Separating DDTs in Edible Animal Fats Using Matrix Solid-Phase Dispersion Extraction with Activated Carbon Filter, Toyobo-KF

Naoto Furusawa
Graduate School of Human Life Science, Osaka City University, Osaka 558-8585, Japan

Abstract

A technique is presented for the economical, routine, and quantitative analysis of contamination by dichloro-diphenyl-trichloroethanes (DDTs) [*pp*'-DDT, *pp*'-dichlorodiphenyl dichloroethylene, and *pp*'-dichlorodiphenyl dichloreothane in beef tallow and chicken fat samples, based on their separation using matrix solid-phase dispersion (MSPD) extraction with Toyobo-KF, an activated carbon fiber. Toyobo-KF is a newly applied MSPD sorbent, and it is followed by reversed-phase high-performance liquid chromatography (HPLC) with a photodiode array detector. The resulting analytical performance parameters [recoveries of spiked DDTs (0.1, 0.2, and 0.4 µg/g) ≥ 81%, with relative standard deviations of ≤ 8% (n = 5), and quantitation limits ≤ 0.03 µg/g], with minimal handling and cost-efficiency, indicate that the present MSPD–HPLC method may be a useful tool for routine monitoring of DDT contamination in meat.

Introduction

pp'-Dichlorodiphenyl trichloroethane (DDT) and its metabolites, *pp*'-dichlorodiphenyl dichloroethylene (DDE) and *pp*'-dichlorodiphenyl dichloreothane (DDD), have especially high lipophilic properties. They are currently viewed with suspicion as primary and important "endocrine-disrupting chemicals" (EDCs) (1), and they are extremely persistent in the environment. The human and environmental pollution caused by EDCs is a global problem in need of a solution. These compounds bioconcentrate in the food chain and are accumulated in animal or marine fats. Persistent DDTs still appear in food-producing animals from environmental contamination of their diet. Human exposure occurs mostly via meat and eatable fish containing a mixture of DDT, DDE, and DDD. To ensure that meat suitable for human consumption is free from contamination by DDTs, the Codex Alimentarius Commission (2) has stated the extraneous maximum residue limit (EMRL) for DDTs in meat (5 µg/g fat). The need for stringent monitoring in meat to guarantee food safety is apparent.

Conventional isolation techniques of contaminating DDTs in edible fats of animal or marine origins (3–11) possess several detriments. The main detriments are that the methods consume a large amount of organic solvents, are labor intensive, time consuming, and expensive. The methods also produce large amounts of waste organic solvents, which is a severe world-wide problem (12–14). The main method of disposal for waste organic solvents (used solvents) is incineration, which has steadily increased over the past 10 years and has become more costly. Matrix solid-phase dispersion (MSPD) extraction, introduced by Barker et al. (15), is a reasonable isolation technique for drug residues in animal tissues. The method has earned a reputation for significantly reducing the analytical time and organic solvent consumption. MSPD has been widely applied to the analysis of veterinary drugs in foods of animal origin (16,17). However, these techniques usually require an expensive MSPD sorbent (C_{18}-silica). The author has reported a successful normal-phase MSPD method with an inexpensive polar MSPD sorbent (acidic aluminum oxide) for the extraction of DDTs in animal fats (18). The redeeming features in traditional MSPD are augmented furthermore by optimizing MSPD conditions for the analyte and sample.

A granular activated carbon has been used as a sorbent for use in column chromatography for the isolation of organochlorine compounds, including DDTs, in various biological samples (19). Solid-phase extraction cartridges, filled with granular-activated carbon-blended silica gel, are commercialized by Wako Pure Chem. Ltd. (Osaka, Japan).

In this study, a novel activated carbon filter, the Toyobo KF (KF), was used as the MSPD sorbent. KF, a novel activated carbon fiber, was first developed by Toyobo (Osaka, Japan). KF possesses the physical and chemical properties described as follows (20): (*i*) the surface area is extremely large, ranging from 1,000 to 1,600 m^2/g, but it is characterized by a micropore structure with an extremely small diameter of 0.5–10 nm, which results in the high speed of adsorption, ranging from 10 to 100 times that of conventional granular activated carbon; (*ii*) the capacity for adsorption is greater than conventional granular-activated carbon; (*iii*) the desorption time is short compared with conven-

tional granular-activated carbon; (*iv*) KF is flexible and has excellent malleability, resulting in good functionality, for example, packing into a column and mixing with samples, such as meats, animal fats, and other materials.

