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Persistence and degradation of metalaxyl, lindane, fenvalerate and deltamethrin during the wine making process

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Abstract

The persistence and degradation of four acaricides (metalaxyl, lindane, fenvalerate and deltamethrin), used to control pests in vineyards, in experimental must and wine samples, have been studied. Commercial formulations of the pesticides were added to non-settled must and their fate during a common vinification method for white and red wines was investigated. Analytes in the extracts were determined by GC/EIMS after a solid-phase extraction on polymeric cartridges to isolate them from the must and wine samples. It was verified that white wines generally contained higher amounts of residues. Moreover, some degradation products, not reported until now, have been observed in the samples in addition to some compounds – byproducts from the manufacturing and excipients – initially present in the commercial formulations.

Keywords: Pesticides; Impurities; Degradation products; Wine; Fate

1. Introduction

The occurrence of diseases in vineyards demands the use of some formulations to control these pests and palliate economic losses. Furthermore, these treatments must guarantee the absence of residues in grapes. Unfortunately, and due to different causes, grapes can contain residual amounts, which could not only alter the vinification process, but also remain in the final product, affecting the wine quality and consumer health (García Cazolar, 1991; Kakalikova, Matisova, & Lesko, 1996; Navarro, Oliva, Navarro, & Barba, 2001; Oliva, Navarro, Barba, Navarro, & Salinas, 1999). Thus, if there are residues, it is necessary to know their evolution during the process. To this end, attention must be paid both, to the active ingredient and to the compounds that go together with it in the formulation. This latter aspect has been scarcely reported in the scientific literature.

This manuscript deals with four acaricides widely used in vineyards: metalaxyl, lindane, fenvalerate and deltamethrin, for which there are few data about their evolution during the vinification (Cabras, Meloni, & Pirisi, 1987; García Cazolar, 1991; Navarro, Barba, Oliva, Navarro, & Pardo, 1999; Navarro et al., 2000) and none about the likely degradation products. However, there is available information on their behaviour in other matrices.

Thus, the degradation of metalaxyl in soil has been established, mentioning the existence of degradation products more polar than the active ingredient (Monkiedje & Spiteller, 2002; Sukul & Spiteller, 2001a; Sukul & Spiteller, 2001b) and the enantioselectivity of the degradation of the stereoisomers (Marucchini & Zadra, 2002). The transformation products resulting from photocatalytic oxidation of metalaxyl-containing waters have been identified, too (Topalov, Molnar-Gabor, & Csanadi, 1999). Moreover, an analytical method to determine the residues that contain the 2,6-dimethylaniline moiety has been reported (Headley, Maxwell, Swyngedouw, & Pudri, 1996).

A few works have been published in the past few years on the degradation and metabolites of lindane. In this

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way, its photodegradation in water (Zaleska, Hupka, Wiergowski, & Biziuk, 2000) and its transformation products in sausage (Abou-Arab, 2002) have been established.

As regards pyrethroids, the decay of deltamethrin and fenvalerate, under different types of radiations (Abdel Aal, Dessouki, & Sokker, 2001; Samsonov & Pokrovoskii, 2001), and the degradation of fenvalerate and other pyrethroids in water, cauliflower and foliage (Gupta & Singh, 2001; Lutnicka, Bogacka, & Wolska, 1999; Sukul, 1995) have been reported. Moreover, analytical methods for the acidic degradation products that contain the aromatic and non-aromatic moieties of some pyrethroids have been optimized for urine, soil, milk, vegetables and animal tissues (Arrebola, Martinez-Vidal, Fernandez-Gutierrez, & Akhtar, 1999; Chen, Fink, & Letinski, 1996; Class, 1992; Colume, Cardenas, Gallego, & Valcarcel, 2001; Jin & Webster, 1998; Mortimer & Shields, 1995). Furthermore, it has been verified that 4-chloro-(1-methylethyl)benzeneacetic acid and 3-phenoxybenzoic acid are the predominant metabolites of fenvalerate in rats (Misra & Sharma, 1997).

For the above-mentioned reasons, the aim of this work has been to study the fate of the four acaricides and those related compounds found in the used commercial formulations during the wine-making process. To this end, typical microvinifications were carried out on musts spiked with formulations of the pesticides. Must and wine samples were subjected to a solid-phase extraction and the extracts were analyzed by GC/MS to determine the residual concentrations of the active ingredients and the possible presence of impurities and degradation products.

2. Materials and methods

2.1. Materials

Pesticide standards (98% minimum purity) were obtained from Promochem (Wesel, Germany) and Riedel de Haën (Hannover, Germany). The commercial formulations Ridomil MZ 72 (metalaxyl 8% and Mancozeb 64%) from Ciba Geigy (Greenboro, NC, USA), Verdane 90 PM (lindane 90%) from Kenogard (Barcelona, Spain), Sumifive (fenvalerate 15%) from Kenogard and Decis (deltamethrin 2.5%) from Agrevo (Cambridge, UK) were also used. Residue analysis grade solvents were provided by Labscan (Dublin, Ireland). Ultrapure water was obtained from a Milli-Q plus apparatus from Millipore Corp. (Bedford, MA). For solid phase extraction, Lichrolut EN 200 mg cartridges were supplied by Merck (Darmstadt, Germany).

