

Development of a Multiresidue Method for the Analysis of 33 Organochlorine Pesticide Residues in Fatty and High Water Content Foods

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Abstract A new multiresidue method has been developed and validated for the determination of 33 organochlorine pesticides (OCPs) in various fatty and high water content food matrices. The OCP residues in foods were extracted with matrix solid-phase dispersion and cleaned up with gel permeation chromatography and Florisil solid-phase extraction. The instrumental determination was carried out by a gas chromatograph coupled to a single quadrupole mass spectrometer (MS) with runtime of 11 min. Besides, negative chemical ionization mode was also studied and evaluated for OCPs' sensitivities. The optimized MS was operated in electron ionization mode and acquiring three selected ions per target compound. The method was validated with various food samples, including edible oil, meat, seafood, eggs, coffee, tree nuts, fruits, vegetables, etc. according to SANCO/12571/2013. An adequate linear relationship was obtained in the studied concentration range (0.5–10.0 $\mu\text{g kg}^{-1}$) in sample; the average spiked recovery values were in the range 70–120 % for the two levels of concentration studied in samples: 0.5 and 2.0 $\mu\text{g kg}^{-1}$. Precision values, expressed as relative standard deviation, were lower than 18 % at aforementioned spiking levels; detection limits and quantification limits were below or equal to 0.1 and 0.5 $\mu\text{g kg}^{-1}$, respectively. Moreover, certified reference materials were used to assess the accuracy of the developed method. Finally, the developed method was successfully applied for the OCPs' determination in real samples for the first Hong Kong Total Diet Study.

Keywords Gas chromatography–mass spectrometry · Matrix solid-phase dispersion · Organochlorine pesticides · Multiresidue · Food

Introduction

Organochlorine pesticides (OCPs) were one of the commonly used pesticides in the mid-twentieth century. Since their mode of action is by targeting systems or enzymes in the pests which may be identical or very similar to systems or enzymes in human beings, they pose risks to human health [1] and the environment. These OCPs are known to have low acute but high chronic toxicity. Through bioaccumulation and biomagnification, some OCPs persist in the environment and accumulate inside living organisms and food stuff. This led to the gradual ban on their use and replacement by other comparatively less hazardous or more easily degradable pesticides.

Aldrin, endrin, chlordane, dichlorodiphenyltrichloroethane (DDT), heptachlor, mirex, toxaphene and hexachlorobenzene (HCB) belong to persistent organic pollutants (POPs) and have been banned for agricultural or domestic uses in many countries in accordance with the Stockholm Convention in 1980s. However, DDT was still used to prevent spreading of malaria and other vector-borne diseases such as dengue, leishmaniasis and Japanese encephalitis through the prevention of growth of mosquito. Lindane (γ -hexachlorocyclohexane, γ -HCH) is another example of widely used OCP which had been used to treat head lice in children [1]. Recently, the scope of POPs was extended to include chlordecone, lindane, α -HCH, β -HCH, pentachlorobenzene (PeCB) and endosulfan, which also belong to OCPs. In addition, public health safety would require constant monitoring of the levels in food of animal origin

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commonly consumed by human as they are the major source of human background exposure to OCPs. Thus, OCPs in foods are commonly monitored by regulatory laboratories.

Nowadays, pesticide residue analysis in foods is usually performed with the QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction. QuEChERS method was primarily designed for low-fat commodities. As shown in the recent review on the determination of OCPs in vegetation, application of QuEChERS in other matrices was still limited [2]. Chung and Chen [3] reviewed the analytical methods for testing OCPs in fatty food. The scope of analysis of published methods was basically matrix specific and none of them can be applied to non-fatty (<2 % lipids), low-fat (2–20 %) and high-fat (>20 %) food simultaneously. Besides, the scope of analysis needs to be broadened as some of the OCPs, including endrin aldehyde, endrin ketone, chlordecone, α -endosulfan, β -endosulfan, nonachlor and *trans*-heptachlor epoxide, etc. were always not tested in those methods. Further, supercritical fluid extraction, pressurized liquid extraction and microwave-assisted extraction cannot extract endrin, endrin aldehyde and/or chlordecone with satisfactory recoveries. Soxhlet extraction, liquid–liquid extraction and matrix solid-phase dispersion (MSPD) are suitable for extraction of those easily degraded OCPs without significant loss [3]. To handle various food matrices, including edible oil, fruits and vegetables, MSPD becomes the first choice amongst the above-mentioned extraction techniques. The main advantage of this technique is that it allows several steps to be performed in the sample preparation simultaneously. Besides, MSPD is versatile and applicable to most food samples regardless of high content of water or lipids. In fact, extraction of OCPs from oil samples had been reported on such technique using acetonitrile saturated with *n*-hexane as extraction solvent [4–7]. Furthermore, the polar nature of acetonitrile (ACN) minimizes the coelution of lipids (fatty acids, hydrocarbons, mono-, di-, and tri-glycerides) present due to the retention of these compounds in the MSPD column [8].

The aim of this work was to develop a method applicable to various types of food items to be tested in a total diet study. The newly developed method involved an MSPD extraction followed by cleanup with gel permeation chromatography (GPC) and Florisil solid-phase extraction. The resulting extract was then applied to the gas chromatography–mass spectrometry (GC–MS) for quantitative and qualitative analysis of 33 OCPs simultaneously with one quantifier and two qualifiers per compound. The target analytes had been spiked to a variety of food matrices such as citric fruits, vegetables, tree nuts, eggs, dairy products, meat, poultry, edible oils, chocolate, coffee, beverages, seafood, etc. Satisfactory spike recovery results were obtained and no significant interference was encountered in these matrices when spiked at the method limit of quantitation (MLOQ) level of 0.5 $\mu\text{g kg}^{-1}$.

Experimental

Chemicals and Standards

All solvents used were of PCB grade or pesticide grade and all reagents were analytical grade. Water was purified through a Milli-Q system (Millipore, Billerica, USA). Hydromatrix was obtained from Agilent Technologies (Santa Clara, USA). Anhydrous magnesium sulphate, Florisil and sodium sulphate were obtained from Fluka, Supelco (Sigma-Aldrich Corp., St. Louis, USA) and UniChem (Haw River, USA), respectively. Lipid Removal Agent media (synthetic calcium silicate hydrate), EXtrelut[®] NT-20 (diatomaceous earth) and Calflo E (synthetic calcium silicate) were obtained from Supelco, Merck and Fluka, respectively. Hydromatrix, magnesium sulphate, sodium sulphate and Florisil were preheated at 550 °C overnight and cooled in dry box prior to use.

Pesticide standards (Table 1) were purchased from Restek (Bellefonte, USA), Chem Service Inc. (West Chester, USA), Cambridge Isotope Laboratory (Tewksbury, USA) and Dr. Ehrenstorfer (Augsburg, Germany). The purities of the pesticide standards were of 95 % or above. Purity-corrected individual pesticide stock solutions (100, 1,000 or 2,000 mg L^{-1}) were prepared in ethyl acetate and kept at -20 °C, protected from light. Two mixed intermediate standard solutions (0.5 and 20 mg L^{-1}) of multiple pesticides were prepared by diluting an appropriate volume of each individual stock standard solution with ethyl acetate. Working standard solutions were freshly prepared by dilution of the mixed pesticide standard solution with isooctane. Isotopically labelled standards, $^{13}\text{C}_{12}$ -aldrin, $^{13}\text{C}_{12}$ -2,4'-DDD, $^{13}\text{C}_{12}$ -endrin aldehyde, $^{13}\text{C}_6$ -hexachlorobenzene (HCB), $^{13}\text{C}_{12}$ -methoxychlor, $^{13}\text{C}_{10}$ -*trans*-nonachlor and $^{13}\text{C}_6$ -pentachlorobenzene (PeCB) were purchased from Cambridge Isotope Laboratory. Individual stock internal standard solutions (50 or 100 mg L^{-1}) were used directly from the ampoules to prepare an intermediate internal standard solution of 0.5 mg L^{-1} . A working internal standard solution mix used for spiking containing all internal standards of 20 ng mL^{-1} was prepared by appropriate dilution of the intermediate internal standard solutions with isooctane and stored at -20 °C. Certified reference materials (CRMs) were purchased from NIST and IRMM.

