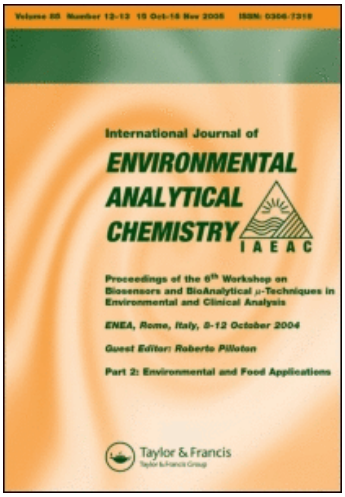

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A simplified method for simultaneous quantitation of alkylphenols and alkylphenol ethoxylates in meat and fish using high-performance liquid chromatography with fluorescence detection

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Nonylphenol (NP), octylphenol (OP), nonylphenol monoethoxylate (NP_1EO) and nonylphenol diethoxylate (NP_2EO) are products of the biodegradation of alkylphenol polyethoxylates (AP_nEO) which are used worldwide as detergents and surfactants. NP and OP are categorized as definitely endocrine disruptors. 2,4-Tert-butylphenol (BP) is extensively used for anti-oxidant of rubber and plastics. This work proposed a simple and stable method for simultaneously determining the concentration of NP, OP, BP, *n*-NP_1EO and *n*-NP_2EO in meat and fish, without requiring the complex pretreatments of current methods. This study used liquid extraction with acetonitrile and hexane and solid extraction using Florisil, in that order to pretreat samples. The sample solutions were analyzed to identify NP, OP, BP, *n*-NP_1EO and *n*-NP_2EO by HPLC with fluorescence detection. The mean recoveries were $85.3 \pm 3.32\%$ for OP, $87.5 \pm 6.01\%$ for BP, $90.9 \pm 4.72\%$ for NP, $86.4 \pm 4.81\%$ for *n*-NP_2EO and $90.9 \pm 4.84\%$ for *n*-NP_1EO. The average coefficients of variation were about 6%. The method's detection limits were $5.4\,ng\,g^{-1}$ for OP, $5.2\,ng\,g^{-1}$ for BP, $8.9\,ng\,g^{-1}$ for NP, $8.7\,ng\,g^{-1}$ for *n*-NP_2EO and $8.1\,ng\,g^{-1}$ for *n*-NP_1EO. This work analyzed 5 kinds of usual foodstuffs of meat and fish that are frequently consumed by residents of Taiwan. All of these samples contained NP, but not detectable levels *n*-NP_1EO. Only salmon was contaminated with *n*-NP_2EO. The NP level was highest in cod ($198.41 \pm 129.34\,ng\,g^{-1}$, wet weight). The fried chicken had the highest BP level ($48.0 \pm 41.3\,ng\,g^{-1}$, wet weight), and the uncooked chicken had the highest OP level ($66.6 \pm 53.0\,ng\,g^{-1}$, wet weight).

Keywords: Alkylphenol; Meat; Fish; HPLC/fluorescence detection

1. Introduction

Alkylphenol polyethoxylates (AP_nEO), a class of nonionic surfactants, are extensively used in industrial, agricultural and household applications [1]. Approximately 650 million kg of AP_nEO are produced annually worldwide [2]. Nonylphenol polyethoxylates (NP_nEO) are the most prevalent of such compounds, being present in about 80% of nonionic surfactants, and the second most common are octylphenol

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polyethoxylates (OP_nEO) [3]. Cheng and Ding [4] reported that the levels of NP_nEO in commercial detergents in Taiwan were 0.2–21%. Wilbur [5] stated that the concentrations of NP_2EO, NP_4EO and NP_9EO were 1–20% in detergents and cosmetics in the USA. Around 60% of used AP_nEO is discharged into the aquatic environment [6, 7], to be biodegraded to very persistent and estrogen-like products, such as nonylphenol (NP), octylphenol (OP), nonylphenol monoethoxylate (NP_1EO) and nonylphenol diethoxylate (NP_2EO), these compounds usually contaminate biota [14, 19]. 2,4-Tert-butylphenol (BP) is extensively used for anti-oxidant of plastics and rubber [8, 9], it is a widespread contaminant in foodstuffs, and is also suspected as estrogen-like product.

