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Residues of the fungicide famoxadone in grapes and its fate during wine production

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Table 1. Concentration of famoxadone during grapes maturation, expressed by means of a relative standard deviation (SD).

Days after treatment	Concentration (mg kg ⁻¹) ± SD	t _{1/2} (days)
0	0.27 ± 0.06	18
3	0.40 ± 0.11	
7	0.30 ± 0.12	
14	0.22 ± 0.11	
21	0.14 ± 0.10	
28	0.16 ± 0.07	

Table 2. Fate of famoxadone during wine production.

Sample		Concentration ($\mu\text{g kg}^{-1}$ or $\mu\text{g l}^{-1} \pm \text{SD}$)	
Grapes		0.27 ± 0.06	
Must		0.33 ± 0.03	
Vinification without maceration		Vinification with maceration	
Cake (33.0%)	0.31 ± 0.04	Wine	<L.O.D.
Wine	<L.O.D.	Cake and lees (32.5%)	0.67 ± 0.17
Lees (5.8%)	1.83 ± 0.44		

Table 3. Results of Tests A (evaporation and thermodegradation) and B (codistillation) for the famoxadone active ingredient, expressed in $\mu\text{g cm}^{-2} \pm \text{SD}$.

	Test A		Test B	
	without waxes	with waxes	without waxes	with waxes
Control sample (c)	2.00 ± 0.06	1.40 ± 0.34	2.10 ± 0.20	1.70 ± 0.15
Vial (v)	<LOD	<LOD	-	-
Membrane (m)	2.00 ± 0.26	1.40 ± 0.29	1.90 ± 0.33	1.80 ± 0.10
c - (v + m)	n.s.	n.s.	n.s.	n.s.

n.s. - not significant

Table 4. Results of Tests A (evaporation and thermodegradation) and B (codistillation) for the commercial product Equation Pro, expressed in $\mu\text{g cm}^{-2} \pm \text{SD}$.

	Test A		Test B	
	without waxes	with waxes	without waxes	with waxes
Control sample (c)	1.34 ± 0.01	1.69 ± 0.07	1.43 ± 0.05	1.63 ± 0.06
Vial (v)	<LOD	<LOD	-	-
Membrane (m)	1.34 ± 0.02	1.61 ± 0.15	1.44 ± 0.05	1.61 ± 0.05
c - (v + m)	n.s.	n.s.	n.s.	n.s.

n.s. - not significant

Table 5. Results of Tests C (sunlight photodegradation) for the famoxadone active ingredient, expressed in $\mu\text{g cm}^{-2} \pm \text{SD}$.

	Test C	
	without waxes	with waxes
$t_{1/2}$ (days)	4.41	4.26
R^2	0.77	0.99

Table 6. Results of Tests C (sunlight photodegradation) for the commercial product Equation Pro, expressed in $\mu\text{g cm}^{-2} \pm \text{SD}$.

	Test C	
	without waxes	with waxes
$t_{1/2}$ (days)	3.75	4.61
R^2	0.91	0.97

Residues of the fungicide famoxadone in grapes and its fate during wine production

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Abstract

Famoxadone is a recently applied fungicide to vines, belonging the oxazolidinedione family. In this work, the fate of famoxadone was studied considering the decay ratio of this fungicide during grapes maturation and wine production. The main factors affecting the presence of fungicide residues such as fruit growth, photodegradation, evaporation, thermodegradation, and co-distillation were studied with model systems.

An experimental field was treated with a commercial product containing famoxadone at the recommended dose. After this application, residues of famoxadone were found in grapes at the concentration of $0.27 \pm 0.06 \text{ mg kg}^{-1}$. In this field experiment, the half-life time ($t_{1/2}$) of famoxadone, described as pseudo-first-order kinetics ($R^2 = 0.74$), was 18 ± 6 days, resulting from the photodegradation. The famoxadone residue levels in grapes were below the established Maximum Residues Level (MRL) for Europe (2 mg kg^{-1}) whilst levels in wine, carried out with and without maceration, were below the calculated limit of detection (LOD) of the method.

Keywords: famoxadone residues; grapes; wine production; model systems.

