Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 1, 2010, pp. 81-87

STUDIES REGARDING THE OPTIMIZATION OF THE SOLVENT CONSUMPTION IN THE DETERMINATION OF ORGANOCHLORINE PESTICIDES FROM GREEN FODDER

Adriana CHIŞ*
Department of Food control, Environment Protection Faculty,
University of Oradea, Romania

ABSTRACT. The determination of organochlorine pesticides from the samples of green fodders submitted to testing was made through the gas chromatography method using capillary columns and detector with electrons capture. In order to separate the contaminants from green fodders, a method specific to non-fat aliments was performed. This method requires the use of three subsequent elutions with 200 ml ethylic ether/light petroleum, in variable proportions. For the purpose of optimizing the method, there were determinations performed on the same samples through extraction in the normal variant and in reduced variants (1:2, respectively 1:5). This way, I could verify the level up to which the solvent consumption can be reduced without affecting the accuracy of the determination. The practical determinations proved that the reduced variant 1:2 has both qualitative and quantitative determination application in the case of green fodders.

Keywords: green fodder, optimisation, organochlorine pesticides

INTRODUCTION

The determination of non volatile chemical contaminants – organochlorine pesticides type – is performed in two phases: the preparation of the samples and the determination per se. The preparation phase consists of the separation of the toxic from the matrix while the purification of the extract is essential in what regards the results obtained. In the same time, it represents the phase with the highest consumption of work time and expensive reagents (Chiş, 2006). For this reason, the studies regarding the possibilities to optimize the methods, without affecting the accuracy of the results obtained presents a real interest. The work will be continued on complex fodders.

MATERIALS AND METHODS

Materials

For the study that makes the object of the present paper, samples have been collected from the grassing area of the Paleu village, nearby the Oradea Municipality. The collection of the samples was made in the months of June and July 2008. The samples consisted of green mass fodders and were taken in plastic water-tight containers. The recipients used for the determination of OCP were rinsed with petroleum ether; after cleaning, they were dried in the drying oven and kept with the lid on in order to prevent contamination.

Methods

The preparation of the samples for the analysis

The preparation for the analysis consisted of the determination of their humidity because the OCP extraction technique from non-fat vegetal products depends on the humidity content of the samples (SR EN 12393-2/2004). The method used was the reference method, that is the drying in thermo-adjustable oven, at 105 □C, for 4 hours; the determination was performed immediately after the collection, in the same day, in the food toxicology laboratory of the Faculty of Environment Protection from Oradea.

The preparation of the samples for chromatography

The way this phase is performed influences seriously upon the results obtained, as in the case of fat products. The technique used is the solvent extraction, followed by the liquid/liquid partition – LLE (Schenck and Hobbs, 2004). The extract is purified on column with activated Florisil ®. The organohalogenated pesticides are eluted with ethylic ether / light petroleum in variable proportions. The extraction and the determination of the contaminants were performed on fresh products in the following 48 hours after the collection, at the Institute of Public Hygiene and Health from Cluj Napoca. All the reagents used were Merck-type with chromatographic purity and there were no additional purifications performed, except for witness samples of the reagents on each work phase.

The phases undertaken are:

- The extraction of the toxic: an amount precisely weighted, as close to 100 g as possible, of the grounded sample was homogenised with 200 ml acetonytril, without adding water, because the humidity was over 75%. The resulting filtrate (F) is extracted through partition with 100 ml light petroleum, in the separation funnel. The extract (P) is passed to the purification phase; the F and P values in the phase of sample preparation are written during the determination because they are used in the calculation of the sample amount that passes through the purification column.

***Correspondence:** Chiş A. M., University of Oradea, Department of Food control, Environment Protection Faculty, no. 26 Gen, Magheru Bd., Oradea, 410048, e-mail : andichis@yahoo.com*
Article received: September 2009; published: February 2010

- Purification of Florisil column: three subsequent elutions are performed with 200 ml mixture ethylic ether / light petroleum for each, in the following V/V proportions: 6/94 (A), 15:85 (B) and 50:50 (C).