The biodegradation products of AP_nEO have been detected in rivers, groundwater, soil, sewage and seawater in many countries because they cannot easily be further degraded [10–13]. Additionally alkylphenols, like NP and OP, can mimic 17-β-estrogen and interfere with it by combining with estrogen receptors. These compounds also bioaccumulate in organisms, including fish, mallards and algae [14], since they become more lipophilic following the degradation of AP_nEO. In the literature [15], the bioconcentration factors (BCF) of NP in fathead minnow was approximately 300, so many European countries have banned the use of NP_nEO. The human exposure routes to the biodegradation products of AP_nEO are various, but ingestion is a major one [2]. NP has been detected in drinking water, duck, seafood and foodstuffs packed with polyvinyl chloride plastics [14, 16, 17].

Previous studies [2, 18, 21] have presented practical methods for analyzing fish or foodstuffs, including high performance liquid chromatography (HPLC) cleanup, gel permeation chromatography (GPC) analysis and modified matrix solid-phase dispersion (MSPD) extraction, but these methods require much time, solvent and are costly or involve complex derivatization. Moreover they cannot simultaneously identify the concentrations of five alkylphenol chemicals, NP, OP, BP, *n*-NP_1EO and *n*-NP_2EO. Therefore, this study aims to develop a simple and stable method for identifying the concentrations of NP, OP, BP, NP_1EO and NP_2EO simultaneously in meat and fish.

2. Experimental

2.1 *Reagents*

4-Nonylphenol (NP), with a purity of over 90%, and 4-tert-octylphenol (OP), with a purity over 90%, were purchased from Fluka (Japan). 2,4-Di-tert-butylphenol (BP) with a purity of over 98% was purchased from Merck (Germany). *p*-*n*-Nonylphenol monoethoxylate (*n*-NP_1EO) and *p*-*n*-nonylphenol diethoxylate (*n*-NP_2EO), at concentration of $100\,mg\,L^{-1}$ in nonane, were obtained from Cambridge Isotope Laboratories, Inc. (USA). Florisil PR, 60/100 mesh, was obtained from Supelco (USA). All other reagents used in extracting the analytes, including acetonitrile, *n*-hexane, acetone and methanol, were LC grade and purchased from Merck (Germany).

2.2 *Apparatus and analytical conditions*

The HPLC system was from Hitachi (Japan), and equipped with an L-7200 autosampler, an L-6200 intelligent pump, a F-1080 fluorescence detector and

a D-6000 manager. The HPLC column was a Luna C18-A (150 × 4.6 mm i.d., 5 μm particle size) and the guard column was a C18 (4 mm × 3.0 mm i.d.). The columns were purchased from Phenomenex (USA). The mobile phase was acetonitrile: water (70 : 25, v/v). The flow rate was 1.0 mL min^{-1}. The excitation wavelength was 275 nm and the emission wavelength was 300 nm. The injection volume was 20 μL. Chromatographic data were collected and processed using a computer with Model D-6000 HPLC Manager version 2 chromatography data station software.

2.3 *Preparation of calibration curves*

Working solutions (0.01–1 mg L^{-1}) were prepared by the serial dilution method and aliquots of NP, BP, OP, NP_1EO and NP_2EO dissolved in methanol.

2.4 *Study subjects*

This study sampled 5 kinds of meat including pig, beef, duck, uncooked chicken and fried chicken, and 2 kinds of fish involved freshwater fish and saltwater fish, including milkfish, tilapia and mullet, salmon, cod, sailfish, mackerel, respectively. All samples were purchased in supermarkets in Taipei city and the mostly samples were obtained locally except salmon, cod and part of beef. The samples were refrigerated in their original packaging at −20°C. The muscles of meat and fish products were analyzed. All samples were analyzed within a week of purchase.