KF is competitively priced (5.7 cents US/g as of 14 March, 2005). Because of its unique properties, it was expected that the application of MSPD with KF for the isolation of DDTs in animal fats could further reduce the operation time, amount of sorbent, KF, and eluent used, resulting in cost saving.

The aim of this study is the development of a rapid and inexpensive method for the routine monitoring of contamination by DDTs in animal fats using a new MSPD with KF as the sorbent followed by high-performance liquid chromatography (HPLC).

Experimental

Materials and reagents

Standards of DDTs, DDT, DDE, and DDD, and other chemicals were obtained from Wako Pure Chemical (Osaka, Japan). Distilled water, ethanol, *n*-heptane, and *n*-hexane were of HPLC grade (Wako). Other organic solvents and anhydrous sodium sulfate were of pesticide residue grade (Wako). Separate stock standard solutions (50 µg/mL for a target compound) were prepared by accurately weighing DDT, DDE, and DDD (5 mg) and dissolving them in heptane (100 mL). As working standard solutions, the mixed solutions of concentrations from 2 to 12.5 µg/mL of each target compound were prepared in heptane. KF-1500, as an activated carbon fiber, was provided kindly by Toyobo Co., Ltd. (Osaka, Japan). The KF was prewashed with heptane and heated at 140°C for 3 h. A 0.45-µm disposable syringe filter unit (hydrophilic cellulose acetate membrane) was obtained from Advantec (Toyo Roshi Co. Ltd., Tokyo, Japan). Beef tallow and chicken fat samples were premelted at 40–50°C and used for blank samples.

Procedure

An accurately weighted 0.5-g sample was mixed sufficiently with 0.2 g of KF-1500 and 2 g of anhydrous sodium sulfate in a porcelain mortar to obtain a homogeneous material. The mixture was transferred to a 20-mL column (syringe barrel) and preplugged with a filter disc. After the column was washed with 20 mL of heptane, the target compounds were eluted with 20 mL of ethyl ether–heptane (6:4, v/v) (flow-rate < 5 mL/min). The eluate was evaporated to dryness, and the residue was dissolved in 1 mL of the HPLC mobile phase. The solution was filtered through a 0.45-µm filter unit, and the filtrate was injected into the HPLC system.

HPLC

The HPLC system included a model PU-980 pump and a DG-980-50 degasser (Jasco Corp., Tokyo, Japan) equipped with a model CO-8010 column oven (Tosoh Corp., Tokyo, Japan) and a model SPD-M10Avp photodiode array (PDA) detector (Shimadzu, Kyoto, Japan).

The operating conditions were as follows: analytical column, a YMC-Pack TMS (75 × 4.6-mm i.d., 5 µm, packed C_1 = methyl-silica, YMC, Kyoto, Japan) equipped with a guard column (5 × 4.6 mm) containing the same packing material. Other conditions were: isocratic mobile phase, 51% (v/v) aqueous ethanol solution; pump flowrate, 1.0 mL/min; column temperature, 40°C; injection volume, 20 µL; and analysis time, < 12 min. The results of the absorption spectra of DDT, DDE, and DDD standard solutions were measured by a PDA detector. The observed maximum absorptions were: DDT, 236 nm; DDE, 245 nm; and DDD, 231 nm. The monitoring wavelengths were adjusted to 231–245 nm. The peak identification of DDT, DDE, and DDD was established by comparing the retention times and their absorption spectra in real samples with those obtained by injection of the standard.

Recovery test

The recoveries and their relative standard deviations (RSDs) from blank animal fat samples spiked at 0.1, 0.2, and 0.4 µg/g, respectively, were determined for DDT, DDE, and DDD. These fortification concentrations were prepared by adding 50 µL of five mixed standard solutions (4, 8, and 16 µg/mL, respectively) to a 2.0 g portion of the sample previously melted at 40–50°C. Fortified samples were fully mixed prior to the test.

