# Dansylation of Endocrine-disrupting Alkylphenolic Compounds on the Solid Phase Used for Extraction from Environmental Water

Bunji Uno,* Jiro Katsumi, and Kazumi Aoki
*Gifu Pharmaceutical University, Mitahora-Higashi, Gifu 502-8585*

(Received September 14, 2004; CL-041080)

The dansylation reaction of endocrine-disruptive alkylphenols on a $C_{18}$-modified silica gel (ODS) instead of organic solvents was investigated with the aim of developing an environment-friendly and efficient derivatization method for the HPLC analyses. The solid-phase aqueous dansylation system has been incorporated into a preconcentration step performed by solid-phase extraction (SPE) from tap water.

---

Development of a method of conventional trace analyses of alkylphenolic compounds such as 4-nonylphenol (**5**) is currently required to estimate the potential estrogenic properties and the environment quantity of these compounds. HPLC analyses with MS,[1] electrochemical,[2] and fluorescence[3] detection as well as GC–MS[4] have been applied to the trace analyses of the phenols. However, the environmental analyses still essentially require an enrichment step such as preconcentration and a derivatization step prior to chromatographic separation because they are performed for trace amounts of analytes in the relatively large volume of sample solutions. The direct derivatization of the phenols preconcentrated on the solid-phase by SPE contributes to simplification of the analytical procedure and overcoming the problem on loss of recovery, and is desirable in view of environmental preservation as an environment-friendly method in aqueous media instead of organic solvents as well.[5] In this study, the dansylation reaction of endocrine-disrupting alkylphenolic compounds in a solid-phase aqueous system has been investigated with the aim of developing an environment-friendly and efficient derivatization method for HPLC analyses of environmental samples. The phenols used were 4-*tert*-butylphenol (**1**), 4-*sec*-butylphenol (**2**), 4-(1,1-dimethylpropyl)phenol (**3**), 4-(1,1,3,3-tetramethylbutyl)phenol (**4**), and **5**.

5-Dimethylaminonaphthalene-1-sulfonyl chloride (Dansyl chloride, DNS-Cl) is entirely used for precolumn derivatization to overcome the disadvantage of the background response. The hard and time-consuming conditions for dansylation of phenols have been significantly improved by a two-phase system consisting of an organic solvent such as heptane or chloroform and water containing a phase-transfer catalytic *tert*-ammonium salt.[6] The conclusion inspires research of environment-friendly derivatization in the solid phase of an ODS instead of the organic solvents. The dansylation reaction in the ODS solid-phase aqueous system was examined in the following procedure. An aqueous sample solution (10 mL) of the alkylphenols was flowed into the ODS cartridge containing dansyl chloride,[7] followed by a run of a buffer solution as a pH control and a reaction initiator. The reaction mixture on the ODS was eluted with 10 mL of $CH_3CN$, and a 10-µL aliquot of the eluate was injected into HPLC with fluorescent detection. Figure 1 shows the dependence of the dansylation yield of **1** on pH of the buffer solutions and on reaction time. The phenols have been easily and quickly converted into the dansyl derivatives with an excellent yield under the mild solid-phase aqueous conditions without a phase-transfer catalyst. The optimal conditions set as the reaction time of 20 min using the buffer solution of pH 11 at room temperature gave the dansyl products of **1**–**5** in high yields of 92.5–98.2%. The solid-phase catalytic reaction has proved to be suitable for dansylation of the phenols under the mild conditions.

The HPLC method involving the solid-phase dansylation was calibrated over the whole analytical procedure and was linear over 2 orders of magnitude ($7.0 \times 10^{-8}$ to $5.0 \times 10^{-6}$ mol/L) for the phenols in water samples with correlation coefficients of 0.9981–0.9997. The wide dynamic range proves that the alkylphenols were quantitatively derived into the dansyl products regardless of the concentrations of sample solutions. Using 10-mL samples without preconcentration, detection limits of $3.5–7.0 \times 10^{-9}$ mol/L were achieved for **1**–**5** in water, and were the same as those obtained for the standards of the dansyl phenols.[8]

An additional advantage of the solid-phase derivatization is simplicity and efficiency of the analytical procedure, combined with an enrichment step performed by SPE of the samples. It is well recognized that the commercially available ODS cartridges for SPE are suitable for extraction and concentration of endocrine-disruptive alkylphenols from environmental water.[1–3] The phenols in water were extracted in the cartridge, and were simultaneously and easily converted into the dansyl derivatives in the same cartridge just running a basic buffer solution. Figures 2a and 2b show typical chromatograms obtained for $10^{-8}$ mol/L of **1**–**5** in tap water using the present solid-phase dansylation method coupled with 100-fold enrichment and without preconcentration, respectively. Even the phenols at concentrations in the vicinity of the detection limit can be efficiently dansylated after enrichment and give a clear chromatogram. As shown in Figure 2c, separation of the products of **1**–**5** dansylated in solution requires careful control of the elution with a gradient pro-