2.5 *Pretreatment of meat samples*

The samples were pretreated according to a method modified from the method of Tsuda *et al*. [19]. The meat and fishsamples were defrosted and the samples were skinned and adsorbed surface water by paper tissues before weighed. Five grams of ground samples were extracted by stirring twice for 8 min with 30 mL of acetonitrile in a power-stir, and the organic layers were filtered. The mixed extracts were concentrated to dryness in a water bath at 50°C using a rotary vacuum evaporator. The residues were dissolved in 10 mL of hexane and shaken for 5 min 3 times with 30, 15 and 15 mL of acetonitrile saturated with hexane. The combined acetonitrile layers were concentrated to dryness at 50°C in a water bath using a rotary vacuum evaporator. The residues were dissolved in 5 mL of hexane and passed through a glass clean-up column packing in 5 g of Florisil (PR, 60/100 mesh) prerinsed with 50 mL of hexane. The analytes were then eluted with 80 mL of hexane and acetone (9 : 1, v/v). The eluates were concentrated to dryness at 50°C in a water bath using rotary vacuum evaporator and dissolved in 3 mL of methanol. The sample solutions were analyzed to determine the concentrations of NP, OP, BP, *n*-NP_1EO and *n*-NP_2EO simultaneously using HPLC with fluorescence detection.

2.6 *Blanks, recovery, precision and detection limits*

The procedural blanks were analyzed with every batch of samples. The surrogates of the samples were subtracted from the corresponding surrogates of blank values (OP, *n*-NP_2EO and *n*-NP_1EO were null, BP was 0–73 ng, NP was 7–96 ng), and then the concentrations of surrogates were calculated in relation to the fresh weight of the samples.

For the calculation of the recovery, the meat or fish samples were homogenously mixed and divided into two portions. These two portions were pretreated with the complete analytical procedures except for the addition of a known amount of the surrogates in one portion. After pre-treatment and HPLC determination, the recoveries were calculated by comparing the concentrations obtained and the added known amount of the surrogates.

The precisions of within-day and between-day for each surrogate were evaluated by assessing five analytes for triple tests and three separate occasions.

The detection limits of the analytical method were determined by spiking the lowest concentrations detectable after sample pretreatment. The determination was repeated 5 times. The detection limit was obtained by the threefold standard deviation of response divided by the mean values for the response and then multiplied by spiking concentration.

3. Results

3.1 *Analytical methods*

The retention times of the OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO standards were 8.7, 10.5, 13.1, 18.8 and 19.2 min (figure 1). The concentrations of the standards were at linear intervals in the range 0.01 to 1 mg L^{-1}. The recoveries of OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO in the meat and fish samples were 85.3 ± 3.32, 87.5 ± 6.01, 90.9 ± 4.72, 86.4 ± 4.81 and $90.9 \pm 4.84\%$ (table 1). Figures 2 and 3 present the chromatograms of fried chicken and fried chicken with spikes OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO. The within-day and between-day coefficients of variation of the five analytes were under 10% (table 2). The limits of detection for OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO in the meat and fish samples were 5.4, 5.2, 8.9, 8.7 and 8.1 ng g^{-1} wet weight.

3.2 *Application of method to meat and fish samples*

This study involved 5 kinds of meat and 7 kinds of fish. Table 3 lists the concentrations of five alkylphenol chemicals in all samples. Of these five alkylphenol chemicals, NP was