To assess the presence of impurities or by-products related to the active ingredients, the commercial formulations were dissolved in acetone at a concentration of 200 mg/l for their injection into the GC system.

2.2. Wine-making process and addition of pesticides

The microvinifications were carried out with only one pesticide each time. For each one, the microvinifications were done in duplicate. Grapes were crushed and pressed by a pneumatic press, and then the SO_2 content of the must was adjusted; in the elaboration of white wine, the must was cold-settled for 24 h. An amount of commercial formulation required to achieve a concentration of 10 mg/l of each pesticide, referred to the total volume of must, was added. Then, the musts were inoculated with dry yeasts to start the alcoholic fermentation: UVAFERM CS2 (*Saccharomyces cerevrisae*) in a ratio of 10 g yeast/100 l must to make a red wine, and UVAFERM BC (*Saccharomyces bayanus*) in a ratio of 5 g yeast/100 l must to make white wine. For red wine elaboration, the lees were removed after the fermentation and a malolactic fermentation was performed; the wine was then obtained by pressing. Red wines were clarified with gelatine/bentonite in a proportion 20/30 g/100 l, while white wines were clarified with casein/bentonite, 20/60 g/100 l. Both wine types were racked several times, stabilized by cold, treated with sodium metabisulfite, and bottled.

The analyzed must samples were collected one day after the addition of the pesticides. Wine samples were taken two days after bottling.

2.3. Solid phase extraction of residues

A wine volume of 10 ml was eluted through a Lichrolut EN cartridge, previously conditioned with 10 ml of methanol and 10 ml of water. Then, the cartridge was eluted with 6 ml of water to clean up the extract and was dried by sucking nitrogen through it for 20 min. Finally, the extract was eluted with 3 ml of ethyl acetate by gravity after soaking the stationary phase for 3 min.

For the analysis of must samples, 10 ml of sample were diluted with 40 ml of water, and the analytes in the mixture were extracted as described above.

2.4. GC/MS determination

Chromatograms and mass spectra were recorded by coupling an Hewlett–Packard (Avondale, PA, USA) 6890 gas chromatograph equipped with a 30 m × 0.25 mm × 0.25 μm HP5MS column to an HP5973 mass selective detector (single quadrupole mass spectrometer). The oven temperature was programmed as follows: initial temperature 50 °C (1 min), a 15 °C/min ramp to 130 °C, a 4 °C/min ramp to 170 °C, then 2.4 °C/min ramp to 240 °C, and finally a 15 °C/min ramp to 270 °C (15 min). The carrier gas (He) flow rate was constant at 1 ml/min, measured at 50 °C. Pulsed splitless injection of a 1 μl sample volume was performed at 230 °C with a pressure pulse of 25 psi for 1 min; the purge valve was also opened at 1 min. The transfer line was heated at 280 °C. Mass spectra were recorded in electron impact mode from m/z 50 to 515 (3.15 scans/s) after a solvent delay of 5.50 min. Source and quadrupole temperatures were 220 and 100 °C, respectively. The electron multiplier voltage was maintained at 235 V above the autotune value.

2.5. Quantitation of the pesticides in the extracts

The residues of the active ingredients were measured in must and wine samples. A linear regression analysis was used to generate the calibration equations (based on peak areas) in the 0.5–40 mg/l range. To avoid quantitation errors derived from the sample matrix, a matrix-matched calibration was done. Aliquots of wine or must (from control microvinifications) were spiked with known amounts of pesticides and subjected to the sample preparation procedure; then the extracts were used as calibration standards. Good fits, $r^2 > 0.990$, were always achieved. The recoveries of the studied pesticides in must and wine samples spiked with 1 and 10 mg/l of each compound were about 95–103% ($n = 7$), with coefficients of variation ranging from 4% to 9%. The detection limits, estimated as a signal-to-noise ratio of 3 on extract chromatograms, varied from 0.05 to 0.15 mg/l. Pesticide stock solutions (1000 mg/l) and working solutions were prepared in acetone.

3. Results and discussion

3.1. Impurities and degradation products found

3.1.1. General

Many of the compounds related to the studied pesticides were not identified by the use of spectral libraries. So, they were characterized according to the fragment ions observed in the electron impact spectra. Brief commentaries about their characterization are made below; related compounds for deltamethrin were not detected. To our knowledge, for the studied compounds, there are no previous data about the degradation products or impurities of the formulations in wines.

In the following discussion, the target-compounds are numbered in the order of elution from the chromatographic column.