Sample Preparation Procedure

Matrix Solid-Phase Dispersion Extraction (MSPD)

For liquid samples, shake vigorously or inverting up and down the liquid content within the container. For solid samples, the entire sample was blended with a high-speed blender. To a 150-mL glass beaker, 10 g of hydromatrix

Table 1 Retention times, time segments, quantifier and qualifier ions and their ratios for GC-MS analysis of OCP analytes

Time window	Start time (min)	Analyte	Internal standard	Rt (min)	Dwell time (ms)	Q0	Q1	Q2	Response ratio 1	Response ratio 2				
1	3.95	PeCB	¹³ C ₆ -PeCB	4.62	40	250	252	215	0.60	0.17				
		¹³ C ₆ -PeCB		4.62	40	256	258		0.61					
		HCB	¹³ C ₆ -HCB	5.84	40	284	286	249	0.81	0.22				
		¹³ C ₆ -HCB		5.84	40	290	292		0.73					
		α-HCH	¹³ C ₁₀ - <i>trans</i> -Nonachlor	5.92	80	219	221	181	0.48	1.10				
		γ-HCH	¹³ C ₁₀ - <i>trans</i> -Nonachlor	6.42	80	219	221	181	0.48	1.32				
		β-HCH	¹³ C ₁₀ - <i>trans</i> -Nonachlor	6.84	80	181	183	219	0.48	0.99				
		Heptachlor	¹³ C ₁₀ - <i>trans</i> -Nonachlor	6.90	80	272	274	237	0.80	0.36				
		δ-HCH	¹³ C ₁₂ - <i>p,p'</i> -DDD	7.16	80	219	221	181	0.50	1.26				
		Aldrin	¹³ C ₁₂ -Aldrin	7.29	80	263	265	293	0.67	0.39				
2	6.20	¹³ C ₁₂ -Aldrin		7.29	80	270	272		0.61					
		<i>o,p'</i> -Dicofof	¹³ C ₁₂ - <i>o,p'</i> -DDD	7.44	80	250	252	139	0.68	3.10				
		Isodrin	¹³ C ₁₂ -Aldrin	7.80	100	263	265	193	0.63	1.37				
		Dichlorobenzophenone	¹³ C ₁₂ - <i>o,p'</i> -DDD	7.88	40	250	252	139	0.67	3.42				
		Oxychlorthane	¹³ C ₁₀ - <i>trans</i> -Nonachlor	7.88	100	387	389	237	0.96	1.38				
		<i>cis</i> -Heptachlor epoxide	¹³ C ₁₀ - <i>trans</i> -Nonachlor	8.00	100	353	355	263	0.81	0.22				
		<i>trans</i> -Heptachlor epoxide	¹³ C ₁₀ - <i>trans</i> -Nonachlor epoxide	8.09	100	253	255	289	0.63	0.46				
		<i>o,p'</i> -DDE	¹³ C ₁₀ - <i>trans</i> -Nonachlor	8.30	30	246	248	318	0.66	0.43				
		<i>trans</i> -Chlordane	¹³ C ₁₀ - <i>trans</i> -Nonachlor	8.36	30	373	375	272	0.96	0.37				
		<i>trans</i> -Nonachlor	¹³ C ₁₀ - <i>trans</i> -Nonachlor	8.40	30	409	411	272	0.64	0.24				
3	7.05	¹³ C ₁₀ - <i>trans</i> -Nonachlor		8.40	30	417	419		0.91					
		<i>cis</i> -Chlordane	¹³ C ₁₀ - <i>trans</i> -Nonachlor	8.45	30	373	375	272	0.97	0.37				
		¹³ C ₁₀ - <i>cis</i> -Chlordane		8.45	30	383	385		0.97					
		α-Endosulfan	¹³ C ₁₀ - <i>trans</i> -Nonachlor	8.51	100	241	243	265	0.74	0.69				
		<i>p,p'</i> -DDE	¹³ C ₁₀ - <i>trans</i> -Nonachlor	8.73	30	246	248	318	0.66	0.94				
		Dieldrin	¹³ C ₁₂ - <i>o,p'</i> -DDD	8.89	100	263	265	277	0.68	0.56				
		<i>o,p'</i> -DDD	¹³ C ₁₂ - <i>o,p'</i> -DDD	8.95	30	235	237	165	0.65	0.35				
		¹³ C ₁₂ - <i>o,p'</i> -DDD		8.95	30	247	249		0.64					
		Endrin	¹³ C ₁₂ - <i>o,p'</i> -DDD	9.27	100	263	265	281	0.76	0.52				
		<i>o,p'</i> -DDT	¹³ C ₁₂ - <i>p,p'</i> -DDD	9.30	30	235	237	165	0.66	0.33				
4	7.60	<i>cis</i> -Nonachlor	¹³ C ₁₂ - <i>o,p'</i> -DDD	9.38	30	409	411	272	0.63	0.16				
		<i>p,p'</i> -DDD	¹³ C ₁₂ - <i>o,p'</i> -DDD	9.49	30	235	237	165	0.65	0.35				
		5	8.20			8.30	30	246	248	318	0.66	0.43		
						8.36	30	373	375	272	0.96	0.37		
						8.40	30	409	411	272	0.64	0.24		
						8.40	30	417	419		0.91			
						8.45	30	373	375	272	0.97	0.37		
						8.45	30	383	385		0.97			
						8.51	100	241	243	265	0.74	0.69		
						8.73	30	246	248	318	0.66	0.94		
8.89	100					263	265	277	0.68	0.56				
8.95	30					235	237	165	0.65	0.35				
6	8.60			8.95	30	247	249		0.64					
				9.27	100	263	265	281	0.76	0.52				
				9.30	30	235	237	165	0.66	0.33				
				9.38	30	409	411	272	0.63	0.16				
				9.49	30	235	237	165	0.65	0.35				
				7	9.10			9.27	100	263	265	281	0.76	0.52
								9.30	30	235	237	165	0.66	0.33
								9.38	30	409	411	272	0.63	0.16
								9.49	30	235	237	165	0.65	0.35