As one can see, the solvent consumption, for a single sample is consistent. For this reason, the method has been tested on the same samples both in the 1:1 variant and in reduced variants: 1:2, respectively 1:2. The reduction referred proportionally to the amounts of sample used, the amounts of solvents used in all the work phases and the dimension of the glassware used. The concrete work method can be seen in table 1, for the variant 1:2.

Table 1

The separation of the pesticides in green fodders, reduced variant, 1:2

A –/ Extraction – U%> 75g/100			
Nr.	**Stage**	**Devices, glass devices**	**Operation, reagents**
1	Homogenization for two min at high speed	Homogenizer	50 g sample + 100 ml acetonitryl
2	Filtration	Funnel with cotton	Gathering in graded cylinder – F (ml) 100ml
B – Partition			
1	Energetic agitation 2 min	Separation funnel 500 ml	Filtrate F + 50 ml light petroleum (ether measured in the cylinder starting from 2)
2	Mixing for 15 sec	Idem	5 ml sat solution of NaCl (1,5g/5ml water) 300 ml water
3	Layer separation	Idem	removal water layer
4,5	2 x washing organic layer	Idem	2 x 50 ml water
6,7	2 x separation	Idem	2 x water layer removal
8	Collection organic layer	Graded cylinder 50 ml	P = volume in the cilinder 50 ml
9	Agitation	With 7,5 g Na_2SO_4 anhydrous	
10	Filtration	Evaporation balloon	
11	Concentration	Evaporator	2,5 - 5ml
C - Purification on Florisil®			
1	Column preparation Florisil ® :min 5h at 130^0C	Glass column 10x200	10 cm Florisil® 1-2cm Na_2SO_4
2	Column washing	Column	25 ml light petroleum, removal
3	Passing the concentrate from B11	Column; Max 5 ml/min	
4,5	2 x Washing recipient B 11	Column; Max 5 ml/min	2 x 2,5 ml light petroleum
6	Washing walls collumn	Max 5 ml/min	A little petroleum ether
7	Elution A: 100 ml SE-A	Evaporation balloon 1	94 ml eter de petrol/ 6 ml petroleum ether
8	Elution B: 100 ml SE-B	Evaporation balloon 2	85 ml eter de petrol/ 15 ml petroleum ether
9	Elution C: 100 ml SE-C	Evaporation balloon 3	50 ml eter de petro / 50 petroleum ether
10	Concentration each eluate separately	Evaporation balloon 1,2,3	Spinning evaporator

Legend: SE - A,B,C – Elution solvent A, B, C

Fig. 1 Layer separation, Partition phase
Source: personal archive

Fig. 2 Elution of column of activated Florisil ®
Source: personal archive

Figure 1 shows one of the partition phases and figure 2 shows one of the elution phases followed for the OCP separation.

The determinations of the residues of organochlorine pesticides in the studies products

We have used the method most recommended by the specialized literature (Tadeo E., 2008; Hura, 2006), as set forth by current legislation for the calculation of pesticide residues in vegetal products. It is the gas chromatography (SR EN 12393-3/2004).

Used Devices

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 1, 2010, pp. 81-87

We have used a GC 2010 Shimadzu gas chromatograph, with the following characteristics:

- Capillary column type RTX -CL- pesticides 30 m length and 0.25 mm diameter. The column works at a temperature between 150 ÷ 3200C with a gradient ofe 30C/2 minutes
- Detector with electrons capture (ECD), nuclid 63Ni – 370 MBq (10mCu)
- Autosampler injection system with 6+2 spaces for vials, type AOC-210

Chromatography conditions:

- Injection temperature (splitting) = 2500C
- Splitting temperature = 163.5 Kpa
- Splitting gas: He with a 124 ml/min flow at scavenging 30 ml/min
- Carrying gas: N2 ultrapure 99.99%
- Detector: = 3200C, detector current of 2 nA, make-up flow = 30 ml/min

The device is connected to a computer and used a specific program for the interpretation of the results. Chromatograms are displayed on a singular monitor for the tested sample or together with the chromatogram of the used sample. The program supplies the retention times, the height of the picks and their surface, through automatic integration.