3.1.2. Metalaxyl

Table 1 shows some fragmentations for the compounds whose structures are related to that of metalaxyl. A few ions were observed in the spectrum of the minor compound, **Met I,** but these seemed to indicate that a decarboxylation had taken place (see Fig. 1). The structure of compound **Met III** was established, assuming that the highest observed m/z ratio (193) was the molecular ion; thus, the fragmentations were coherent with the assigned structure.

The most abundant ion for **Met IV** was detected at m/z 234. This ion was not indicative of the compound structure, as it can come from the two substituents attached to the amine group: the loss of methoxymethyl (CH_2OCH_3) or the loss of CH_3 and H_2CO in the methoxycarbonylethyl group, $CH(CH_3)CO_2CH_3$. Perhaps, **Met IV** might be a hydroxylated compound and, in consequence, its molecular ion would not be detected. In the same way, a structure for **Met II** was not proposed; however, the ion at m/z 162 could be explained by the formation of $(CH_3)_2(C_6H_3)$ N (CO) CH_3, in which the nitrogen atom of the isocyanate group was bonded at a methyl group.

The ion at m/z 234 was also observed in the **Met V** spectrum, together with two other ions: 117 and 174. The ion at m/z 117 is common to all the compounds derived from metalaxyl but the second ion, attributed to the loss of methanol, could involve the presence of a metoxycarbonylethyl moiety.

Table 1
Retention times and fragment ions for the compounds related to metalaxyl found in musts, wines and commercial formulation

Time (min)	Ion (m/z)	Fragmentation
12.30 min (**Met I**)	207	M^+; N-(1-methoxycarbonylethyl)-2,6-dimethylaniline
	148	$M^+ - COOCH_3$
	132	$M^+ - COOCH_3 - CH_3 - H$
12.52 min (**Met II**)	162	Higher m/z, not identified
14.92 min (**Met III**)	193	M^+ ; N-(methoxyacetyl)-2,6-dimethylaniline
	163	$M^+ - H_2CO$
	148	$M^+ - CH_2OCH_3$
	134	$M^+ - H_2CO - CO - H$
	120	$M^+ - CH_2OCH_3 - CO$
20.10 min (**Met IV**)	234	Higher m/z, not identified
20.42 min (**Met V**)	234	Higher m/z, arisen from N-carboxy-N-(1-methoxycarbonylethyl)-2,6-dimethylaniline
	206	$(CH_3)_2C_6H_3NCH(CH_3)(COOCH_3)$
	174	$(CH_3)_2C_6H_3NCH(CH_3)(COOCH_3) - CH_3OH$
	148	$(CH_3)_2C_6H_3NH(CO)$
	132	$(CH_3)_2C_6H_3NCH$
	117	$(CH_3)_2C_6H_3N - H$
	105	$(CH_3)_2C_6H_3$
22.72 min (**Met VI**)	279	M^+; metalaxyl
	249	$M^+ - H_2CO$
	234	$M^+ - CH_2OCH_3$ or $-CH_3 - H_2CO$
	220	$M^+ - COOCH_3$
	160	$M^+ - CH_2OCH_3 - COOCH_3 - CH_3$
	146	$(CH_3)_2C_6H_3(NCHCH_2)$
	132	$(CH_3)_2$ C_6H_3 (NCH)

M^+: molecular ion.

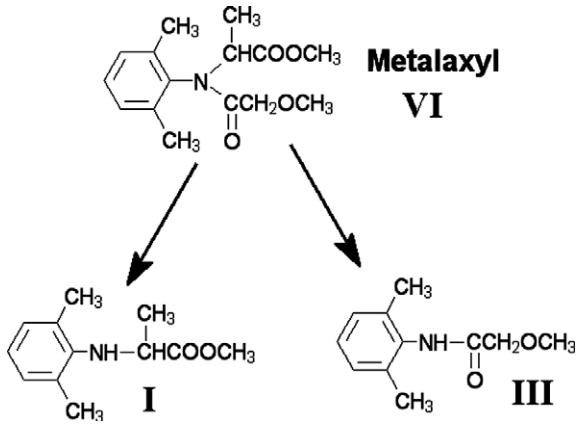

Fig. 1. Structures of metalaxyl and related compounds.

The structures of the metalaxyl related compounds found in musts and wines seem to be similar to those of the degradation products of metalaxyl reported in other matrices (Roberts & Hutson, 1999).

3.1.3. Lindane

The structures of compounds **Lin I**, **Lin II** and **Lin IV** were correctly assigned by the use of spectral libraries (see Table 2 and Fig. 2). The identity of **Lin VI**, lindane isomer, was elucidated by comparing its retention time with those of different isomers.

The highest *m*/*z* ratios in the spectrum of **Lin III** (trichlorinated at least) were centred at *m*/*z* 183. It is plausible that **Lin III** is an isomer of **Lin II,** from the similarity of spectra and retention times. **Lin IV** and **Lin V** could also be isomers. The spectrum of **Lin V** was similar to that of **Lin IV,** from the fragments at low *m*/*z* up to the isotopic profile centred at *m*/*z* 217.