Results and Discussion

HPLC operating conditions

The short packed-C_1 column selected for this study was a highly purified silica-based reversed-phase (nonpolar) column. The medium was end-capped in order to reduce polar secondary interactions associated with surface silanol groups. The C_1 should remarkably reduce the volume of elution of organic solvents as the mobile phase required and provide a more rapid separation.

A YMC-Pack TMS, packed-C_1 and short (length, 75 mm), column with an ethanol solution as the mobile phase was examined. A chromatogram, with complete separation of target compounds and clear and short retention time, was obtained by using the column and an isocratic mobile phase of a 51% (v/v) ethanol solution. Without the gradient system to improve the separation, simple, and isocratic reversed-phase HPLC conditions enabled rapid and complete separation of DDT, DDE, and DDD in animal fat samples as illustrated in Figure 1A. The target compounds were successfully detected within 12 min (Figure 1A) when the flow-rate was 1.0 mL/min at 40°C. There was no requirement of precolumn washing immediately after a sample analysis to remove the interfering compounds of animal fat sample origin.

The chromatographic repeatability was determined using a spiked (0.4 µg/g of each compound) beef tallow sample obtained from the present MSPD with KF method. The repeatability was found from the RSDs of areas and retention times calculated for 10 replicate injections of the spiked sample. The repeatabilities for DDT, DDE, and DDD were 0.08–0.10% for area and 0.50–0.58% for the retention time, respectively. Similar results were obtained using a spiked chicken fat sample.

MSPD with KF-1500

The present method was developed on the basis of experience

gained in use of the normal-phase solid-phase extraction (SPE) or MSPD for the isolation of organochlorine compounds in egg yolk fat or animal fat samples (18,21).

First, the retention profiles of DDT, DDE, and DDD from the present MSPD with KF column, when several nonpolar solvents (diethyl ether, hexane, and heptane) that are used frequently in the analysis of drug residue, were used as the washing eluent. Their volumes were standardized at 20 mL. In a recent paper, the ability of normal-phase MSPD, with acidic aluminum oxide (AAO) as the sorbent (AAO-MSPD), to selectively retain DDTs in heptane was shown. In comparison with other solvents, the KF showed a highly selective retention of DDTs in heptane. Because the target compounds were not eluted with heptane from the MSPD with KF column, heptane was used as the washing eluent to eliminate fats of sample origin. Prewashing of the MSPD with KF column using heptane as a prior eluate was, therefore, introduced into the procedure. This provided the isolation of fat constitutes, except for DDTs, from the column.

The possibility of eluting DDTs from this new MSPD method by other solvents was evaluated. The sorbent, KF, was de-retained by adding ethyl acetate to the eluent. The effect of the concentration of ethyl acetate in the eluent (ethyl acetate–heptane, v/v) on the recoveries of DDTs from the MSPD with KF column was then determined, and the results are given in Table I. DDT, DDE, and DDD were satisfactorily eluted (the recoveries of DDTs ≥ 80%, Table I) with ethyl acetate–heptane (60:40, v/v). The eluting curves of DDT, DDE, and DDD from the MSPD with KF column using ethyl acetate–heptane (60:40, v/v) as the eluate are presented in Table II. In this study, the target compounds in 5 mL of each collected fraction were determined by HPLC. All compounds were eluted with an elution volume of 20 mL. The average recoveries (n = 3) of DDT, DDE, and DDD were 85%, 86%, and 81%, respectively, under these conditions. No increase in DDT recoveries was obtained by increasing the elution volume (> 20 mL).

In the MSPD with KF preparation, the sample and KF were mixed with anhydrous sodium sulfate to remove the moisture in the sample completely, allowing the matrix–sorbent (i.e., sample KF) mixture to become uniform readily and easily to pack into the column for MSPD. The fat sample is dispersed over an extremely large surface area (0.2–0.32 m^2 per 0.2 g of KF), with a micropore structure that has an extremely small diameter (0.5–10 nm), exposing the entire sample to the isolation operation. The surface areas (0.2–0.32 m^2 per 0.2 g of KF) used as the present MSPD sorbent is 2–4 times larger when compared with those of the previous MSPD sorbents [such as C_{18} (15–17) and aluminum oxide (18)], suggesting a more efficient dispersion of the sample entirety to KF, which results in sorbent saving.