Qualitative and quantitative calculations

For the qualitative and quantitative calculation of the contaminants possibly present in the tested products, we used a standard produces by the RESTEK Company No 32292, Lot nr A021837, type "Organochlorine pesticide Mix ÁB" having a concentration of 200 ppb. Notice the fact that the standard contains, with one exception (hexachlorobenzen) all the organochlorine compounds under sanitary-veterinary surveillance in vegetal and animal foods. The standard was used at the 50 ppb dilution.

Green fodder tests submitted to the verification from the point of view of the contamination with organochlorine pesticides have undergone the procedures explained in "Test preparation for chromatography" part of the paper. The extract purified, retaken in petroleum ether was submitted to the 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.

For the quantitative determinations, we have used the value of the surface of the drops (compounds that have been previously identified as being present in the tested sample). In order to prevent possible calculation errors, we introduced the data we obtained through the automatic integration of the unknown tests and the used standard in EXCELL calculation sheets, selecting only the surface of the picks we need, that is the compounds that were found from the qualitative point of view.

In the case of the non-fat products (fodders) the calculation of the organochlorinated pesticide residues concentration refers to the amount of sample that passes through the Florisil purification column (S). The S value is calculated according to the moisture of the products and using parameters that result from the development of all the work procedure for non-fat products (SR EN 12393-2/2004).

$S = (m \times F \times P)/ T \times 100$, g, where:

Where:

S: the amount of sample, g, passed through the purification column

m: the mass of the sample, g

F: the measured volume of the filtrate after the aceteonytril extraction, ml

T: total volume, ml; T = ml water from the sample plus ml of acetonytril added, less the empiric contraction volume, in green fodders; T = ml of water plus ml of extraction mixture added, less ml correction for volume contraction, in complex fodders, in ml;

P – the measured extract of light petroleum, ml

100 – the volume of the light petroleum used in the extraction, ml

The contraction volume is considered to be equal with 5 ml for sample with a moisture between 80 and 95% when 200 ml acetonytril are used for the extraction

In the case of non-fat products, the values obtained are not corrected with the recovery degree (SR EN 12393,3-2004) .

RESULTS

For the qualitative determination, we compared the retention times for the significant picks that appear on the chromatogram of the unknown tests with the ones of the compounds from the standard sample. This way, we can determine which of the compounds that are present in the standard can also be found in the tested sample (Gocan, 1998). The results are written in table 2, 3 and 4, for the three variants applied separately for the three elution solvents used.

Since the compounds appear mostly in the A type eluent, figures 3, 4 and 5 show examples of chromatograms of the FV1 samples in the 1:1, 1:2 and 1:5 variants, elution A.

For the quantitative determinations, I calculated the amounts of samples passed through the purification column, in the three work variants applied to the tested samples. The values are found in table 5.

The results of the quantitative determination are shown in tables 6, 7 and 8, for the three work variants applied. The concentration written in the table represents the amount of the values at the three elutions applied, if case. The expression of the concentration as isomer sum for α and β HCH, endosulfan and DDT complies with the European laws (EFSA 2006, 2005) and the national laws (Legis).

Table 2

Qualitative calculation of the OCP residues, green fodder, variant 1:1

A	B	C	D - FV1			D – FV2			D – FV3		
			E_A	E_B	E_C	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		
3	β HCH	8.886			8.791					8.818	
11	α endosulfan	16.753	16.730			16.744			16.751		
13	Endrin	18.878	18.831			18.848			18.838		
14	4,4' DDD	19.853	19.809			19.814			19.822		
16	4,4' DDT	21.305	21.273		21.464	21.288		21.378	21.398		21.380
19	Sulfat endosulfan	24.693	24.798								

Legend: A – Elution order; B – Organochlorine compound from the sample; C – Retention time in the standard; D – FV1, FV2, FV3 –retention time in green fodder 1, 2,3; EA, EB, EC – Elution solvents used: A, B, C

