THE DEGREE OF PESTICIDE CONTAMINATION OF WINE GRAPES PRODUCED IN 2009

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Abstract
Because carcinogenic, mutagenic, toxic pesticides assigned endocrine system is necessary to investigate the degree of contamination with these substances to food. To establish the extent of pesticide contamination of wine grapes were analyzed 16 samples of wine in Mehedinti County, of six wine companies in the area. Samples of wine were analyzed by gas chromatography method, pesticide residues were extracted with the mixture of acetone: methylene chloride: petroleum ether, centrifuged, the supernatant evaporated and pesticide residues resumed izooctan mixture: toluene 9:1, then injected into gas chromatograph. From the grape samples analyzed were not detected organophosphorus pesticide residues in group nor the synthesis pyrethroid present in standard mixture. The pesticide group organoclorurate pesticides present in standard mixture of folpet residues were detected, but in amounts below the maximum permissible legislation..

Keywords: grapes, pesticides, gas chromatograph, MRLs.

1. INTRODUCTION

Nowadays, over 300 comercial products are known, based on 50 active products used for the phytopatogen agent control in vineyard. There are many fungicide products for viticulture on the market. Their diversity and efficiency allow a good prevention and control of the main vineyard deseasses.
The significance of profilactic use of pesticides is the minimization of grape damages. Nevertheless, the risk of pesticide residue on grapes have to be considered in the case of wrong treatment procedure. When the pesticide residues are present in wine, there are a lot of negative consequences such as: the quality of wine diminishes, the fermentation slow down, and throubles with the malolactiv fermentation.
The growing interest for the study of pesticide presence in grapes is justified by oenologic point of view by the possible interferences with the fermentative microflora used for the wine prodution [4]. Even if the pesticide mobility from grapes to wine is generaly reduced due to the winery process (especialy the alcoholic fermentation drasticaly reduce the pesticide level) there is always a risk for wine contamination and, therefore a toxic source for the consumers [5].

2. MATERIALS AND METHODS

For determination of pesticide residues in grapes were used in these pesticides analytical standards: ά, β, γ HCH, HCB, chlorothalonil, heptachlor, aldrin, captan, folpet, endosulfan, dieldrin, endrin, iprodione, pp DDE, cypermethrin (sum of 4 isomers), bifenthrin, cyhalothrin (sum of 4 isomers), fenvalerate, esfenvalerat, deltamethrin, dichlorvos, phorate, dimethoate, omethoate, disiston, diazinon, parathion, methyl parathion, chlorpyrifos, pirimiphos methyl, chlorpyrifos methyl, azinphos methyl, malathion, methidathion, manufactured by Riedel de Haen, purity > 99%. Reagents used were: acetone, methylene chloride, petroleum ether, toluene, HPLC purity izooctan produced by Merck (Germany). Stock solutions of standards were prepared in toluene and stored in glass flasks at - 18 ^{0} C. Working solutions were prepared in mixed izooctan: toluene 9:1 and kept everything in the freezer. For quantitative determination of pesticide residues we used a Varian CP 3800 gas chromatograph with autosampler and detectors ECD and TSD.

Working conditions of the device [2], [3]:

- Carrier gas: He 2 ml / min,

- Make-up: N2 30 ml / min;
- Type of injection: split
- Injection volume: µl;
- Injection temperatur: 250 ^{0}C,
- Split flow: 40 ml / min;
- Detector temperatur: 300 ^{0}C,
- Chromatographic column: VF 5 ms 30 m x 0.25 id, 0.5 µm;
- Oven temperature: 50 ^{0}C(1min) - 30 ^{0}C/min - 215 ^{0}C(3min) - 5 ^{0}C/min - 280 ^{0}C (5min).

Pesticide residues were extracted with acetone: methylene chloride: petroleum ether, centrifuged, the supernatant evaporated and pesticide residues resumed izooctan mixture: toluene 9:1, then injected into the device.

Procedure:

- is finely powder sample and homogenize well;
- are weighed in a centrifuge tube, 15 g of homogenized sample;
- add 30 ml acetone, homogenize 20 sec.;
- add 30 ml methylene chloride and 30 ml petroleum ether, mix 20 sec.
- nearly evaporate in seconds rotavapor and to dry at room temperature;
- residue is dissolved in 3 ml mixture izooctan - toluene (9: 1, v / v) containing internal standard 0.03 mg / l (Mirex / ethion) by sonication.