The phenolic degradation products of lindane described in some matrices were not detected in this work (Aizawa, 1982).

3.1.4. Fenvalerate

From the five fenvalerate-related compounds detected, only two structures have been unequivocally elucidated. They are **Fen II** and **Fen V** (see Table 3). A structure is proposed for **Fen I** whose highest *m*/*z* ratio (139) suggests that it is related to the acidic moiety of fenvalerate. As regards

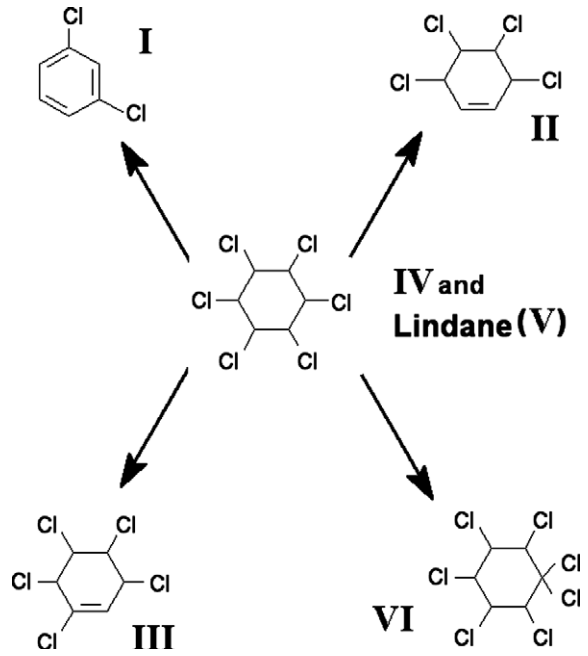

Fig. 2. Structures of lindane and related compounds.

Table 2
Retention times and fragment ions for the compounds related to lindane found in musts, wines and commercial formulation

Time (min)	Ion (*m*/*z*)	Fragmentation
5.56 min (**Lin I**)	146	M^+; Dichlorobenzene
	111	$M^+ - Cl$
	75	$M^+ - HCl - Cl$
9.67 min (**Lin II**)	218	M^+; 3,4,5,6-tetrachlorocyclohex-1-ene
	183	$M^+ - Cl$
	147	$M^+ - Cl - HCl$
10.46 min (**Lin III**)	183	Higher *m*/*z*, likely tetrachlorocyclohex-1-ene isomer
11.53 min (**Lin IV**)	252	M^+ ; 1,2,3,4,5-pentachlorocyclohex-1-ene
	217	$M^+ - Cl$
	181	$M^+ - Cl - HCl$
	111	C_6H_4Cl
14.34 min (**Lin V**)	217	Higher *m*/*z*, likely pentachlorocyclohex-1-ene isomer
16.71 min (**Lin VI**)	288	M^+; α-Hexachlorocyclohexane
18.30 min (**Lin VII**)	288	M^+ ; Lindane (γ-hexachlorocyclohexane)
	252	$M^+ - HCl$
	217	$M^+ - HCl - Cl$
	181	$M^+ - HCl - HCl - Cl$
	145	$M^+ - HCl - HCl - HCl - Cl$
	109	$M^+ - HCl - HCl - HCl - HCl - Cl$
20.85 min (**Lin VIII**)	322	M^+; 1,1,2,3,4,5,6-heptachlorocyclohexane
	287	$M^+ - Cl$
	251	$M^+ - HCl - Cl$
	215	$M^+ - HCl - HCl - Cl$

Table 3
Retention times and fragment ions for the compounds related to fenvalerate found in musts, wines and commercial formulation

Time (min)	Ion (*m*/*z*)	Fragmentation
9.85 min (**Fen I**)	139	Higher *m*/*z*, atributted to $ClC_6H_4(CH_2{=}CH_2)$
	111	$M^+ - CH_2CH_2$
10.46 min (**Fen II**)	202	M^+; 4-(1-chloro-2-methyl)-propylchlorobenzene
	167	$M^+ - Cl$
	159	$M^+ - CH(CH_3)_2$
	125	$M^+ - CH(CH_3){=}CH_2 - Cl$
12.18 min (**Fen III**)	193	Higher *m*/*z*, atributted to $(CH_3)_2C{=}C(ClC_6H_4)(CO)$
	151	$ClC_6H_4CH{=}CH(CH_3) - H$
	165	$ClC_6H_4CH{=}CH(CH_3)_2 - 2\,H$
	178	$(ClC_6H_4)(CO)C{=}C(CH_3)$
16.23 min (**Fen IV**)	193	Higher *m*/*z*, atributted to $(CH_3)_2C{=}C(ClC_6H_4)(CO)$
16.70 min (**Fen V**)	198	M^+; 3-phenoxybenzaldehyde
	181	$M^+ - HCO$
	169	$M^+ - OH$
	141	$M^+ - HCO - CH_2CH_2$
50.59 min and 51.17 min (**Fen VI**)	419	M^+; fenvalerate (isomers 1 and 2)
	389	$M^+ - HCN$
	225	$C_6H_5OC_6H_4CH(CN)OH$
	209	$C_6H_5OC_6H_4CH_2(CN)$
	181	$C_6H_5OC_6H_3CH$
	167	$ClC_6H_4CH{=}CH(CH_3)_2$
	152	$ClC_6H_4CH{=}CHCH_3$
	125	$ClC_6H_4CH_2$