Table 1 continued

Time window	Start time (min)	Analyte	Internal standard	Rt (min)	Dwell time (ms)	Q0	Q1	Q2	Response ratio 1	Response ratio 2
8	9.60	β -Endosulfan	$^{13}\text{C}_{12}$ - <i>o,p'</i> -DDD	9.66	100	241	239	339	0.96	0.59
		<i>p,p'</i> -DDT	$^{13}\text{C}_{12}$ -Methoxychlor	9.82	30	235	237	165	0.65	0.33
		Endrin aldehyde	$^{13}\text{C}_{12}$ -Endrin aldehyde	9.92	100	345	347	250	0.65	0.75
		$^{13}\text{C}_{12}$ -Endrin aldehyde		9.92	100	357	359		0.64	
9	10.10	Endosulfan sulphate	$^{13}\text{C}_{12}$ - <i>o,p'</i> -DDD	10.22	100	272	274	239	0.80	0.50
		Methoxychlor	$^{13}\text{C}_{12}$ -Methoxychlor	10.64	40	227	228	212	0.18	0.06
		$^{13}\text{C}_{12}$ -Methoxychlor		10.64	100	239	238		0.19	
10	10.70	Endrin ketone	$^{13}\text{C}_{12}$ - <i>o,p'</i> -DDD	10.80	100	317	319	281	0.64	0.35
		Mirex	$^{13}\text{C}_{10}$ - <i>trans</i> -Nonachlor	11.16	40	272	274	237	0.81	0.48

and 10 g of magnesium sulphate were added. About 10 g of sample was accurately weighed and transferred into the beaker. One mL of spike internal standard solution was then added onto the sample. Another 5 g of hydromatrix was finally added to the mixture before being mixed thoroughly with a Teflon stick and stood for at least 10 min to allow equilibration. The sample mix was transferred from the beaker into a glass column containing 15 g of sodium sulphate at the bottom. The side of the column was tapped gently to settle this layer. An additional 10 g of sodium sulphate was added on top of the sample mix and the glass column was tapped gently again. OCPs were extracted from the sample by passing 150 mL of acetonitrile (saturated with *n*-hexane) at a rate of about 1–2 drops per second. Five mL of toluene was added to the extract as trapping agent before the resulting extract was evaporated to around 1 mL by a Buchi Syncore evaporator (Postfach, Switzerland) at 55 °C and 300 rpm.

Gel Permeation Chromatography (GPC)

The extract concentrate obtained from MSPD extraction was filtered through a syringe filter, and made up to about 5.5 mL with the GPC mobile phase (cyclohexane:dichloromethane (DCM) = 1:1). The sample was automatically introduced into the 5 mL sample loop of the GPC system (composed of an Alltech 301 isocratic pump, Gilson GX-271 liquid handler, 402 syringe pump and Trilution software) equipped with an Envirosep-ABC column (350 mm \times 21.2 mm) and a guard column (60 mm \times 21.2 mm). GPC was performed with cyclohexane–DCM (1:1, v/v) as the mobile phase with a flow rate of 5 mL min⁻¹. The dump and collection times of 14 and 31 min were optimized with *trans*-chlordane and HCB, which are among the first- and last-eluted OCP compounds, respectively. Three mL of isooctane was added to the eluate as trapping agent before the resulting eluate was evaporated to around 1 mL by the Buchi Syncore Polyvap operated at 50 °C and 300 rpm.

Florisil Solid-Phase Extraction (SPE) Cleanup

A 1 g portion of activated Florisil was dry packed in a glass column (1.0 cm id) and then covered with 2 g MgSO₄. The SPE column was conditioned with 2 mL of isooctane and then 6 mL of hexane. The resulting extract obtained from GPC cleanup was dissolved with 1 mL *n*-hexane and placed on the Florisil column, and the analytes were eluted with 10 mL of acetone:*n*-hexane (1:9). After adding 0.5 mL of isooctane as trapping agent to the eluate, the eluate was concentrated by evaporation by a nitrogen stream at 40 °C to around 0.5 mL in an amber GC vial. Aliquots were analysed by GC–MS for the determination of OCPs with internal standardization.

Gas Chromatography–Mass Spectrometry (GC–MS)

The analysis of the residues was carried out on an Agilent 6890 gas chromatograph equipped with a Series 5973 Network mass selective detector, a Series 7683A automatic sampler and a data processing system with ChemStation software (Version B.03.02) (Agilent, Avondale, USA). GC separation was performed on a Zebron ZB-MR-2 fused-silica capillary column (30 m, 0.25 mm I.D., 0.20 μm film thickness, Phenomenex, Torrance, USA) with a guard column (5 m, 0.25 mm). Ultra-high-purity helium was used as the carrier gas at constant flow of 1.5 mL min^{-1} .

A split–splitless injection system operated in pulsed splitless mode with quartz Gooseneck splitless injector liner (4 mm id, 6.5 mm od and 78.5 mm length) was employed. Injection volume was 2 μL . The temperature of the injector was set at 220 $^{\circ}\text{C}$. One min after the injection, the split valve was activated to a total flow rate of 70 mL min^{-1} for 1 min. Afterwards, the total flow was set to 1.5 mL min^{-1} . The initial column temperature was set at 120 $^{\circ}\text{C}$. After the sample was injected for 0.75 min, it was increased to 200 $^{\circ}\text{C}$ with ramp rate of 20 $^{\circ}\text{C}$ per min then to 300 $^{\circ}\text{C}$ with ramp rate of 15 $^{\circ}\text{C}$ per min. Total runtime was 11 min.

The mass spectrometer was operated in electron ionization mode at 70 eV. The temperatures of the ion source, the quadrupole and the transfer line were set at 230, 150 and 280 $^{\circ}\text{C}$, respectively. Qualitative and quantitative analysis was carried out by selectively monitoring the detector response of characteristic ions in time segments with two and three scan events designated for each internal standard and target analyte, respectively. The quantitative ion and secondary (identification) ions measured for each analyte are listed in Table 1.

Quantitation and Identification

Calibration curves were constructed for all target analytes by injecting 10 calibration standard solutions directly into the GC, at the concentrations 0.5, 1, 2, 5, 10, 15, 20, 50, 75 and 100 $\mu\text{g L}^{-1}$, for pesticides with a method limit of quantitation (MLOQ) of 0.5 $\mu\text{g kg}^{-1}$. Calibration curve was constructed each time a new sample set was analysed to accurately compensate for the day-to-day variation of the control standards. For simultaneous quantitation and identification purposes, two secondary ions were used to avoid false positives at trace pesticide levels. According to the SANCO guidelines 12571/2013 [9], identification of an analyte above the MLOQ in the sample is made when the following interpretation criteria are fulfilled:

1. A minimum of three identification points is required, i.e. when the two selected product ions are present;

2. The tolerance criteria for retention time should be within ± 0.2 min when comparing the unknown peak (in the test sample) with that of the corresponding analyte peak in the calibration standard;
3. The quantitative ion and the two identification ions should be present with signal-to-noise (S/N) ratio greater than 3; and
4. The identification ion/quantification ion ratios in the sample and the previously injected standard should not differ by more than the percentage as stipulated in the SANCO guidelines 12571/2013 [9].

If the above criteria were met, then identification of the analyte in the sample could be confirmed.

Recovery Tests and Matrix Effect

The proposed method is meant to be a versatile multiresidue method for many common OCPs in different variety of food matrices of wide range of lipid and moisture contents. To test the performance of the method for a wide range of matrices, we used the same grouping of commodities as specified in the European SANCO guidelines 12571/2013 [9] as well as selected food items in Hong Kong's first total diet study. A total of 17 food items were studied and some of these food items were grouped depending on their properties, e.g. apples, bananas, hairy gourd, lettuce, celery and carrot were grouped under 'high water content' samples. In each commodity group, the most representative species were selected and tested for recovery (Table 2). Recovery tests were conducted three times at MLOQ and 4 \times MLOQ level, respectively. Recovery tests were done with all 33 pesticide residues in all listed matrices, regardless of whether all pesticides are normally applied in these food items. Matrix effect, expressed as the percent deviation of slope of relative response ratio against relative concentration of the pesticide in matrix from the corresponding slope in isooctane, was also evaluated in 17 selected matrices.