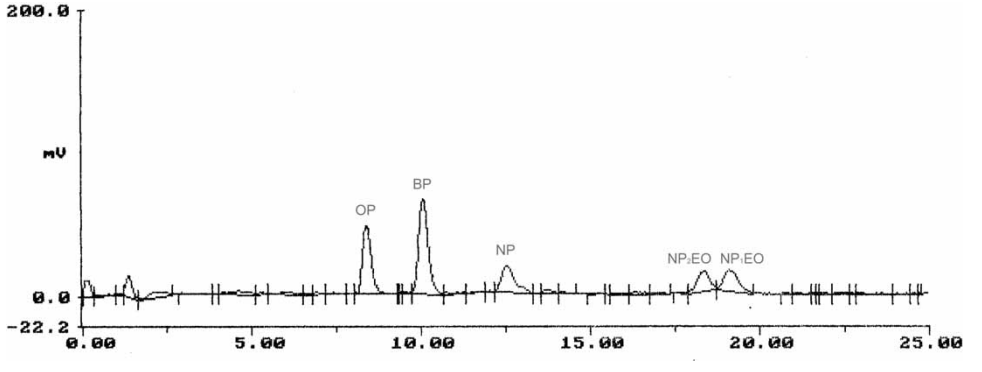

Figure 1. Chromatogram of OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO.

Table 1. The recoveries of meat and fish samples with spikes OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO.

	Spike weight (μg)	Number (*N*)	Recovery (%) Mean ± SD
OP	0.15	5	85.8 ± 5.65
	0.30	5	73.3 ± 1.75
	0.90	8	78.6 ± 2.37
	1.50	3	93.3 ± 3.74
	3.00	4	107.29 ± 2.08
	Mean ± SD	25	85.3 ± 3.32
BP	0.15	3	88.6 ± 7.61
	0.30	3	82.3 ± 7.15
	0.90	3	78.6 ± 6.20
	1.50	3	86.3 ± 1.74
	3.00	5	96.1 ± 5.76
	Mean ± SD	17	87.5 ± 6.01
NP	0.15	3	89.7 ± 4.65
	0.30	5	90.7 ± 6.24
	0.90	4	84.2 ± 1.37
	1.50	3	94.4 ± 2.95
	3.00	4	96.1 ± 5.48
	Mean ± SD	19	90.9 ± 4.72
n-NP_2EO	0.15	3	86.5 ± 7.80
	0.30	5	79.0 ± 7.27
	0.90	8	81.7 ± 2.87
	1.50	5	88.2 ± 2.46
	3.00	11	92.3 ± 4.58
	Mean ± SD	32	86.4 ± 4.81
n-NP_1EO	0.15	5	92.0 ± 6.14
	0.30	6	82.2 ± 7.31
	0.90	7	77.9 ± 3.41
	1.50	4	103.49 ± 1.81
	3.00	9	100.78 ± 3.72
	Mean ± SD	31	90.9 ± 4.84

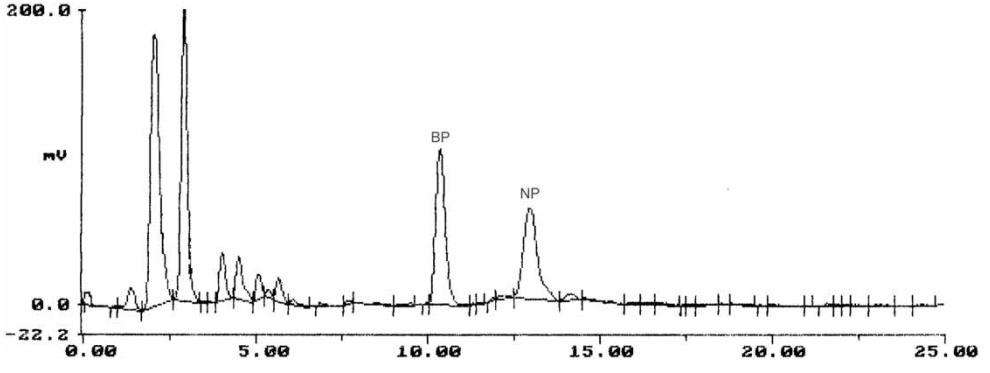

Figure 2. Chromatogram of OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO in fried chicken.

most often detected (100%), followed by BP. However, no sample contained a detectable level of *n*-NP_1EO. Only salmon contained *n*-NP_2EO, at a concentration of $323.72 \pm 298.77\,ng\,g^{-1}$ wet weight. The cod had the most NP at $198.41 \pm 129.34\,ng\,g^{-1}$ wet weight, followed by the mullet with an NP concentration

