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Short communication

Gas chromatographic–mass spectrometric determination of 4-nonylphenols and 4-*tert*-octylphenol in biological samples

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Abstract

A simple and rapid method is described for the GC–MS determination of 4-nonylphenols (NOs) and 4-*tert*-octylphenol (OC) in biological samples. The NOs and OC in the sample are extracted with acetonitrile and the lipid in the sample extract is eliminated by partitioning between hexane and acetonitrile. After Florisil PR column clean-up, the sample extract is analyzed by GC–MS in the selected ion monitoring (SIM) mode. Average recoveries in pale chub (fish) and corbicula (shellfish) are 86.0 and 93.4% for NOs, and 95.8 and 96.4% for OC, respectively, spiked at the levels of 1.0 μg of NOs and 0.1 μg of OC per 5 g of fish and shellfish samples. The detection limits are 20 ng/g for NOs and 2 ng/g for OC.

Keywords: 4-Nonylphenols; 4-*tert*-Octylphenol

1. Introduction

Alkylphenolpolyethoxylates (APEOs) have been widely used in the last 50 years for a variety of industrial, household and commercial applications. Concern has increased recently about the wide usage of APEOs, because their biodegradation metabolites nonylphenol and octylphenol are stable and nonylphenol has been demonstrated to be toxic to both marine and freshwater species [1,2], to induce estrogenic responses in male trout [3,4] and may accumulate in freshwater organisms [5,6].

Surveys of nonylphenol and octylphenol in water samples of rivers, sewage effluents and estuaries have been widely carried out in many countries [7–14]. However, few reports [6,15,16] have been published for the analyses of these compounds in marine or freshwater biological samples. In the survey by Ahel et al. [15], tissue samples were extracted, employing concurrent steam-distillation and solvent extraction in a specially designed apparatus and the extracts were analyzed by normal-phase HPLC. In the bioaccumulation experiments of 4-nonyl-[^{14}C]-phenol (^{14}C-NO) [6], ^{14}C-NO was extracted with high recovery from tissue samples which were pretreated with pancreatic enzymes and the samples were scintillation counted. Further in the survey by Wahlbarg et al. [16], nonylphenol an nonylphenol ethoxylates in mussels were analyzed as their pentafluorobenzoates.

In the above methods [6,15,16], accurate and high sensitive analyses are possible but special apparatus is necessary or the operations such as enzyme

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treatment and derivatization are tedious and time-consuming. Therefore we have developed a simple and rapid GC–MS method for the determination of nonylphenol and octylphenol in biological samples such as fish and shellfish.

2. Experimental

2.1. Apparatus

The GC–MS systems were equipped with MAGNUM ion trap mass spectrometer (Finnigan mat, USA) and 3300/3400 gas chromatograph (Varian, USA). As GC column, J&W DB-1701 (30 m×0.53 mm I.D., film thickness 1.0 μm) was used.

2.2. Operating conditions

With MS: operating mode, electron impact ionization mode; manifold temperature, 220°C; multiplier voltage, 1600 V; emission current; 10 μA. With GC: injection port temperature, 250°C; oven temperature, 60°C (1 min) 10°C/min to 200°C (0 min) 2°C/min to 220°C (0 min) 10°C/min to 270°C (10 min); Carrier gas (helium) flow-rate, 10 ml/min.

2.3. Reagents

4-Nonylphenols (NOs), a mixture of compounds with branched sidechains, and 4-*tert*-octylphenol (OC), purity more than 93%, were purchased from Tokyo Chemical Industry (Tokyo, Japan). Florisil PR from Katayama Industries Co. Ltd. (Osaka, Japan) after activation at 130°C for 16 h, and Sep-Pak Florisil from Waters (Massachusetts, USA) were used for column clean-up. Pesticide-grade solvents and chemicals were used throughout.

2.4. Standard solution

Standard stock solutions (1000 μg/ml) were prepared by dissolving 100 mg of each NOs and OC in 100 ml of acetone. Working standards of mixtures of NOs (0.2–2.0 μg/ml) and OC (0.02–0.2 μg/ml) were prepared by diluting aliquots of the stock solutions in hexane.

