THE STUDY OF THE MULTIPLE ELUTIONS FOR THE QUANTITATIVE DETERMINATION OF ORGANOCHLORINE PESTICIDES IN GREEN FODDER

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Abstract

This paper presents the results of the determination (qualitative and quantitative) of organochlorine pesticides in green fodders by gas chromatography. The applied method requires the use of three subsequent elutions with 200 ml ethylic ether/light petroleum each, in variable proportions. The paper analyses the apparition of the compounds in relation with the used eluents in order to identify if this massive consumption of solvent is really necessary. The practical determinations proved that the use of one particular eluent is enough for a specific level of certainty.

Keywords: green fodder, organochlorine pesticide contamination, solvent consumption

INTRODUCTION

The determination of the contamination degree of the fodders is extremely important for the counterbalance of the contamination of the animal foods. This aspect is more important with toxics which are bioaccumulable such as the organochlorine pesticides (OCP in this paper). Because of their persistence in air, water and soil and their traceability, this kind of contaminant is under surveillance in environmental factors as well as in food-stuff and human food in all European Union (Neamțu and al., 2009, Koci and al, 2007, Chiş et al., 2010, 2008,a, b) as well as in non EU countries such as Serbia (Škrbič and Predojevič, 2008).

Moreover, there are areas on the Globe, such as Asia and South America, where they still use certain organochlorinated pesticides, especially for malaria control. Therefore in this area there were many studies regarding the contamination of fodder (Nag and Raikwar, 2011, Gill et al., 2001, Singh et al., 1997 Kanaan et al., 1992) and food (Salem et al., 2009, Nag and Raikwar 2008, Katagi, 2010, Sarkar and al., 2008, Bakore et al, 2004 and 2002, Waliszewski and al., 2004) as well as the impact of the pesticide contamination on the humans' health state.

The pollution has no border and food travels around the world, so it is very important to monitor the level of contaminants even if in the European Union, the use of this type of pesticide was gradually decreased up to the total interdiction of the last product admitted – Endosulfane, starting with 2008. The residues are found at smaller and smaller concentration levels but

it continues to be supervised at European level for reasons of food safety, in order to prevent further sanitary-veterinary problems (EFSA, a, b, 2005, EFSA, a, b, 2006, EU 2006).

Since we know that the decrease of the concentration makes the analytic determination more difficult, we chose the application of a multiple-elution method, which has high chances to highlight all the compounds present, even at ppb concentrations (SR EN 12393-2/2004). However, at the same time, we wished to highlight the use of the three elutions practices according to the purpose followed.

MATERIALS AND METHODS

Materials

For the study that makes the object of the present paper, three series of green fodder samples were tested. Since this paper is a research study, not the result of a control activity, the name of the village from where the samples have been taken, was not revealed. They were coded by letters and numbers FV1, FV2 and FV3 (Tables 1 to 5). From geographical point of view it is the area near the municipality of Oradea.

Methods

The OCP are extracted from the matrix by liquid/liquid technique using a mixture of acetonitril/water (65:35 V/V), followed by liquid/liquid partition with light petroleum. The extract is purified on an activated Florisil column. The OCP are eluted with mix of ethylic ether/light petroleum in variable proportions. Three subsequent elutions of 200 ml mixture ethylic ether / light petroleum for each, was used in the following V/V proportions: 6/94 (eluent A), 15:85 (eluent B) and 50:50 (eluent C). Further on this paper, letters A, B and C refers at the specific eluent above nominated.

Like in other researchers, the gas chromatography method was used (Zhao and al., 2011, Tadeo, Ed., 2008, Hura, 2006, Yan and al., 2005) with the help of a GC 2010 type Shimadzu gas chromatograph equipped with capillary column type RTX -CL- pesticides and detector with electrons capture (ECD). The chromatography conditions were : Injection temperature (splitting) = 250^0C, Splitting temperature = 163,5 Kpa, Splitting gas : He with a 124 ml/min flow at scavenging 30 ml/min, Carrying gas : N_2 ultrapure 99,99%.