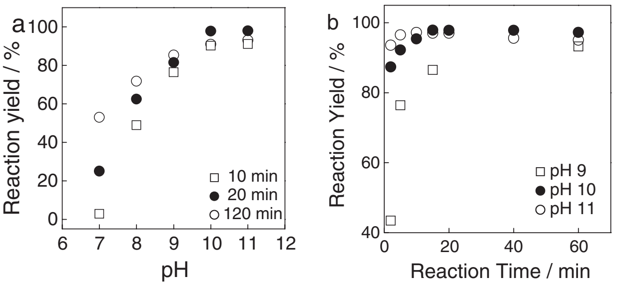

**Figure 1.** Dependence of the dansylation yields of **1** on pH of the buffer solutions (a) and reaction time (b). The reaction yields are estimated for the reaction time of 10 (□), 20 (●), and 120 min (○) at room temperature in Figure a, and for pH 9 (□), 10 (●), and 11 (○) at room temperature in Figure b.

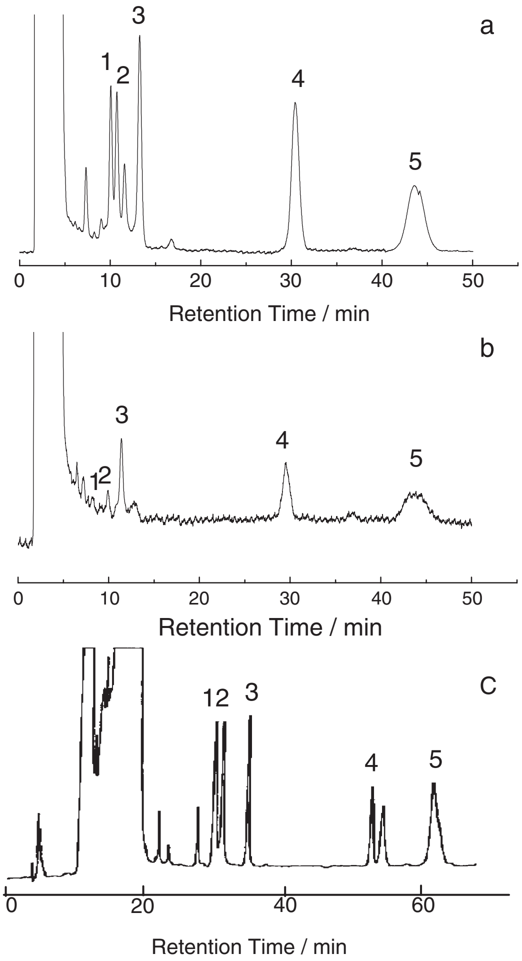

**Figure 2.** Elution profile of the dansyl derivatives of **1**–**5**, separated on a $C_4$-modified silica gel column with fluorescent detection at 510 nm (Ext. 340 nm). Figures **a** and **b** show the chromatograms of **1**–**5** ($10^{-8}$ mol/L) dansylated on the ODS solid-phase with 100-fold preconcentration and without preconcentration, respectively. The separation was performed by the isocratic mobile phase consisting of an acetate buffer (pH 4.8) and methanol (1:3). Figure c shows the chromatogram of **1**–**5** dansylated in solution, separated with the gradient mobile phase consisting of an acetate buffer (pH 4.8) and $CH_3OH$. The gradients are as follows: 30% ($CH_3OH$) to 70% for 0 min to 10 min, 70 to 20% for 10 to 80 min.

**Table 1.** Recovery of **1** spiked in tap water, measured with the present solid-phase dansylation method

| [**1**] / mol dm$^{-3}$ (sample volume / L) | Preconcentration ratio | Recovery / % | RSD / % |
|---|---|---|---|
| $5.0 \times 10^{-8}$ (0.1) | 10 | 97.5 | 1.8 ($n = 10$) |
| $5.0 \times 10^{-9}$ (0.1) | 10 | 90.5 | 4.7 ($n = 5$) |
| $5.0 \times 10^{-9}$ (1.0) | 100 | 92.5 | 3.7 ($n = 5$) |
| $5.0 \times 10^{-10}$ (5.0) | 500 | 72.0 | 12.7 ($n = 3$) |
| $2.5 \times 10^{-10}$ (10.0) | 1000 | 73.5 | 13.5 ($n = 3$) |