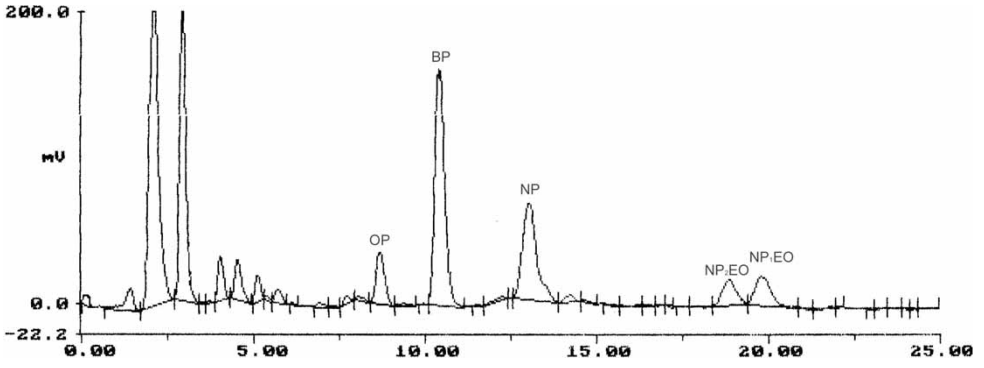

Figure 3. Chromatogram of fried chicken muscle with spikes OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO.

Table 2. The within- and between-day precisions of OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO.

	Concentration ($mg\,L^{-1}$)	Within-day ($n=3$) C.V. (%)	Between-day ($n=9$) C.V. (%)
OP	0.01	4.38	8.17
	0.05	2.52	7.87
	0.10	3.05	6.93
	0.50	0.81	3.45
	1.00	1.10	4.23
	Mean ± SD	2.37 ± 1.46	6.13 ± 2.16
BP	0.01	4.23	4.26
	0.05	1.75	6.65
	0.10	2.59	6.27
	0.50	0.23	3.65
	1.00	1.41	5.12
	Mean ± SD	2.04 ± 1.49	5.19 ± 1.28
NP	0.01	8.22	12.66
	0.05	4.60	3.81
	0.10	2.60	4.73
	0.50	1.85	3.78
	1.00	0.51	4.59
	Mean ± SD	3.56 ± 3.00	5.92 ± 3.80
n-NP_2EO	0.01	3.51	7.61
	0.05	4.26	9.35
	0.10	0.91	6.83
	0.50	0.77	3.08
	1.00	0.50	3.10
	Mean ± SD	1.99 ± 1.76	5.99 ± 2.80
n-NP_1EO	0.01	2.17	11.68
	0.05	2.98	5.54
	0.10	1.46	7.80
	0.50	0.45	2.77
	1.00	0.45	3.31
	Mean ± SD	1.50 ± 1.10	6.22 ± 3.64

Table 3. The concentrations of OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO in meat and fish samples.

