Normal-Phase High-Performance Liquid Chromatographic Determination and Identification of Aldrin, Dieldrin, and DDTs in Eggs

Naoto Furusawa

Faculty of Human Life Science, Osaka City University, Osaka 558-8585, Japan

Abstract

A method for the routine monitoring of residual aldrin, dieldrin, pp'-DDT, *op*'-DDT, *pp*'-DDE, and *pp*'-DDD in eggs by high-performance liquid chromatography (HPLC) with a photo-diode array (PDA) detector is described. The lipids extracted from a whole egg are cleaned by a solid-phase extraction (SPE) ISOLUTE NH_2 column with a diethyl ether–hexane (5:95, v/v) eluent. The HPLC separation is carried out using a normal-phase (LiChrosorb NH_2) column, a heptane–hexane eluent (97:3, v/v), and a PDA detector. The average recoveries from fortified target compounds (0.1, 0.2, 0.3, and 0.4 µg/g, respectively) are ≥ 83%, with standard deviations (SDs) between 2 and 5%. The interassay variabilities and their SDs are ≤ 4.8% and ≤ 0.7%, with intra-assay variabilities of 2.1–3.3%. The limits of determination for these compounds range from 0.04 to 0.08 µg/g.

Introduction

Aldrin (AD), dieldrin (DD), the DDTs *op*'-DDT and *pp*'-DDT, and DDT's major metabolites *pp*'-DDE and *pp*'-DDD (Figure 1) have been receiving much attention as "endocrine-disrupting chemicals" (1–3) and carcinogenic substances (4), especially their high-lipophilic properties. A main problem concerns the persistence of these compounds in the fatty part of the food chain; therefore, there is a possible risk to human beings through various foods.

Eggs are an important food because they are highly nutritious, cheap, and readily available. They are consumed by most children and adults every day. In order to ensure the safety of food for consumers, the Food and Nutrition Division of the Food and Agriculture Organization of the United Nations (FAO) (Codex Alimentarius Commission, 1997) have set up the Extraneous Maximum Residue Limit (EMRL) of AD, DD, and DDT at 0.1 µg/g in eggs (whole egg). Analytical methods for the routine residue monitoring of these compounds should be rigorous and economical in regards to time and cost in order to permit the monitoring of large numbers of samples and the capability of detecting the residues below the EMRL, hence the need for better analytical techniques.

For drug residue analyses in eggs (including abundant fats and proteins) the difficulties are caused by the formation of an emulsion that hinders the recovery, thus interfering with coextracts when target compounds are isolated (which require more effective clean-up techniques). For the purpose of using a relatively rapid and simple clean-up technique to replace the conventional liquid–liquid partition followed by Florisil (a normal-phase type) macrocolumn chromatography (5,6), commercial solid-phase extraction (SPE) cartridges and columns have been applied to the clean-up of DD or DDTs in various foods of animal origin (7–10). This technique has substantially reduced analysis time and organic solvent consumption.

The concentrations of organochlorine pesticides (OCPs) including AD, DD, and DDTs have been classically detected by gas chromatography (GC) with electron-capture detection (ECD) (5–10). However, there are disadvantages with the GC determination. A longer determining time occurs, and the presence of poly-

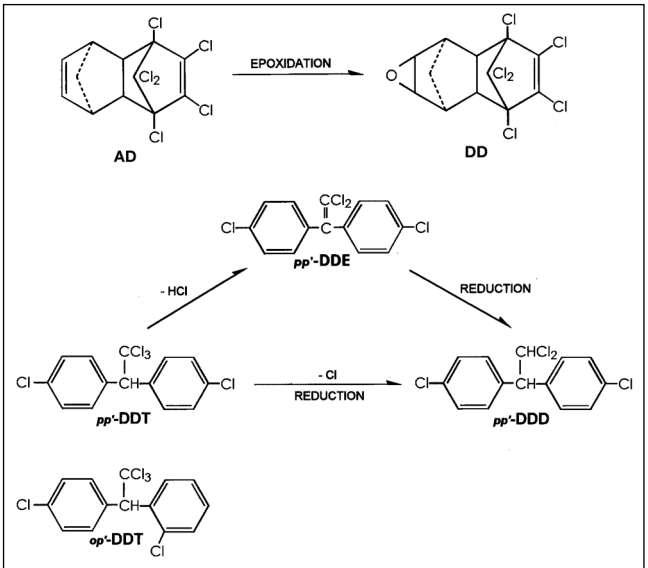

Figure 1. Structures and biotransformations of AD and DDTs.

merized compounds or low volatility leads to column and detector contamination (12). The total time required for the measurement of one sample was 1–2 h (i.e., run time plus cooling time; that is, a return to the initial temperature). In order to prevent the problems with GC–ECD, high-performance liquid chromatography (HPLC) has recently been used to determine OCP levels in water and food samples (11,12), except for eggs.

