

MULTICOMPONENT DETERMINATION OF ORGANOCHLORINATED COMPOUNDS IN MILK BY GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION AFTER LIQUID-LIQUID EXTRACTION

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Abstract: *This paper reports a multiresidue analytical method based on liquid-liquid extraction and capillary gas chromatography with electron-capture detection (LLE-GC-ECD). The determined analytes belong to two organochlorinated compound classes: polychlorinated biphenyls (PCBs), and organochlorine pesticide (OCPs). This procedure can be used in routine determination of OCPs and PCBs in milk.*

Keywords: liquid-liquid extraction, GC, PCBs, OCPs, milk

INTRODUCTION

Worldwide production and use of polychlorinated biphenyls (PCBs) and organochlorine pesticide (OCPs) has resulted in their widespread occurrence in the environment [1, 2].

These compounds are persistent and toxic, lipophilic and rapidly metabolized. These organic compounds are intensively studied due to their accumulation in high concentrations in the environment, food, milk and blood. Environmental exposure of living organisms to these toxic compounds led in their accumulation and persistence in fat tissues [3]. Food, particularly dairy products, meat and fish has been identified as the primary intake route of organochlorine pesticides and PCB congeners for the general population. Exposure to these pollutants has the toxic effects in human and especially in infants [4].

Milk-producing animals accumulate residues of these pesticides contaminated feed, grass/hay, and inhaled air. Bovine milk has been used as an indicator of the persistence of chemical environmental contaminants [5, 6]. Considering the toxicity of organochlorine compounds and the importance of milk and dairy products for human nutrition, especially for children, the aim of this work was to determine the level of individual PCB congeners and organochlorine pesticides in milk.

MATERIAL AND METHOD

1. Sampling

Three cow milk samples were collected in the rural area situated in the north-eastern adjacent area of Cluj-Napoca city, in September 2009. Samples (50 ml) were collected and transported to the laboratory in clean glass bottles and stored at -20 °C until analysis.

2. Chemicals

The solvents (n-hexane, acetonitrile and ethanol) used in this study were gas chromatography grade of quality (Merck, Darmstadt, Germany).

Anhydrous sodium sulphate, silica gel and Florisil (Merck) were used after heating overnight at 120°C.

Standard solution (Mix Standard solution for EN ISO 6468 CERTAN, NE7550) for organochlorine pesticides, polychlorinated biphenyls and chlorobenzenes was purchased from LGC Promochem (Germany) and contained 36 compounds: α -, β -, γ -, δ -, ϵ -isomers of hexachlorocyclohexane (expressed as HCHs), 4,4'-DDE, 2,4'-DDE, 4,4'-TDE, 2,4'-TDE, 2,4'-DDT and 4,4'-DDT (expressed as DDTs), 4,4'-methoxychlor, aldrin, dieldrin, endrin, heptachlor, heptachlorepoxyde (isomer A), heptachlorepoxyde (isomer B), alfa-endosulfan, beta-endosulfan, 1,2,3-trichlorobenzene, 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene, 1,2,4,5-tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene and the following PCB congeners: tri (28), tetra (52), penta (101), hexa (138, 153), hepta (180) and octa (194).

Stock standard solution was 10 $\mu\text{g}/\text{ml}$ for each analyte and working standard solutions were prepared diluting accurate volumes of mix standard solution in dichloromethane.

3. Instrumentation

In order to separate, detect and quantify the OCPs, CBs and PBCs gas chromatography-electron-capture detection (GC-ECD) was used. An Agilent Technologies 6890N gas chromatograph with electron capture detector (μECD) equipped with a 30 m L \times 0.32 mm ID \times 0.50 μm (DB-608 made by Agilent J&W) was used in the study. Helium was used as carrier and nitrogen as make up gas. The ion source was ^{36}Ni .

For solvents evaporation was used a rotary evaporator, Laborota 4010 (Heidolph, Germany) coupled with a vacuum pump (Ilmvac, Germany).

4. Method

In this study the extraction and clean-up of milk samples were carried out according to method described by Ennaceur et al., (2007). The method consists of extraction with n-hexane, acetonitrile and ethanol, clean-up on florisil column and elution with dicloromethane and n-hexane, evaporation with rotavapor and then GC-ECD analysis [7].

The milk samples were left to defrost at ambient temperature and homogenized. Twenty milliliters of milk were extracted three times with a solvent mixture (20 ml n-hexane, 5 ml acetonitrile and 1 ml ethanol). The hexane layers were filtered on anhydrous sodium sulfate and evaporated to 5 ml by a rotating evaporator. Exactly 1 ml was pipetted into a pre-weighed bottle and evaporated to dryness. The flask was weighed until a constant weight.

The difference between this weight and the original weight of the flask was used to calculate the fat content of the sample. The 4 ml extract was purified in a chromatographic mini-column filled with activated florisil and anhydrous sodium sulfate. The extract was eluted with 30 ml of elution mixture (dichloromethane and n-hexane, 1:9; v/v). The eluate was evaporated using a rotary evaporator to 1.0 ml and was ready for GC analysis. One μ l was injected in the GC-ECD. The operation conditions for inlet, column, oven and detector are shown in Table 1.

