

## THE IDENTIFICATION of SOME PESTICIDES through STATISTICAL METHODS

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### **Abstract**

*As it is known, the qualitative determination of substances through gas-chromatography (GC) is performed by comparing the retention times for the compounds came out in the test with those in the used standard. This paper shows a statistical approach for the identification of some organochlorine compounds (OCP) under sanitary veterinary surveillance (SVS) in milk and fat dairies..*

**Key words:** organochlorine pesticides, t-test, qualitative identification

### **INTRODUCTION**

The determination of the food contamination level with pesticides, as a component of the chemical contamination determination is directly connected with the food safety issue. This kind of contamination is extremely dangerous because, in most cases, it does not affect the sensorial quality of the food at the concentration levels which may cause harm. Due to the impact on the public health, many scientific researches in this field of activity focus on the development of multi-residue methods for the determination of pesticides at lower and lower levels of concentration, both in food and raw material food. (Lehotay, 2010, Caykra et al, 2007, Paya, 2007).

Nowadays, the qualitative and quantitative determination of this contaminants is performed by means of instrumental methods, due to the lower and lower level of residues which must be determined in such complex matrix as food is. This is especially about gas chromatography and high performance liquid chromatography. (Tadeo, 2008)

Beyond the use of state-of-the-art devices and techniques, the problem is how to interpret the obtained results so that the subjectivity be eliminated to the highest possible extent.

### **MATERIALS AND METHODS**

### **Materials**

The tested material comes from three different dairy producers from Bihor county and consists of:

- three types of raw material milk having a fat content between 3,2% and 4,4%;
- three types of sour cream having a fat content of 15%, 20% and 25%;
- three types of “Dalia” type pressed cheese, having a fat content in dry substance between 40,9% and 69%.

### **Methods**

The following methods were used in the preparation of the samples:

- extraction with a n-hexane/acetone mixture on a glass column filled with sea sand/ anhydrous sodium sulphate, for the separation of fat from milk (SR EN 1528-2/2004);
- Soxhlet extraction with light petroleum for the separation of fat from sour cream and pressed cheese (Popescu et al., 1986);
- Liquid/liquid partition with acetonitril and elution from Florisil column for the purification of the fat extract that contains the pesticides residues (Lehotay, 2007, Diez et al, 2006, Cuniff, 1995)

The determination of the organochlorine pesticides was performed by the gas chromatography method on non-polar capillary column using the electron capture detector, due to its high sensitivity to the halogens. The injection of the extract was performed by the PTV system (Programmable Temperature Vaporization) which is the most recommended for the traces' analysis (Grob, 2001, Tadeo, 2008).

For the qualitative 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 AB #2” having a concentration of 200 ppb.

As known, the qualitative identification is based on the comparison of the retention times of the compounds in the used standard with the retention times recorded in the analysed tests (Gocan, 1998). This kind of approach is useful only if the standard and the samples are tested under the same conditions and the injection of the standard is performed in the same time as the samples. The dilution of the standard from the basic concentration of the used ones was made with the same solvent as the one used in the preparation of the samples phases, including the final retake of the pesticide extract, respectively light petroleum. In the case of complex matrix such as foods, no matter how good the preparation phase is made, regardless of the chromatographic technique used, the retention times will not overlay perfectly in the standard and in the samples chromatograms.

The question is to obtain the highest degree of safety in the identification issue. The graphic processing, peak by peak, at different zoom level is a very laborious activity and it cannot help when a great number of samples needs to be processes. The comparison of numbers that represent the retention times, on decimal expression in the chromatograms, by mathematical means of statistic, leads to the verification of the deviation's size for a positive identification.

For the organochlorinated pesticides under sanitary veterinary surveillance we talk about approximately 10 compounds but four of them are checked as isomers sum, each having different retention time values (Order 23/2007 and Order 12/2006).

For this reason we practiced a mathematical verification through statistical calculation based on "t" – Student distribution (Ardelean, 2005). As reference values we used the retention time values for the respectively compound in different concentration standards (25, 40 and 50 ppm) and they were compared with the retention time values for the same presumed compound in milk and pressed cheese tested samples, values obtained from the computer' automatic software. The obtained results were statistically processed using the "t- test" in *Prisma 5 for Windows*. The values were compared with the theoretical ones of the required freedom degree in the scope of framing of the mean difference at "irrelevant", "relevant", "distinct relevant" or "very relevant" deviation. For sour cream the verification principle is the same as for milk, the reference value being the retention time in the used standard. As comparison mean we used the values of the retention time at the presumed compound in the 15%, 20% and 20% fat sour cream samples.

