ELSEVIER

Journal of Chromatography A, 830 (1999) 473–476

JOURNAL OF CHROMATOGRAPHY A

Short communication

Simple and rapid extraction method of total egg lipids for determining organochlorine pesticides in the egg

Naoto Furusawa[a,*], Asako Ozaki[a], Miyuki Nakamura[a], Yukari Morita[a], Kunio Okazaki[b]

[a]*Faculty of Human Life Science, Osaka City University, Sumiyoshi-ku, Osaka 558-8585, Japan*

[b]*Osaka City Institute of Public Health and Environmental Sciences, Tennoji-ku, Osaka 543-0026, Japan*

Received 12 August 1998; received in revised form 13 October 1998; accepted 15 October 1998

Abstract

A simple and rapid extraction method of total egg lipids for determination of organochlorine pesticides (OCPs) in the egg was developed. After being mixed with anhydrous sodium sulphate, the extraction of lipids in egg yolk was performed using *n*-hexane–acetonitrile (2:1, v/v). Troublesome emulsions did not occur. Using the present method, an average of 3.03 g of egg lipids was collected from 10 g of egg yolk. Compared with classical methods, the present method is handy; needs much shorter analysis time and less requirement of solvents and has higher efficiency of egg lipid extraction and higher recoveries of OCPs.

Keywords: Lipids; Organochlorine pesticides

1. Introduction

For determination of residual organochlorine pesticides (OCPs) in high-lipid-content foods of animal origin, the starting point will be the extraction of lipid from the samples [1–3,5]. In the routine monitoring of OCPs in eggs, the lipid extraction has been done by liquid–liquid partition from the whole egg in accordance with the AOAC's Official Methods of Analysis [2], Japanese official method [5] that conformed to the AOAC method, and that in the US Food and Drug Administration's (FDAs) Pesticide Analytical Manual [4] (referred to as AOAC, JO and FDA, respectively). AOAC/JO used acetonitrile followed by petroleum ether. The FDA method used alcohol followed by ethyl ether and petroleum ether. These methods possess the following three problems: (1) low recovery for the lipid; (2) easy to emulsify; (3) time consuming (e.g., phase separation times). These problems may be certainly due to the components of an egg and their physico–chemical properties. At present, a method, which is accurate, fast and economical in terms of time, is required.

In physico–chemical studies on the lipids, Folch et al. [6] have established that a relatively simple approach to lipid extraction using chloroform–methanol (2:1, v/v) as an extraction solvent has been found effective for the collecting total lipids in foods. However, it is not certain whether or not the method is applicable to the analysis of OCPs in eggs.

In the present study, we developed a simple, rapid and reliable method of total lipid extraction in eggs for the determination of residual OCPs, and evalu-

*Corresponding author. Tel.: +81-6-605-2864, Fax: +81-6-605-2914, E-mail: furusawa@life.osaka-cu.ac.jp

0021-9673/99/$ – see front matter
PII: S0021-9673(98)00873-5

ated comparative performance data for the four extraction techniques (AOAC/JO, FDA, Folch and the present method).

2. Experimental

2.1. Materials and reagents

Egg yolks and albumen from chicken eggs, which were purchased from a supermarket in Osaka city, Japan, were separated immediately. The egg yolk served as samples. All solvents and anhydrous sodium sulfate used in this study were of residual pesticide grade (Wako Pure Chemicals, Osaka, Japan). Ten organochlorine pesticide standards, i.e., α-BHC, β-BHC, γ-BHC, δ-BHC (BHC=mixed isomers of 1,2,3,4,5,6-hexachlorocyclohexane), aldrin, dieldrin, *p,p′*-DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene), *o,p′*1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), *p,p′*-DDD, *p,p′*-DDT, were obtained from Wako. As working standard solutions, the mixed solutions of concentrations from 1.0 to 2.5 μg/ml of each pesticide were prepared in acetone.