Table 1

Operation characteristics for GC-ECD

Inlet (split/splitless)	Column	Oven	Detector (μ ECD)
Mode: Splitless	Capillary Column	Maximum temp: 325°C	Temperature: 300 °C
Initial temp: 280 °C	Model Number: J&W 123-1730	Initial temp: 80 °C	Mode: Constant makeup flow
Pressure: 9.03 psi	Max temperature: 280 °C	Initial time: 8.00 min	Makeup flow: 30.0 mL/min
Purge flow: 60.0 mL/min	Nominal length: 30.0 m	Equilibration time: 1.00 min	Makeup Gas Type: Nitrogen
Purge time: 1.00 min	Nominal diameter: 320.00 μ m	Ramps: 4 (shown in Table 2)	Electrometer: On
Total flow: 64.7 mL/min	Nominal film thickness: 0.50 μ m	Post temp: 120 °C	
Gas saver: On	Mode: constant flow	Post time: 0.00 min	
Saver flow: 20.0 mL/min	Initial flow: 1.6 mL/min	Run time: 85.75 min	

The oven temperature program is shown in table 2.

Table 2

The oven temperature program

Ramp	Rate	Final temperature, °C	Final time, min
1	4.00	196	2.00
2	4.00	224	2.00
3	4.00	240	5.00
4	4.00	275	20.00

The values were calculated after integration of chromatograms using Chemstation soft. To obtain the values for OCPs and PCBs for each sample, we summed the obtained concentrations for each class of compounds. Thus, for OCPs concentration we summed the concentrations obtained for: α -, β -, γ -, δ -, ϵ -isomers of hexachlorocyclohexane, 4,4'-DDE, 2,4'-DDE, 4,4'-TDE, 2,4'-TDE, 2,4'-DDT and 4,4'-DDT, p,p'-methoxychlor, aldrin, dieldrin, endrin, heptachlor, heptachlorepoxyde (isomer A), heptachlorepoxyde (isomer B), alfa-endosulfan, beta-endosulfan and for PCBs the concentrations of: tri (28), tetra (52), penta (101), hexa (138, 153), hepta (180) and octa (194).

Multi-level calibration curves were created for the quantification and good linearity ($r^2 > 0.999$) was achieved for tested intervals that included the whole concentration range found in the samples. The analyte identification was based on their relative retention times to the standard used for quantification (for ECD).

RESULTS AND DISCUSSION

The levels of the concentrations (in ng/kg fat weight) for the analyzed compounds are shown in table 3.

Table 3

The concentrations (ng/kg fat weight) for the analyzed milk samples

Analyte	Sample 1	Sample 2	Sample 3
α -HCH	ND	2.66	1.78
γ -HCH	2.88	1.31	1.94
β -HCH	ND	4.53	2.97
heptachlor	0.69	0.21	1.11
δ -HCH	ND	ND	3.43
ϵ -HCH	ND	ND	0.11
Aldrin	ND	2.33	1.14
Heptachlor epoxide beta	ND	0.41	ND
Heptachlor epoxide alfa	ND	0.17	0.22
Endosulfan α	0.18	ND	0.11
2,4'-DDE	1.77	ND	2.01
4,4'-DDE	0.55	3.55	5.07
Dieldrin	6.21	4.18	6.08
2,4'-DDT	ND	0.33	0.81
Endosulfan β	0.08	ND	0.10
Total	12.36	19.68	26.88
PCB28	ND	0.77	0.07
PCB52	1.15	0.08	1.55
PCB101	1.33	ND	0.88
PCB 138	1.88	0.42	1.17
PCB 153	ND	ND	1.08
PCB 180	ND	1.74	3.44
PCB 194	ND	1.55	2.89
Total	4.36	4.56	11.08
ND = not detected			

γ -Isomers of hexachlorocyclohexane, 4,4'-DDE, Dieldrin, heptachlor, PCB 52 and PCB 138 were identified in all milk samples. The most preponderant OCP compound was Dieldrin and the most predominant PCB was PCB 138 among analyzed milk samples.

The sum of OCPs in milk samples were: 12.36, 19.6 and 26.88 ng/kg fat weight, respectively. The sum of PCBs in the studied samples were: 4.36, 4.56 and 11.08, respectively. The obtained values for PCBs were lower than those obtained by Heck et al.

(2007) in cow milk samples collected from Rio Grande do Sul State, Brazil [4] and also lower than those obtained by Ennaceur et al. (2008) in breast milk collected from various locations in Tunisia [1].

CONCLUSIONS

This study reports a multiresidue analysis procedure which permits the identification and quantification of twenty-two compounds, structurally different, in one single GC injection, within 85.75 min. The investigated method is simple, reliable, reproducible and sensitive.

The practical utility of the method was shown by the determination of OCPs and PCBs in milk samples. The obtained results suggest a need to investigate the levels of OCPs and PCBs in other food products and their effects on human health.

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