This study describes the optimization of normal-phase SPE columns for the clean-up of AD, DD, DDT, *op*'-DDT, DDE, and DDD in eggs and a quantitative HPLC method for the target compounds.

Experimental

Materials

AD, DD, *pp*'-DDT, *op*'-DDT, *pp*'-DDE, and *pp*'-DDD were obtained from Wako Pure Chemicals International (Osaka, Japan). Other chemicals were also obtained from Wako. Acetonitrile, diethyl ether, and anhydrous sodium sulfate were of pesticide residue grade. Distilled water, *n*-heptane, and *n*-hexane were of HPLC grade. A stock standard solution containing 100 µg/mL (in heptane) was prepared for each target compound. Working mixed standard solutions containing 5, 10, 20, and 40 µg/mL of each compound were prepared in the HPLC eluent from the stock standard solutions. The following apparatuses were used in the sample preparation: an autohomogenizer (Model PH-91-1, Mitsui Denki-Seiki, SMT Company, Chiba, Japan), a microcentrifuge (Model H-103N, Kokusan Enshinki Co., Tokyo, Japan), a vacuum rotary evaporator (Model EYELA N-1M, Tokyo Rika-kikai Co., Tokyo, Japan), a funnel formed with a fitted disc (Buchner type RYREX 11G3, Iwaki Glass, Funabashi, Japan), and a 0.5-µm disposable syringe filter unit equipped with a polypropylene membrane (Iwaki Glass). Used as SPE columns were nine polar sorbent types from the ISOLUTE series (500-mg sorbent mass, 3-mL reservoir volume). ISOLUTE-ALa (alumina acidic), ISOLUTE-ALn (alumina neutral), ISOLUTE-ALb (alumina basic), ISOLUTE-FL (Florisil), ISOLUTE-NH_2 (aminopropyl), ISOLUTE-DIOL (2,3-dihydroxypropoxypropyl), ISOLUTE-CN (cyanopropyl), ISOLUTE-PSA (ethylenediamine-*N*-propyl), and ISOLUTE-SI (silica) were obtained from International Sorbent Technology (Hengoed, Mid Glamorgan, U.K.). These columns were preconditioned by washing them with 3 mL of hexane.

HPLC analysis

HPLC analysis of the target compounds was conducted using a Jasco (Tokyo, Japan) HPLC (Model PU-980 pump and DG-980-50 degasser) equipped with an SPD-M10AVP photo-diode array (PDA) detector (Shimadzu, Kyoto, Japan) interfaced with a Fujitsu (Tokyo, Japan) FMV-5133D7 personal computer. The separation was performed on a LiChrosorb NH_2 (7 µm) column (250- × 4.6-mm i.d.) (Kanto Chemical Co., Tokyo, Japan) equipped with a guard column (5 × 4.6 mm) containing the same packing material using a heptane–hexane mixture (97:3) as the eluent at a flow rate of 1.0 mL/min at ambient temperature. The injection volume was 20 µL.

Sample preparation

Chicken eggs that were removed from their shells were homogenized and used as the egg samples.

First, lipid in the egg sample was extracted by using the procedure of Furusawa et al. (13). Namely, an accurately weighed 1.0-g amount of egg sample was mixed with sufficient amounts of anhydrous sodium sulfate. Afterwards, the mixture was homogenized in 10 mL of acetonitrile and 20 mL hexane (saturated with acetonitrile) with an autohomogenizer for 2 min. After centrifugation at 3500 rpm for 5 min the supernatant was put into a separating funnel through a funnel packed with anhydrous sodium sulfate. The dried hexane layer was collected and then evaporated to dryness, and the egg lipid was obtained.

