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Determination of organochlorine pesticide residues in different pork tissues using GS

Crina Muresan*, Maria Tofana, C. Laslo

University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca, Faculty of Agriculture , 3-5 Manastur Street, 400372 – Cluj Napoca, Romania

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Abstract

The aim of this work was to develop an efficient method for the pesticides organochlorines method , in pork meat and fat tissues. The fat and the organochlorine compounds were extracted from test portions. The organochlorine compounds isolated by cryogenic extraction and cleaned up in two successive, using C18 and Florisil SPE cartridges, respectively.

To determine the organochlorine pesticides, was used: a GC-2010 gas chromatograph equipment with a electron capture detection (ECD) and a RESTEK model capillary chromatographic column.

The method was validated by fortification sample with standard solutions, and determinate the recovery coefficient and repeatability of measurements expressed as RSDs.

Keywords: GS, pork, OCPs

1. Introduction

The presence and occurrence of organochlorine pesticides (OCPs) in the environment is of great concern due to their persistency, long-range transport ability, and to their toxic effects. One common feature of OCPs is that their bioaccumulation in the food chain and in the human body, and, as a consequence, represent a potential risk for the human health. The main sources of OCPs in the human diet are foods of animal origin. It has been concluded that humans are exposed to toxic compounds via diet in a much higher degree compared to other exposure routes such as inhalation and dermal exposure[4,7].

Low volatility and high stability, together with lipophilic behavior, are responsible for their persistence in the environment and concentration in fatty tissues, therefore, it's important to identify levels of OCPs in food of animal origin.

In the EU, starting September 1, 2008, a new legislative framework has been applied (Regulation (EC) no. 396/2005 of the European Parliament and Council) on pesticide residues. It complements, harmonize and simplify MRLs for pesticides, while ensuring better consumer protection throughout the EU [1]. Due to their structure and physical-chemical properties, the analysis of OCPs is usually carried out by gas chromatography coupled with electron capture detection (GC/ECD) or with mass spectrometry GC-MS.

The analytical methods developed for the determination of OCPs involve traditional extraction techniques such as extraction by Soxhlet and sonication as well as modern extraction techniques such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), accelerated solvent extraction (ASE) extraction by Polytron [2].

Corresponding author: *e-mail:* crinartn@yahoo.com

SFE, MAE and ASE techniques work automatically and require low quantities of solvent, with a consequent reduction of solvent waste. On the other hand, a purification step is required, so to remove co-extracted fatty acids, sterols and glycerides incompatible with those detectors usually employed for the trace analysis of OCPs such as the electron capture detector (ECD), the mass detector (MS), and the tandem mass detector (MS/MS). The clean-up of the extracts is accomplished by liquid-liquid partition, gel permeation chromatography (GPC) [2] adsorption chromatography on different adsorbents such as silica, florisil or alumina, solid phase extraction (SPE) on ready-to-use cartridges and solid-matrix dispersion partition (SMDP) [8].

In this study, we measured levels of OPCs in some animal products resulting from slaughter from a farm in the county of Cluj, Romania. Organochlorine compounds were isolated in a cryogenic extraction and cleaned by two successive SPE cartridges: C18 and florisil. Organochlorine compounds were identified and determined by gas chromatography [5].

2. Materials and methods

*Chemicals and materials.*The pesticide standard 97-99% pure (HCH, aldrin, heptachlor, endosulfan I and II, endrin aldehyde, endosulfan sulphate, p, p-DDT, p, p-DDE, methoxychlor, hepachlor epoxide, α,γ, chlordane, dieldrin, endrin, endrin aldehyde, endrin ketona) used in this study from achieve the calibration curve. A standard stock mixture solution were preparated in hexane and stored at 4 ° C. The calibration graph of each pesticide was constructed using samples with six different concentration(0.001 ppm and 0.5 ppm) of the standard mixture solution. The analyte peaks obtained were integrated and plotted as functions of the concentration. The standard mixture solutions were analysed in duplicate by GC-ECD at each concentration level.

