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Research Article

Development of Low Density Miniaturized Homogeneous Liquid–Liquid Extraction for Determination of Organochlorine Pesticide Residues in Cow's Milk by Gas Chromatography/Electron Capture Detector

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Abstract

A simple, rapid, sensitive procedure based on a technique called Low Density Miniaturized Homogeneous Liquid-Liquid Extraction (LDMHLLE) is introduced for the extraction of selected organochlorine pesticides from cow's milk. The organochlorine pesticides were extracted from cow's milk by mixture of methanol and n-hexane. The calibration curves were linear in the ranges of 1.0 to 20.0 ng mL^{-1} and the limits of detection were in the range of 0.03-0.7 ng mL^{-1}. The recovery and percent Relative Standard Deviations (RSDs) varied from 81-111% and 2-11%, respectively. The method was validated through the routine analysis of organochlorine pesticides in cow milk samples.

Keywords: Low density miniaturized homogenous liquid-liquid extraction; Organochlorine pesticides; Cow's milk; Clean up

Introduction

In the world, alarming levels of pesticides have been reported in air, water, soil, as well as in foods and biological materials because they are used widely to control pests that affect agricultural crops and pests in the home, yards, and gardens. Our interest was focused on analysis of Organochlorine Pesticide Residues (OCPs) in (cow) milk because they are persistent in the environment. OCPs tend to bioaccumulate in the food chain, however, and are stored in fat because of their persistent and lipophilic characteristics [1]. Among foods, milk stands out as participant at the top of the trophic food chain and as biomarker of environmental pollution. The examination of cow milk can be used to indicate the general level of contamination, and thus of potential health risk. Milk is an ideal liquid to dissolve environmental contaminants such as pesticides, because most of them are fat-soluble. Bovine milk may contain high levels of pesticide residues as a result of concentration of residues in the tissues following the cattle dipping, when they feed on contaminated feedstuffs or when they drink water which is contaminated by the pesticides residues [2]. Qualitative and quantitative analysis includes a procedure of sample preparation. Sample preparation for fatty food analysis is often the most time consuming step in analysis and involves following steps: extraction of the analytes, removal of co-extracted compounds, concentration and chromatographic analysis. The extraction step is the least evolved part of most analytical procedures. Extraction procedures in food-producing animals (such as milk) are labor-intensive and solvent consuming [3]. Liquid-liquid extraction is the most widely used method of sample pre-treatment for extraction of OCPs from biological sample matrices. To determine the traces of lipophilic compounds, such as organochlorine pesticides, their separation from bulk of fatty materials should be achieved. Because of the complexity of the biological matrices mentioned above, the presence of interfering compounds in the extract requires intensive cleanup before samples can be submitted to the separation and determination step (clean up). However, due to the existence of only one clean-up stage, the detection limits are still too high for the trace levels of OCPs in cow's milk. For this type of samples, in addition to desired extraction efficiency and sufficient selectivity, the subsequent purification steps may also be simplified.

In recent years, with the developing interest in miniaturization in analytical chemistry, resultant solvent and sample savings, some newer miniaturized procedures to liquid extraction have been reported [4]. Some examples of miniaturization in sample preparation techniques are: Liquid-phase micro-extraction [5], solid phase micro-extraction, [6] dispersive liquid-phase microextraction (DLPME) [7], Hollow fiber liquid-phase microextraction [8] and Matrix Solid-Phase Dispersion (MSPD) [9].

Recently, we reported using of Miniaturized Homogenous Liquid–Liquid Extraction (MHLLE) for determination of polyaromatic hydrocarbons in sediment and aflatoxins in pistachio and wheat samples [10-12]. The aim of this study is to evaluate and develop the Miniaturized Homogenous Liquid–Liquid Extraction (MHLLE) method for extraction and clean up of pesticide residues in cow milk to minimize the using of organic solvent and the analysis time with GC/ECD determination. To the best of authors our knowledge, this study may be the first report describing the application of the MHLLME method for the determination of OCPs in cow milk.

Experimental

Chemicals and reagents

Analytical grade methanol, n-hexane and sodium sulfate were purchased from Merck (Darmstadt, Germany) and were used without further purification. Standard mixture of 17 OCPs (α-BHC

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Table 1: Limit of Detections (LOD), regression equations, correlation coefficients (R^2), and dynamic linear ranges of the developed extraction method for pesticides analytes.