M^+ : molecular ion.

the other two non-identified compounds, **Fen III** and **Fen IV**, they could be related to the (4-chlorobenzene)-(1-methylethyl) acetoxy moiety, as seen by the detection of the ions at *m/z* 151, 177/178 and 193. The ion at *m/z* 193 could be attributed to $(CH_3)_2C{=}C(ClC_6H_4)(CO)$. The ion at *m/z* 178 could arise from the loss of CH_3 in the ion at *m/z* 193, and the ion at *m/z* 151 could originate from the loss of H in the substructure $(ClC_6H_4)HC{=}CH(CH_3)$. Higher *m/z* ratios were not found in the spectra. This suggests that **Fen III** and **Fen IV** could be acidic or hydroxylated compounds, for which molecular ions are not commonly observed in electron impact spectra. Here, it is noteworthy that common fenvalerate degradation products, such as 3-phenoxybenzoic acid, (4-chlorobenzene)-(1-methylethyl)-acetic acid and cyanohydrin, have not been observed in this work. Fig. 3 shows the elucidated structures.

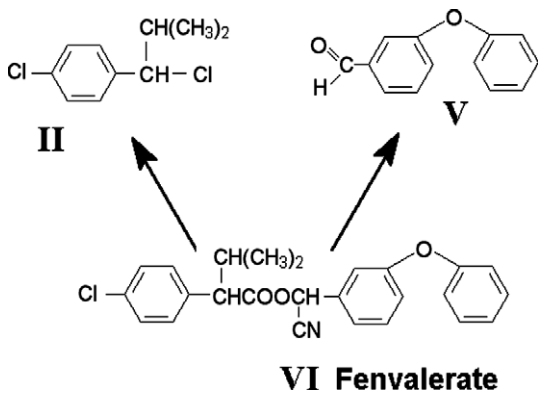

Fig. 3. Structures of fenvalerate and related compounds.

3.2. Occurrence of the degradation products and impurities in must and wine

Table 4 shows the peak areas for the compounds detected in must and wine samples. Many of the data are only qualitative, because the corresponding chromatographic peaks could not be integrated in the total ion chromatogram. Some compounds could only be monitored through the extracted ion chromatograms, owing to their

Table 4
Occurrence of pesticides and related compounds in musts and wines: peak area (thousands of counts) of the compounds in the total ion current chromatograms of the extracts. Concentrations (mg/l) for the active ingredients are expressed in brackets ($n = 2$)

Compound	Formulation occurrence	Red wine			White wine		
		Peak area in must	Peak area in wine	Peak area in clarified wine	Peak area in must	Peak area in wine	Peak area in clarified wine
Metalaxyl							
(**Met I**)	No	xx	xx	xx	xx	xx	xx
(**Met II**)	Yes	–	–	–	–	–	–
(**Met III**)	No	xx	xx	xx	xx	xx	xx
(**Met IV**)	No	xx	xx	xx	xx	xx	xx
(**Met V**)	Yes	108	xx	xx	176	332	310
(**Met VI**) Metalaxyl	Yes	117057 (9.5)	271374 (1.8)	223225 (1.6)	123069 (9.3)	189002 (4.0)	166103 (3.2)
Lindane							
(**Lin I**)	Yes	–	–	–	–	–	–
(**Lin II**)	Yes	–	–	–	–	–	–
(**Lin III**)	Yes	–	–	–	–	–	–
(**Lin IV**)	Yes	–	–	–	–	xx	xx
(**Lin V**)	Yes	–	–	–	–	–	–
(**Lin VI**)	Yes	–	–	–	–	xx	xx
(**Lin VII**) Lindane	Yes	35558 (4.5)	10479 (1.5)	6944 (1.1)	52589 (6.0)	24231 (4.5)	22712 (4.2)
(**Lin VIII**)	Yes	–	–	–	–	–	–
Fenvalerate							
(**Fen I**)	Yes	xx	xx	–	xx	xx	–
(**Fen II**)	Yes	–	–	–	–	–	–
(**Fen III**)	Yes	–	–	–	xx	xx	xx
(**Fen IV**)	Yes	–	–	–	–	–	–
(**Fen V**)	Yes	198	–	–	xx	–	–
(**Fen VI**) Fenvalerate	Yes	(0.7)			(0.4)		
isomer 1		165	–	–	85	–	–
isomer 2		112	–	–	67	–	–
Deltamethrin							
3-phenoxy benzaldehyde	No	158	–	–	81	–	–
Deltamethrin	Yes	2845 (1.4)	–	–	325 (0.4)	–	–

–: not detected. xx : detected, not quantified.

very low concentrations in the experimental musts and wines.