2.2. Apparatus

Gel-permeation chromatography (GPC) operating conditions were as follows: Waters Model 600E equipped a 300 mm×19 mm Envirogel GPC column (Waters, Milford, MA, USA) and a 150 mm×19 mm Envirogel GPC guard column; column temperature, 30°C; elution solvent, ethyl acetate–cyclohexane (1:1, v/v); flow-rate, 5 ml/min; fractions, 8 min dumped (40 ml), 1 collect/min from 41 to 110 ml.

Gas chromatography (GC) operating conditions were as follows: HP-5890 series (Hewlett-Packard, Palo Alto, CA, USA) equipped with ^{63}Ni electron-capture detectors; 30 m×0.25 mm, 0.25 μm SPB1 (Supelco, Bellefonte, PA, USA) capillary column; He carrier gas flow, 20 cm/s; detector make-up gas, N_2 at 10 cm/s; column temperature program: 140°C for 2 min, 10°C/min to 200°C, 2.5°C/min to 260°C; splitless injection; injector temperature, 200°C; detector temperature, 300°C; injection volume, 2 μl.

2.3. Lipid extraction procedure

2.3.1. The present method

Ten g of the egg yolk was weighed accurately into a homogenizer-cup and mixed sufficiently with a sufficient amounts of anhydrous sodium sulphate. Afterwards, the sample was homogenized in 20 ml of acetonitrile and 40 ml of *n*-hexane (saturated with acetonitrile) with an autohomogenizer (Model PH-91, SMT; Mitsui Denki-Seiki, Tokyo, Japan). After centrifugation at 3500 rpm for 5 min, the supernatant was poured into a separating funnel through a funnel (Buchner type with Fritted disc 11G3, Pyrex; Iwaki Glass, Funabashi City, Japan) packed with anhydrous sodium sulphate. The dried hexane layer was collected and then evaporated to dryness, and the egg lipid was obtained. The mass was measured as described previously [7].

2.3.2. AOAC/JO, FDA and Folch methods

These procedures are briefly presented in Fig. 1.

2.4. Recoveries of OCPs

To demonstrate that the lipid extraction technique proposed here will efficiently extract incurred OCPs residues from egg yolk, we extracted the OCPs spiked egg yolks by the present method and also by AOAC/JO, FDA and Floch methods (Fig. 1), respectively. Egg yolk samples (10 g) were fortified with 250 μl of working standard solution (10 mixed OCPs). That is, the spiked level of OCPs were 0.0025–0.0125 ppm. Fortified samples were allowed to stand at room temperature for 12 h after pesticide addition followed by mixing.

Each lipid that was extracted by using the four extraction techniques, respectively, was dissolved in 5 ml ethyl acetate–cyclohexane (1:1, v/v). Using a 2-ml volume, clean-up–gas chromatographic determination was performed by a procedure described previously [9]. Namely, the 2-ml volume was injected into the GPC system. The OCP fraction, 70–110 ml, was evaporated to dryness. The residue was dissolved in 2 ml hexane and applied to a Florisil (packed 2 g) mini-column. OCPs were eluted with 30 ml of 15% (v/v) diethyl ether–hexane (flow-rate<3 ml/min). The elute was evaporated to dry-