gram due to elution of a large amount of 5-dimethylaminonaphthalene-1-sulfonic acid (aqueous decomposition product of DNS-Cl).[3c] However, the sufficient separation has been given by an isocratic mobile phase in the present dansylation method because the decomposition product is removed from the ODS solid phase during the reaction, as shown in Figure 2a. Table 1 lists the recovery of **1** ($10^{-8}$ to $10^{-10}$ mol/L) spiked in tap water. The enrichment in the ratio of less than 100 gave high recovery with the relative standard deviation (RSD) less than 5%. Indeed, the $10^{-10}$ mol/L level of **1** in tap water is easily detected by the proposed method. However, an increase in the ratio lowered the recovery, probably due to insufficient retainment of the phenols resulting from the prolonged run of a large volume of the sample water into the SPE cartridge. The recovery of **2**–**5** spiked in tap water ($10^{-8}$ to $10^{-9}$ mol/L) was also examined with the solid-phase dansylation method involving an enrichment step at a ratio of less than 100. High recovery rates (90.2–96.6%) were obtained for all sample solutions with a RSD less than 7% ($n = 5$).

In conclusion, we have proposed a new approach to give the dansyl derivatives of alkylphenolic compounds on the ODS solid-phase. The solid-phase aqueous dansylation coupled with SPE is the desirable method for environmental analyses from the point of view of environmental conservation as well as efficiency in the analytical procedure. The solid-phase derivatization is applicable to the other systems involving conversion into the detectable products linked by the sulfonyl group.

This work was supported by a Grant-in-Aid for Scientific Research No. 15590046 from Japan Society for the Promotion of Science.

## References and Notes

1 a) S. N. Pedersen and C. Lindholst, *J. Chromatogr., A*, **864**, 17 (1999). b) A. Motoyama, A. Suzuki, O. Shirota, and R. Namba, *Rapid Commun. Mass Spectrom.*, **13**, 2204 (1999).

2 a) K. Inoue, Y. Yoshimura, T. Makino, and H. Nakazawa, *Analyst*, **125**, 1959 (2000). b) K. Inoue, K. Kato, Y. Yoshimura, T. Makino, and H. Nakazawa, *J. Chromatogr., B*, **749**, 17 (2000). c) B. Uno and Y. Kato, *Chem. Lett.*, **2002**, 652.

3 a) T. Tsuda, K. Suga, E. Kaneda, and M. Ohsuga, *J. Chromatogr., B*, **746**, 305 (2000). b) M. Ahel, W. Giger, E. Molnar, and S. Ibric, *Croat. Chem. Acta*, **73**, 209 (2000). c) B. Uno, Y. Kato, and S. Miwa, *Anal. Sci.*, **18**, 685 (2002).

4 a) H. G. J. Mol, S. Sunarto, and O. M. Steijger, *J. Chromatogr., A*, **879**, 97 (2000). b) D. Jahr, *Chromatographia*, **47**, 49 (1998). c) R. A. Rudel, S. J. Melly, R. W. Geno, G. Sun, and J. G. Brody, *Environ. Sci. Technol.*, **32**, 861 (1998).

5 a) G. Bartoli, M. Bartolacci, M. Bosco, G. Foglia, A. Giuliani, E. Marcantoni, L. Sambri, and E. Torregiani, *J. Org. Chem.*, **68**, 4594 (2003). b) K. Manabe, Y. Mori, T. Wakabayashi, S. Nagayama, and S. Kobayashi, *J. Am. Chem. Soc.*, **122**, 7202 (2000).

6 C. De Ruiter, R. R. Otten, U. A. Th. Brinkman, and R. W. Frei, *J. Chromatogr.*, **436**, 429 (1988).

7 The ODS cartridges containing DNS-Cl for SPE were prepared as follows. A 1-mL aliquot of $10^{-2}$ mol/L DNS-Cl in $CH_3CN$ was run in the inlet of the Waters Sep-Pak® cartridges packed with ODS, and then the cartridges were washed with 20 mL of water.

8 The standard of the dansyl phenols was synthesized by addition of a phosphate buffer (pH 12) to a mixture of equimolar DNS-Cl and **1**–**5** in $CH_3CN$. The reaction mixture was refluxed at 60 °C for 120 min, followed by extraction with ether. The structure and purity were checked by MS and elemental analyses.

Published on the web (Advance View) November 13, 2004; DOI 10.1246/cl.2004.1584