The concentration of metalaxyl in must scarcely decreased, (less than 10%), but, after vinification, the decrease was notable, the remaining concentration was 18% with respect to the added amount. The concentrations were somewhat lower in the wines subjected to the clarification step and they were higher in white wine than in red wine.

Among impurities found in the formulation, **Met II** was not detected in any extract, whereas **Met V** was observed in the extracted ion chromatograms of must and wine extracts; the content of **Met V** in red wines was very small in relation to that in must, but, for white wines, the content was higher than that in must and the clarification did not seem to modify its concentration. So, **Met V** might be a degradation product, as well, with remarkable incidence in white wines.

Met I, **Met III** and **Met IV** were degradation products from metalxyl found in musts and wines. They were also observed in the clarified wines. As a result of their very low concentrations, they were monitored in the extracted ion chromatograms but not in the total ion current chromatograms. Thus, the areas of the corresponding chromatographic peaks are not shown in Table 4. Fig. 4a shows a chromatogram for a red wine extract.

The residues of lindane in white wine were higher than those in red wine. The concentration in the wines elaborated without a clarification step decreased by 55% and 85% for white and red wines, respectively. When clarifica-

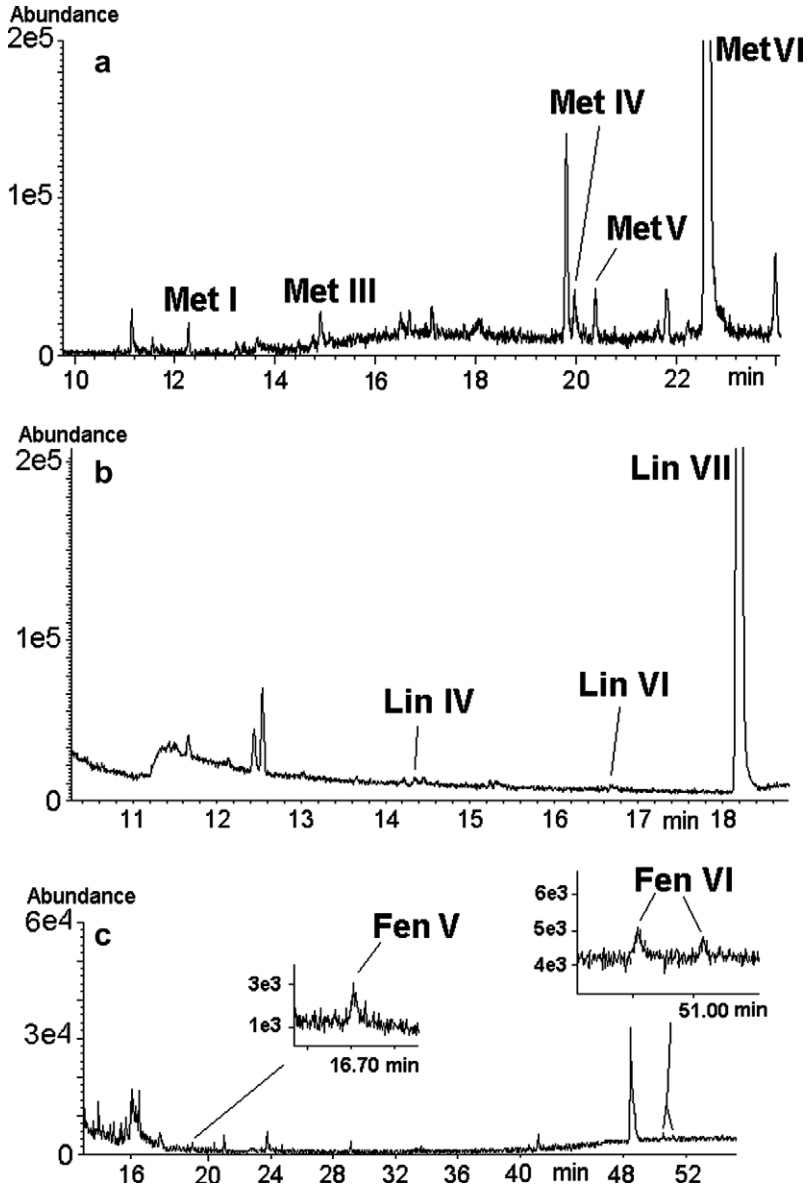

Fig. 4. Chromatograms from wine and must extracts. See tables for peak identification. (a) Extract from a red wine whose must was treated with metalaxyl (reconstructed ion chromatogram), *m/z* 149, 193 and 234. (b) Extract from a white wine whose must was treated with lindane (total ion current chromatogram). (c) Extract of a red grape must treated with fenvalerate (total ion current chromatogram).

tion was carried out, the decrease was about 4% more. In musts, the concentration had already decreased considerably: 40% and 55% for white and red grape musts, respectively. Two degradation products – present in the used formulation, too – were found in the experimental white wine: **Lin IV** and **Lin VI**. One of them was the α-isomer, the other was the compound resulting from HCl elimination. They were found in very low concentrations. Fig. 4b shows the chromatogram of a red wine. Other formulation impurities were not detected.