Peak No.	Pesticide	Retention time (min)	Equation	R^2	LOD (ng g^{-1})
1	α-BHC (alpha-HCH)	35.2	y = 58.3x - 115.33	0.9988	0.10
2	β-BHC (beta-HCH)	37.4	y = 38.8x - 68	0.9997	0.15
3	δ-BHC (delta-HCH)	37.1	y = 16x + 42.667	0.9915	0.38
4	Heptachlor	39.1	y = 9x - 10	0.9985	0.67
5	Aldrin	44.1	y = 214.3x - 268	0.9992	0.03
6	Heptachlor epoxide (isomer B)	46.6	y = 99.5x - 181	0.9981	0.06
7	γ -Chlordane	48.1	y = 105.6x - 156	0.9965	0.06
8	a -Chlordane	49.0	y = 78.8x + 19	0.9998	0.08
9	Endosulfan I	49.8	y = 121.8x - 191.67	0.9925	0.05
10	4,4'-DDE	50.5	y = 95.4x - 191	0.9923	0.06
11	Dieldrin	50.3	y = 124.2x + 40	0.9944	0.05
12	Endrin	51.8	y = 20.6x + 21.667	0.998	0.29
13	4,4'-DDD	52.5	y = 26.1x - 22.333	0.9918	0.23
14	Endosulfan II	53.3	y = 56.7x - 84	0.9979	0.11
15	4,4'-DDT	53.9	y = 36.8x - 56	0.9924	0.16
16	Methoxychlor	57.3	y = 45.3x - 93.333	0.9973	0.13
17	Endrin ketone	60.2	y = 31.4x + 192.67	0.9951	0.19

RSD= Relative Standard Deviation (n=3)

Table 2: Recovery and corresponding Relative Standard Deviation (RSD) of OCPs for the spiked cow's milk samples.

Peak No.	Pesticide	Recovery ± (RSD)%		
		1.0 (ng mL^{-1})	2.0 (ng mL^{-1})	4.0 (ng mL^{-1})
1	α-BHC (alpha-HCH)	87 ± 6	93 ± 4	97 ± 2
2	β-BHC (beta-HCH)	87 ± 5	110 ± 7	97 ± 8
3	δ-BHC (delta-HCH)	93 ± 11	101 ± 6	88 ± 5
4	Heptachlor	103 ± 6	89 ± 4	87 ± 9
5	Aldrin	81 ± 9	111 ± 2	93 ± 5
6	Heptachlor epoxide (isomer B)	96 ± 5	95 ± 4	110 ± 4
7	γ -Chlordane	103 ± 3	94 ± 5	105 ± 3
8	a -Chlordane	83 ± 8	91 ± 8	103 ± 8
9	Endosulfan I	91 ± 7	88 ± 3	95 ± 7
10	4,4'-DDE	97 ± 6	96 ± 7	96 ± 7
11	Dieldrin	98 ± 3	106 ± 8	98 ± 6
12	Endrin	94 ± 8	110 ± 5	89 ± 3
13	4,4'-DDD	93 ± 8	103 ± 2	99 ± 7
14	Endosulfan II	96 ± 2	97 ± 10	96 ± 8
15	4,4'-DDT	106 ± 7	96 ± 4	93 ± 8
16	Methoxychlor	89 ± 5	92 ± 8	98 ± 2
17	Endrin ketone	96 ± 9	103 ± 4	110 ± 4

(alpha-HCH), β-BHC (beta-HCH), Heptachlor, δ-BHC (delta-HCH), Aldrin, Heptachlor epoxide (isomer B), γ –Chlordane, a –Chlordane, Endosulfan I, 4,4'-DDE, Dieldrin, Endrin, 4,4'-DDD, Endosulfan II, 4,4'-DDT, sulfate, Methoxychlor, Endrin ketone) at the concentration level of 2000 mg L^{-1} in hexane/toluene (1:1) were purchased from Ultra Scientific Co. The working solutions were prepared at appropriate concentration from stock solutions and stored in a refrigerator at 4 °C. Fresh cow milk samples were collected from farmer (Tehran, Iran) and was packaged in polyethylene bags and the samples were transported in cooling boxes containing ice packs to the laboratory where they were immediately stored in a freezer at -20 °C until further analysis.