Processed samples were injected as a gas chromatograph Varian CP 3800. Injecteat after stabilizing signal to 1 µl of working standard solution and 1 µl of sample. Identify the components of the sample was made according to retention time compared with the retention time of standard substance. Determination is based on an automatic integration system, realized the automatic marking of retention times and integration of chromatographic peaks, being directly their range and 3 ml extract concentration.

Pesticide residues x, expressed in mg / kg in case of using internal standard method, is calculated using the formula:

$x = CA * V1 * V2 / V3 * m$ where:

x - concentration of pesticide in mg / kg,
CA - standard concentration in mg / kg
V1 - volume of extract in ml;
V2 - volume of solvent to dissolve the extract in ml;
V3 - the amount that evaporates in ml,
m - the quantity of samples taken in work in grams.

3. RESULTS AND DISCUSSIONS

Between 26.08 - 08.10.2009 were analyzed 16 samples of grape wine Dutch MRM method. After injecting the standard mixture concentrations 25, 50, 75, 100 and 150 µg / kg was plotted calibration curve, then grape samples was injected. The result analysis is presented in Table 1.

Maximum permissible limit (MRLs) of the EC nr.149/2008 for folpet is 5 mg / kg [1]. Folpet dispose of product after 40 days of application. Samples containing folpet of 2.4 and 2.89 mg / kg were examined in August 26, two weeks before harvesting living samples, were analyzed in September increasingly content less than the minimum limit up folpet detection method, so to phase out the product as required by the manufacturer.

Chloride pesticides standard mixture chromatograms and two samples containing folpet at 2, 4 and 0.1 mg / kg are presented in the figure 1-3:

Table 1. The degree of pesticids contamination of samples of grape production in 2009

NO	SAMPLES OF GRAPES	COMPANY OR VITICULTURAL AREA	RESULTS IN MG / KG		
			Chloride pesticides	Organophosphorus pesticides	Piretroizi Synthesis
1.	1. White wine rapes 2. Black grape wine	SC Vintera, Mehedin\i		1. undetectable 2. undetectable	

2.	1. White grape wine 2. Black grape wine	SC Vinarte, loc.Rogova, Mehedin\i	1. folpet 0,11	2. undetectable	2. undetectable
3.	Wine grapes, 2009 1. Merlot 2. Cabernet Sauvignon 3. Chardonay 4. Pinot Noir 5. Syrah	Viticola Corcova, Mehedin\i	1. folpet 2,4 2. folpet 2,89	3. undetectable 4. undetectable	5. undetectable
4.	Wine grapes, 2009 1. White grape wine 2. Black grape wine 3. White grape wine	SC Vie Vin, V`nju Mare	1. folpet 0,02	2. undetectable	3. undetectable
5.	1. Table grapes, 2009 2. Wine grapes, 2009	SC Viticola Dealul Viilor	1. folpet 0,017 2. folpet 0,025	1. undetectable 2. undetectable	
6.	Wine grapes, 2009 1. Chardonay 2. Merlot	SC Carl Reh Winery, Crama Opri]or	1. undetectable 2. undetectable	1. undetectable	2. undetectable

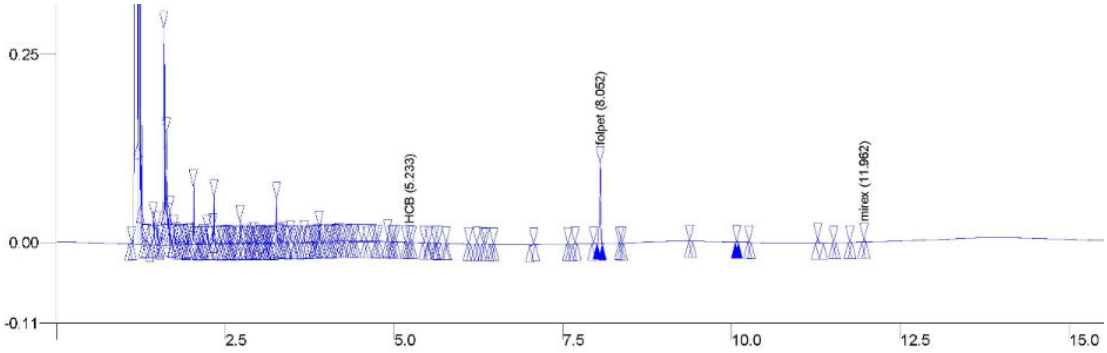

Fig. 1. Chromatogram of grape samples with a concentration of folpet of 2.4 mg / kg