Fenvalerate was not detected in wines. In musts, its concentration already decreased by more than 93% after the addition. In red grape must, the concentration was slightly higher. A chromatogram from the red grape must extract is shown in Fig. 4c.

Two byproducts of the formulation were observed in red grape must and three byproducts were detected in white grape must. From these compounds, 3-phenoxybenzaldehyde (**Fen V**) was the most abundant, mainly in the elaboration of red wines. Impurities **Fen II** and **Fen IV** were not seen, either in must or in wine extracts.

Fen III was also a degradation product only detected in white wine. Its concentrations in the wines were higher than those in musts, even for the clarified wine. On the other hand, traces of **Fen I** (already present in the formulation) were recorded in the extracts. For white wine, the peak areas of its characteristic ions were maintained with respect to must extracts whereas, for red wine, the peak areas increased slightly after fermentation. According to our experience with the other compounds, the formulation impurities present at such a low concentration are not detected in elaborated wines or even in must. So, **Fen I** is probably a degradation product.

As in the case of metalaxyl, the degradation products of fenvalerate in wines seemed to originate mainly from hydrolysis reactions of the active ingredient.

Deltamethrin was not detected in wines, either. However, the residues in red grape must were higher than those of fenvalerate. The decrease of concentration in red grape must was about 86% and in white grape must, about 96%. The concentration in the red one was three times higher. 3-Phenoxybenzaldyde, not present in the commercial formulation, was found in must, confirming that it is a common degradation product of the pyrethroids which contain this moiety. **FEN V**, in the previous study, was 3-phenoxybenzaldyde, as well. As in the case of fenvalerate, the amount of 3-phenoxybenzaldehyde seemed to be higher in red grape must than in white. Bromated compounds, related to the acidic moiety of deltamethrin, were not observed, in contrast with the observation of chlorophenyl derivatives of fenvalerate.

Finally, it is interesting to note the finding of traces of alkylbenzenes – ethyldimethylbenzenes or methyl(methylethyl)benzenes, whose spectra are characterized by the ions at m/z 77 (9), 91 (18), 105 (11), 117 (11), 119 (100) and 134 (39) – at the beginning of the chromatograms obtained from red and white wines spiked with deltamethrin, between 5.50 and 6.3 min, and arising from the commercial formulation.

4. Conclusions

The impurities from the commercial formulations can be found in musts, and some of them can persist through the vinification process and still be observed in the final wines, besides some degradation products. The clarification of the wines decreases the amounts of pesticides and related compounds detected in the final wine. Most of the degradation products are present in wines in lower amounts than are the residues of the pesticides.

The pyrethroids, fenvalerate and deltamethrin, do not persist in the experimental wines, while metalaxyl is found in relatively high concentrations. Compounds related to the 2,6-dimethylaniline moiety for metalaxyl, isomerization and dehydrochlorination products for lindane, and hydrolysis derivatives of the pyrethroids appear to be the degradation products found in must and wine extracts.

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References

Abdel Aal, S. E., Dessouki, A. M., & Sokker, H. H. (2001). Degradation of some pesticides in aqueous solutions by electron beam and gamma-radiation. *Journal of Radioanalytical and Nuclear Chemistry, 250*, 329–334.

Abou-Arab, A. A. K. (2002). Degradation of organochlorine pesticides by meat starter in liquid media and fermented sausage. *Food and Chemical Toxicology, 40*, 33–41.

Aizawa, H. (1982). *Metabolic maps of pesticides*. Academic Press.

Arrebola, F. J., Martinez-Vidal, J. L., Fernandez-Gutierrez, A., & Akhtar, M. H. (1999). Monitoring of pyrethroid metabolites in human urine using solid-phase extraction followed by gas chromatography-tandem mass spectrometry. *Analytica Chimica Acta, 401*, 45–54.

Cabras, P., Meloni, M., & Pirisi, F. M. (1987). Pesticide fate from vine to wine. *Reviews of Environmental Contamination and Toxicology, 99*, 83–111.

Chen, A. W., Fink, J. M., & Letinski, D. J. (1996). Analytical methods to determine residual cypermethrin and its major acid metabolites in bovine milk and tissues. *Journal of Agricultural and Food Chemistry, 44*, 3534–3539.

Class, T. J. (1992). Environmental analysis of cypermethrin and its degradation products after forestry applications. *International Journal of Environmental Analytical Chemistry, 49*, 189–205.

Colume, A., Cardenas, S., Gallego, M., & Valcarcel, M. (2001). A solid-phase extraction method for the screening and determination of pyrethroid metabolites and organochlorine pesticides in human urine. *Rapid Communications in Mass Spectrometry, 15*, 2007–2013.