Apparatus.(a) Soxhlet apparatus, SPE vacuum manifold and accessories - Shimadzu 2010 gas chromatograph equipement with an electron capture detection (ECD) was used: the oven temperature was initiated at 150°C for 2 min, raised to 200°C at 4°C/min, kept at 200°C for 5 min, raised to 230°C at 5°C/min, kept at 230°C for 5 min, raised to 300°C at 2°C/min, and then kept at 300°C.

The temperatures of the injector and detector were 250°C and 315°C, respectively. The injection volume was 1 μL. The flow rates of carrier gas (N_2) and make up gas (N_2) were maintained at 19.2 and 3.0 mL/min, respectively. A RESTEK model capillary chromatographic column (phase RTx-5, with 0,4 μm thick film layer, 20 m length and inside diameter of 0,18 mm) was used.

(b) SPE florisil cartriges (2000mg) and SPE column Chromabond C18 cartiges(6 ml,500mg).

*Samples collection and storage.*15 samples of meat and fat tissue (about 100 g each) were taken after slaughter pigs(lard, mesenteric tissue, pork chops) at a farm in the Cluj-Romania in July 2007. After sampling all samples were stored at -17°C until analysis.

Before analysis, each sample was homogenized and split in two. One portion was used to detect and quantify the compounds of interest in experimental conditions. The other part was further divided into 10 g subsamples and used to verify method performance.

Extraction by automatic extractor. Ten g of meat sample were accurately weighed accurately and homogenized 10 g of Celite. The mixture was quantitatively transferred into an extraction thimble and covered with wool. Before use, the extraction thimbles was washed with 15 ml of acetone and 15 ml of hexane.

Extraction was performed with 90 ml of petroleum ether at 70 ◦ C by placing the cursor in the extract of "diving" position for 20 min successively in "wash" position for 60 min. After recovery and drying solvent extracted fat is passed to the analysis itself.

Cryogenic extraction. They weigh 0.5 grams of fat obtained as above and add 3 ml of methylene chloride-acetonitrile.Was mixed vigorously and centrifuged tube and its contents at 3000 rpm at - 15 ° C for 20 min. The first top layer supernatant was transferred to a separate tube. Then slowly heated in the bottom-centrifuge tube in a water bath set at 40 ° C to melt fat. Extraction was repeated with another 3 ml of acetonitrile-methylene chloride and centrifuging was repeated. Second layer was added above the first supernatant from the tube.

Organic phase evaporated to approx. 35 ° C under nitrogen to about 2 ml to 3 ml remains to obtain solution A.

Clean-up on a C18 SPE cartridge. C18-SPE cartridge was prepared by eluting twice with 5 ml of petroleum ether, then 5 ml of acetone and finally 5 ml of methanol, (above the meniscus stops every time). Eluted solutions were discarded. The solution was added in cartridge and was left for 3 min. Then elueat solution A 10 ml acetonitrile at a flow rate of 1 drop /3s. The eluted solution was collected in a suitable container. Nitrogen solution thus obtained was evaporated to about 35° C carefully. Was diluted in 2 ml solution evaporated to 3 ml of n-hexane and obtained solution B.

Clean-up on a Florisil SPE cartridge. Florisil SPE cartridge was prepared by elution with 10 ml of n-hexane until the liquid meniscus reaches the upper frit. Was added to solution B and left for 3 min. Solution B was eluted with 10 ml of ethyl ether-petroleumether (98:2), with a rate of 1 drop/ s.The eluted fraction was collected in an evaporation flask. Then eluted with 12 ml of petroleum ether-diethyl ether III (85:15), with a rate of 1 drop every 3s.Collect fraction eluted in the same glass and mix the first part. Finally the elution mixture was brought to dryness and taken up with hexane at a volume of 5 ml. After the optimal working conditions were 1µl solution injected into GS- EDC.

Quality assurance. Multi-level curves were created for the quantification and good linearity (r^2>0.99) was achieved for the whole concentration range found in the samples. The identification of compounds was based on retention times and, for samples with high concentrations, results were confirmed by GC-MS. Procedural blanks were performed every 8 samples and the mean values were subtracted from the values found for each compound in the samples. Method limits of quantification (LOQ) for individual OCPs ranged between 0.005 and 0.1 ppm lipid weight and were calculated as 3x standard deviation of the procedural blanks, resulting in a certainty of more than 95% for results given for the samples.

