APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Simultaneous Determination of Pesticides Residues and Illegal Additives in Wine

Introduction

Wine derived from grapes can often contain pesticides and fungicides that have been sprayed on the fruits during their growing period. In addition to these contaminants, wine can also contain additives that have been deliberately added during production processes to improve its flavor and color. Both pesticides and illegal additives, if present in significant levels in wine, can pose health risk to consumers. Currently, pesticides and illegal additives are tested using different methodologies[1,2,3,4]. In this study, a simple and sensitive LC-MS/MS method has been developed and applied for the determination of both pesticides and pigments in a single analytical run.

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Experimental

Hardware/Software

Chromatographic separation was conducted on a PerkinElmer UHPLC system, while detection was achieved using a PerkinElmer QSight™ 220 triple quadrupole mass spectrometer with a dual ionization source. All instrument control, data acquisition and data processing was performed using the Simplicity 3Q™ software.

Method

Sample Preparation

1.0 mL of test sample was accurately pipetted into a centrifuge tube and then 9.0 mL of water was added and blended. After centrifugation for five minutes at 6000 rpm, the supernatant was transferred directly into an auto sampler vial without further filtration for LC-MS/MS analysis.

LC Conditions

The analytes were separated using a PerkinElmer Brownlee SPP C18 column (4.6 x 100 mm, 2.7 µm). The temperature of the column oven was set at 30 °C. The mobile phases consisted of (A) 5 mM ammonium acetate in water and (B) acetonitrile. The flow rate was 0.8 mL/min and the mobile phase gradient is shown in Table 1. The injection volume was 10 µL.

Mass Detection Parameters

The mass spectrometer source conditions are listed in Table 2, the compound dependent parameters such as collision energy (CE) and the entrance voltage (EV) were optimized for each analyte by flow injection analysis and their values are shown in Table 3, in which the values of limit of quantification (LOQ) for all the analytes determined under the optimized conditions are also listed.

Results and Discussion

A new UHPLC-MS/MS method was successfully developed for simultaneous quantification of 23 pesticides residues and nine illegal additives of pigments. As illustrated in Figure 1, all target compounds were detected with good peak shape and sensitivity. Using this method, the LOQs of the target compounds ranged from 0.5 to 50 µg/L in wine samples as shown in Table 3.

The effects of sample matrices and dilution factors on the analysis were studied during sample preparation process. Wine samples with different dilution factors (1:2, 1:5, 1:10, and 1:20) and spiked with the same amount of analytes (pigments: 100µg/L, pesticides: 10 µg/L) were analyzed and their responses were compared. As shown in Figures 2 and 3, the responses increased with the increase of the dilutions, indicating that sample matrix effects (mainly ions suppressions) could be reduced by sample dilutions. Thus, a 1:10 dilution of the sample was used in this study for wine analysis. Sample clean up treatments using PSA, C18 and GCB were also investigated for the spiked samples (pigments: 200 µg/L, pesticides: 20 µg/L) with 1:10 dilution of the samples. The results showed that better responses and recoveries were obtained for most of the analytes studied without clean-up, as shown in Figures 4 and 5. The lower responses were obtained from the samples after clean-up steps, especially from samples treated with GCB and C18, possibly because these compounds contain nonpolar components, which could be lost due to retention on the materials during clean up steps. PSA (primary secondary amines) could be used to remove sugars, fatty acids, organic acids, and anthocyanin pigments; C18 was used to remove nonpolar interferences and GCB (carbon) was used to remove pigments, sterols, and nonpolar interferences.

Table 1. Mobile phase gradient.

	Time (min)	A%	B%
1	0.0	95	5
2	3.0	60	40
3	5.0	50	50
4	8.0	20	80
5	9.0	5	95
6	11.0	5	95
7	11.1	95	5
8	13.0	95	5

Table 2. MS Source Conditions.

ESI Voltage (Positive)	5500 V
Drying Gas	70 arbitrary units
Nebulizer Gas	200 arbitrary units
Source Temperature	500 °C
HSID Temperature	320 °C
Detection Mode	Time-managed MRM™

Table 3. Optimized MRM Parameters and the Limit of Quantifications (LOQs).