		Mean ± SD[a] (range) (ng g^{-1} wet weight)				
Samples	*N*	OP	BP	NP	*n*-NP_2EO	*n*-NP_1EO
Meat						
Pig	6	n.d.[b]	n.d.	79.4 ± 33.4 (49.4–148.8)	n.d.	n.d.
Beef	6	n.d.	3.30 ± 1.64 (n.d.–6.95)	143.59 ± 20.0 (111.34–169.44)	n.d.	n.d.
Duck	6	n.d.	n.d.	26.7 ± 13.4 (11.7–50.5)	n.d.	n.d.
Uncooked chicken	6	66.6 ± 53.0 (30.2–152.15)	26.1 ± 3.50 (16.3–31.2)	144.27 ± 81.4 (61.9–238.47)	n.d.	n.d.
Fried chicken	4	n.d.	48.0 ± 41.3 (17.7–124.42)	114.6 ± 66.2 (51.9–262.91)	n.d.	n.d.
Fish						
Freshwater fish						
Milkfish	10	n.d.	7.84 ± 5.70 (n.d.–17.2)	96.6 ± 51.2 (22.7–166.02)	n.d.	n.d.
Tilapia	11	n.d.	13.8 ± 19.6 (n.d.–60.2)	83.5 ± 32.0 (34.7–159.23)	n.d.	n.d.
Saltwater fish						
Mullet	3	n.d.	28.2 ± 1.37 (27.2–30.5)	168.10 ± 29.6 (129.76–196.20)	n.d.	n.d.
Salmon	6	43.3 ± 15.9 (27.1–68.9)	32.1 ± 2.70 (28.2–37.1)	161.74 ± 25.2 (133.73–213.56)	323.72 ± 298.77 (91.2–916.95)	n.d.
Cod	5	66.1 ± 42.7 (25.1–132.8)	28.0 ± 1.25 (30.3–26.1)	198.41 ± 129.34 (63.6–399.55)	n.d.	n.d.
Sailfish	6	n.d.	n.d.	51.2 ± 6.59 (40.7–60.4)	n.d.	n.d.
Mackerel	6	n.d.	n.d.	54.6 ± 10.9 (37.7–77.1)		n.d.

[a] Calculated assuming that non-detected congener concentrations are equal to half of the limit of detection.
[b] OP: n.d. < 5.4 ng g^{-1}, BP: n.d. < 5.2 ng g^{-1}, NP: n.d. < 8.9 ng g^{-1}, *n*-NP_2EO: n.d. < 8.7 ng g^{-1}, *n*-NP_1EO: n.d. < 8.1 ng g^{-1}.

of 168.10 ± 29.6 ng g^{-1} wet weight. The BP level was highest in fried chicken, at the concentration of 48.0 ± 41.3 ng g^{-1} wet weight. The uncooked chicken had the highest OP level, 66.6 ± 53.0 ng g^{-1} wet weight.

To avoid inaccuracies in the results, use of paper tissues to absorb surface water of the fish or meat samples before weighing was required. There were no significant differences in the concentrations between samples using paper tissues to absorb surface water and samples not using paper tissues.

4. Discussion

The degradations of AP_nEO have led to high bioaccumulation of some low-molecular weight products of these classes of compounds and these have been identified as having endocrine-disrupting potential. Therefore, according to relative oestrogenic potencies of the active compounds, nonylphenol and octylphenol are categorized as definitely endocrine disruptors [20]. NP_nEO is present as the most heavily-used nonionic surfactant. Hence, this work proposes a simple and stable method for pretreating and determining levels of NP, OP, BP, *n*-NP_1EO and *n*-NP_2EO in meat and fish

simultaneously. Previous studies on the analysis of alkylphenol residues in biota involve time-consuming and costly extractions or cleanup steps. Datta *et al.* [18] employed aminopropyl silica to isolate NP and NP_1EO–NP_5EO from fish extracts, but did not detect the concentrations of OP or BP. Schmitz-Afonso *et al.* [22] used ASE apparatus and aminopropyl cartridges to extract NP, NP_1EO–NP_5EO and OP, OP_1EO–OP_5EO from fish tissue, but not BP. Zhao *et al.* [21] used modified matrix solid-phase dispersion (MSPD) to extract NP, OP and $NP_{10}EO$ from fish but not BP, NP_1EO or NP_2EO. This method saved on extraction time and consumed less solvent, but column-packing affected the reproducibility of the elution profile and optimal recovery. Guenther *et al.* [2] utilized steam distillation/solvent extraction and clean-up using HPLC to determine only the NP content, but the time-consuming and complex derivatization involved was uneconomical. The present work, however, uses liquid and solid extraction to extract OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO simultaneously.