For the qualitative and quantitative calculation of the contaminants possibly present in the tested products, we used a standard produced by the RESTEK company No 32292, Lot nr A021837, type “Organochlorine pesticide Mix AB ≠2” having a concentration of 200 ppb. The standard was used at 50 ppb dilution.

The purified extract, retaken in petroleum ether underwent chromatography under the same conditions as the standard test, as well as the blanks-test of the used reagents, according to the separation/purification method.

RESULTS AND DISCUSSIONS

The results are written in table 1 for the qualitative determination, separated on the three elutions performed. Only the positively identified compounds are mentioned.

Table 1

Qualitative calculation of the organochlorine pesticide residues in green fodder, (n=6)

A	B	C	D - FV1			D - FV2			D – FV3		
			E_A	E_B	E_C	E_A	E_B	E_C			
1	α HCH	7.194	7.179			7.183			7,188		
2	γ HCH	8.426	8.409	8.413		8.410	8.415		8,418	8.422	
3	β HCH	8.886			8.791						8.765
11	α endosulfan	16.753	16.73			16.744			16.723		
13	Endrin	18.878	18.831			18.848			18.840		
14	4,4' DDD	19.853	19.809			19.814			19.901		
16	4,4' DDT	21.305	21.273		21.464	21.288		21.378	21.277		21.389
19	Sulfat endosulfan	24.693	24.798						24.803		

Legend:
A – Elution order; B – Organochlorine compound from the sample; Retention time in the standard; D - FV1, FV2 – retention time in green fodder 1, respectively 2;
E_A, E_B, E_C – Elution solvents used: A, B, C

Figure 1 presents, as an example, the scanned chromatogram of elution A, where most of the compounds are eluted, by overlaying of the tested sample and the standard at 50 ppb.

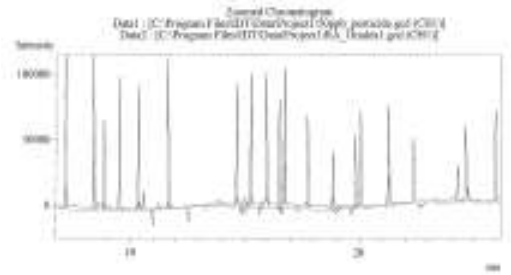

Figure 1 - Green fodder chromatogram

The results of the quantum determinations are presented in table 2 for the three series of samples tested. The concentration written in the table

represents the amount of the values from the three elutions applied, where it was necessary.

Table 2

Qualitative calculation of the organochlorine residues in samples of green fodders (n=6)

Nr crt *	Organochlorine compound found in the sample	MRL ppm	Concentration in the sample, ppm		
			FV1	FV2	FV3
1	α HCH	0,01	0.0007	0,0005	0,0009
3	β HCH		0,0004		0,0004
2	γ HCH	0,01	0,0009	0,0012	0,0034
11 15 19	Endosulfan (Sum of α and β and endosulfan expressed in endosulfan)	0,05	0,0018+ 0,0003= 0,0021	0,0021	0,0032+ 0,0006= 0,0038
13	Endrin	0,01	0,0008	0,0013	0,0016
10 14 16	DDT (Sum of DDT, DDE and DDD isomers expressed in DDT)	0,05	0,0011+ 0,0006= 0,0017	0,0002+ 0,0004= 0,0006	0,0008+ 0,0005= 0,0013

Legend :
(*) – it refers to the elution order of the compounds in the sample
MRL – maximum residue level

In order to analyze the role and the contribution of the three elutions, tables 3, 4 and 5 present the intake of residues divided on the three employed eluents in each series of the tested samples. In the calculation we was took into account the values of the surfaces of the significant peaks appeared in the witness samples, separated in the three elutions practiced.