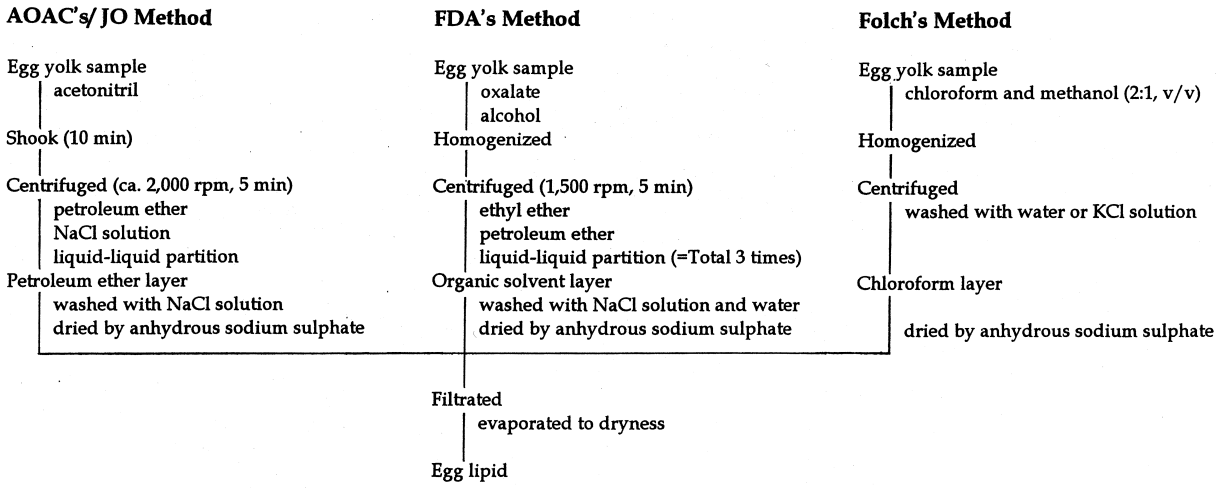

Fig. 1. Current procedures for extracting total lipids in an egg. AOACs/JO [2,5]; FDA [4]; Folch [6] methods.

ness, and the residue was dissolved in 2 ml hexane. A 2-μl portion was injected into the GC system.

In this clean-up procedure, average recoveries of 10 OCPs from spiked lipids (0.0025–0.0125 ppm) ranged from 85 to 102%, with relative standard deviations (R.S.D.s) between 1 and 8% ($n=3$).

3. Results and discussion

The lipids in an egg are concentrated in the yolk. One-third of the yolk is lipid, while the lipid content in the albumen is almost equal to zero [8]. The present study, therefore, only the yolk was used in the analysis procedure.

Table 1
Amounts of total lipids extracted from 10 g of egg yolk

Method	Extracted (g)	Theoretical[a] (g)
Present study	3.03±0.09	3.12
AOAC[b]/JO[c]	0.94±0.07	
FDA[d]	1.48±0.08	
Folch[e]	2.93±0.06	

Data are expressed as mean±standard deviation ($n=3$).
[a] Total lipid amounts in 10 g of an egg yolk: Ref. [8].
[b] Ref. [2].
[c] Ref. [5].
[d] Ref. [4].
[e] Ref. [6].

3.1. Lipid extraction efficiency

Table 1 lists amounts of total lipids extracted from egg. The theoretical value from the data of Ref. [8] is also given in this table. The average extraction amount of lipids from 10 g of egg yolk by the procedure presented here was 3.03 g ($n=3$), which is equal to 97% of the theoretical lipid content in 10 g of egg yolk. The result was much better than that for other reference methods as can be seen in this table. Additionally, to evaluate purity of the lipid extract (3.03 g, Table 1), re-extraction by the method of Folch et al. [6] was undertaken. As a result, the average amount of extract that was re-extracted was 2.99 g. There was no a significant difference between the two data (3.03 and 2.99 g), suggesting that the present method is an efficient method for lipid extraction in egg.

With the simple method that proposed here, the extract did not form an emulsion that would hinder the recoveries of the target compounds. After centrifugation, two layers for hexane and acetonitrile were separated completely. On the other hand, the AOAC/JO and FDA procedures [2,4,5] are complicated and required longer phase separation times (Fig. 1). The proposed method can be evaluated in terms of the minimal steps and times which are required without phase separation times. Analysis time was also extremely reduced.