The lipid extract was dissolved in 1 mL of hexane and was applied to an ISOLUTE-NH_2 SPE column. AD, DD, and DDTs were eluted with 3 mL of diethyl ether–hexane (5:95) (flow rate < 3 mL/min). The eluate was evaporated to dryness and the residue was dissolved in 1 mL of the HPLC eluent. The solution was filtered through a 0.5-µm filter unit. A 20-µL volume of the filtrate was injected into the HPLC system.

Recovery test

The recoveries of AD, DD, DDT, *op*'-DDT, DDE, and DDD from

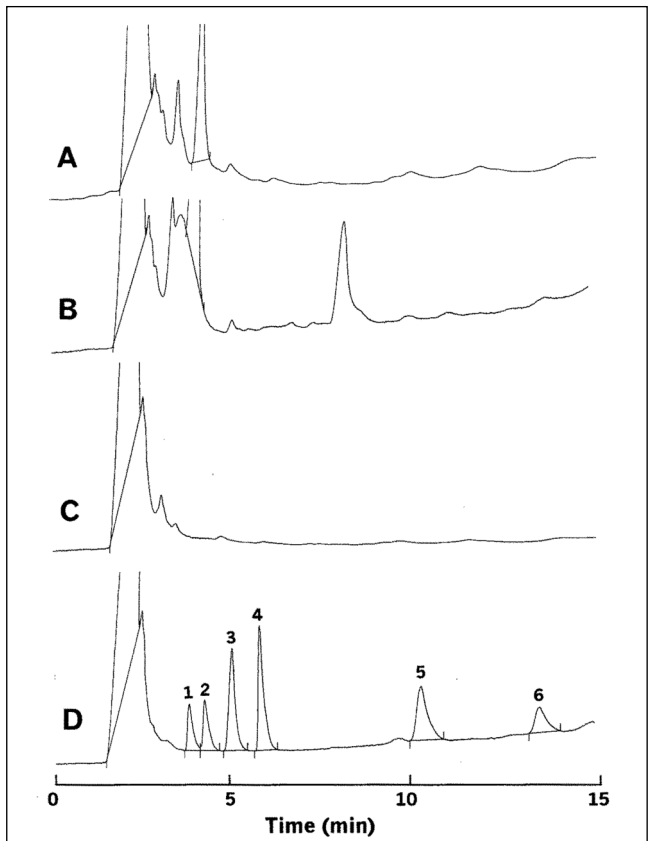

Figure 2. HPLC chromatograms obtained from egg samples (PDA detector set at 210 nm): (A) blank egg sample treated by an ISOLUTE-DIOL, (B) blank egg sample treated by an ISOLUTE-FL, (C) blank egg sample treated by an ISOLUTE-NH_2, and (D) spiked egg sample (0.4 µg/g of each compound) treated by an ISOLUTE-NH_2. The peaks obtained were (1) AD with a 3.7-min retention time, (2) DDE with a 4.2-min retention time, (3) *op*'-DDT with a 5.1-min retention time, (4) DDT with a 5.8-min retention time, (5) DDD with a 10.4-min retention time, and (6) DD with a 13.6-min retention time.

blank samples spiked at 0.1, 0.2, 0.3, and 0.4 µg/g were determined. These fortification concentrations were prepared by adding 10 µL of four mixed standard solutions of these compounds (10, 20, 30, and 40 µg/mL, respectively) to a 1.0-g portion of the sample. Fortified samples were mixed prior to the test. In the test, relative standard deviations (RSDs) determined for each spiked concentration were then averaged resulting in a mean ± the standard deviation (SD). This was defined as interassay variability. Intra-assay variability was defined as the RSD for the mean of five replicates of the same sample and represents the variability associated with the analytical procedure used.

Results and Discussion

The focus of this study was to establish a rapid and simple determining/identifying procedure for AD, DD, and DDTs in eggs. Therefore, samples were cleaned by a commercial SPE column. The extracts containing the target compounds were determined by HPLC equipped with a PDA detector.

HPLC and sample preparation

The absorption spectra of AD, DD, DDT, *op*'-DDT, DDE, and DDD standard solutions were measured by a PDA detector. The obtained maximum absorptions were 210 nm for AD, 214 nm for DD, 203 nm for DDT, 205 nm for *op*'-DDT, 222 nm for DDE, and 207 nm for DDD. The monitoring wavelength for the target compounds was adjusted to 210 nm, which was an average maximum for all of the compounds.