Validation Study

The validation study was performed on the basis of the SANCO guidelines [9]. Analytical characteristics evaluated were sensitivity, mean spiked recovery, accuracy (as a measure of trueness), precision (expressed as repeatability and reproducibility), and selectivity. With this objective, recovery experiments with spiked blank samples were performed at 4 \times MLOQ during the total diet study (see Table 3). At least one pair of replicate was used for each sample matrix and at each spiking level (MLOQ and 4 \times MLOQ). Linearity was studied using standards, not matrix matched, across the ten concentrations between 0.5 and 100 $\mu\text{g L}^{-1}$.

Table 2 Spiked recoveries results in different food matrices conducted at MLOQ and four times MLOQ level, i.e. 0.5 and 2.0 $\mu\text{g kg}^{-1}$, respectively

Pesticide	Average recovery, % (RSD, %)															
	High water content		Citrus fruit		Cereal grain products		Milk		Eggs		Meat and seafood		High oil content		Difficult commodities	
	Fruits and vegetables*		Oranges		Pastas		Cow milk		Chicken eggs		Chicken, pig liver and salmon		Peanuts, olive oil and butter		Coffee	
	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ
Pentachlorobenzene	108 (4)	107 (6)	109 (2)	106 (2)	112 (4)	102 (6)	108 (8)	105 (7)	102 (9)	105 (7)	110 (2)	103 (5)	109 (7)	105 (3)	109 (1)	106 (7)
Hexachlorobenzene	108 (3)	108 (6)	110 (3)	110 (2)	107 (7)	105 (6)	115 (3)	93 (4)	102 (5)	101 (2)	106 (7)	113 (5)	101 (8)	104 (5)	91 (7)	107 (4)
α -HCH	92 (4)	87 (6)	88 (13)	86 (7)	94 (7)	93 (9)	105 (8)	91 (5)	100 (2)	81 (9)	109 (6)	96 (8)	106 (8)	97 (2)	108 (4)	89 (1)
γ -HCH	95 (8)	99 (5)	108 (12)	86 (4)	108 (2)	105 (6)	103 (9)	93 (3)	99 (8)	86 (6)	101 (4)	100 (6)	104 (6)	112 (2)	106 (11)	102 (7)
β -HCH	96 (4)	95 (7)	83 (5)	97 (10)	110 (11)	114 (0)	111 (7)	94 (2)	107 (8)	107 (2)	109 (7)	104 (7)	99 (10)	109 (8)	108 (4)	114 (3)
Heptachlor	106 (6)	111 (3)	108 (5)	114 (3)	113 (5)	112 (2)	115 (5)	116 (2)	112 (5)	110 (8)	104 (8)	105 (6)	103 (6)	112 (4)	116 (4)	115 (4)
Oxychlorodane	107 (4)	104 (4)	104 (7)	109 (8)	117 (2)	117 (1)	113 (2)	104 (10)	109 (6)	105 (1)	106 (6)	107 (5)	110 (3)	110 (3)	115 (2)	111 (1)
<i>cis</i> -Heptachlor epoxide	103 (6)	103 (3)	92 (3)	98 (2)	115 (1)	98 (5)	104 (5)	98 (8)	94 (4)	93 (4)	105 (2)	98 (4)	101 (4)	105 (3)	105 (6)	100 (2)
<i>trans</i> -Heptachlor epoxide	101 (8)	91 (5)	112 (5)	89 (10)	104 (5)	99 (7)	114 (5)	106 (3)	98 (3)	90 (11)	106 (5)	98 (9)	107 (5)	101 (5)	93 (3)	100 (6)
2,4'-DDE	95 (4)	97 (5)	104 (11)	100 (2)	110 (4)	107 (2)	107 (5)	104 (7)	97 (4)	93 (2)	101 (5)	97 (5)	106 (6)	106 (1)	110 (7)	100 (3)
<i>trans</i> -Chlordane	104 (8)	107 (2)	106 (5)	109 (2)	106 (4)	111 (2)	112 (5)	103 (9)	105 (1)	109 (5)	109 (4)	106 (5)	108 (5)	108 (1)	112 (3)	112 (2)
<i>trans</i> -Nonachlor	104 (5)	106 (2)	112 (5)	114 (4)	114 (6)	111 (2)	114 (7)	102 (5)	109 (5)	112 (2)	109 (5)	102 (4)	110 (3)	107 (1)	114 (5)	114 (2)
<i>cis</i> -Chlordane	99 (7)	98 (4)	97 (7)	100 (3)	108 (4)	98 (5)	107 (6)	95 (5)	97 (4)	93 (5)	99 (7)	95 (2)	101 (3)	100 (1)	106 (7)	98 (3)
α -Endosulfan	101 (6)	92 (5)	112 (6)	95 (9)	102 (7)	97 (4)	92 (5)	90 (6)	89 (4)	84 (10)	99 (9)	99 (6)	109 (9)	109 (3)	95 (11)	91 (10)
4,4'-DDE	105 (5)	106 (4)	104 (7)	106 (4)	117 (3)	109 (3)	96 (8)	100 (2)	97 (9)	106 (5)	101 (8)	109 (4)	104 (7)	108 (4)	114 (1)	115 (2)
Mirex	108 (6)	105 (5)	112 (3)	108 (6)	113 (0)	91 (8)	111 (7)	92 (5)	100 (6)	106 (4)	108 (6)	103 (7)	98 (5)	90 (3)	110 (7)	102 (9)
δ -HCH	98 (7)	101 (7)	108 (7)	106 (4)	105 (9)	108 (4)	108 (5)	91 (4)	108 (5)	111 (5)	99 (6)	105 (5)	109 (7)	108 (3)	115 (5)	109 (7)
2,4'-Dicofof	94 (6)	97 (8)	102 (6)	102 (7)	108 (5)	105 (9)	111 (3)	104 (7)	100 (7)	97 (5)	108 (5)	109 (4)	102 (6)	108 (6)	103 (11)	109 (3)
4,4'-Dichlorobenzophenone	96 (8)	95 (8)	98 (2)	101 (9)	97 (9)	95 (10)	99 (5)	96 (2)	103 (12)	89 (5)	109 (6)	105 (4)	98 (8)	106 (5)	117 (3)	102 (5)
Dieldrin	101 (8)	99 (10)	112 (4)	96 (5)	105 (10)	94 (7)	108 (3)	91 (8)	95 (3)	92 (6)	105 (6)	94 (8)	109 (5)	101 (3)	111 (3)	110 (3)
2,4'-DDD	99 (4)	100 (3)	100 (1)	98 (1)	108 (10)	107 (6)	113 (6)	111 (5)	101 (7)	99 (4)	104 (4)	106 (3)	104 (4)	104 (1)	101 (8)	107 (2)
Endrin	105 (6)	106 (3)	108 (5)	101 (2)	111 (4)	115 (1)	114 (4)	106 (1)	103 (5)	109 (4)	105 (7)	108 (5)	107 (4)	108 (2)	108 (8)	116 (1)
2,4'-DDT	97 (3)	96 (5)	95 (3)	95 (3)	108 (4)	102 (3)	110 (6)	97 (1)	96 (5)	96 (4)	102 (4)	103 (4)	106 (6)	105 (3)	103 (5)	107 (3)
<i>cis</i> -Nonachlor	91 (3)	93 (3)	101 (2)	97 (3)	103 (2)	94 (1)	97 (1)	89 (2)	93 (5)	98 (4)	93 (4)	93 (4)	94 (3)	92 (3)	97 (5)	99 (4)
4,4'-DDD	106 (6)	110 (5)	104 (8)	102 (4)	111 (6)	107 (8)	104 (6)	99 (7)	101 (10)	107 (1)	108 (7)	110 (5)	108 (4)	108 (2)	113 (4)	115 (5)
β -Endosulfan	100 (9)	95 (8)	105 (10)	101 (5)	103 (2)	92 (3)	107 (4)	92 (4)	98 (9)	91 (8)	102 (9)	101 (6)	100 (7)	97 (5)	106 (8)	96 (2)
Endosulfan sulphate	104 (6)	106 (7)	99 (2)	100 (7)	94 (8)	97 (8)	115 (2)	99 (1)	108 (3)	101 (11)	99 (5)	105 (7)	105 (7)	100 (3)	105 (13)	104 (4)