The recoveries of OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO from meat and fish samples in this work, were 85.3 ± 3.32, 87.5 ± 6.01, 90.9 ± 4.72, 86.4 ± 4.81 and $90.9 \pm 4.84\%$. Except the recoveries of *n*-NP_2EO, these values were higher than those obtained by Tsuda *et al.* [19], who found recoveries of 77.1–83.2%, 71.0–73.4%, 81.2–84.3%, 90.5–96.2% and 83.5–84.8%, respectively. Additionally, the recovery of NP in this work was more stable than that obtained by Guenther *et al.* [2], who obtained recoveries of 12–126%, indicating that the pretreatment method used herein was more efficient. However, the detection limits of the method used herein for OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO were 5.4, 5.2, 8.9, 8.7 and 8.1 ng g^{-1} wet weight, which were worse than those of Tsuda *et al.* [19], whose method had a detection limit for NP, NP_2EO and NP_1EO of 2 ng g^{-1} and for OP and BP of 1 ng g^{-1}, wet weight. In this work, NP had 100% of detection rate in all of meat and fish samples. In addition, the analytic apparatus, HPLC with a fluorescence detector, is in widespread use. Therefore, although the detection limits of the method were somewhat higher than reported by others, the method of this work was adequate for identification of the concentration of OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO in meat and fish from markets in Taipei city, Taiwan.

The OP, BP, NP, *n*-NP_2EO and *n*-NP_1EO levels of all of the samples in this work were n.d.–152.15 ng g^{-1}, n.d.–124.42 ng g^{-1}, 11.7–399.55 ng g^{-1}, n.d.–916.95 ng g^{-1} and n.d. (wet weight), values that greatly exceeded those of Tsuda *et al.* [19], who determined levels of n.d., n.d., n.d.–19 ng g^{-1}, n.d.–6.2 ng g^{-1} and n.d.–21 ng g^{-1} wet weight in aquatics, respectively. Datta *et al.* [18] investigated NP values of freshwater fish in USA rivers; their values of 119–1842 ng g^{-1} wet weight exceeded those in this work and their values for *n*-NP_2EO and *n*-NP_1EO, 18–567 ng g^{-1} and 87–2075 ng g^{-1} wet weight greatly exceeded those in this work, perhaps because the sediment in the rivers obtained much higher concentrations of *n*-NP_2EO and *n*-NP_1EO. The mean NP concentrations in uncooked chickens in this study were 144.27 ± 81.4 ng g^{-1} wet weight, higher than the corresponding values obtained by Guenther *et al.* [2], of 3.8 ng g^{-1} wet weight. The uncooked chicken and fried chicken may come from different sources, so the OP concentrations were 66.6 ± 53.0 ng g^{-1} and n.d. (wet weight), respectively.

The concentrations of NP in fish samples correlated with fat contents due to the strong lipophilic properties of NP. The mean of crude fat of mullet, salmon and cod was 12.3 g/100 g, the milkfish and tilapia was 7.1 g/100 g, and the sailfish and mackerel

was 6.2 g/100 g [23]. Therefore, the range of NP in mullet, salmon and cod were 63.6–399.55 ng g^{-1} higher than milkfish, tilapia and sailfish, mackerel, the range of NP were 22.7–166.02 ng g^{-1} and 37.7–77.1 ng g^{-1}, respectively. Furthermore, all of the samples were wrapped by plastic packaging materials so that contaminants could migrate into samples [17] except due to the exposure to the growth environment of the samples.

The procedure for pretreating the samples used in this work was modified from that of Tsuda *et al*. [19]. Anhydrous sodium sulfate, used by Tsuda *et al*. to absorb water [19], was not used herein, because the NP concentration in the sodium sulfate was approximately 1 μg g^{-1}, and thus, seriously interfered with the analytic results. The liquid–liquid extraction step was performed 3 times with 30, 15 and 15 mL of acetonitrile saturated with hexane in this work, which replaced 2 times in the method of Tsuda *et al*. [19] to improve the recovery of alkylphenol compounds. The solid extraction performed by Tsuda *et al*. [19] involved the elution of NP, BP and OP with 80 mL of hexane and ethyl ether (9 : 1, v/v) and that of *n*-NP_1EO and *n*-NP_2EO with 40 mL of hexane and acetone (7 : 3, v/v). In this work, the elution solutions were 80 mL of hexane and acetone (9 : 1, v/v) to elute all of the analytes, NP, BP, OP, *n*-NP_1EO and *n*-NP_2EO, simultaneously. The temperature of the water bath was increased to 50°C during concentration and a rotary vacuum evaporator was used, rather than a concentration in a stream of nitrogen, to concentrate the residue to dryness since the concentration of the analytes was not influenced for recovery and could accelerate the time of concentrated process.