2.5. Determination of NOs and OC in biological samples

About 5 g of the biological sample was suspended twice with 30 ml of acetonitrile after addition of 5 g of anhydrous sodium sulfate, and the organic layer was filtered through anhydrous sodium sulfate. The combined filtrate was rotary-vacuum evaporated to dryness at 40°C. The residue was dissolved in 10 ml of hexane and shaken twice with 30 ml of acetonitrile saturated with hexane. The combined acetonitrile layer was rotary-vacuum evaporated to dryness at 40°C. The residue was dissolved in 5 ml of hexane and passed through a 30×1.0 cm I.D. glass clean-up column containing 5 g of hexane-rinsed Florisil PR and 1 g of anhydrous sodium sulfate. The column was eluted with ethyl ether and hexane (10+90). The first 20 ml was discarded and the next 20–70 ml was collected for NOs and OC. The eluate was rotary-vacuum evaporated to about 2 ml, transferred to a graduated test-tube (rinsing the flask with hexane) and adjusted to 1 ml under a stream of nitrogen at 40°C. A 3-μl volume of the sample solution was injected into the GC–MS for analysis. The GC–MS measurement was performed by monitoring the ions at m/z 107, 135, 149 and summed (107+135+149) for NOs and at m/z 107 and 135 for OC. Peak areas of ions at m/z (107+135+149) for NOs and at m/z 135 for OC were integrated and evaluated by the use of calibration curves. NOs was a mixture of compounds with branched sidechains, so determination was performed by integrating the cluster of isomers seen at m/z (107+135+149).

2.6. Calibration curves

A 3-μl volume of each standard mixture of NOs and OC was injected into the GC–MS, and calibration curves were constructed by plotting the peak areas of ions at m/z (107+135+149) against NOs (0.2–2.0 μg/ml) and at m/z 135 against OC (0.02–0.2 μg/ml).

3. Results and discussion

3.1. GC–MS determination of NOs and OC

Capillary GC–MS determination of organic compounds in the sample using SIM mode is generally performed by the internal standard method because of matrix effects or bad reproducibilities of measurement. A wide-bore capillary GC column was used in this study, so matrix effects were little observed and good reproducibilities of measurements were obtained by the external standard method. From the results, the external standard method was adopted in the present GC–MS determination. Calibration curves of NOs and OC had straight lines as follows: For NOs, $y=46456x+1663.3$, $r=0.9975$ ($m/z=107+135+149$); $y=16013x+492.24$, $r=0.9947$ ($m/z=107$); $y=24334x-224.56$, $r=0.9960$ ($m/z=135$); $y=11786x+276.06$, $r=0.9984$ ($m/z=149$). For OC, $y=73677x-147.08$, $r=0.9995$ ($m/z=135$). Linearity range was 0.2–10.0 μg/ml for NOs and 0.02–1.0 μg/ml for OC. Detection limits of standard NOs and OC were 0.1 and 0.01 μg/ml, respectively.

3.2. Florisil column clean-up

At first, Sep-Pak Florisil was applied to the clean-up of fish extracts (crucian carp and pale chub). The clean-up procedure was as follows: The fish extract in hexane was passed through Sep-Pak Florisil and the column was eluted with 10 ml of ethyl ether and hexane (10+90) after washing with 10 ml of hexane. As shown in Fig. 1, the clean-up was adequate for the extracts of crucian carp (muscle) but impossible for the extract of pale chub (whole body). Here Florisil PR was applied to the clean-up of the pale chub extract. The elution patterns of standard NOs and OC (each 10 and 1 μg) and sample NOs and OC (equally added to the extract) are shown in Fig. 2. Both samples of NOs and OC (20–50 ml) were slightly more rapidly eluted than standard NOs (20–60 ml) and OC (30–50 ml). Therefore the first 20 ml