Table 2 continued

Pesticide	Average recovery, % (RSD, %)															
	High water content		Citrus fruit		Cereal grain products		Milk		Eggs		Meat and seafood		High oil content		Difficult commodities	
	Fruits and vegetables*		Oranges		Pastas		Cow milk		Chicken eggs		Chicken, pig liver and salmon		Peanuts, olive oil and butter		Coffee	
	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ	MLOQ	4 × MLOQ
Endrin ketone	92 (6)	95 (6)	88 (3)	87 (1)	100 (7)	83 (8)	89 (10)	84 (4)	95 (8)	84 (4)	95 (3)	97 (8)	102 (5)	94 (5)	100 (11)	91 (10)
Aldrin	108 (4)	108 (5)	108 (3)	99 (3)	111 (4)	100 (1)	118 (2)	97 (3)	102 (7)	101 (5)	97 (5)	96 (6)	104 (4)	99 (2)	110 (7)	109 (1)
Isodrin	102 (7)	101 (6)	104 (5)	112 (6)	114 (2)	104 (10)	103 (3)	91 (2)	106 (4)	106 (13)	103 (5)	107 (10)	105 (7)	98 (5)	114 (4)	114 (3)
Methoxychlor	109 (4)	107 (4)	111 (1)	108 (1)	106 (3)	109 (4)	114 (4)	105 (4)	104 (2)	109 (3)	110 (4)	105 (2)	111 (3)	108 (2)	114 (2)	111 (2)
4,4'-DDT	107 (5)	105 (3)	107 (4)	104 (3)	109 (5)	102 (6)	114 (4)	112 (3)	108 (7)	103 (6)	111 (4)	105 (5)	103 (4)	99 (1)	115 (1)	108 (6)
Endrin aldehyde	106 (4)	103 (5)	107 (9)	100 (2)	108 (10)	90 (2)	104 (7)	87 (4)	111 (7)	95 (2)	101 (6)	96 (4)	104 (7)	100 (6)	117 (3)	97 (6)

* Commodities included apples, bananas, hairy gourd, lettuce, celery and carrot

The MLOQ was established as the lowest quantifiable concentration tested, for which recovery and precision were assessed in accordance with the criteria established for analysis of pesticide residues in foods [9]. The trueness was validated with CRMs including cod liver oil (SRM 1588c), spiked milk powder (BCR 188), Lake Superior fish tissue (SRM 1946) and animal feed (BCR 115) as summarized in Table 4.

Results and Discussion

Extraction of OCPs

For extracting OCPs from various food matrices, including beverage, cereal products, edible oil, animal tissues, etc. using MSPD, a number of different sorbent materials with large surface area were tested, including Lipid Removal Agent (LRA) media [5], EXTrelut® NT-20 and Calflo E. However, trace amount of impurities were found to be co-extracted that produced noisy background signal in GC-MS analysis and/or their lipids absorption capacities were found to be ineffective. Thus, these materials were considered not fit-for-purpose. Another material, namely Hydromatrix, was then tested as it had been reported that it can absorb water efficiently. Even if 10 g edible oil was used, we found that the amount of fatty substances left after the MSPD step was around 0.3–0.4 g only. Besides, amount of trace impurities were found to be reduced significantly. Therefore, Hydromatrix together with 10 g of magnesium sulphate was employed as the MSPD sorbent for the extraction of OCPs in various food matrices including high water content foods. To our understanding, this is the first successful case that used hydromatrix as absorbent in MSPD for removing both water and fatty substances from food so as to analyse OCPs.

To achieve the low detection limit and quantitation limit, this method necessitates at least 10 g of sample to start with. This in turn requires considerable amount of solvent to elute all extractable analytes from the sample/sorbent mixture packed in the column. Keeping environmental concern in mind, we had tried using different volumes of eluting solvent to find out the lowest possible volume (to minimize solvent usage) with reasonable recovery (to attain good recovery). It was noted that after packing the column with the sample/sorbent mixture, a minimum of 50 mL eluting solvent was needed to wet the column. Another 100 mL would be needed to extract and elute most of the desired analytes from the column.

Cleanup

As OCPs are fat-soluble compounds, other fatty substances would be co-extracted from the sample at the same time.

Table 3 Comparison of found concentrations with the certified values of CRMs

Pesticide	Certified reference materials							
	BCR 115		BCR 188		NIST SRM 1588c		NIST SRM 1946	
	Animal feed		Spiked milk powder		Cod liver oil		Lake Superior fish tissue	
	Certified value	Found value (RSD, %)	Certified value	Found value (RSD, %)	Certified value	Found value (RSD, %)	Certified value	Found value (RSD, %)
Aldrin	–	14.2 (5.4)	–	–	–	–	–	–
<i>cis</i> -Chlordane	–	–	–	–	12.34 ± 0.38	12.4 (1.7)	32.5 ± 1.8	31.1 (1.5)
<i>trans</i> -Chlordane	48 ± 6	49.1 (3.2)	–	–	11.98 ± 0.95*	11.8 (4.7)	8.36 ± 0.91	8.4 (3.5)
<i>o,p'</i> -DDD	–	9.3 (3.6)	–	–	9.546 ± 0.072	9.6 (0.4)	2.20 ± 0.25	2.1 (7.0)
<i>o,p'</i> -DDE	–	–	–	–	6.61 ± 0.33*	6.5 (2.8)	1.04 ± 0.29*	0.9 (4.2)
<i>o,p'</i> -DDT	46 ± 5	43.7 (0.6)	–	–	–	–	22.3 ± 3.2*	21.1 (3.9)
<i>p,p'</i> -DDD	–	57.3 (2.3)	–	–	65.97 ± 0.99	66.3 (0.8)	17.7 ± 2.8	15.4 (3.9)
<i>p,p'</i> -DDE	47 ± 4	47.1 (2.4)	51 ± 4	53.2 (2.4)	104.0 ± 1.6	103 (1.2)	373 ± 48	383 (4.1)
<i>p,p'</i> -DDT	–	36.3 (7.4)	69 ± 5	67.0 (1.6)	71.5 ± 1.6	71.5 (1.2)	37.2 ± 3.5	37.7 (4.1)
Dieldrin	18.1 ± 2.3	18.2 (6.8)	36.1 ± 2.5	35.7 (3.4)	–	–	32.5 ± 3.5	32.6 (1.6)
α-Endosulfan	46 ± 4	45.6 (4.4)	–	–	–	–	–	–
Endrin	46 ± 6	45.5 (4.8)	6.2 ± 0.9	6.0 (8.0)	–	–	–	–
Endrin ketone	–	2.0 (6.4)	–	–	–	–	–	–
Heptachlor	19.0 ± 1.5	19.1 (1.4)	–	–	–	–	–	–
<i>cis</i> -Heptachlor epoxide	–	15.6 (7.0)	32.0 ± 1.9	31.9 (3.8)	–	–	5.50 ± 0.23	5.6 (1.4)
HCB	19.4 ± 1.4	18.4 (1.4)	37.4 ± 2.7	38.3 (3.7)	–	6.0 (17)	7.25 ± 0.83	7.6 (1.6)
α-HCH	–	17.0 (5.4)	–	11.3 (6.7)	–	–	5.72 ± 0.65	5.6 (5.6)
β-HCH	23.4 ± 2.6	23.5 (3.2)	12.0 ± 1.2	12.7 (5.6)	–	–	–	–
γ-HCH	21.8 ± 2.0	21.1 (4.4)	45.4 ± 2.9	44.3 (2.8)	–	–	1.14 ± 0.18	1.1 (5.5)
Methoxychlor	–	2.0 (4.2)	–	–	–	–	–	–
Mirex	–	–	–	–	–	–	6.47 ± 0.77	6.0 (5.8)
<i>cis</i> -Nonachlor	–	–	–	–	14.64 ± 0.49	14.7 (2.0)	59.1 ± 3.6	57.4 (2.5)
<i>trans</i> -Nonachlor	–	–	–	–	17.01 ± 0.50	17.0 (1.9)	99.6 ± 7.6	93.7 (2.4)
Oxychlordane	–	–	–	–	1.78 ± 0.17	1.8 (5.5)	18.9 ± 1.5	17.8 (9.7)