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References

[1] C.G. Naylor, J.P. Mieure, W.J. Adams, J.A. Weeks, F.J. Castaldi, L. Ogle, R.R. Romano. *J. Am. Oil Chem. Soc*., **69**, 695 (1992).
[2] K. Guenther, V. Heinke, B. Thiele, E. Kleist, H. Prast, T. Raecker. *Environ. Sci. Technol*., **36**, 1676 (2002).
[3] R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, M.G. Parker. *Endocrinology*, **135**, 175 (1994).
[4] C.Y. Cheng, W.H. Ding. *J. Chromatogr. A*, **968**, 143 (2002).
[5] J. Wilbur. *Int. J. Toxicol*., **18**(Suppl. 1), 11 (1999).
[6] C.G. Naylor, J.P. Mieure, W.J. Adams, J.A. Weeks, F.J. Castaldi, L. Ogle, R.R. Romano. *J. Am. Oil Chem. Soc*., **69**, 695 (1992).
[7] M. Sole, M.J. Lopez de Alda, M. Castillo, C. Porte, K. Ladegaard-Pedersen, D. Barcelo. *Environ. Sci. Technol*., **34**, 5076 (2000).
[8] C. Nerin, C. Fernandez, C. Domeno, J. Salafranca. *J. Agric. Food Chem*., **51**, 5647 (2003).
[9] M. Mutsuga, Y. Kawamura, C. Wakui, T. Maitani. *J. Food Hyg. Soc. Japan*, **44**, 103 (2003).
[10] W.H. Ding, S.H. Tzing. *J. Chromatogr. A*, **824**, 79 (1998).
[11] R. Thibaut, L. Debrauwer, D. Rao, J.P. Cravedi. *Mar. Environ. Res*., **46**, 521 (1998).
[12] V. Jorgen, T. Marianne, C. Lars. *Sci. Total Environ*., **296**, 105 (2002).
[13] P.L. Ferguson, C.R. Iden, B.J. Brownwell. *Environ. Sci. Technol*., **35**, 2428 (2001).
[14] M. Ahel, J. McEvoy, W. Giger. *Environ. Pollut*., **79**, 243 (1993).
[15] S.A. Snyder, T.L. Keith, S.L. Pierens, E.M. Snyder, J.P. Giesy. *Chemosphere*, **44**, 1697 (2001).

[16] L.B. Clark, R.T. Risen, T.G. Hartman, J.B. Louis, I.H. Suffet, R.T. Lippincott, J.D. Rosen. *Int. J. Environ. Anal. Chem.*, **47**, 167 (1993).
[17] J. Gilbert, J.R. Startin, J.D. McGuinness. *Food Addit. Contam.*, **3**, 133 (1986).
[18] S. Datta, J.E. Loyo-Rosales, C.P. Rice. *J. Agric. Food Chem.*, **50**, 1350 (2002).
[19] T. Tsuda, K. Suga, E. Kaneda, M. Ohsuga. *J. Chromatogr. B*, **746**, 305 (2000).
[20] S. Jobling, J.P. Sumpter. *Aquat. Toxicol.*, **27**, 361 (1993).
[21] M. Zhao, F. van der Wielen, P. de Voogt. *J. Chromatogr. A*, **837**, 129 (1999).
[22] I. Schmitz-Afonso, J.E. Loyo-Rosales, M. de la Paz Aviles, B.A. Rattner, C.P. Rice. *J. Chromatogr, A*, **1010**, 25 (2003).
[23] Department of Health, Taiwan. *The nutrition database of foodstuffs in Taiwan* (2002), http://www.doh.gov.tw