The MSPD with KF procedure had a minimal number of steps, used a tiny amount of the KF (0.2 g), and had low solvent consumption (total 40 mL/sample: heptane and ethyl acetate used as the washing eluent and eluate). Based on MSPD with the KF procedure, transfer of the sample KF mixture, dispersion of the fat sample onto the KF, washing and eluting operation, selective

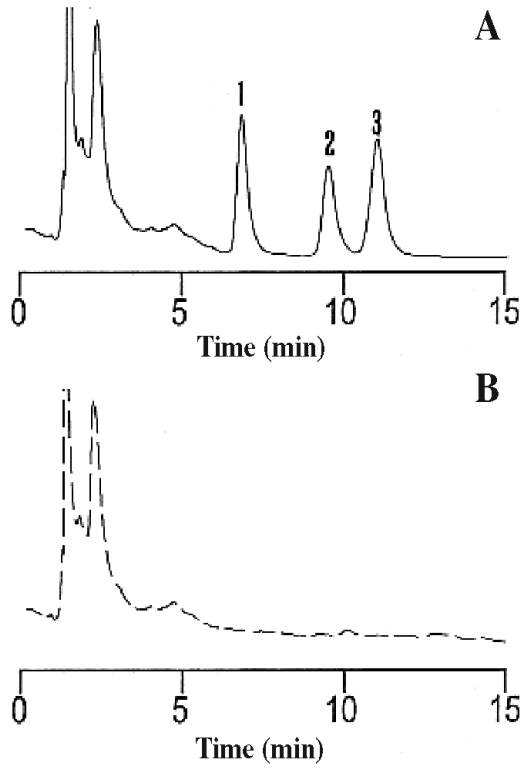

Figure 1. HPLC chromatograms obtained from beef tallow samples (PDA detector set at 232 nm): spiked (0.4 µg/g of each compound) beef tallow sample (A); blank beef tallow sample (B). Peaks numbers: DDD (Retention time (t_R) = 7.0 min), 1; DDT (t_R= 9.0 min), 2; DDE (t_R = 11.0 min), 3.

Table I. Effect of the Ethyl Acetate Concentration in the Eluent on the Recoveries of DDTs from MSPD with KF for DDT-Fortified Animal Fat Samples*

	% Recovery ethyl acetate—heptane (v/v)							
Compound	10:90	20:80	30:70	40:60	50:50	60:40	70:30	80:20
DDT	32	73	75	81	84	86	84	83
DDE	28	29	35	56	68	85	85	84
DDD	21	56	62	80	82	80	78	80

* Data are averages (n = 3). A 0.5 g spiked beef tallow sample (0.4 µg/g for each compound) was applied to the present MSPD with KF system. The volume of the eluent was standardized at 20 mL.

Table II. Elution Volumes of DDTs from MSPD with KF for DDT-Fortified Animal Fat Samples*

		%Recovery		
Fraction	Volume (mL)	DDT	DDE	DDD
1st	5	10	22	25
2nd	5	53	42	40
3rd	5	14	15	11
4th	5	6	6	5
Total	**20**	**85**	**86**	**81**

* Data are averages (n = 5). A 0.5 g spiked beef tallow sample (0.4 µg/g for each compound) was applied to the present MSPD with KF column.

elution of the nonpolar lipid constitutes with heptane, and the subsequent elution of DDTs with ethyl acetate–heptane (60:40, v/v) were easy and smooth.

The described findings result in a rapid and simple method, with considerable savings in analytical cost as well as extracts free from interferences for detection and identification as displayed in Figures 1A (blank beef tallow) and 1B(DDTs-fortified beef tallow). Similar HPLC traces were obtained from chicken fat samples.

In order to evaluate the defatting efficiency of the present procedure here, the residual sample amount after the MSPD with KF treatment followed by evaporation was measured as described previously (22). The obtained efficiency was compared with those of other MSPD (18) and SPE (19) techniques. The MSPD or SPE procedure (18) were as follows.

For MSPD, the sample was mixed with 2 g of acidic aluminum oxide and 1 g of anhydrous sodium sulfate. The mixture was packed into a syringe barrel. DDTs were directly eluted with 15 mL of heptane without the prewashing.