Grice et al. (12) previously reported that the three compounds AD, DD, and DDT extracted from medicinal plants could be determined by HPLC using a reversed-phase ODS column and an acetonitrile–water mixture as the eluent. In preliminary experiments when the similar HPLC conditions were used, AD and DDE could be separated, but not DDT and *op*'-DDT. The present study therefore tried to separate the six compounds (AD, DD, DDT, *op*'-DDT, DDE, and DDD) using a normal-phase NH_2 column and a combination of heptane and hexane as the eluent. The best separation of the six compounds was obtained with the NH_2 column and heptane–hexane (97:3) as the eluent. The target compounds were successfully detected within 14 min (Figure 2D) when the flow rate was 1.0 mL/min.

Using the mixed standard solutions, the minimum detectable drug levels (signal-to-noise ratio greater than 3) under the established conditions were 0.01 µg/mL (0.2 ng) for *pp*'-DDT and *op*'-DDT; 0.02 µg/mL (0.4 ng) for AD, DDD, and DDE; and 0.03 µg/mL (0.6 ng) for DD. The precision of the HPLC procedure was obtained from the RSD of areas calculated for ten 0.2-µg/mL replicate injections (4 ng) of each target compound. Values between 0.8 and 1.3% were obtained.

Previous studies have indicated that a normal-phase SPE Alumina or Florisil cartridge is effective in terms of retaining the interfering compounds that remain in extracts from a cheese and animal diet (10,14). In this study, nine ISOLUTE columns packing normal-phase materials were used for the SPE column. The retention and elution of the target compounds in these columns were compared. A mixture of diethyl ether and hexane (10:90, v/v) was used as the eluent and all the SPE columns were prewashed by hexane.

Table I summarizes the recovery profiles of AD, DD, and DDTs from ISOLUTE SPE columns when the elution volume was standardized at 3 mL. ISOLUTE-DIOL, -FL, and -NH_2 gave good recoveries (> 80%) of AD, DD, and DDTs simultaneously. Table II

Table I. Comparison of the Recoveries of AD, DD, and DDTs from ISOLUTE SPE Columns Using a Diethyl Ether–Hexane (10:90, v/v) Eluent*

Packing material	Recovery (%)					
	AD	DD	DDT	*op*'-DDT	DDE	DDD
CN	94	58	85	98	101	99
DIOL	92	84	88	89	93	86
NH_2	98	96	103	102	97	98
PSA	0	12	92	105	99	103
SI	43	0	78	85	65	95
FL	80	85	102	90	94	92
ALa	90	21	36	96	90	81
ALn	0	0	72	58	65	50
ALb	0	5	0	0	0	0

* Data expressed as an average (n = 3). A mixed standard solution containing 0.4 µg/L of all the target compounds was applied to the column.

Table II. Effect of the Diethyl Ether Concentration in the Eluent on the Recoveries of AD, DD, and DDTs from Selected ISOLUTE SPE Columns*

Packing material	Recovery (%)			
	Diethyl ether–hexane (v/v)			
	0:100	5:95	10:90	Total
NH_2				
AD	6	91	0	97
DD	23	73	0	96
DDT	0	101	0	101
op'-DDT	12	87	0	99
DDE	90	6	0	96
DDD	11	86	0	97
DIOL				
AD	0	91	0	91
DD	44	41	0	85
DDT	39	50	0	89
op'-DDT	28	60	0	88
DDE	64	28	0	92
DDD	33	55	0	88
FL				
AD	0	3	78	81
DD	0	83	0	83
DDT	5	81	18	104
op'-DDT	51	47	0	88
DDE	49	45	0	94
DDD	63	27	0	90

* Data expressed as the average (n = 3). A mixed standard solution containing 0.4 µg of all the target compounds was applied to the column.

presents the effect of a diethyl ether concentration in the eluent (diethyl ether–hexane, v/v) on the recoveries of AD, DD, and DDTs from the selected three SPE columns. The target compounds in 3 mL of each eluted fraction were determined by the HPLC. The better eluents that recovered all compounds from the three columns were a 5% (v/v) solution of diethyl ether in hexane for the DIOL and NH_2 columns and a 10% (v/v) solution of diethyl ether in hexane for the FL column. The average recoveries ($n = 3$) of AD, DD, and DDTs were in excess of 81% under these conditions. As can be seen in Figures 2A and 2B, the extracts that were treated by using the DIOL and FL columns showed a chromatographic interference that hindered the confirmation of AD. Figures 2C and 2D demonstrated that the NH_2 column can effectively eliminate the matrix-interfering components and provide a reliable determination and identification of AD, DD, and DDTs. The present method made it unnecessary to use the gradient system to improve the separation and did not require "precolumn washing" after an analysis.

