLC, and calibration curves were constructed by plotting the peak heights against the concentrations for each phenol. The calibration graph obtained had linearity with correlation coefficients more than 0.99; the calibration limits were 1–3 × 10⁻⁶ mol/L for UV detection and 5 × 10⁻⁸ mol/L for fluorescent detection. Detection limits for a 20

μL injection of the sample solution of **1** were 4×10^{-7} mol/L (60 ppb) for UV detection and 7×10^{-9} mol/L (1 ppb) for fluorescent detection.

SPE and recoveries of the phenols 1 – 7 from tap water

Many sample preparation methods involving clean-up and preconcentration have been already published for the determination of 4-nonylphenol and 4-octylphenol in water and sediment samples.^{1-5,8-11} We demonstrate here that the alkylphenolic compounds **1** – **7** are efficiently preconcentrated by SPE to show that the published methods based upon SPE are applicable to the simultaneous determination of **1** – **7**. Recovery tests were performed by adding 1 mg of each phenol to 500 mL of tap water. The tap water was passed through an ODS column for SPE, and the alkylphenols retained in the column were extracted by organic solvents. The recoveries were checked by the proposed derivatization method. Good recoveries were obtained by using hexane followed by ether; the results are listed in Table 1.

Conclusions

In conclusion, we have proposed a simultaneous HPLC method for determination of **1** – **7** as the dansyl derivatives. The detection limit of the fluorescent detection involving the dansylation is several times higher than that of the direct fluorescent detection (4.5×10^{-8} mol/L).¹⁰ We believe that the proposed method is a simple and highly sensitive one for simultaneous determination of a trace amount of alkylphenolic compounds in environmental and biological samples, coupled with the sample preparation techniques published.

References

1. H. G. J. Mol, S. Sunarto, and O. M. Steijger, *J. Chromatogr. A*, **2000**, 879, 97.
2. D. Jahr, *Chromatographia*, **1998**, 47, 49.
3. R. A. Rudel, S. J. Melly, R. W. Geno, G. Sun, and J. G. Brody, *Environ. Sci. Technol.*, **1998**, 32, 861.
4. S. N. Pedersen and C. Lindholm, *J. Chromatogr. A*, **1999**, 864, 17.
5. A. Motoyama, A. Suzuki, O. Shirota, and R. Namba, *Rapid Commun. Mass Spectrom.*, **1999**, 13, 2204.
6. K. Inoue, Y. Yoshimura, T. Makino, and H. Nakazawa, *Analyst*, **2000**, 125, 1959.
7. K. Inoue, K. Kato, Y. Yoshimura, T. Makino, and H. Nakazawa, *J. Chromatogr. B*, **2000**, 749, 17.
8. J. Ruana, I. Urbe, and F. Borrull, *J. Chromatogr. A*, **1993**, 655, 217.
9. M. Ahel and W. Giger, *Anal. Chem.*, **1985**, 57, 1577.
10. T. Tsuda, K. Suga, E. Kaneda, and M. Ohsuga, *J. Chromatogr. B*, **2000**, 746, 305.
11. M. Ahel, W. Giger, E. Molnar, and S. Ibric, *Croat. Chem. Acta*, **2000**, 73, 209.
12. A. Marcomini and W. Giger, *Anal. Chem.*, **1987**, 59, 1709.
13. J. F. Lawrence, U. A. Th. Brinkman, and R. W. Frei, in "Reaction Determination in Liquid Chromatography", ed. I. S. Krull, **1987**, Chap. 6, Marcel Dekker, New York, Basle.
14. C. De Ruiter, R. R. Otten, U. A. Th. Brinkman, and R. W. Frei, *J. Chromatogr.*, **1988**, 436, 429.
15. A. Takadate, H. Hirage, H. Fuyino, and S. Goya, *Chem. Pharm. Bull.*, **1985**, 33, 5092.
16. H. Tsuchiya, M. Tatsumi, N. Takagi, T. Koike, H. Yamaguchi, and H. Hayashi, *Anal. Biochem.*, **1986**, 155, 28.
17. M. Frei-Häusler and R. W. Frei, *J. Chromatogr.*, **1973**, 79, 209.