For SPE, the sample was dissolved in 1 mL of hexane and was applied to an ISOTUTE-NH_2(aminopropyl) SPE column (500-mg sorbent mass, 3-mL reservoir volume, Internal Sorbent Technology, Hengoed, Mid Glamorgan, UK). The SPE column was preconditioned by washing it with 3 mL of hexane. DDTs were eluted with 3 mL of diethyl ether–hexane (5:95, v/v) (flow-rate < 3 mL/min).

In this comparative study, beef tallow samples of 0.2 g were used. These results are given in Table III. For the present MSPD with KF method, the average amount of residual fat constituents was 1.96 mg, which is equal to 0.98% of the beef tallow sample (0.2 g) applied. Similar findings were obtained from the chicken fat sample. Although the sorbent amount in the MSPD with KF was half of those in the previous MSPD and SPE, the residual sample amount of the present MSPD with KF is significantly lower than those of the previous MSPD and SPE methods (Table III). The finding indicates that the present MSPD with KF method possesses the ability to be refined. There was no requirement for purify from the sample extract obtained from the MSPD with KF to HPLC, which minimized sample preparation time and eliminated potential sample preparation artifacts.

The present procedure enabled the simple, rapid, economical, and reliable determination and identification of DDTs by HPLC with PDA detection. The total time, solvent consumption, and budget required for the analysis of one sample were: less than 45 min, less than 50 mL (ethyl acetate, heptane, and ethanol), and roughly $1.72 US funds (1.28 and ¥180 as of 14 March, 2005), respectively.

Table III. Comparison for Defatting Abilities after the MSPD and SPE Treatments Using Animal Fat Samples*

	Sorbent (amount, g)	Defatting ability: Residual amount (mg, mean ± SD, *n* = 3)	Defatting ability: Defatting rate† (%, mean)
MSPD with KF	KF (0.2 g)	1.96 ± 0.08**	99.0
MSPD with ALa§	Acidic aluminum oxide (2 g)	5.60 ± 0.11**	97.2
SPE with NH_2**	Aminoppropyl (0.5 g)	9.10 ± 0.54**	95.5

* A 0.2-g beef tallow sample was applied to the present MSPD with KF system.
† 100-(Residual amount/sample amount used) × 100.
‡ There are significant differences among opposite signs ($p < 0.01$).
§ See the literature (18).
** See the literature (21).

Method qualification

The analytical performance parameters assessed for the complete procedure were linearity, accuracy, precision, sensitivity, and selectivity for each compound and animal fat sample. Blank animal fat samples (known drug-free) used in these examinations were spiked with standard solutions of the target compound at 0.05, 0.1, 0.4, 1.0, or 5.0 µg/g, respectively. The results obtained are summarized in Table IV.

A calibration curve was generated by plotting the peak area of the spiked sample extracts ranging from 0.05–5.0 µg/g. The line was constructed from five points, and each point represented the mean of five injections into the present HPLC system. The resulting correlation coefficients were greater than or equal to 0.997 ($P < 0.01$), demonstrating significant linearity for the examined lines.

The average recoveries from samples at three different spiking levels (0.1, 0.2, and 0.4 µg/g) were 81–85%, with RSDs of 4–8%. These values are well within the "acceptable criteria" for residue analysis of the Codex setup. The recommended criteria are as follows: average recoveries of 80–110% with RSDs less than 15% when the EMRL for the analyte is greater than or equal to 0.1 µg/g and recoveries of 70–110% with RSDs less than 20% when the EMRL is 0.01–0.1 µg/g (23).

The quantitation limits (QLs) for DDT, DDE, and DDD were calculated by measuring the analytical background response.

Table IV. Accuracy, Precision, Linearity, and Sensitivity Data

Spiked (µg/g)	%Recovery* DDT	DDE	DDD
Beef tallow			
0.1	84 (6)	82 (8)	82 (7)
0.2	82 (8)	83 (6)	84 (5)
0.4	85 (6)	85 (6)	81 (8)
r†	0.999	0.998	0.998
QL (µg/g)‡	0.02	0.03	0.025
Chicken fat			
0.1	83 (5)	82 (7)	81 (8)
0.2	81 (7)	83 (6)	82 (6)
0.4	84 (5)	85 (4)	82 (6)
r	0.999	0.997	0.998
QL	0.02	0.03	0.025

* Data are average recoveries and their RSDs ($n = 5$) in parentheses.
† *r* is the correlation coefficient. Mean from three determinations using spiked samples for calibration curves, ranging from 0.05 to 5 µg/g.
‡ QL as the concentration of analyte giving a signal-to-noise ratio > 10.