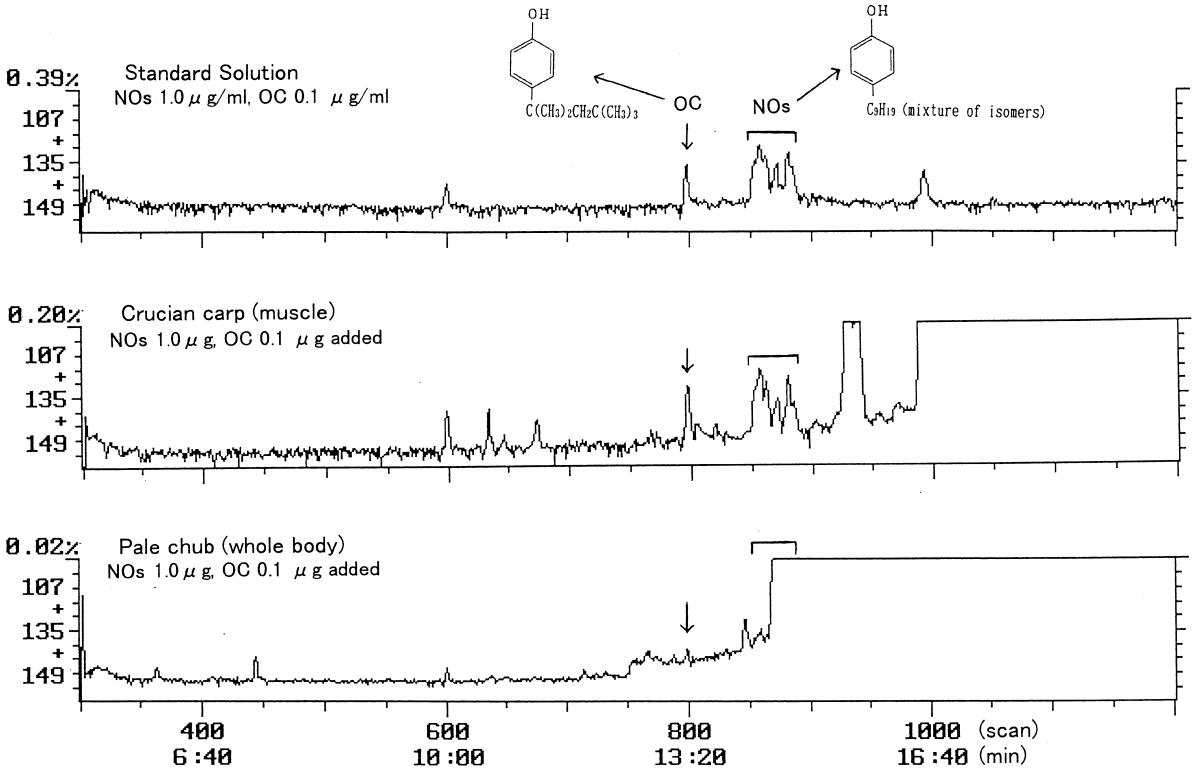

Fig. 1. Sep-Pak Florisil clean-up of the extracts of crucian carp (muscle) and pale chub (whole body).

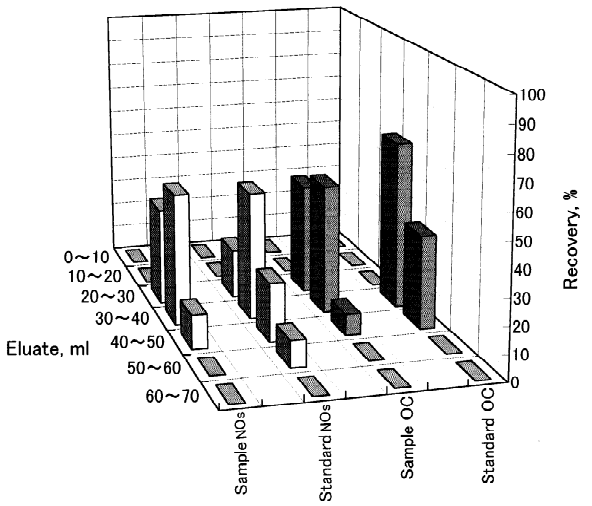

Fig. 2. Elution patterns of 4-nonylphenols and 4-*tert*-octylphenol from Florisil PR. NOs: 4-nonylphenols; OC: 4-*tert*-octylphenol.

eluate was discarded and the next 20–70 ml (slightly wide range) was collected for the GC–MS analysis of NOs and OC. As shown in Fig. 3, GC–MS chromatograms at m/z 149, 135, 107 and (107+135+149) were all adequately made clean-up. Fish muscle or some kinds of fish samples can be adequately made clean-up by Sep-Pak Florisil, but Florisil PR will probably give better results of clean-up and recovery than Sep-Pak Florisil.