* Reference value

These fatty substances are highly soluble in organic solvent and tend to adsorb in the GC system resulting in poor chromatographic performance and shorten the lifetime of the GC column. Besides, co-extracted substances might also induce matrix enhancement/suppression effect. Furthermore, the remaining lipids would also affect the efficiency of solid-phase extraction (SPE) cleanup. Therefore, the success of the analysis of OCP critically relies on the efficiency of lipids removal.

Based on the above-described MSPD extraction, the maximum amount of lipids left was <0.5 g for 10 g lipids. As gel permeation chromatography (GPC) can separate OCPs from high molecular mass compounds such as lipids, chlorophylls, etc. and remove most of the residual lipids, GPC was selected to remove both the remaining lipids and other high molecular weight substances. In comparison,

other alternative lipid-removing techniques, such as liquid-liquid extraction, lipid-removal SPE, etc. could not achieve the same level of lipid-removing capacity as well as separation of the lipid-soluble OCPs from other co-extractives. However, one drawback of GPC is the consumption of considerable amount of GPC mobile phase. To tackle this issue, we noticed that though most authors used Bio-beads SX-3[®] column as the GPC phase for OCPs analysis, the use of Envirosep-ABC[®] GPC column could reduce the solvent usage to half. Another advantage is that the whole run time could also be reduced to about half an hour per sample.

Doong and Lee [7] compared the cleaning efficiency of ready-to-use cartridge filled with three different adsorbents (C₁₈, alumina and Florisil) for shellfish extract. Their results demonstrated that out of the 14 OCPs tested, two were retained in the C₁₈-cartridge. As for alumina and Florisil

Table 4 Spiked recoveries results of 49 different food matrices at 2 µg kg⁻¹ level and matrix effect

Mean recovery (%) and precision (%)	Overall recoveries amongst 49 matrices			Overall RSD amongst 49 matrices			Mean matrix effect (%)
	Mean (%)	Min (%)	Max (%)	Mean (%)	Min (%)	Max (%)	
PeCB	106	80	114	3	0	12	6
HCB	106	75	115	2	0	21	8
α-HCH	91	74	115	5	0	15	-14
γ-HCH	101	80	114	5	0	15	-3
β-HCH	101	87	116	7	0	23	-1
Heptachlor	104	80	118	5	0	26	10
Oxychlordane	101	80	118	4	0	14	8
<i>cis</i> -Heptachlor epoxide	103	73	118	4	0	15	-4
<i>trans</i> -Heptachlor epoxide	103	81	119	6	0	17	-13
2,4'-DDE	108	83	119	3	0	8	-3
<i>trans</i> -Chlordane	104	76	116	2	0	10	11
<i>trans</i> -Nonachlor	100	80	109	2	0	5	3
<i>cis</i> -Chlordane	101	79	112	3	0	9	-7
α-Endosulfan	105	80	119	4	0	12	-11
4,4'-DDE	106	81	117	4	0	24	4
Mirex	96	72	116	5	0	18	-6
δ-HCH	101	75	115	4	0	14	4
2,4'-Dicofol	100	79	115	6	0	20	5
4,4'-Dichlorobenzophenone	105	80	119	5	0	19	-4
Dieldrin	98	73	115	5	0	14	-9
2,4'-DDD	100	74	115	3	0	11	-4
Endrin	108	89	119	5	0	18	5
2,4'-DDT	104	77	116	4	0	15	0
<i>cis</i> -Nonachlor	93	71	107	3	1	12	-5
4,4'-DDD	100	72	116	5	1	13	5
β-Endosulfan	96	76	113	8	1	19	-7
Endosulfan sulphate	95	71	111	6	1	19	2
Endrin ketone	89	71	108	5	0	20	-15
Aldrin	102	82	114	3	1	9	-3
Isodrin	98	72	119	7	0	20	5
Methoxychlor	103	81	117	2	0	9	8
4,4'-DDT	104	81	118	4	0	29	4
Endrin aldehyde	94	74	110	4	0	17	-5

Food matrices included rice, corn, pasta, bread, oat meal, potato, broccoli, petiole cabbage, celery, Chinese lettuce, mung bean sprout, bitter melon, hairy gourd, wax gourd, zucchini, onion, eggplant, green string bean, mung bean vermicelli, banana, kiwi, longan/lychee, melon, peach, pear, pineapple, water melon, beef, pork, soy-sauced chicken, horse-head fish, sole fish, daced minced fish, skim milk, yoghurt, tea, malt drink, soy bean drink, carbonated drink, chrysanthemum tea, siu mei, Chinese soup, plain steamed rice roll, table salt, soya sauce, potato chip, oyster sauce, grouper fish & tomato sauce/ketchup

SPE, though all 14 tested OCPs could be recovered, Florisil provided better results in terms of recoveries, repeatability and removal of interfering substances. Besides, Yague et al. [10, 11] reported that β-HCH, β-endosulfan and endosulfan sulphate were believed to retain on the neutral alumina SPE cleanup. Based on above-mentioned literatures, Florisil is considered the most suitable sorbent for SPE cleanup of OCPs.

During the initial stage of method development, cleanup performance of Florisil packed in glass column

and SPE cartridge were studied, against various eluting solvents commonly used including *n*-hexane, DCM, cyclohexane and toluene. Only the combination of Florisil with *n*-hexane (with 1 % methanol) could recover all targeted OCPs. Any other combinations would lose some of the polar OCPs such as endrin, endrin aldehyde and/or chlordecone. For ready-to-use SPE cartridge, additional interferences were coeluted and interfered the GC-MS analyses.