Recovery, variability, and limit of determination

The recoveries from egg samples at four different spiking levels (0.1, 0.2, 0.3, and 0.4 µg/g of each drug), the correlation coefficients of standard graphs, and the inter- and intra-assay variabilities of five compounds isolated from spiked egg samples are summarized in Table III. Generally, excellent recoveries and assay variabilities were obtained. Average recoveries were greater than 83% with SDs between 2.3 and 4.8%. Inter- and intra-assay variabilities ranged from 3.2 to 4.8%. The standard graphs were generated by plotting peak areas of fortified sample extracts ranging from 0.1 to 0.4 µg/g. The graph was constructed from five points and each point represented the mean of the five injections. The resulting correlation coefficients for all of the compounds were highly significant statistically ($P < 0.01$) (Table III). A good linearity and reproducibility of the determination were obtained in the concentration range examined. For the target compounds respectively, the standard spiking graph and its pure standard (aqueous) graph were able to pool statistically, indicating that the slope of the standard spiking graph was similar to that of the pure standard. The calibration can be performed with the simplest procedure using pure standards. The data given in Table III indicate that the present method may be precise and accurate.

To properly characterize the practical residue monitoring, the limit of determination (LOD) for target compounds were calculated. Based on the peak areas in HPLC chromatograms, the LOD was defined as the average background plus three times the SD. Four different blank egg samples were analyzed in duplicate. In a practical analysis for the residue monitoring, the LODs for the six compounds ranged from 0.04 to 0.08 µg/g (Table III). These LODs were well below the EMRL.

Table III. Average Recoveries, Correlation Coefficients of Standard Graphs, Inter- and Intra-assay Variabilities, and LODs for AD-, DD-, and DDTs-Fortified Eggs

Spiked (µg/g)	%Recovery (average ± SD, $n = 5$)					
	AD	DD	DDT	*op*'-DDT	DDE	DDD
0.1	83 ± 3	89 ± 4	91 ± 3	85 ± 3	89 ± 4	85 ± 4
0.2	85 ± 4	92 ± 3	95 ± 4	85 ± 2	87 ± 3	83 ± 5
0.3	86 ± 2	91 ± 3	95 ± 2	90 ± 4	87 ± 3	84 ± 4
0.4	83 ± 3	86 ± 3	90 ± 2	89 ± 3	88 ± 3	89 ± 4
Standard graph correlation coefficient						
($n = 4$)*	0.999	0.999	0.998	0.997	0.998	0.997
%Assay variance interassay						
($n = 4$)	3.6 ± 0.7	3.5 ± 0.4	3.2 ± 0.6	3.5 ± 0.4	3.3 ± 0.5	4.8 ± 0.6
Intra-assay						
($n = 5$)	2.8	2.5	2.1	2.8	3.0	3.3
LOD						
(µg/g)	0.07	0.08	0.04	0.05	0.07	0.06

* Mean of five determinations using spiked samples for standard curves (range of concentration between 0.1 and 0.4 µg/g.

Identification

In HPLC analysis for residual chemical monitoring, the PDA detector generally gives spectral information and is an easy way for the confirmation of the chemical. HPLC combined with the PDA system proved to be able to detect a wide range of molecules and ensure the identification of target compounds. The retention time and spectrum provided strong evidence of its identity. The target compounds examined could be identified in the egg sample with their retention times and absorption spectra. Their spectra obtained from a sample were practically identical with those of the standard. The present sample preparation allowed for a reliable confirmation.

Conclusion

The proposed procedure for the simultaneous HPLC determination and identification of AD, DD, DDT, *op*'-DDT, DDE, and DDD in eggs offers the following advantages: it is simple, it requires a shorter analysis time (< 1 h per sample) and less use of organic solvents (< 55 mL per sample), and it is highly precise and economical. Therefore, it may be useful for the routine monitoring of these compounds in eggs.

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