3.3. Recoveries of NOs and OC from biological samples

The recoveries of NOs and OC added to fish and shellfish samples are given in Table 1. Recovery tests were performed by Sep-Pak Florisil clean-up for fish 1 (crucian carp muscle) and fish 2 (killifish whole body), and by Florisil PR clean-up for fish 3 (pale chub whole body) and shellfish (corbicula whole body). In the column clean-up of whole body fish samples, better results were obtained for Florisil PR (fish 3) than for Sep-Pak Florisil (fish 2). That is, the same or higher recoveries (NOs 86.0% and OC 95.8%) and lower RSD (NOs 3.1% and OC 2.6%) were obtained for Florisil PR, compared with those of (NOs 87.0% and OC 87.3%) and (NOs 5.7% and OC 8.8%) for Sep-Pak Florisil. For fish 3 and shellfish, GC–MS determination of NOs were performed by m/z 149, 135, 107 and summed (107+135+149). There were no wide differences in the results of recovery tests among the four determinations but GC–MS determination by m/z (107+135+149) had the highest sensitivity in the four determinations. Therefore this determination was selected in this method. Detection limits were 20 ng/g for NOs and 2 ng/g for OC.

3.4. Comparison of the proposed method with the other methods

The method by Ekelund [6] is widely different from the other chromatographic methods, so this method is excluded in this comparison. For the recoveries of NOs from biological samples, the proposed GC–MS method (86.0–93.4%) is not widely different from the HPLC method (100%) by Ahel et al. [15] and the GC–MS or ECD–GC method (93%) after derivatization by Wahlbarg et al. [16]. Next the detection limit of the proposed method (20 ng/g wet weight) is higher than those of the two

Table 1
Recovery of 4-nonylphenols and 4-*tert*-octylphenol from biological samples

Sample	Added amount (μg)	Recovery (%)* of 4-nonylphenols				Added amount (μg)	Recovery (%) * of 4-*tert*-octylphenol
		m/z=149	m/z=135	m/z=107	m/z=Summed		m/z=135
Fish 1[a]	1.0	–	–	–	102±4.2	0.1	92.3±8.8
Fish 2[b]	1.0	–	–	–	87.0±5.0	0.1	87.3±7.7
Fish 3[c]	1.0	95.4±7.3	106±10	82.3±4.9	86.0±2.7	0.1	95.8 ±2.5
Shellfish[d]	1.0	103±1.6	105±4.5	99.2±6.9	93.4±6.7	0.1	96.4±1.1

* Mean+SD n=5 GC–MS determination was carried out using m/z=149, 135, 107 and summed (107+135+149).
[a] Crucian carp muscle, 5 g.
[b] Killifish whole body, 5 g.
[c] Pale chub whole body, 5 g.
[d] Corbicula whole body, 5 g.