Apparatus

The extracted compounds were analyzed on an Agilent 6890 N gas chromatograph equipped with an electron capture detector

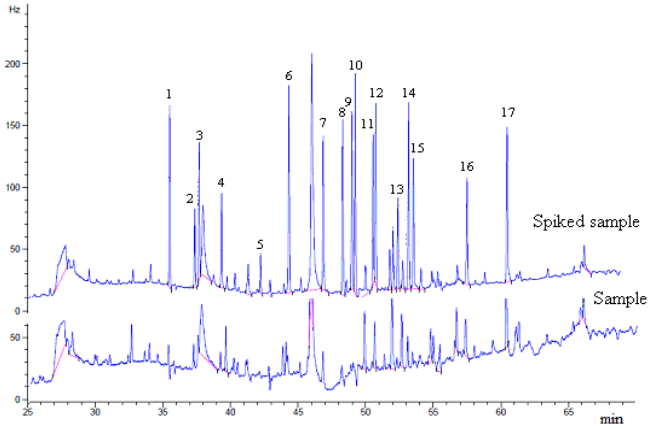

Figure 1: GC-ECD chromatograms obtained from a real sample extract (lower) and an extract obtained from spiked samples with a certified concentration of 17 pesticides (upper): Condition: 5.0 g milk sample was vortexes with 10 mL methanol, and then 5.0 mL of methanolic phase was extracted with 1.0 mL of *n*-hexane. 2.0 µL of hexane layer was injected to GC/ECD.

(µ-ECD, Agilent Technologies, Avondale, PA, USA) and 2 µL of the sample were injected in the splitless mode at 270 °C into a 30 m × 0.25 mm × 0.32 µm DB-5 capillary column and operated by Chemstation Software (Agilent Technologies). The detector temperature was 300 °C. The temperature program used for the chromatographic separation of OCPs was as follows: 70 °C for 2 min, temperature increased at 3 °C min^{-1} to 260 °C and hold for 10 min. The carrier gas was helium (99.999%) and was kept at a constant flow of 1.0 mL min^{-1} and make up gas was nitrogen (99.999%, Roham Gas Company, Tehran, Iran) at flow rate of 30 mL min^{-1}.

Extraction procedure

5.0 g of defrosted and homogenized milk sample and 10 mL of methanol were added into 15 mL centrifuge tube; the tube was vortexes for 60 seconds and centrifuged at 3500 rpm for 5 min. An aliquot of 5.0 mL methanolic phase was placed into a 10 mL volumetric flask containing 1.0 mL of extraction solvent (n-hexane) and then vigorously shaken for 30 seconds. The saturated sodium sulfate solution (4 mL) was added into the extract solution and then n-hexane phase (the top layer) separated. The extraction solvent was drawn out by a Hamilton syringe and transferred into a conical vial and 2.0 µL was injected to GC/ECD.

Results and Discussion

The proposed method was found to be effective in determination organochlorine pesticide in cow's milk. This extraction technique is fast, inexpensive, and robust. The denaturization of milk protein and fat was done by addition of methanol and shaking. Removal of the fat from the extract was sufficiently accomplished in a single-step by methanol/hexane partitioning for cow's milk. Samples that do not bear residues at or above the LOD are referred to as blank milk samples. An external calibration plot was constructed in triplicate (n = 3) for analysis of blank milk samples fortified by addition of standard solutions of the OCPs pesticide in the range of 1.0–20.0 ng mL^{-1}. The calibration curves were obtained by plotting the peak areas versus the concentrations of analytes in the milk sample. The curve equations are listed in Table 1 and used to evaluate the Limits of Detection (LODs). LODs were calculated on the basis of a signal-to-noise (S/N) ratio of 3. All calibration curves were linear with correlation coefficients better than 0.990. The figure of merits of the method for the extraction and determination of pesticides from milk samples are summarized in Table 1.

Finally, the applicability of the developed method was evaluated by analyzing different samples of cow milk. The relative recoveries were studied in the blank milk sample by spiking at three different levels of pesticides. (1.0 ng mL^{-1}, 2.0 ng mL^{-1} and 4.0 ng mL^{-1}). The cleanup procedure was evaluated in terms of recovery and the lack of interfering compounds in the final extracts. Acceptable recoveries and repeatability data are summarized in Table 2. The calculated detection limits of the developed LDMHLLE were in the range of 0.03-0.67 ng mL^{-1}. Figure 1 shows the obtained chromatograms for sample and spiked milk sample at the concentration level of 4.0 ng mL^{-1} of each OCPs.

Conclusion

LDMHLLE method as an extraction and clean-up procedure has been successfully applied in the determination of OCPs residues in cow's milk samples. This method efficiently supplemented most of the laborious and expensive analytical procedures for the determination of OCPs in fatty food matrixes. The whole sample preparation process did not take more than 5 min per sample, where a single operator could run several samples at the same time. The elimination of the use of SPE column for clean up not only lowers the cost of the analysis but also shortens the analytical time. The method proposed is rapid and inexpensive, and reduces consumption of organic solvents, which are toxic to health and the environment.

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