Introduction

Famoxadone (3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione) is a recently developed highly active and broad spectrum fungicide used to control different crop diseases. In viniculture, the commercial formulation, named Equation Pro and manufactured by Dupont Crop Protection, is applied to bunch and leaves to control downy mildew, which is an important grapevine disease induced by the *Plasmopora viticola* (Joshi and Sternberg 1996). The chemistry and biochemical mode of action of famoxadone is close to the known class of strobilurins (Bartlett et al. 2002). The fungicidal activity of famoxadone consists in the inhibition of the mitochondrial respiration by binding at the so-called Q₀ site of cytochrome b, located in the inner mitochondrial membrane of the fungi (Jordan et al. 1999).

Famoxadone active ingredient can be used on grapes in Europe at a maximum application rate of 0.05 kg ha⁻¹ (France and Greece), 0.09 kg ha⁻¹ (Italy and Spain) or 0.14 kg ha⁻¹ (Germany). Spain and Greece allow a maximum of 6 applications per season and the other countries a maximum of 3 applications, except Germany (8). In Italy the maximum residue level (MRL) for famoxadone is 2 mg kg⁻¹ with a safety interval (PHI) of 28 days

(http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/006/Y5221E/y5221e0e.htm).

The first analytical method reported in the literature to determine famoxadone in grapes and wine used liquid-liquid extraction (LLE) and gel permeation chromatography (GPC) before a gas chromatographic separation with electron capture detection (GC-ECD) (Jernberg 2003). Our group validated one method by GC/MS, using a simple and fast LLE procedure (de Melo Abreu et al. 2005). This method was used in the present work. To our knowledge no studies reported the fate of

famoxadone from vine to wine. The application of the active ingredient close to the grapes harvest could lead to the presence of famoxadone residues in wine.

Different papers (Cabras et al. 1997, 1998, 2001; Garau et al. 2002) deal with the persistence and the study of the mechanism of disappearance of pesticides with model systems in grapes and wine production. Following treatment, pesticides spread into the epicuticular waxes present on fruits surface and could affect the degradative process of the active ingredient (Cabras et al. 1997). This paper aims to contribute to the knowledge of the persistence of famoxadone in grapes and its behaviour during wine production.

Experimental

Fungicide treatment

The trial was carried out in a farm located in Santadi (Cagliari, Italy), in 240 vines of the vitis variety Carignano, with a plant spacing of 1.9 m × 0.8 m. The treatment with the commercial formulation Equation Pro, containing 22.5% of famoxadone, was done September 2, 2004. A random block scheme with 4 replications for each test was used. Each block contained 60 vines. The fungicide application was carried out with the dose recommended by the manufacturer (480 g ha⁻¹), using a Carpi portable motor sprayer (Modena, Italy).

The weather conditions were continuously recorded with a Silimet AD-2 automatic station (Modena, Italy), with an average total rainfall of 0.7 mm, a minimum temperature of 17.1 °C, a maximum temperature of 30.2 °C, and an average solar radiation of 167 watt m².

Grapes material and wine production

Samplings of 2 kg of dry stemmed black grapes were taken. The first collection was carried out on September 2, before and after fungicide treatment. Then, the grapes were collected after 3, 7, 14, 21 and 28 days. In the wine production, each grape sample was divided in two portions, both of them pressed and added with 200 mg of sodium metabisulphite. One portion was fermented with skin, for the vinification with maceration process, and the other without skin, being called vinification without maceration process. To improve the fermentation process, 20 mg kg⁻¹ of active dry yeasts was added.

The amount of the famoxadone residues during the grapes maturation and wine-making was determined. Crushed grape samples were directly analysed as described below. An aliquot of 100 g of must was centrifuged at 4000 rpm for 5 min, the amount of cake and the concentration of famoxadone residues in the cake and in the clear must were measured. Fermentation had been a regular course in all samples and, after 15 days, the obtained wines were centrifuged in the same manner as must and analysed. At the end of the vinification process without maceration, lees amount and famoxadone residues were measured. In vinification process with maceration, after the wine had been made, the amount of cake with lees, and famoxadone residues were measured.

Chemicals

The commercial formulation Equation Pro (22.5% of famoxadone and 30.0% of cimoxanil) and the analytical standard of famoxadone (99.4%) were obtained from Dupont Crop Protection (Newark, USA). Acetonitrile LiChrosolv isocratic grade for GC (≥99.8%) and chloroform Normapur for GC (≥99.0%) were purchased from Merck (Darmstadt, Germany). Hexane (≥95.0%), ethyl acetate (≥99.8%) and acetone (≥99.8%) were Pestanal Grade for residues analysis from Riedel-de Haën (Seelze, Germany). The water was bidistilled and filtered with a Millipore Milli-Q apparatus (Milan, Italy).