García Cazolar, J. (1991). Los residuos desde la uva al vino. In Ayuntamiento de Haro (Ed.), Residuos de tratamientos vitícolas en vinos. (pp 123–151).

Gupta, A., & Singh, B. (2001). Dissipation of fenvalerate residues in cauliflower. *Bulletin of Environmental Contamination and Toxicology, 66*, 24–28.

Headley, J. V., Maxwell, D. B., Swyngedouw, C., & Pudri, J. R. (1996). Determination of combined residues of metalaxyl and 2,6-dimethyl-

aniline metabolites in urine by gas chromatography-mass spectrometry. *Journal of AOAC International, 79*, 117–123.

Jin, H., & Webster, G. R. B. (1998). GC-ECD determination of cypermethrin and its major metabolites in soil, elm bark, and litter. *Fresenius' Journal of Analytical Chemistry, 360*, 573–579.

Kakalikova, L., Matisova, E., & Lesko, J. (1996). Analysis of metalaxyl residues in wines by SPE in combination with HRGC and GC/MS. *Zeitschrift fur Lebensmittel Untersuchung und Forschung, 203*, 56–60.

Lutnicka, H., Bogacka, T., & Wolska, L. (1999). Degradation of pyrethroids in an aquatic Ecosystem model. *Water Research, 33*, 3441–3446.

Marucchini, C., & Zadra, C. (2002). Stereoselective degradation of metalaxyl and metalaxyl-M in soil and sunflower plants. *Chirality, 14*, 32–38.

Misra, S., & Sharma, C. B. (1997). Metabolism and bioaccumulation of fenvalerate and its metabolites in rat organs. *Biomedical Chromatography, 11*, 50–53.

Monkiedje, A., & Spiteller, M. (2002). Sorptive behavior of the phenylamide fungicides, mefenoxam and metalaxyl, and their acid metabolite in typical Cameroonian and German soils. *Chemosphere, 49*, 659–668.

Mortimer, R. D., & Shields, J. B. (1995). Total, free and glucose-conjugated acid metabolites from permethrin and cypermethrin in foods by gas chromatography-electron capture detection. *Journal of AOAC International, 78*, 1302–1307.

Navarro, S., Barba, A., Oliva, J., Navarro, G., & Pardo, F. (1999). Evolution of residual levels of six pesticides during elaboration of red wines. Effect of wine-making procedures in their dissappearance. *Journal of Agricultural and Food Chemistry, 47*, 264–270.

Navarro, S., Oliva, J., Barba, A., Navarro, G., García, M. A., & Zamorano, M. (2000). Evolution of chlorpyrifos, fenarimol, metalaxyl, penconazol and vinclozolin in red wines elaborated by carbonic maceration of Monastrell grapes. *Journal of Agricultural and Food Chemistry, 48*, 3537–3541.

Navarro, S., Oliva, J., Navarro, G., & Barba, A. (2001). Dissipation of chlorpyrifos, fenarimol, mancozeb, metalaxyl, penconazole and vinclozolin in grapes. *American Journal of Enology and Viticulture, 52*, 35–40.

Oliva, J., Navarro, S., Barba, A., Navarro, G., & Salinas, R. (1999). Effect of pesticide residues on the aromatic composition of red wines. *Journal of Agricultural and Food Chemistry, 47*, 2830–2836.

Roberts, T., & Hutson, D. (Eds.). (1999). *Metabolic pathways of agrochemicals, part two, insecticides and fungicides*. The Royal Society of Chemistry.

Samsonov, Y. N., & Pokrovoskii, L. M. (2001). Sensitized photodecomposition of high disperse pesticide chemicals exposed to sunlight and irradiation from halogen or mercury lamp. *Atmospheric Environment, 35*, 2133–2141.

Sukul, P. (1995). Dissipation of deltamethrin and fenvalerate residues in green gram (Vigna radiata Wilczek) under Indian climatic condition. *Bulletin of Environmental Contamination and Toxicology, 55*, 562–567.

Sukul, P., & Spiteller, M. (2001a). Influence of biotic and abiotic factors in dissipating metalaxyl on soil. *Chemosphere, 45*, 941–947.

Sukul, P., & Spiteller, M. (2001b). Persistence, fate, and metabolism of [14C]metalaxyl in typical Indian soils. *Journal of Agricultural and Food Chemistry, 49*, 2352–2358.

Topalov, A., Molnar-Gabor, D., & Csanadi, J. (1999). Photocatalytic oxidation of the fungicide metalaxyl dissolved in water over TiO_2. *Water Research, 33*, 1371–1376.

Zaleska, A., Hupka, J., Wiergowski, M., & Biziuk, M. (2000). Photocatalytic degradation of lindane, p,p-DDT and methoxychlor in an aqueous environment. *Journal of Photochemistry and Photobiology A: Chemistry, 135*, 213–220.