Table V. Average DDT, DDE, and DDD Concentrations in the Real Adipose Tissues of Laying Hens Who Had Been Treated with DDT

	Concentrations (µg/g) (*n* = 3)		
	DDT	DDE	DDD
Present method	0.91	0.33	nd*
Approved method –1†	0.93	0.34	nd
–2‡	0.93	0.35	nd

* nd = not detected (< 0.025 µg/g).
† See the literature (17) (QL for DDD = 0.05 µg/g).
‡ See the literature (19) (QL for DDD = 0.06 µg/g).

The QL was defined as 10 standard deviation (SD) obtained by replicate analysis at a subsequently low spiked concentration (0.1 µg/g). Five different spiked blank animal fat samples known to be near the QL were analyzed in duplicate. The QLs in beef tallow and chicken fat samples were less than or equal to 0.03 µg/g, which was far below the tolerance or EMRL of 5 µg/g (Table IV).

The PDA detector chosen provides an easy means of confirming peak identity, and it enables the separation and identification of target compound in the sample by the retention time and its spectrum. The "identification criteria" of the EU regulation decision 2002/657/EC for residue analysis in animal products (24) for the full scan UV–vis detection were accomplished for all animal fat samples. The DDTs examined could be identified in the beef tallow and chicken fat samples by their retention times and absorption spectra. The DDT, DDE, and DDD spectra obtained from the beef tallow and chicken fat samples were practically identical with those of the standards. The present MSPD with KF technique allowed a reliable confirmation.

"Real" samples

In order to prove the validity of the present method for routine monitoring, adipose tissues with residual DDTs from three White Leghorn laying hens (aged 30 weeks, and weighing 1.4–1.9 kg), which were treated with a single oral dose of DDT (1 mg/kg body weight) were used as "real" samples. The dose was absorbed onto a small amount of the layer diet and delivered orally, enclosed in a gelatin capsule. All hens were sacrificed by decaptitation at the third day after dosing, and adipose tissues were quickly collected. These samples were used as the real chicken fat.

The quantitative analyses for DDTs in the real samples were performed by the present method and also by the approved methods, respectively. The obtained concentrations of DDTs were summarized in Table V. There are no significant differences among the data of the four methods. Table V demonstrates that the present method is valid for routine monitoring (practical usage).

Monitoring contamination in marketed adipose

Thirty-three different beef tallow or chicken fat samples that were available in Osaka and Ibaraki, Japan were analyzed by the present method. No samples contained detectable concentrations of DDT, DDE, and DDD. The resulting chromatograms were free from interference.

Conclusion

In the present study, KF-1500, an activated carbon fiber, was applied as the MSPD sorbent for the determination of residual chemicals in foods. The proposed MSPD with KF method followed by HPLC is a useful tool for routine residue monitoring of DDT, DDE, and DDD in beef tallow and chicken fat for the following reasons: using MSPD with KF makes easy, rapid, smooth, effective, and economical sample preparation for the target compounds possible. The procedure has a total analytical time of less than 45 min/sample and solvent consumption of less than 50 mL/sample.

Acknowledgments

The author is grateful to AC Operations Department Toyobo for providing KF-1500 activated carbon fiber.