3. Results and discussion

Organochlorine pesticide residues were found present in the samples analyzed in different proportions.

Generally, a small variation between individual samples was observed.

The mean levels of organochlorine pesticide residues in pork tissue are presented in tabel1. It can see a decrease in MRL edosulfan from 0.1ppm.. at 0.05 ppm and 1 ppm of γHCH to 0.02 ppm.

Multi-level curves were created for the quantification and good linearity was achieved for the whole concentration range found in the samples. The identification of compounds was based on retention times and for samples with high concentrations (table 2). Pesticide residues found were: α,β,γ HCH, Aldrin, pp DDE, pp DDD, pp DDT, endosulfan, heptachlor epoxide, methoxychlor. It can be observed that organochlorine pesticides (HCHs, DDTs and methoxychlor) are the principal contaminants in all samples.

Moreover, similar or higher concentrations were already measured in Romanian farmed animals [3,6], showing that the food chain contamination with pesticides is still an acute problem. Levels of organochlorine pesticides in pork fat and muscle were reported to be below 0,015 ppm in Croatia [9] and below 0,330 ppm in Czech Republic [4]. In the table 3 values are obtained for all residues identified. It shows a higher proportion of OCPs residues in the mesenteric tissue and decreasing values in fat tissue (table 3). However, all samples contained relatively high concentrations of pollutants suggesting that OCP pollution is a potential problem in Romania.

Even with the reduction in the levels of DDT in the environment, there are still areas of concern where heavy applications of DDT during past legal uses of the pesticide have resulted in high concentrations of residual DDT and its metabolites [10].

Regarding the residual content of αHCH is apparent that the share is highest in mesenteric tissue. Isomer of HCH is found in greater proportion in the fat tissue sample compared to pork, other isomers are higher in proportion to sample the pork chops β-HCH was the most persistent HCH isomer in all tissues, accounting for 40–50% of sum HCHs. Values of situations is between 0.027 ppm and 0.040 ppm in fat tissue in the mesenteric tissue of 0.1 ppm compared to how the legislation provides. The greater accumulation of b-HCH in tissues is expected since this isomer is known to be metabolized more slowly [11].

Table 1: MRL admitted in meat and fat tissue

No crt.	**Pesticide residues**	**Maximum limits in (ppm) of fat contained in meat, offal and animal fat, fish and fishery products**	**Maximum limits in (ppm) of fat contained in meat, offal and animal fat, fish and fishery products covered in 2008**
1.	Aldrin	0,2	0,2
2.	Dieldrin		
3.	Chlordan (sum cis şi trans	0,05*)	0,05*)
4.	DDT (sum pp'DDT,op' DDT, pp,DDE şi pp'DDD)	1	1
5.	Endrin	0,05*)	0,05*)
6.	Heptaclor (sum heptaclor şi heptaclor epoxid)	0,2	0,2
7.	Methoxychlore	0,01*)	0,01*)
8.	Hexaclorhexan (HCH)		
	- Izomer alfa	0,2	0,2
	- Izomer beta	0,1	0,1
	- Izomer gama (Lindan)	2,0sheep meat 1,0 other	0,02
9.	Endosulfan (sum alfa beta	0,1	0,05*)

* analytic detection lower limit

Table2. Retention time (RT), average recoveries % and correlation coefficients (r^2)