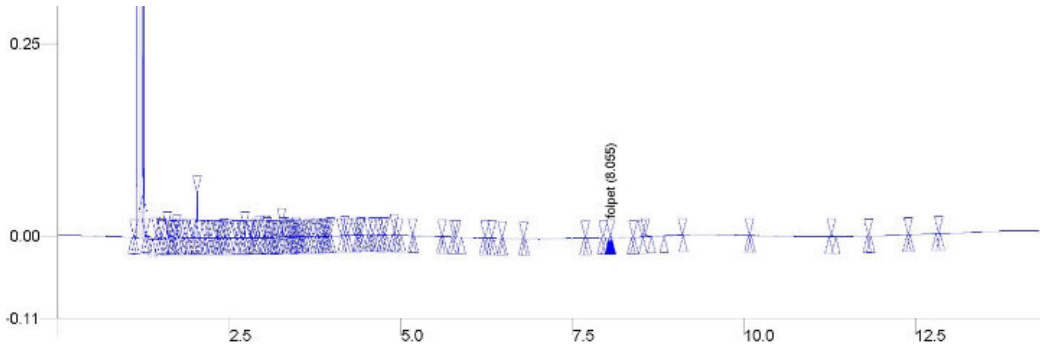

Fig.2.Chromatogram of grape samples with a concentration of folpet of 0,1 mg/kg

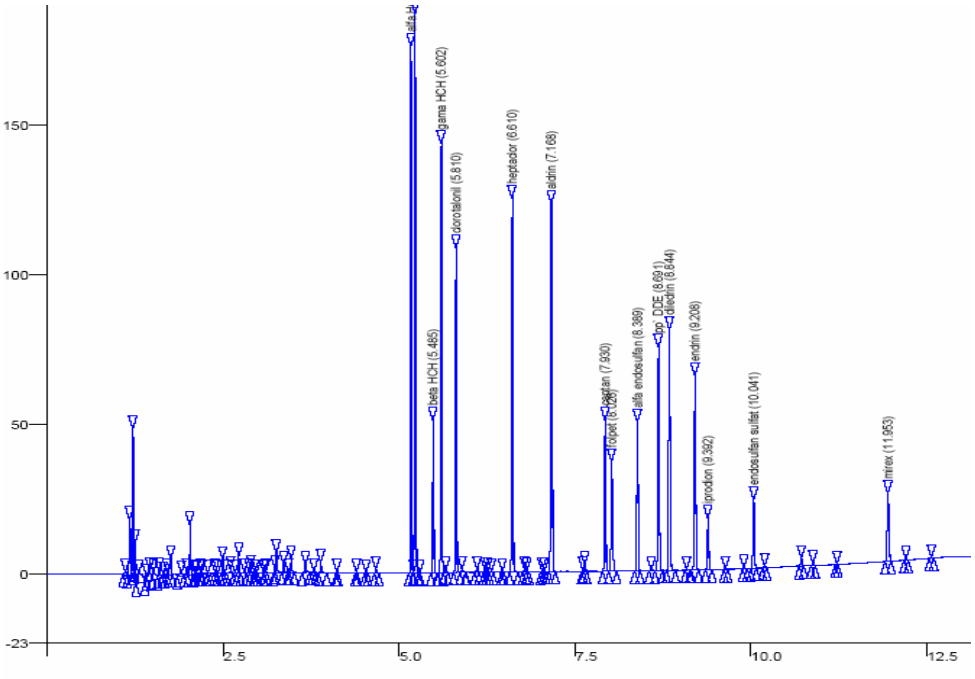

Fig. 3. Chromatogram of standard mixture of chloride pesticides

4. CONCLUSIONS

The significance of profilactic use of pesticides is the minimization of grape damages. Nevertheless, the risk of pesticide residue on grapes have to be considered in the case of wrong treatment procedure.

When the pesticide residues are present in wine, there are a lot of negative consequences such as: the quality of wine diminishes, the fermentation slow down, and throubles with the malolactiv fermentation.

The growing interest for the study of pesticide presence in grapes is justified by oenologic point of view by the possible interferences with the fermentative microflora used in winemaking.

Wine grapes produced in 2009 in the Mehedinti not hazardous to wine quality and population health.

5. REFERENCES

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