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Standards preparation

A fungicide stock solution (1 g l^{-1}), and a working solution (100 mg l^{-1}) were prepared in acetonitrile and stored in dark glass vials, and kept respectively at -20°C and 4°C . Appropriate standards solutions for control quality assessment were made daily as described elsewhere (de Melo Abreu et al., 2005).

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Sample extraction

For extraction, crushed grapes (5 g), must and wine (5 ml) samples were weighed in 40 ml screw-capped tubes and 10 ml of ethyl acetate/hexane (50:50 v/v) was added. Tubes containing the mixture were agitated for 15 min in a rotatory shaker, the organic phase was removed and injected in the chromatographic system. The same procedure was used to analyse cake and lees.

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Apparatus and chromatographic conditions

GC/MS analysis of famoxadone was carried out on a Hewlett-Packard (Palo Alto, USA) HP 5890 Series II gas chromatograph system, equipped with a HP 7673 GC/SFC autosampler, a 19251A capillary split-splitless injector and a GC-MS HP 5971 Series detector. The analytical column used was a J&W Scientific DB-17MS fused silica capillary column (Folsom, USA) with 15 m of length, 0.25 mm of internal diameter and $0.15 \mu\text{m}$ of film thickness. The injector temperature was set at 300°C . The oven temperature program was as follows: initial temperature was kept at 200°C for 1 min, increased to 300°C at $10^\circ\text{C min}^{-1}$ and held at this temperature for 2 min. Total run time was 13.0 min. The volume of sample was $2 \mu\text{l}$, injected in splitless mode with splitless time of 1 min. Helium (99.9999% purity) was used as carrier gas at constant flow rate of 0.8 ml min^{-1} . The mass spectrometer was operated in electron impact (65 eV of ion energy), with 10 minute solvent delay, the interface temperature was kept at 300°C , and the ion source temperature was kept at 180°C .

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2
3 149 The dwell time for the ion monitoring was 100 ms per ion. Selected monitoring ion (SIM) mode
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6 150 was used. The ions utilized for quantification of famoxadone were 196, 224, 330 and 374 *m/z*.
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10 152 *Extraction procedure for grape waxes*
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13 153 The extraction of the grapes waxes was carried out as previously (McDonald et al. 1993). Grape
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15 154 wax extraction was performed by the immersion of a known weight of untreated grapes in a known
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17 155 volume of chloroform, for 1 min. The amount of waxes of the grape surface, expressed in $\mu\text{g cm}^{-2}$,
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20 156 was determined by evaporating an aliquot of 10 ml of the chloroform extract to dryness and
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22 157 calculating the surface area of the fruit.
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27 159 *Model systems*
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29 160 The model systems for photodegradation, codistillation, evaporation and thermodegradation were
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32 161 studied according to Garau et al. 2002. Briefly, three tests, presenting below, were done:
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34 162 *Test A - Evaporation and Thermodegradation.* In a membrane of regenerated cellulose there was
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36 163 placed a solution of famoxadone with known concentration. After solvent evaporation, the
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39 164 membrane was placed in a 10 ml dark screw-capped vial. This vial was putted in a thermostatic
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41 165 stove at 50 °C for 24 h. Then, the vial was removed and placed in freezer for 5h at -20 °C to allow
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43 166 the condensation of famoxadone. After removing the vial from the freezer, the membrane was
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46 167 transferred to another vial with 5 ml of ethyl acetate/hexane (50:50 v/v) for the extraction of the
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49 168 remained residues of famoxadone. The first vial was also washed with the same amount of the
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51 169 solvent solution, allowing to measure the residues of famoxadone evaporated from the membrane.
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53 170 At the same time, a control sample stored in the dark at room temperature was analysed with the
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55 171 same procedure. After the GC/MS analysis of these extracted samples, the difference between the
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58 172 amount of famoxadone of the control sample and the sum of the amounts of the famoxadone found
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in the membrane and in the vial of the heated sample was calculated. This value indicates the amount of thermo-degradation of famoxadone.