GC Analysis of OCPs

Overlapping of peaks commonly occurs if the number of target OCPs is more than 20, especially when isomers of BHC, α -endosulfan, *trans*-nonachlor and α -chlordane are involved. In our first attempt to separate 33 targeted OCPs, Stx-CLPesticides columns (by Restek) were used and could not completely separate all 33 OCPs after trying many different GC running conditions. Examples of chromatographic overlapping OCPs included heptachlor epoxide, *o,p'*-DDE, *trans*-nonachlor, *cis*-chlordane, *p,p'*-DDE, α -endosulfan, chlordecone and *cis*-nonachlor, depending on the running parameters. The Rtx-CLPesticides columns (by Restek) were found to be better and could basically separate almost all 33 targeted OCPs in a run of half an hour. Recently, another model of capillary columns, Zebtron-MultiResidue, i.e. ZB-MR1 and ZB-MR2 (by Phenomenex), were introduced and found to provide almost baseline separation for the 33 targeted OCPs in 12 min (Fig. 1a). Although *p,p'*-dichlorobenzophenone overlapped with oxychlordane, their molecular masses are different and can be easily distinguished by GC-MS.

DDT and endrin are easily degraded in the GC injection port, especially when the liner is contaminated with high-boiling residue from samples [12] or the inertness of flow path is not well controlled. When the injector temperature is higher than 150 °C, these OCPs would start to degrade noticeably [13]. However, low injector temperature would significantly reduce the sensitivities for other OCPs. In recent years, highly inert deactivated liners and connectors are commercially available that can greatly reduce the decomposition of these OCPs. Using the Siltek® double gooseneck splitless liner, it was found that there was almost no breakdown product for DDT and endrin at 180, 200 or even 220 °C. Thus, an optimum temperature of 220 °C was selected with sufficient sensitivities for all other OCPs. Another commonly encountered problematic analyte in OCPs analysis is dicofol. Only *o,p'*-dicofol and *p,p'*-dichlorobenzophenone could be detected by GC analysis. The major isomer, namely *p,p'*-dicofol, was found to degrade almost completely during GC analysis even though the solution was prepared freshly. Hence, this compound is likely degraded at the injection port or inside the column and cannot be analysed.

Electronic and Negative Chemical Ionization MS

Although GC hyphenated with a number of different detectors have been used for the detection of OCPs, confirmation with mass spectrometric (MS) detector was normally required. Single quadrupole MS detector running in electron ionization (EI) mode with target analytes monitored by selective ion monitoring (SIM) is a common monitoring

tool for OCPs [13–15]. However, some OCPs such as dieldrin, endrin, endrin aldehyde, chlordecone, oxychlordane, α - and β -endosulfan were found to have lower signal responses using EI mode when compared to other OCPs.

Since some of the OCPs are electronegative in nature, GC-MS detector under negative chemical ionization (NCI) mode with methane as reagent gas could provide better sensitivity [16]. The signal responses of 33 targeted OCPs, using EI and NCI as ionization modes, respectively, have been compared. It was found that NCI generally gives better sensitivity, both in terms of absolute counts and signal-to-noise (S/N) ratio. As Fig. 1b shows, some of the OCPs gained more than an order increase in sensitivity when switching from EI to NCI mode. The drawback of NCI is that methoxychlor is very insensitive and cannot be detected in parts-per-billion level. Besides, the signal responses for some OCPs, notably *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT, are actually reduced. Furthermore, chlordecone had been found to be quite insensitive, both in NCI and EI mode. Therefore, EI was finally chosen and chlordecone was eventually excluded from the scope of analysis.

Matrix Effect

In this work, seventeen matrices were selected for the evaluation of matrix effect at MLOQ and 4× MLOQ levels. The slopes obtained in the calibration with matrix-matched standards were compared with those obtained with standard solutions. Evaluation data of matrix effect are presented in Table 3. Mild matrix effect (suppression or enhancement of 0–25 %) were found for most of the analyte–matrix pairs. Medium matrix effect of between 26 and 29 % was only found for six analyte–matrix pairs distributed in six different matrices. Therefore, we did not perform quantitation using calibration with matrix-matched standards as the matrix effect was <30 %. This eliminated the trouble of finding representative blank matrices similar to various types of food samples.

Schenck and Donoghue [17] reported matrix enhancement effect for DDT metabolites when analysing OCPs in eggs with GC-ECD. Similarly, Garrido-Frenich et al. [18] reported that corrected spike recoveries were close to 100 % only after matrix-matched calibration was applied. Hence, our study provided a low matrix effect cleanup so that matrix-matched calibration is not necessary.

Matching of Internal Standards

Although more than 10 targeted OCPs have commercially available isotopically labelled standards, minimum number of labelled standards was employed to reduce the cost of analysis. Stefanelli et al. [19] suspected that the low recovery of HCB was due to its high volatility and loss

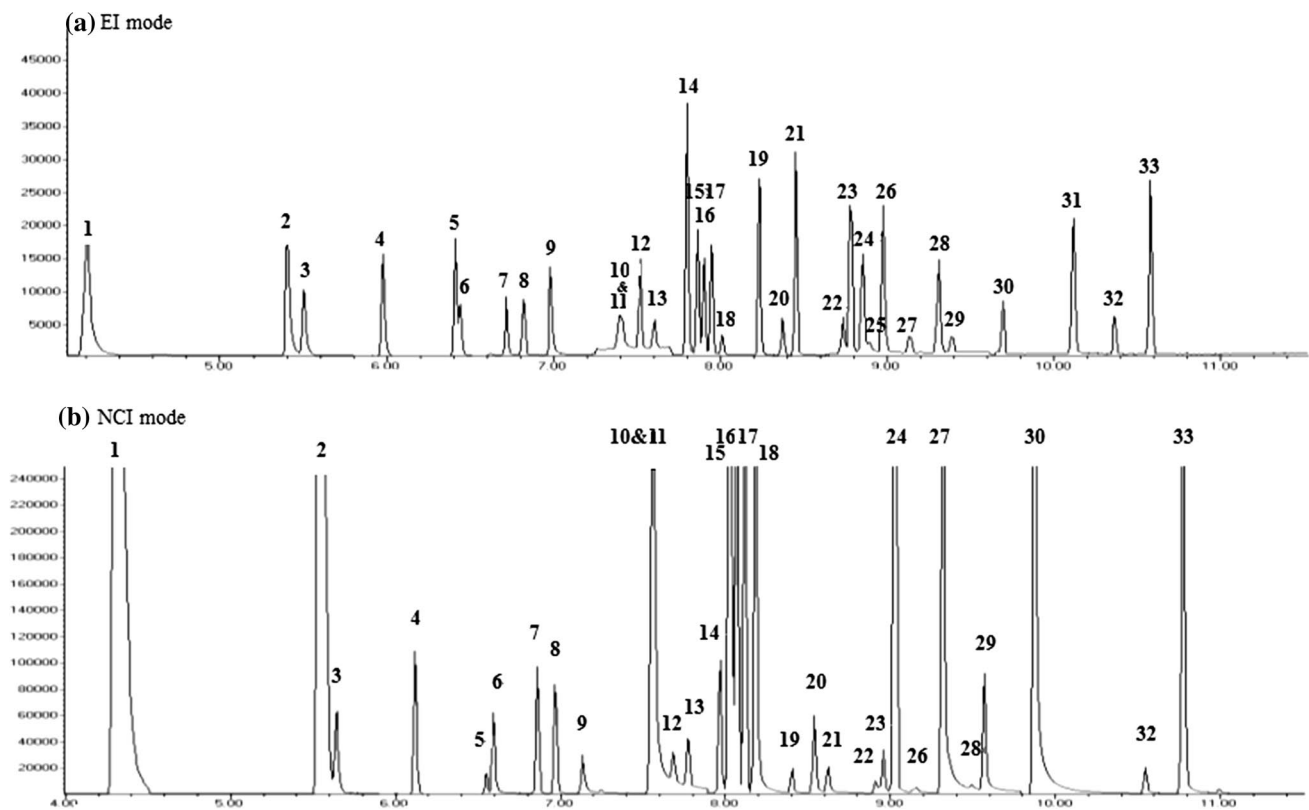


Fig. 1 Different OCPs at $20 \mu\text{g kg}^{-1}$ level analysed by GC–MS with a ZB-MR2 column under SIM mode. **a** EI mode and **b** NCI mode. Note The 33 OCPs are as follows: 1 PeCB, 2 HCB, 3 α -HCH, 4 γ -HCH, 5 β -HCH, 6 heptachlor, 7 δ -HCH, 8 aldrin, 9 *o,p'*-dicofol, 10 *p,p'*-dichlorobenzophenone, 11 oxychlordan, 12 *cis*-heptachlor epoxide, 13 *trans*-heptachlor epoxide, 14 *o,p'*-DDE, 15 *trans*-chlor-