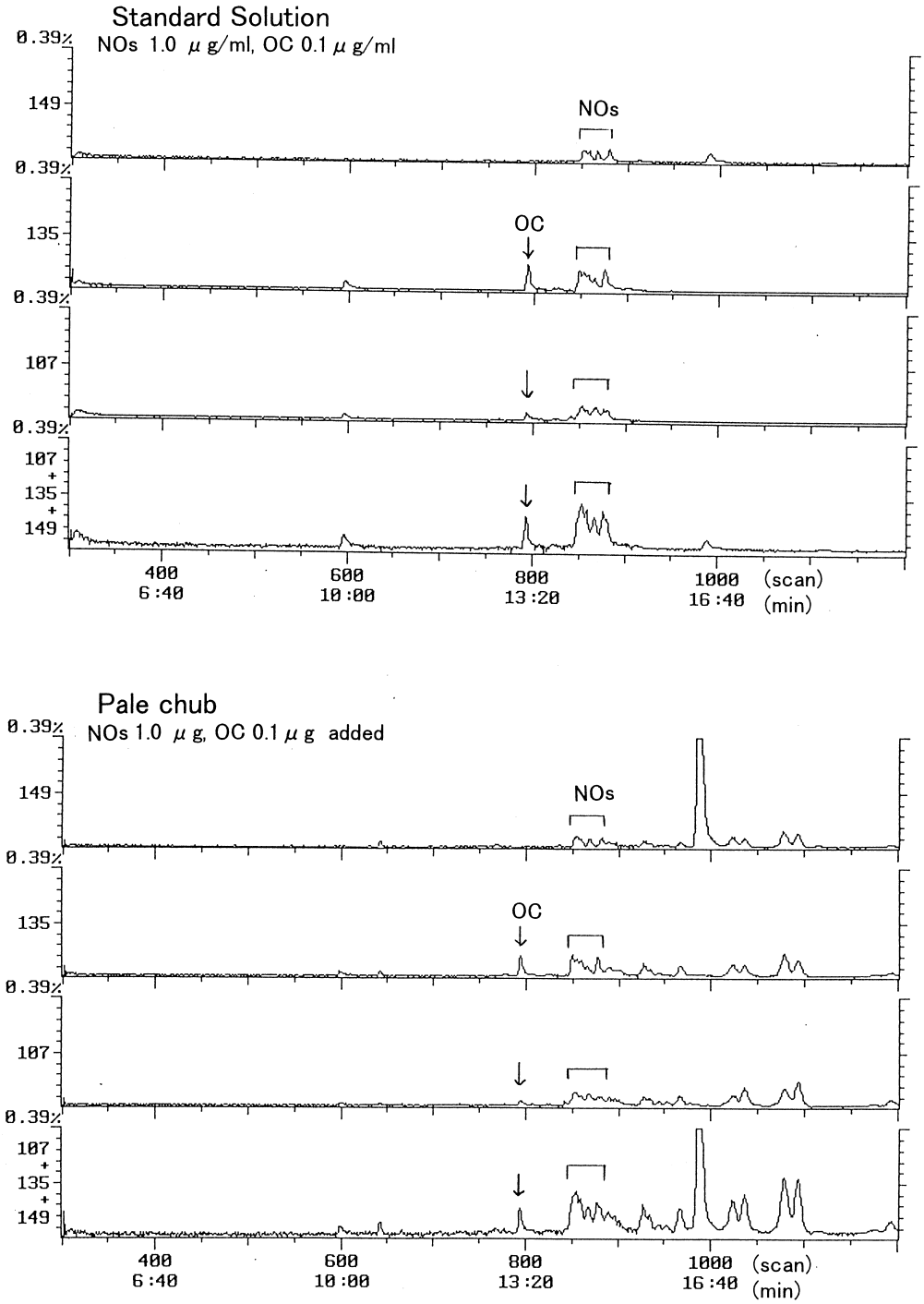

Fig. 3. GC–MS chromatograms of standard and recovered NOs and OC from pale chub after Florisil PR clean-up.