## RESULTS AND DISSCUSIONS

Tables 1, 2 and 3 show the results of the statistical calculation depending on the identified compounds and the used material. The freedom degree is not the same for all compounds because they do not have the same frequency appearance in all the tested samples, as we previously reported (Chiş, 2008 a and b).

Table 1

The synthesis of the statistic analysis at the verification of the deviation of the retention times in  $\alpha$  endosulfan, endrine and 4,4' DDT – sour-cream

	Retention times							
	$\alpha$ endosulfan		Endrin		4,4 DDT		Sulfat endosulfan	
	Stan- dard	Sample	Stan- dard	Sample	Stan- dard	Sample	Stan- dard	Sample
Mean	16.088	16.060	18.160	18.141	20.543	20.501	23.822	23.784

Mean deviation	0.001	0.011	0.028	0.019	0.030	0.044	0.033	0.071
T		0.7544		1.1202		1.225		0.6822
Sg		ns		ns		ns		ns

Table 2

The synthesis of the statistical analysis at the verification of the deviation of the retention times for  $\alpha$  endosulphane, Endrine and 4,4' DDT  
- raw material milk, and cheese

	Retention times					
	$\alpha$ Endosulfan		Endrin		4,4' DDT	
	Standard	Sample	Standard	Sample	Standard	Sample
<b>raw milk</b>						
Mean	16.069	16.078	18.160	18.166	20.543	20.528
Mean deviation	0.026	0.009	0.028	0.006	0.030	0.006
T		1.4889		1.1202		1.3693
Sg		ns		ns		ns
<b>cheese</b>						
Mean	16.069	18.160	18.163	20.543	20.528	16.069
Mean deviation	0.011	0.028	0.007	0.030	0.008	0.011
T		2.3155		0.2806		1.2883
Sg		ns		ns		ns

Table 3

Synthesis of the statistical analysis in the verification of the deviation of the retention times for the HCH isomers – raw material milk, sour-cream and cheese

	Retention times					
	$\alpha$ HCH		$\gamma$ HCH		$\beta$ HCH	
	Standard	Sample	Standard	Sample	Standard	Sample
<b>raw material milk</b>						
Mean	6.803	6.833	7.978	7.961	8.394	8.380
Mean deviation	0.012	0.066	0.001	0.014	0.013	0.007
T		1.0793		1.6283		1.9585
Sg		ns		ns		ns
<b>sour-cream</b>						
Mean	6.803	6.781	7.978	7.940	8.394	8.456
Mean deviation	0.012	0.038	0.001	0.068	0.013	0.182
T		0.759		0.7497		-0.4568
Sg		ns		ns		ns
<b>cheese</b>						
Mean	6.803	-	7.978	7.958	8.394	8.383
Mean deviation	0.012	-	0.001	0.008	0.013	0.004
T				2.2361		1.4721
Sg		-		ns		ns

Legend for table 1, 2 and 3 :

ns –irrelevant

Sg - Signification of the mean difference

In order to frame as “irrelevant”, the statistic method foreseen for “t” factor a maximum value 2,776 at freedom degree “4”, 2,571 at freedom degree “5” and 2,447 at freedom degree “6” (Ardelean, 2005).

The obtained results marked in tables 1, 2 and 3 show for each of the contaminants found in the samples values for “t” less than the respective maximum. So the deviations of the retention times are qualified as “irrelevant” (ns) for every one of the positively identified compounds in the tested samples, respectively HCH isomers,  $\alpha$  Endosulfan, Endosulfan sulphate, Endrin and 4,4’DDT .

## CONCLUSIONS

The use of statistic methods to process experimental values has the scope to offer certainty for the qualitative determination. This way the deviation of the value of the retention time is clearly limited in the tested samples towards the standard in order to have an acceptable identification of the interest compounds.

In the same time, in the concrete case of the chromatography, it shortens the needed time for the visual comparison of the picks in the sample, respectively in the standards.

Such a approach is useful especially when no confirmation methods are available using other columns, other detectors (Mondello, 2008) or other work techniques (bidirectional chromatography). Our aim is that the next experiments shall be focused on comparison of the present work, a current technique, with MS detection, especially in the scope of verifying the sensibility at organohalogenated compounds at ECD detector compared to MS at the concentration levels ppm-ppb specifics to the chemical contaminants residues in food.

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