Table 3

Quantitative calculation of the OCP residues, in green fodder, FV (1) series

Nr	Organochlorine compounds	Concentration of OCP in the sample						
		eluent A		eluent B		eluent C		Total
		ppm	%	ppm	%	ppm	%	ppm
1	α HCH	0.0007	100	-	-	-	-	0.0007
2	γ HCH	0,0007	78	0,0002	22	-	-	0,0009
3	β HCH	-	-	-	-	0,0004	100	0,0004
11	α Endosulfan	0,0018	100	-	-	-	-	0,0018
13	Endrin	0,0008	100	-	-	-	-	0,0008
14	4,4' DDD	0,0006	100	-	-	-	-	0,0006
16	4,4' DDT	0,0008	73	-	-	0,0003	27	0,0011
19	Endosulfan Sulfate	0,0003	100	-	-	-	-	0,0003

Table 4

Quantitative calculation of the OCP residues, in green fodder, FV (2) series

Nr	Organochlorine compounds	Concentration of OCP in the sample						
		eluent A		eluent B		eluent C		Total
		ppm	%	ppm	%	ppm	%	ppm
1	α HCH	0,0005	100	-	-	-	-	0,0005
2	γ HCH	0,0009	75	0,0003	25	-	-	0,0012
3	β HCH		-	-	-		-	-
11	α Endosulfan	0,0018	100	-	-	-	-	0,0018
13	Endrin	0,0008	100	-	-	-	-	0,0008
14	4,4' DDD	0,0002	100	-	-	-	-	0,0002
16	4,4' DDT	0,0003	75	-	-	0,0001	25	0,0004
19	Endosulfan Sulfate	0,0003	100	-	-	-	-	0,0003

Table 5

Quantitative calculation of the OCP residues, in green fodder, FV (3) series

Nr	Organochlorine compounds	Concentration of OCP in the sample						
		eluent A		eluent B		eluent C		Total
		ppm	%	ppm	%	ppm	%	ppm
1	α HCH	0,0009	100	-	-	-	-	0,0009
2	γ HCH	0,0027	79	0,0007	21	-	-	0,0034
3	β HCH	-	-	-	-	0,0004	100	0,0004
11	α Endosulfan	0,0032	100	-	-	-	-	0,0032
13	Endrin	0,0016	100	-	-	-	-	0,0016
14	4,4' DDD	0,0005	100	-	-	-	-	0,0005
16	4,4' DDT	0,0007	87	-	-	0,0001	13	0,0008
19	Endosulfan Sulfate	0,0006	100	-	-	-	-	0,0006

The analysis of the results obtained leads to two discussion pathways:

Regarding the types of organochlorinated pesticides identified (table 1) and they way of appearing in the used eluents:

- the great majority of the compounds, that is 87.5% are found in eluent A;
- from them, 62% are found only in eluent A and 25% appear in two eluents, A and B for γ HCH respectively A and C for 4,4' DDT;
- the compounds found in all the samples appear in the same type of eluent;
- only one compound, β HCH, are eluted in eluent C;

Regarding the concentration of the identified organochlorinates pesticide residues, as well as their repartition in the used eluents, we can make the following considerations (tables 2, 3, 4 and 5)

- Most residues are fully eluted in the A eluent; it is about the following compounds: αHCH, Endrin, 4,4' DDD, α Endosulfan, Endosulfan sulphate.
- γHCH eluted both in A and B eluent, but in all series the A eluent reveals the significant quantity, between 82% and 96%;
- 4,4'DDT is eluted both in A and C eluent, but in different proportion: % to % in series (1), % to % in series (2) and: % to % in series (3).

CONCLUSIONS

For quick routine quantum analysis, the type A solvent use may be considered sufficient especially since β HCH which is found constantly only in the C eluent, is an isomer which is dosed only together with the γ HCH according with the European (EFSA a,b 2005 and EFSA a,b, 2006) and national legal norms (Legis: Order 12/2006,118/2007, 160/2007) referring to the contamination of the fodders with organochlorinated pesticide.

For γ HCH, dosed alone as Lindan, the fact that it appeared in both A and B eluent has very low impact on the amount of the calculated residues.

The only problem seems to be referring to 4,4' DDT which does not have the same behavior in all the tested series. However, considering the low impurification of green fodder by this OCP compound, the impact of renouncing of elution B and C seem to be insignificant.

In order to diminish the amount of used solvent, the three elution should be used only if the A elution shows values closed to the MRL ones.

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