References

1. U.S. Environmental Protection Agency. Special report on environmental endocrine disruption. US EPA, Washington, DC, 1997.
2. Codex Alimentarius Commission. Commission decision of 12 August 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. commun.* **17(8):** L221 (2002).
3. *Official Methods of Analysis,* Section 970.520, 15th eds. AOAC International, Arlington, VA, (1990).
4. *Pesticide Analytical Manual*, Vol. I, US Food and Drug Administration, Washington, DC, 1991.
5. *Standard Methods of Analysis for Hygienic Chemists—With Commentary,* 2nd ed. Pharmaceutical Society of Japan, Tokyo, Japan, 1990.
6. B.G. Luke, J.C. Richards, and E.F. Dawes. Recent advances in cleanup of fats by sweep co-distillation. 1. Organochlorine residues. *J.—Assoc. Off. Anal. Chem.* **67:** 295–98 (1984).
7. R.L. Brown, C.N. Farmer, and R.G. Millar. Optimization of sweep codistillation apparatus for determination of coumaphos and other organophosphorus pesticide-residues in animal fat. *J.—Assoc. Off. Anal. Chem.* **70:** 442–45 (1987).
8. Codex Alimentarius Commission. "Recommendations for methods of analysis of pesticide residues", Document CAC/PR8-1989. Food and Agriculture Organization, Roma, Italy, 1989.
9. M. Miyahara, M. Murayama, T. Suzuki, and Y. Saito. Silica-gel chromatographic cleanup procedure for organochlorinedpesticide analysis with capillary gas-chromatography. *J. Agr. Food Chem.* **41:** 221–26 (1993).
10. J.R. Patterson. Reducing solvent consumption in automated gel-permeation chromatographic cleanup for pesticide-residue analysis—a modified GPC autoprep 1002. *J.—Assoc. Off. Anal. Chem.* **74:** 1016–18 (1991).
11. P. Armishaw and R.G. Millar. Comparison of gel-permeation chromatography, sweep codistillation, and florisil column adsorption chromatography as sample cleanup techniques for the determination

of organochlorine pesticide-residues in animal fats. *J. AOAC Int.* **76:** 1317–22 (1993).
12. M. Ogawa. The selection of organic solvents for food analyses that considered influence to the environment. *J. Food Hyg. Soc. Jpn.* **37:** J289–90 (1996).
13. R. Malish, B. Bourgeois, and R. Lippold. Multiresidue analysis of selected chemotherapeutics and antiparasitics. *Dtsch. Lebensm-Rundsch.* **88:** 205–16 (1992).
14. P.T. Anastas and J.C. Warner. *Green Chemistry: Theory and Practice*, P.T. Anastas and J.C. Warner, eds. Oxford University Press, Oxford, UK, 1998.
15. B.A. Barker, A.R. Long, and C.R. Short. Isolation of drug residues from tissues by solid phase dispersion. *J. Chromatogr.* **475:** 353–62 (1989).
16. A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short, and S.A. Barker. Matrix solid-phase dispersion isolation and liquid chromatographic determination of oxytetracycline in catfish muscle tissues. *J.—Assoc. Off. Anal. Chem.* **73:** 864–67 (1990).
17. S. Le Boulaire, J.-C. Bauduret, and F. Andre. Veterinary drug residues survery in meat: an HPLC method with a matrix solid phase dispersion extraction. *J. Agri. Food Chem.* **45:** 2134–42 (1997).
18. N. Furusawa. A toxic reagent-free method for normal-phase matrix solid-phase dispersion extraction and reversed-phase liquid chromatographic determination of aldrin, dieldrin, and DDTs in animal fats. *Anal. Bioanal. Chem.* **378:** 2004–2007 (2004).
19. B. Jansson, R. Andersson, L. Asplund, A. Bergman, K. Litzèn, K. Nylund, L. Reutergårdh, U. Sellström, B. Uvemo, C. Wahlberg, and H. Wideqvist. Multiresidue method for the gas chromatographic analysis of some polychlorinated and polybrominated pollutants in biological samples. *Fresenius' J. Anal. Chem.* **340:** 439–45 (1991).
20. http://www.toyobo.co.jp/e. (Date Accessed 12/23/05)
21. N. Furusawa. Normal-phase high performance liquid chromatographic determination and identification of aldrin, dieldrin, and DDTs in eggs. *J. Chromatogr. Sci.* **39:** 183–87 (2001).
22. G. Rouser, G. Kritchevsky, and A. Yamamoto. *Lipid Chromatographic Analysis*, 2nd Ed., G. Rouser, G. Kritchevsky, and A. Yamamoto, eds. Marcel Dekker Press, New York, NY, 1976.
23. Codex Alimentarius Commission. Joint FAO/WHO food standards program. *Residues of Veterinary Drugs in Food*. vol. 3, 2nd ed. Codex Alimentarius Commission, Roma, Italy, 1993.
24. EC Decision 2002/657. *Off. J. Eur. Commun.* L221/8, 2002.

Manuscript received March 21, 2005;
revision received April 28, 2006.