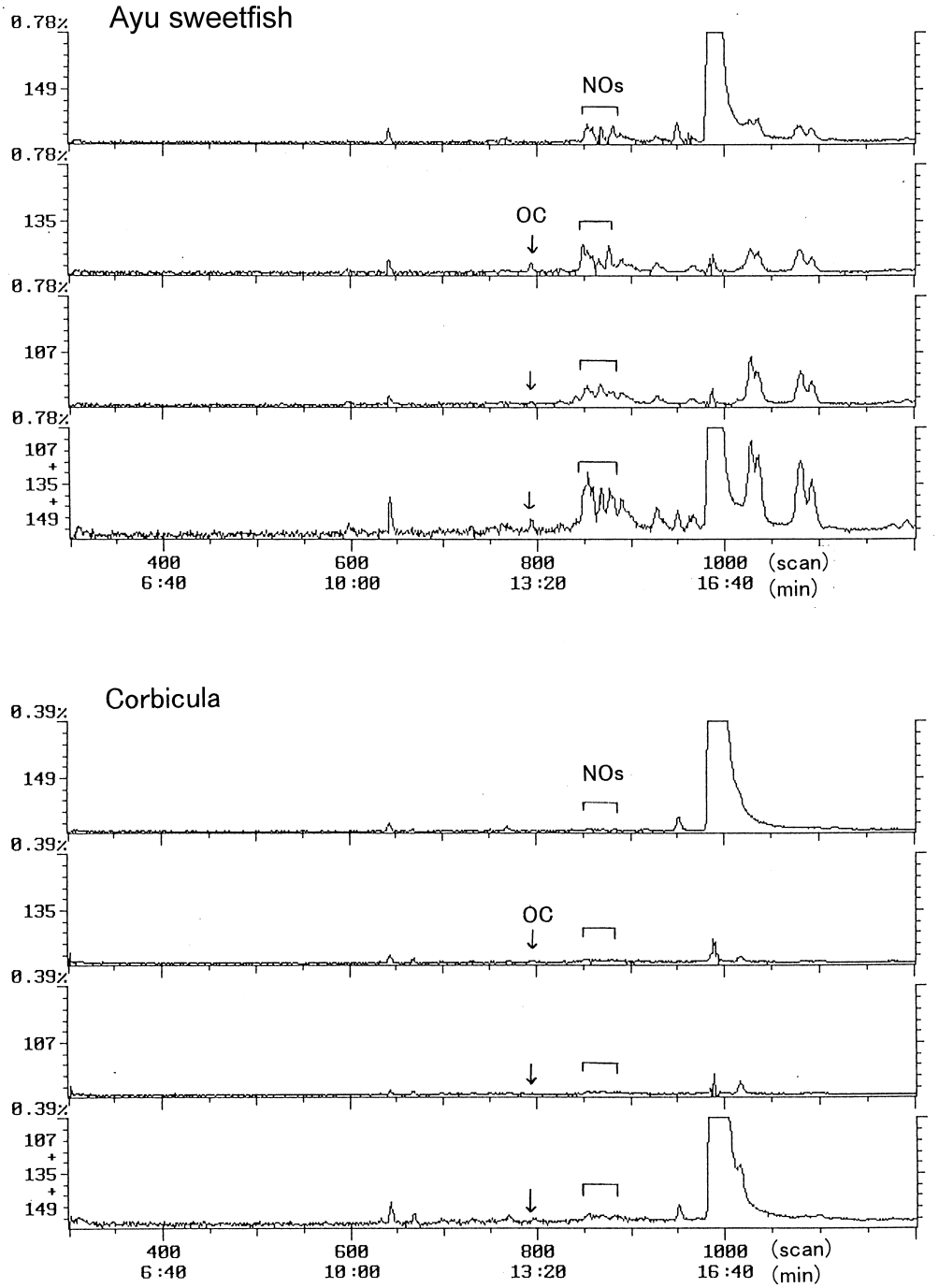

Fig. 4. GC–MS chromatograms of NOs and OC in ayu sweetfish from River F and in Corbicula from Lake Biwa.

Table 2
Concentrations of 4-nonylphenols and 4-*tert*-octylphenol in environmental biological samples

Sample	Part	Sampling date	Location*	4-Nonylphenols ng/g, wet weight	4-*tert*-Octylphenol ng/g, wet weight
Pale chub	Whole body	1998.4.13	River A	20	<2
Crucian carp	Whole body	1998.4.13	River A	30	<2
Dark chub	Whole body	1998.4.13	River B	<20	<2
Bluegill	Muscle	1998.4.13	River C	<20	<2
Large-mouth bass	Muscle	1998.5.7	River D	<20	5
Ayu sweetfish	Whole body	1998.5.7	River E	<20	<2
Ayu sweetfish	Whole body	1998.5.7	River F	110	6
Crucian carp	Muscle	1997.4.18	Lake Biwa	<20	<2
Corbicula	Meat and viscera	1998.4.16	Lake Biwa	<20	<2

* Rivers A–F are all flowing into Lake Biwa.

methods (about 10 ng/g and 1 ng/g wet weight). Finally the time consumed in the operations of the three methods is presumed to be Ahel et al. [15]≤the proposed method<Wahlbarg et al. [16]. The method by Wahlbarg et al. [16] is highly sensitive and accurate but slightly tedious and time-consuming. The proposed method is simple, rapid and there is no need for special apparatus. As shown in Figs. 3 and 4, the proposed clean-up is effective in spite of less selectivity and will be satisfactorily applicable to the screening analyses of NOs and OC in biological samples such as fish and shellfish.

3.5. Application to environmental biological samples

Environmental biological samples were analyzed by the proposed method. Typical GC–MS chromatograms of NOs and OC in fish from River F and in shellfish from Lake Biwa (Shiga Prefecture, Japan) are shown in Fig. 4. For ayu sweetfish (fish), a small interfering peak was observed near the peak of NOs but little influence was given on the determination of NOs. For corbicula (shellfish), no interfering peaks were observed near the peaks of NOs and OC. The application data ($n=9$) were calculated and are summarized in Table 2. For example, OC was detected in 5 ng/g in large-mouth bass (muscle) from River D and 6 ng/g in ayu sweetfish (whole body) from River F and NOs were 110 ng/g in ayu sweetfish (whole body) from River F.

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