OPs	RT min	Mean recovery± SD	Correlation coefficients(r^2)
a-BHC	14.377	89.26±9.17	0.9979
g -BHC lindan	15.867	97.72±1.99	0.9975
b-BHC	16.177	92.46±1.04	0.9972
d-BHC	17.462	113.84±1.08	0.9972
heptaclor	19.925	97.44±1.09	0.9953
aldrin	21.681	70.62±8.45	0.9977
heptaclor epoxide	23.701	83.50±1.10	0.9955
g-clordan	25.455	77.78±9.38	0.9965
a-chlordane	25.607	73.62±1.10	0.9958
4,4 DDE	25.865	70.52±9.35	0.9964
endosulfanI	26.654	77.20±9.27	0.9981
dieldrin	26.716	67.98±1.22	0.9951
endrin	27.748	100.62±1.64	0.9957
4,4`-DDD	28.184	74.24±1.21	0.9957
endosulfanII	28.620	67.94±1.57	0.9957
4,4 DDT	29.085	83.20±1.21	0.9950
endrin aldehyde	30.182	77.78±1.57	0.9951
methoxyclor	30.350	102.50±1.15	0.9972
endosulfan sulfate	32.327	111.00±1.27	0.9939
endrin cetona	32.987	77.26±2.71	0.9925

Table 3. Concentration (mg/kg lipid) of pesticides residue in meat and fat tissue

samples	**N**	**%lipid**	**HCH sum**	**DDT sum**	**Aldrin+ endrin**	**Heptachlor sum**	**Endosulfan sum**	**Clordan sum**	**Methoxyclor**
lard	5	100	0.054±0.005	0,048±0.011	0.018±0.001	0.007± 0.003	0.031±0.006	0.016±0.003	0.051±0.044
mesenteric tissue	5	100	0.084 ±0.002	0,053±0.018	0.026±0.001	0.007±0.005	0.040±0.008	0.021±0.005	0.054±0.016
pork chops	5	8,2	0.058±0.002	0,045±0.021	0.015±0.003	0.005±0.004	0.025±0.008	0.009±0.006	0.050±0.032

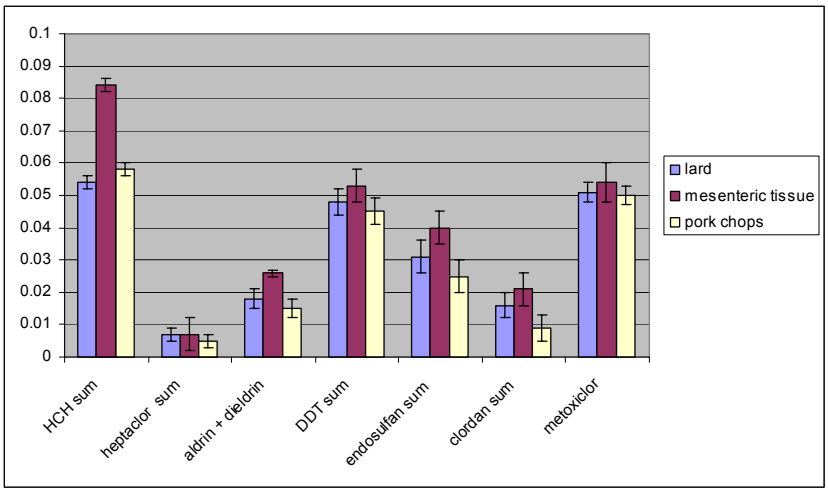

Figure 1. Pesticides residue in meat and fat tissue

Table 4. Concentration (mg/kg lipid) of HCHs in meat and fat tissue

samples	**α HCH (ppm)**	**β HCH (ppm)**	**γHCH (ppm)**
lard	0.004±0.003	0.027±0.002	0.023±0.001
mesenteric tissue	0.006±0.002	0.040±0.002	0.038±0.003
pork chops	0.002±0.001	0.030±0.002	0.026±0.003