Test B – Co-distillation. A regenerated cellulose membrane prepared in the same way of the Test A was placed on the top of a 10 ml dark vial, with a screw-closed cap with a hole in the middle, containing 5 ml of ultra-pure water previously weighted. This vial was placed in a thermostatic stove in the same conditions of Test A, allowing the evaporation of the water and the co-distillation of the famoxadone. After 24 h, the vials were weighted to determine the amount of water evaporated. The control sample was stored in the dark at room temperature. The loss of famoxadone by codistillation was calculated by the difference between the amount of famoxadone in the control sample and the amount of the famoxadone found in the membrane of the heated sample estimated by GC-MS. For a suitable evaluation of codistillation, the possible losses of famoxadone due to evaporation and thermo-degradation, determined with the Test A, must be considered.

Test C - Sunlight Photodegradation. An aliquot of famoxadone solution at known concentration was poured into Petri dishes with 5 cm of diameter and the solvent was allowed to evaporate, at room temperature. The dishes were exposed to direct sunlight, removed at prefixed intervals and, after re-dissolving the remained residues with 5 ml of ethyl acetate/hexane (50:50 v/v) injected for GC- MS analysis. The control sample was stored in the dark at room temperature.

The Tests A, B and C were carried out with and without the presence of the epicuticular waxes extracted from grapes, at the concentration of $0.37 \mu\text{g cm}^{-2}$. Each experiment was replicated 4 times.

Results and discussion

During grapes maturation and before the treatment with Equation Pro, famoxadone was not detected at the limit of 0.03 mg kg^{-1} in grapes. The limit of detection (LOD) for famoxadone, calculated as three times the signal-to-noise ratio, was 0.02 mg l^{-1} for ethyl acetate/hexane (50:50 v/v), 0.03 mg kg^{-1} for grapes and 0.04 mg l^{-1} for wine. The concentration of famoxadone residues before treatment was, as expected, below the limits of detection of the method. After the referred treatment, as reported in the Table 1, the concentration of famoxadone was $0.27 \pm 0.06 \text{ mg kg}^{-1}$, below the European LMR. It should be noted that three days after treatment levels of famoxadone were $0.40 \pm 0.11 \text{ mg kg}^{-1}$ and this is probably caused to a sample-to-sample variability. A decline in residue levels was not observed until day 14 and then levels of famoxadone decreased until the last determination near harvesting for wine production. The half-life time ($t_{1/2}$) of famoxadone, calculated as pseudo-first order kinetics ($R^2 = 0.74$), was 18 ± 6 days.

In wine production it was also important to understand the possible mechanism of disappearance of famoxadone residues. For this study, grapes after treatment, with a concentration of famoxadone of $0.27 \pm 0.06 \text{ mg kg}^{-1}$, were used. Before fermentation, the concentration of famoxadone residues in must ($0.33 \pm 0.03 \text{ mg kg}^{-1}$) was the same of the grapes, indicating that it was distributed between liquid and solid parts (Table 2).

After the fermentation process with and without the maceration process, famoxadone was not detected in wines at the limit of 0.04 mg kg^{-1} . During wine production without maceration, part of the famoxadone residues were removed with the cake ($33\% \times 0.31 \pm 0.04 \text{ mg kg}^{-1} = 0.10 \pm 0.01 \text{ mg kg}^{-1}$), and the remained part, after fermentation, was also removed with the lees ($5.8\% \times 1.83 \pm 0.44 \text{ mg kg}^{-1} = 0.11 \pm 0.02 \text{ mg kg}^{-1}$). In wine production with maceration process, all the famoxadone residues were separated with the cake and lees $32.5\% \times 0.67 \pm 0.17 \text{ mg kg}^{-1} = 0.22 \pm$

0.06 mg kg⁻¹) at the end of the fermentation process. In both cases, all the residues of famoxadone were adsorbed to the solid parts.

Model system studies (Tests A, B and C) were carried out with and without grape epicuticular waxes (0.37 µg cm⁻²) because the latter could have an influence on the fate of pesticides on fruits (Cabras et al. 1997) enhancing or reducing $t_{1/2}$ of the active ingredient. Each test was carried out with the active ingredient and with the commercial formulation. The results obtained for the Test A, provide an insight into the mechanisms of evaporation and thermodegradation, and Test B, provide insight into co-distillation, and these are presented in the Tables 3 and 4 for famoxadone and for the commercial product, respectively. Concerning the Test A for famoxadone and for the commercial product, no evaporation phenomena occurred in either case, with and without waxes. In fact, at 50 °C, which is on average the higher temperature found in the field during grape maturation, all the residues remained in the membrane (m). Considering the LOD of the method, residues of famoxadone were not found in the vial (v) which contained the membrane. There were no observed differences between the concentration of famoxadone in the control sample (c) and the sum of the concentration in the vial and in the membrane indicating that there were no losses by thermodegradation. In the Test B for the active ingredient and for the commercial product, it was shown that co-distillation did not occur, either in the presence or absence of waxes. As shown in Tables 3 and 4 no differences were observed comparing c and m values.