Table 3

Qualitative calculation of the OCP residues, green fodder, variant 1:2

A	B	C	D - FV1			D – FV2			D – FV3		
			E_A	E_B	E_C	E_A	E_B	E_C	E_A	E_B	E_C
1	α HCH	7.194	7.190			7.191			7.175		
2	γ HCH	8.426	8.381	8.422		8.424	8.419		8.412		
3	β HCH	8.886			8.694					8.833	
11	α endosulfan	16.753	16.738			16.732			16.730		
13	Endrin	18.878	18.866			18.851			18.851		
14	4,4' DDD	19.853	19.822			19.833			19.845		
16	4,4' DDT	21.305	21.244			21.294			21.381		21.388
19	Sulfat endosulfan	24.693									

Legend: A – Elution order; B – Organochlorine compound from the sample; C – Retention time in the standard; D – FV1, FV2, FV3 –retention time in green fodder 1, 2,3; EA, EB, EC – Elution solvents used: A, B, C

Table 4

Qualitative calculation of the OCP residues, green fodder, variant 1:5

A	B	C	D - FV1			D – FV2			D – FV3		
			E_A	E_B	E_C	E_A	E_B	E_C	E_A	E_B	E_C
1	α HCH	7.194	7.179			7.183					
2	γ HCH	8.426	8.409			8.410			8.418		
3	β HCH	8.886									
11	α endosulfan	16.753							16.751		
13	Endrin	18.878	18.831						18.838		
14	4,4' DDD	19.853				19.814					
16	4,4' DDT	21.305	21.273			21.288			21.398		
19	Sulfat endosulfan	24.693									

Legend: A – Elution order; B – Organochlorine compound from the sample; C – Retention time in the standard; D – FV1, FV2, FV3 –retention time in green fodder 1, 2,3; EA, EB, EC – Elution solvents used: A, B, C

DISCUSSION

The discussions consist of the comparison if the results obtained in the normal 1:1 work variants, with the ones obtained in the three reduces variants applied – 1:2, respectively 1:5. The comparison refers both at the types of organochlorinated pesticides revealed and at the value of the concentration of the residues identified quantitavely.

The comparison of the 1:1 variants with the 1:2 variant

From the qualitative point of view, it is noteworthy that the same types of organochlorinated types of compounds have been identified through both methods, respectively the λ, β and γHCH isomers, αendosulfan, endrin, 4,4'DDD and 4,4'DDT in all the tested samples (Tables 2 and 3). Only for the FV1 type of sample, the endosulfan sulfate was identified through the 1:1 work method. This is a metabolite of endosulphane and it is quantitavely expressed as isomer sum.