Table 2
Comparison for recoveries of OCPs using different lipid extraction operations on OCP analysis in egg yolk[a]

Pesticide	Lipid extraction method			
	Present study	AOAC[b]/JO[c]	FDA[d]	Folch[e]
α-BHC	80 (7)	79(10)	55 (8)	5 (9)
β-BHC	93 (8)	82(11)	72 (10)	13 (10)
γ-BHC	82 (5)	74(12)	64 (16)	0
δ-BHC	92 (2)	88(8)	74 (10)	31 (22)
Aldrin	86 (8)	44(21)	80 (19)	23 (30)
Dieldrin	91 (12)	80(17)	79 (21)	71 (13)
p,p′-DDE	98 (13)	54(18)	91 (12)	80 (18)
o,p′-DDT	99 (8)	91(9)	89 (8)	89 (7)
p,p′-DDD	96 (6)	90(15)	93 (11)	83 (11)
p,p′-DDT	95 (8)	92(13)	92 (15)	86 (10)

Data are expressed as means. Figures in parentheses are relative standard deviations (%, $n=3$).
[a] Egg lipids in the OCP-spiked egg yolk were extracted by using the four methods. Each lipid underwent clean-up–GC using the same method described previously [9].
[b] Ref. [2].
[c] Ref. [5].
[d] Ref. [4].
[e] Ref. [6].

3.2. Recoveries of OCPs

In order to determine recoveries of OCPs, the lipid extract needed to be cleaned up followed by GC determination. The lipid extracts obtained by the four lipid extraction techniques were analyzed using a combination procedure of clean-up and GC analysis described previously [9]. Table 2 shows that the recoveries of 10 OCPs obtained by using the four methods give comparable results. In the data for the recoveries of OCPs and their R.S.D.s shown in Table 2, the differences in the same column (among OCPs) depend on the clean-up procedure used. On the other hand, the differences in the same row (among the four extraction methods) must be due to the extraction solvents and the operability, indicating that the complicated procedures (AOAC/JO and FDAs, see Fig. 1) and the different extraction solvents (Folch, see Fig. 1) give low recoveries for some OCTs.

Consequently, good results were obtained with the present method. Average recoveries of 10 OCPs ranged from 80 to 99%, with R.S.D.s between 2 and 13%. These results were much better than those for the AOAC/JO [2,5], FDA [4] and Folch [6] methods, indicating that the proposed method has a good precision and may be accurate.

In conclusion, the method presented here has a technical improvement over the previous procedures [2,4–6]. Characteristics of the proposed procedure are: higher efficiency of total lipid extraction; higher recoveries of OCPs; shorter analysis time; easy. Therefore, this procedure of total lipid extraction in an egg may be useful as first step for monitoring residual OCPs in the table egg.

References

[1] P.A. Mills, J.H. Onley, R.A. Gaither, J. Assoc. Off. Agric. Chem. 46 (1963) 186.
[2] Official Methods of Analysis, Vol. 1, AOAC International, Arlington, VA, 15th ed., 1990, Ch. 10.
[3] Pesticide Analytical Manual, Vol. I, US Food and Drug Administration, Washington, DC, 1991, Sec. 212.13a(2).
[4] Pesticide Analytical Manual, Vol. I, US Food and Drug Administration, Washington, DC, 1991, Sec. 304E4.
[5] Standard Methods of Analysis for Hygienic Chemists – With Commentary, Pharmaceutical Society of Japan, Tokyo, 2nd ed., 1990, p. 621.
[6] J. Folch, I. Ascoli, M. Lees, J.A. Meath, B.N. LeBaron, J. Biol. Chem. 191 (1951) 833.
[7] G. Rouser, G. Kritchevsky, A. Yamamoto (Eds.), Lipid Chromatographic Analysis, Marcel Dekker, New York, 2nd ed., 1976, p. 713.
[8] A. Kagawa, Standard Tables of Food Composition in Japan 1991, Resources Survey Meeting, Science and Technology Agency.
[9] N. Furusawa, K. Okazaki, S. Iriguchi, H. Yamaguchi, M. Saitoh, J. AOAC Int. 81 (1998) 1033.