The results obtained for the Test C, which examined the mechanisms of sunlight photodegradation, are presented in Table 5 for the famoxadone and for the commercial product. Half-lives of famoxadone ranged from 3.75 to 4.61 days with a standard error (SE) ranging from 0.02 to 0.99 and a R^2 ranging from 0.78 to 0.99. R^2 correlates the natural logarithm (Ln) of the concentration with the time of exposure to solar radiation. In the Test C for the active ingredient it was observed

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3 246 that the presence of waxes did not influence the photodegradation process. Nevertheless,
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5 247 photodegradation of the commercial product with waxes was slightly lower than without waxes.
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8 248 This means that waxes in the presence of co-formulations could adsorb the radiation responsible
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10 249 for the photodegradation.
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15 251 **Conclusions**

16
17 252 This field degradation trial showed that famoxadone residues at the pre-harvest interval and during
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19 253 vinification were below the MRL (2 mg kg⁻¹). During the wine-making process the fungicide is
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22 254 completely adsorbed in the cake and lees. Data from the models system experiments suggest that
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25 255 in real conditions evaporation, thermodegradation and co-distillation processes do not affect
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27 256 residue levels of famoxadone and the mechanism responsible for the disappearance of famoxadone
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29 257 was essentially that of photodegradation.
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34 259 **Acknowledgements**

35
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Table 1. Concentration of famoxadone during grapes maturation and half-life time..

Days after treatment	Concentration (mg kg ⁻¹) ± SD	t _{1/2} (days ± SE)
0	0.27 ± 0.06	18 ± 6
3	0.40 ± 0.11	
7	0.30 ± 0.12	
14	0.22 ± 0.11	
21	0.14 ± 0.10	
28	0.16 ± 0.07	

SD – standard deviation; SE – standard error

Table 2. Fate of famoxadone during wine production.

Sample		Concentration (mg kg ⁻¹ or mg l ⁻¹ ± SD)	
Grapes		0.27 ± 0.06	
Must		0.33 ± 0.03	
Vinification without maceration		Vinification with maceration	
Cake (33.0%)	0.31 ± 0.04	Wine	<0.04
Wine	<0.04	Cake and lees (32.5%)	0.67 ± 0.17
Lees (5.8%)	1.83 ± 0.44		

SD – standard deviation

Table 3. Results of Tests A (evaporation and thermodegradation) and B (codistillation) for the famoxadone active ingredient, expressed in $\text{mg kg}^{-1} \pm \text{SD}$.

	Test A		Test B	
	without waxes	with waxes	without waxes	with waxes
Control sample (c)	2.00 ± 0.06	1.40 ± 0.34	2.10 ± 0.20	1.70 ± 0.15
Vial (v)	<0.02 (LOD)	<0.02 (LOD)	-	-
Membrane (m)	2.00 ± 0.26	1.40 ± 0.29	1.90 ± 0.33	1.80 ± 0.10
c - (v + m)	n.s.	n.s.	n.s.	n.s.

n.s. - not significant

Table 4. Results of Tests A (evaporation and thermodegradation) and B (codistillation) for the commercial product, expressed in $\text{mg kg}^{-1} \pm \text{SD}$.

	Test A		Test B	
	without waxes	with waxes	without waxes	with waxes
Control sample (c)	1.34 ± 0.01	1.69 ± 0.07	1.43 ± 0.05	1.63 ± 0.06
Vial (v)	<0.02 (LOD)	<0.02 (LOD)	-	-
Membrane (m)	1.34 ± 0.02	1.61 ± 0.15	1.44 ± 0.05	1.61 ± 0.05
c - (v + m)	n.s.	n.s.	n.s.	n.s.

n.s. - not significant

Table 5. Results of Tests C (sunlight photodegradation) for the famoxadone and commercial product, expressed in days $\pm$ standard error (SE).

	Test C			
	without waxes		with waxes	
	Famoxadone	Equation Pro	Famoxadone	Equation Pro
$t_{1/2}$ (days)	4.41 ± 0.99	3.75 ± 0.58	4.26 ± 0.02	4.61 ± 0.43
R^2	0.78	0.91	0.99	0.97

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