dane, 16 *trans*-nonachlor, 17 *cis*-chlordan, 18 α -endosulfan, 19 *p,p'*-DDE, 20 dieldrin, 21 *o,p'*-DDD, 22 endrin, 23 *o,p'*-DDT, 24 *cis*-nonachlor, 25 chlordecone, 26 *p,p'*-DDD, 27 β -endosulfan, 28 *p,p'*-DDT, 29 endrin aldehyde, 30 endosulfan sulphate, 31 methoxychlor, 32 endrin ketone and 33 mirex

during solvent evaporation. Similarly, PeCB was found to be lost easily during evaporation without adding some “high-boiling” solvent as a trapping agent into the sample before being concentrated. Therefore, for quantitation purpose, the matching of internal standard with target analytes was based on the volatility and overall recovery of targeted analytes.

Analytical Performance

The accuracy and repeatability of the method were studied by means of recovery experiments at two spiking levels, MLOQ and $4 \times$ MLOQ (i.e. 0.5 and $2.0 \mu\text{g kg}^{-1}$). Apples, bananas, hairy gourd, lettuce, celery, carrot, orange, pasta, peanuts, olive oil, butter, chicken meat, pig liver, salmon, cow milk, chicken eggs and coffee were selected as the representative matrices for different commodity groups in the validation. All recovery experiments were performed three times at each level as suggested by the SANCO guidelines. The overall performance of initial validation was summarized into 17 food matrices in Table 2. The average

recoveries were ranged from 81 to 118 % and average coefficients of variation (CV) were below 13 %, which fulfilled the SANCO recommendations.

The trueness of the method was demonstrated by analyzing four different matrix CRMs, including animal feed, milk powder, fish tissue and cod liver oil. The results of analyses of each OCP are given in Table 3 and were shown to comply well with the certified ranges as specified by the producers. Using our analytical method, some of the OCPs were actually found in these CRMs but not reported in the certificates, especially in the animal feed sample.

On-going performance of the method was monitored by recovery experiments of real sample spikes at $4 \times$ MLOQ during the total diet studies. Average recoveries of 89–108 % were achieved, which were not much different from the initial validation data (Table 4). Despite the wide variety of sample matrices spiked in this study, the worst individual single recoveries still fell within the range of 71–119 %, which matched the generalized acceptable range for routine multiresidue analysis mentioned in the SANCO guidelines. Within-laboratory reproducibility, expressed as

standard deviation on on-going performance of the method, was found to be <10 % for 33 analytes. Hence, the robustness of the method was also demonstrated.

Codex Alimentarius Commission's Requirements

With reference to the maximum residue level (MRL) or extraneous maximum residue level (EMRL) set by Codex Alimentarius Commission (Codex) for OCPs, most of them are on lipid basis. For liquid milk samples with around 3.5 % lipid, the quantitation limit has to be lowered by around 30 times when whole weight basis is applied. Furthermore, Codex's procedure manual [20] also established working instruction for the implementation of the criteria approach which specified that the limit of detection (LOD) and limit of quantitation (LOQ) should be equal to or lower than 1/5 and 2/5 of the MRL/EMRL, respectively, for analyte level lower than 0.1 mg kg⁻¹. For example, the MRL for DDT in milk is 0.02 mg kg⁻¹, the corresponding LOQ for DDT in milk should be lower than 0.3 µg kg⁻¹ on whole weight basis. Owing to the fact that DDT is defined as the sum of 4 isomers in the Codex system, the LOQ for individual isomer should be better than 0.1 µg kg⁻¹ on whole weight basis. Therefore, the developed method is not applicable at first glance. But in practice, since OCPs and lipids of milk samples can be first extracted into *n*-hexane before subjecting to GPC procedure, a 20-fold lower of LOQ can easily be achieved which would fulfil Codex's requirements. Another way to get around this limitation is concentrate the final eluate from the Florisil SPE column from 1 mL to 50 µL before subjecting to GCMS analysis.

Application of the Method to Real Samples

The developed method was applied to the analysis of 600 total diet study samples containing different types of food samples. To assure the quality of the results, reagent blank (obtained by performing the whole procedure without sample) was used to remove any possibility of false positive due to contamination in the instruments or reagents employed. Replicates of spiked samples at 4× MLOQ were also performed to assess the extraction efficiency, spike recovery as well as precision. Results of spiked recoveries ($n = 133$) were shown in Table 3 and all fell within the range of 70–120 %.

Traces of OCPs, most of them were of <10 µg kg⁻¹, were detected in some of the analysed samples. Only four out of 600 samples were found to contain residues of DDT of levels >100 µg kg⁻¹, three of them were found to contain endosulfan with level of >50 µg kg⁻¹ while one of them contained HCH content >20 µg kg⁻¹. None of them

was found to be greater than the MRL/EMRL as set by the Codex Alimentarius Commission [21]. It was very rare to detect 5 OCPs simultaneously in the same sample except for pomfret fish, which showed traces of chlordane, endosulfan, HCB, HCH and PeCB of <2 µg kg⁻¹ and DDTs in the range of 40–72 µg kg⁻¹. In our study, 32 % of the samples, mainly fish, were found to contain trace amount of DDTs. In addition, 30 and 22 % of the samples were found to contain trace amounts of HCB and endosulfan, respectively.

Conclusions

A novel analytical method employing MSPD extraction, GPC and Florisil cleanup that allows efficient and matrix effect-free extraction and enrichment of 33 OCPs from various food samples has been developed and validated. Combined with GC–MS, the method achieves MLODs in the sub µg kg⁻¹ concentration range for all target analytes in a wide range of dietary matrices. Compared to previously described methods for OCP analysis, the method presented here represents a significant step forward with respect to:

- **Applicability.** The rigorous extraction cum cleanup approach exploiting the lipophilic properties of OCPs makes the method applicable to a wide range of (food) matrices, including food with high water or fat content. Solids and liquids can be analysed alike.
- **Sensitivity.** MLOQs are a factor of 5–100 (depending on the homologue) lower than in earlier studies [3]. This improvement in method performance enabled the first detection and quantification of 33 OCPs in the total diet study samples.
- **Reliability of results.** This is the first study to demonstrate with results of four different CRMs of OCPs in complex matrices using non-fortified samples.

Besides, the method has successfully included metabolites/derivatives of OCPs as analytes in the various food matrices. Hence, the quantified value for each OCP with different residue definitions could be assessed. Furthermore, the method performance of this method also satisfied with the method performance of guidelines of SANCO. In conclusion, up to now, the developed method is one of the only very few reported methods that can determine most of the OCPs in foods, of both fatty and high water content nature, and fulfil the required method performance criteria as set by the Codex.

Conflict of interest The authors declare that they have no conflict of interest.

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