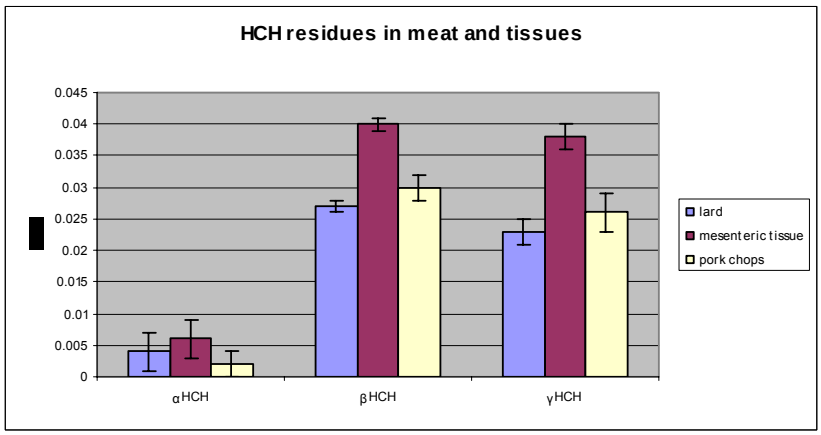

Figure 2.Variation HCHs in meat and fat tissue

Value ranges between 0.023ppm γHCH in adipose tissue and 0.038 ppm in the mesenteric test. It can be seen exceeding the MRL (0.02 ppm) in all samples.

4. Conclusion

The method used is much faster, is achieved with a low consumption of reagents and the recovery rate is good for almost all compounds analyzed.

All identified compounds is below the MRL, except lindane which shows higher values than the MRL between 15-90%.

The presence of these pollutants in food requires to continue the analyzed of these in the aim respected MRL and the reduction their presence in food.

References

1. Regulation (EC) No 299/2008 of the European Parliament and of the Council of 11 March, 2008
2. Garrido Frenich J.L. Martínez Vidal, A.D. Cruz Sicilia, M.J. González Rodríguez, P. Plaza Bolaños Multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken, pork and lamb by gas chromatography–triple quadrupole mass spectrometry, *Analytica Chimica Acta*, **2006**, *558*(1-2), 42-52, doi:10.1016/ j.aca.2005.11.012

3. Covaci, A., Gheorghe, A., Schepens, P., Distribution of organochlorine pesticides, polychlorinated biphenyls and α-HCH enanatiomers in pork tissues, *Chemosphere,* **2004**, *56*(8), 757–766, doi:10.1016/j.chemosphere. 2004.02.014
4. Ullrich, R., Raszky, J., Variations in environmental contamination by polychlorinated biphenyls (PCBs) and chlorinated pesticides (lindane, DDT) on pig farms in Hodonin district (Czech Republic) in 1994 to 1999. *Vet.Med. Czech,* **2002**, *47*(6), 159–168
5. Hura Carmen, *Ghid de laborator*, Cermi Iasi, 343-367, 2006
6. Hura Carmen, *Chemical contaminanti in Romania*, Ed. Cermi, Iasi, 2002
7. DiMuccio A., Generali T., Attard Barbini D.,Pelosi P., Ausili A., Vergori F., Girolimetti S., Single-step separation of organochlorine pesticide residues from fatty materials by combined use of solid-matrix partition and C18 cartridges, *J. Chromatogr., A,* **1997**, *765*(1), 61-68, doi:10.1016/S0021-9673(96)01006-0
8. Feei Sun, Feng-Yi Lin, Sue-Sun Wong And Gwo-Chen Li, The Screening of Organophosphorus, Organochlorine and Synthetic Pyrethroid Pesticides Residues in Beef Fat byTandem Solid-Phase Extraction Technique, *Journal of Food and Drug Analysis*, **2003**, *11*(3), **2003**, 258-265
9. Kipcic, D., J. Vukusic, and B. Sebecic, Monitoring of chlorinated hydrocarbon pollution of meat and fi sh in Croatia, *Food Technology and Biotechnology*, **2002**, *40*(1), 39–47
10. Harris, M.L., L.K. Wilson, J.E. Elliott, C.A. Bishop, A.D. Tomlin, and K.V. Henning, Transfer of DDT and metabolites from fruit orchard soils to American robins (Turdus Migratorious) twenty years after agricultural use of DDT in Canada, *Archives of Environmental Contamination and Toxicology*, **2000**, *39*(2), 205-220
11. Willett, K. L., Ulrich, E. M., and Hites, R. A.: Differential toxicity and environmental fates of hexachlorocyclohexane isomers, Environ. Sci. Technol., **1998**, *32*(15), 2197–2207, doi: 10.1021/ es9708530