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 1, 2010, pp. 81-87

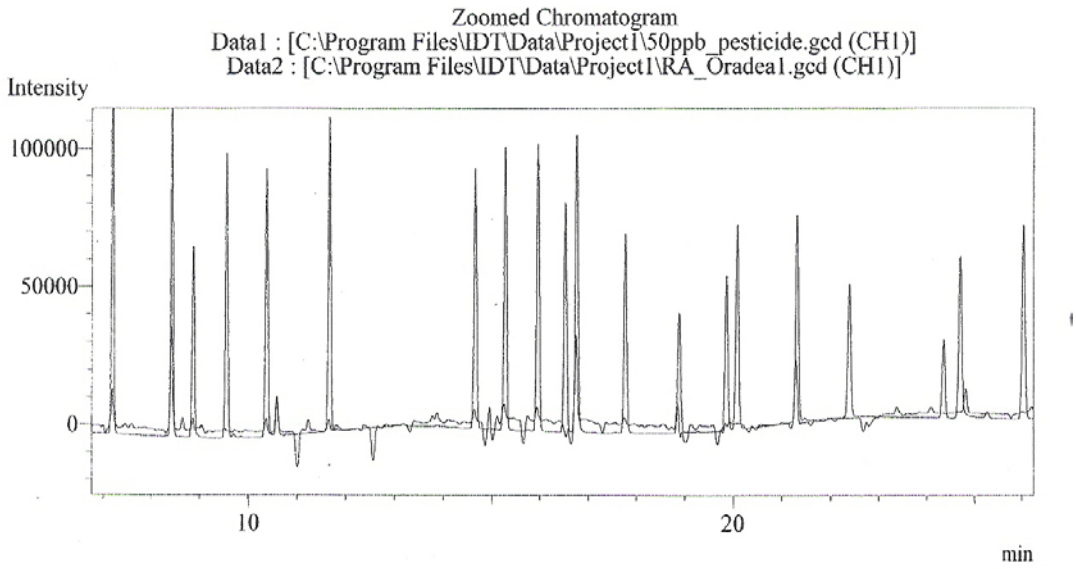

Fig. 3 Green fodder chromatogram, Elution A, 50 ppb standard, 1:1

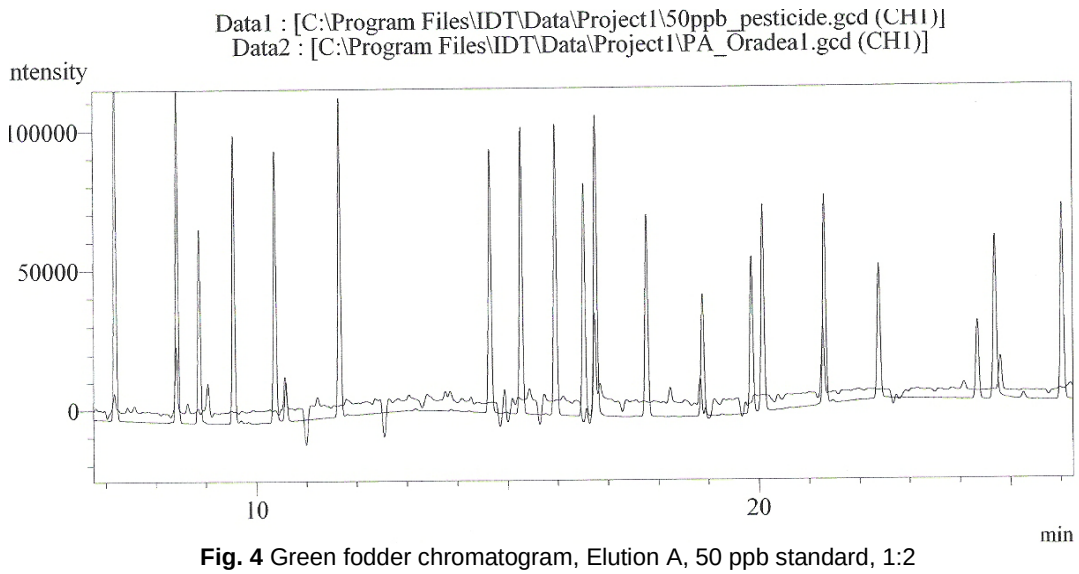

Fig. 4 Green fodder chromatogram, Elution A, 50 ppb standard, 1:2

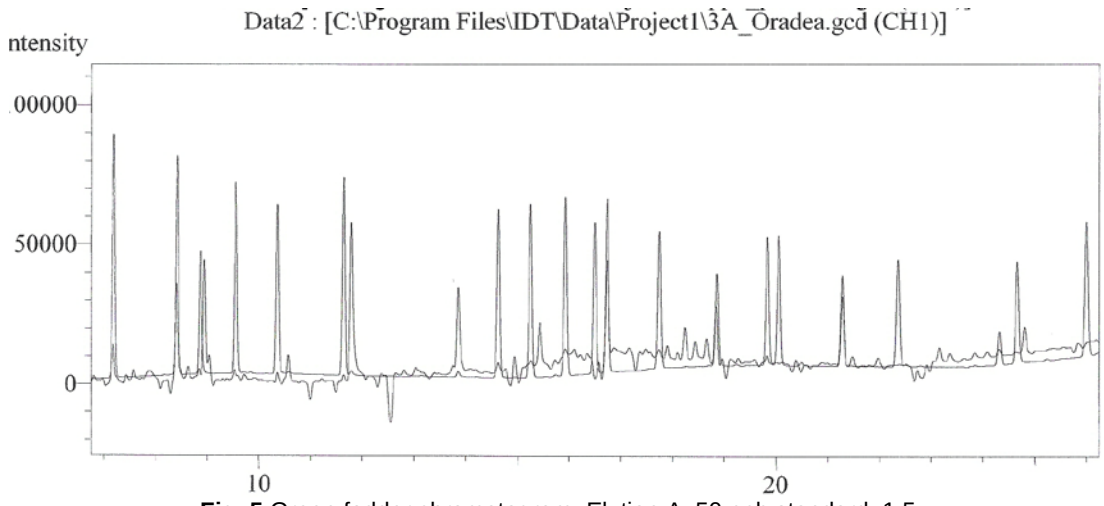

Fig. 5 Green fodder chromatogram, Elution A, 50 ppb standard, 1:5

Table 5

The sample amount submitted to purification, green fodder, variant 1:1, 1:2 and 1:5

Sample, variant	U,%	M,g	F,ml	P, ml	Water, ml	T,ml	S,g
FV1 1:1	88.1	98.65	239	85	86.91	281.91	71.09
1:2		51.66	125	47	45.51	143.01	21.22
1:5		21.19	42	15	18.67	57.67	2.31
FV2 1:1	86.7	101.06	240	83	87.62	282.62	71.23
1:2		52.32	123	46	45.36	142.86	20.72
1:5		19.04	40	13	16.51	55.51	1.78
FV3 1:1	90.0	97.44	240	86	87.70	282.70	71.14
1:2		49.76	122	46	44.78	142.28	19.63

Table 6

Quantitative calculation of the OCP residues, green fodder, variant 1:1

Nr (*)	Organochlorine compound found in the sample	Concentration in the sample, ppm		
		FV1	FV2	FV3
1,3	HCH (sum of izomer α şi β)	0.0011	0,0005	0.0016
2	γ HCH	0,0009	0,0012	0.0023
11,15,19	Sum of α, β and endosulfan sulphate, expressed in Endosulfan	0,0021	0,0021	0.0038
13	Endrin	0,0008	0,0013	0.0006
10,14,16	Sum of DDT, DDE and DDT isomers, exressed in DDT	0,0007	0,0002	0.0012

*Legend: * refers to the elution order of the compounds in the samples; FV1, FV2 – green fodder 1, respectively 2*

Table 7

Quantitative calculation of the OCP residues, green fodder, variant 1:2

Nr (*)	Organochlorine compound found in the sample	Concentration in the sample, ppm		
		FV1	FV2	FV3
1,3	HCH (sum of izomer α şi β)	0.0009	0.0004	0.0014
2	γ HCH	0.0008	0.0009	0.0019
11,15,19	Sum of α, β and endosulfan sulphate, expressed in Endosulfan	0.0017	0.0022	0.0042
13	Endrin	0.0009	0.0011	0.0006
10,14,16	Sum of DDT, DDE and DDT isomers, exressed in DDT	0.0005	0.0002	0.0010

*Legend: * refers to the elution order of the compounds in the samples; FV1, FV2 – green fodder 1, respectively 2*

Table 8

Quantitative calculation of the OCP residues, green fodder, variant 1:5

Nr (*)	Organochlorine compound found in the sample	Concentration in the sample, ppm		
		FV1	FV2	FV3
1,3	HCH (sum of isomers α şi β)	0.0003	0.0001	-
2	γ HCH	0.0002	0.0002	0.0005
11,15,19	Sum of α, β and endosulfan sulphate, expressed in Endosulfan	-	-	0.0008
13	Endrin	0.0003	-	0.0002
10,14,16	Sum of DDT, DDE and DDT isomers, exressed in DDT	0.0004	-	0.0003

*Legend: * refers to the elution order of the compounds in the samples; FV1, FV2 – green fodder 1, respectively 2*

From the quantitative point of view (tables 6 and 7) the differences of values obtained through the two work methods range between 0 and 20%, with one exception 28% for the sum of DDT isomers in samples FV1. In the range order where the contaminants were revealed, the differences are irrelevant.

The comparison of the 1:1 variants with the 1:5 variant

From the qualitative point of view, it is noteworthy the fact that between the two variants there are relevant differences not only because of the fact that the compounds found in the 1:1 variant are to be found in the 1:5 variant, but their apparition is random in the three tested samples (tables 2 and 4). So, in sample FV1 βHCH, α Endosulfan, Endosulfan sulfate and 4,4'DDD are not to be found , in sample FV2 - βHCH,

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 1, 2010, pp. 81-87

α Endosulfan and Endrin are not found and in sample FV3 - α and βHCH and 4,4'DDD are not found..

From the quantitative point of view (tables 6 and 8) the differences are noteworthy for the compounds that can be found quantitavely and for which the integrated area is enough to allow the calculation. Therefore, for the FV1 sample the values found in the 1:5 variant differ from the 1;1 variant with 43% up to 80% for the FV2 sample with 80% until 83% and for the FV3 the differences are between 66% and 200%.

In what regards the apparition method and the compounds identified quantitavely, one can observe that except for the βHCH isomer, all the compounds appear in the A type eluent, for the three types of samples both in the 1:1 variant and in the 1:2 variant. The γHCH and 4,4'DDT isomers show a signal repetition in the C eluent for most samples. In the 1:5 variant the compounds identified appear only in the A eluent.

CONCLUSIONS

In conclusion, we can state the fact that the 1:2 reduced variant has qualitative and quantitative applications in the determination of the organichlorinated pesticide residues from green fodders, which leads to important economies of solvents. In the same time, the duration of the determinations is shortened without affecting the accuracy, by maintaining the maximum elution speed at 5 ml/minute. This is highly important in the case of monitoring programs when there's the need of working a high number of samples. On the other hand, the 1:5 variant does not have a qualitative or quantitative applicability. This thing can be put on behalf of the solvent and sample amount used, which prove to be insufficient in the field of 10^{-3} – 10^{-4} ppm concentrations.

Moreover, I consider that it is important to continue the testing in other intermediary variants, as well as other types of fodders, since they represent the most likely source of contamination of animal foods with bioaccumulable toxics (Savu and Georgescu, 2004).

REFERENCES

Chiş A., Analytic methods for confirming the presence of chlorinated pesticide residues in high-fat foods, The 4th International Symposium „Natural resources and sustenable developement", Oradea 10- 11 October 2006, p.599

EFSA, a, 2005, Opinion of the Scientfic Panel on Contaminants in the Food Chain on a request from the Commission related to Gamma – HCH and other hexachlorocyclohexanes as undesirable substances in animal feed, The EFSA Journal, 250, pp. 1-39

EFSA, a, 2006, Opinion of the Scientfic Panel on Contaminants in the Food Chain on a reques tfrom the Commission related to Endosulfan as undesirable substances in animal feed, The EFSA Journal, 234, pp. 1-31

EFSA, b, 2005, Opinion of the Scientfic Panel on Contaminants in the Food Chain on a request from the Commission related to Endrin as undesirable substances in animal feed, 2005, The EFSA Journal, 286, pp. 1-31

EFSA, b, 2006, Opinion of the Scientfic Panel on Contaminants in the Food Chain on a reques tfrom the Commission related to DDT as undesirable substances in animal feed, The EFSA Journal, 433, pp. 1-69

Gocan S., 1998, High-performance cromatography, part I, gas chromatography, Dacia Publishing House, Cluj Napoca

Hura C., 2006, Laboratory guide – Analysis methods for food products, Editura Cermi, Iaşi

LEGIS – Romanian legislation

Savu C., Georgescu N., 2004, Food safety, "Semne" Publishing House, Bucharest

Schenck, F.J. and J. Hobbs, 2004, Evaluation of a quick, easy, cheap, effective, rugged and safe (QuEChERS) approach to pesticide residue analysis, Bull. Environ. Contam. Toxicol.73(1), pp. 24-30

SR EN 12393-2, 2003, Produse alimentare negrase. Metode multireziduu pentru derminarea gaz-cromatografică a reziduurilor de pesticide, Partea 2 : metode de extracţie şi purificare

SR EN 12393-3, 2003, Produse alimentare negrase. Metode multireziduu pentru derminarea gaz-cromatografică a reziduurilor de pesticide, Partea 3 : Determinare şi teste de confirmare

Tadeo, J. L., (ed.), 2008, Analysis of Pesticides in Food and Environmental Samples, CRC Press, Taylor & Frances Group Boca Raton , London, New York