No.	Analyte	MRM Transition Quantifier / MRM Transition Qualifier		RT/min	CE/eV	EV/V	LOQ/ μg/L
1	Tartrazine	468.9	451.0	1.45	-22	23	50
		468.9	200.1		-33	23	
2	New red	545.9	504.0	1.85	-20	24	50
		545.9	341.1		-34	24	
3	Acid Red-27	538.8	348.1	1.99	-41	27	50
		538.8	223.0		-37	27	
4	Carmine	538.9	158.2	2.29	-49	30	50
		538.9	223.1		-37	30	
5	Sunset Yellow	408.7	392.1	2.56	-26	25	50
		408.7	236.1		-29	25	
6	Allura Red AC	452.9	217.1	2.80	-30	17	10
		452.9	202.2		-54	17	
7	Azorubine	458.8	223.2	3.26	-34	20	10
		458.8	442.0		-22	20	
8	Brilliant Blue	749.2	306.1	3.45	-59	75	10
		749.2	171.2		-71	75	
9	Erythrosin B	836.7	583.0	3.81	-69	67	10
		836.7	329.0		-86	67	
10	Methamidophos	142.0	94.0	1.97	-11	22	10
		142.0	125.0		-18	22	
11	Thiamethoxam	292.0	211.0	3.48	-17	20	0.5
		292.0	181.0		-30	20	
12	Carbendazim	192.0	160.0	3.97	-24	28	0.5
		192.0	132.0		-40	28	
13	Dimethoate	230.0	125.0	4.09	-29	22	0.5
		230.0	199.0		-12	22	
14	Acetamiprid	223.0	126.0	4.19	-29	30	0.5
		223.0	99.0		-54	30	
15	Thiabendazole	202.2	175.2	4.40	-45	33	0.5
		202.2	131.2		-57	45	
16	Dimethomorph	388.0	301.0	6.87/7.11	-26	40	0.5
		388.0	165.0		-41	40	
17	Pyrimethanil	200.0	107.0	7.48	-32	50	5
		200.0	82.0		-32	50	
18	Fenhexamid	302.1	97.2	7.97	-32	57	5
		302.1	55.2		-71	77	
19	Azoxystrobin	404.0	372.0	7.99	-19	25	0.5
		404.0	344.0		-33	25	
20	Epoxiconazole	330.0	121.0	8.04	-22	25	0.5
		330.0	101.0		-50	25	
21	Triadimefon	294.0	197.0	8.08	-20	30	0.5
		294.0	225.0		-16	30	
22	Boscalid	343.0	307.0	8.09	-25	25	1
		343.0	140.0		-28	25	
23	Fluquinconazole	376.0	349.0	8.16	-26	25	1
		376.0	307.0		-34	25	
24	Hexaconazole	314.0	70.0	8.44	-24	25	0.5
		314.0	159.0		-36	25	
25	Imazalil	297.1	159.1	8.49	-42	30	1
		299.1	161.1		-42	30	
26	Penconazole	283.8	70.1	8.51	-23	20	0.5
		283.8	159.1		-48	20	
27	Malathion	331.0	127.0	8.51	-10	20	5
		331.0	285.0		-16	20	
28	Prochloraz	376.0	308.0	8.84	-16	20	0.5
		376.0	70.0		-37	20	
29	Cyprodynil	226.3	93.2	8.87	-51	66	0.5
		226.3	108.3		-35	56	
30	Phoxim	299.0	77.0	9.40	-46	20	1
		299.0	129.0		-18	20	
31	Trifloxystrobin	409.2	186.1	9.52	-43	31	0.5
		409.2	206.2		-33	21	
32	Chlorpyrifos	350.0	198.0	10.00	-23	25	0.5
		350.0	97.0		-47	25	

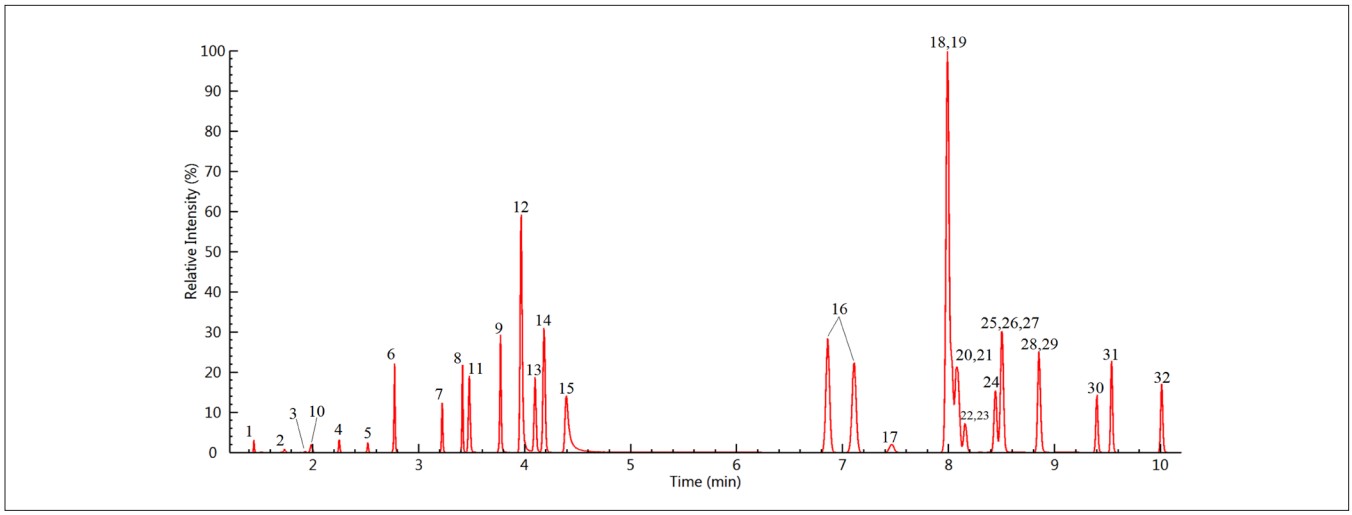

Figure 1. LC–MS/MS chromatograms of the 9 pigments (100 µg/L) and 23 pesticides (10 µg/L) spiked to a wine sample (compound names are shown in Table 3).

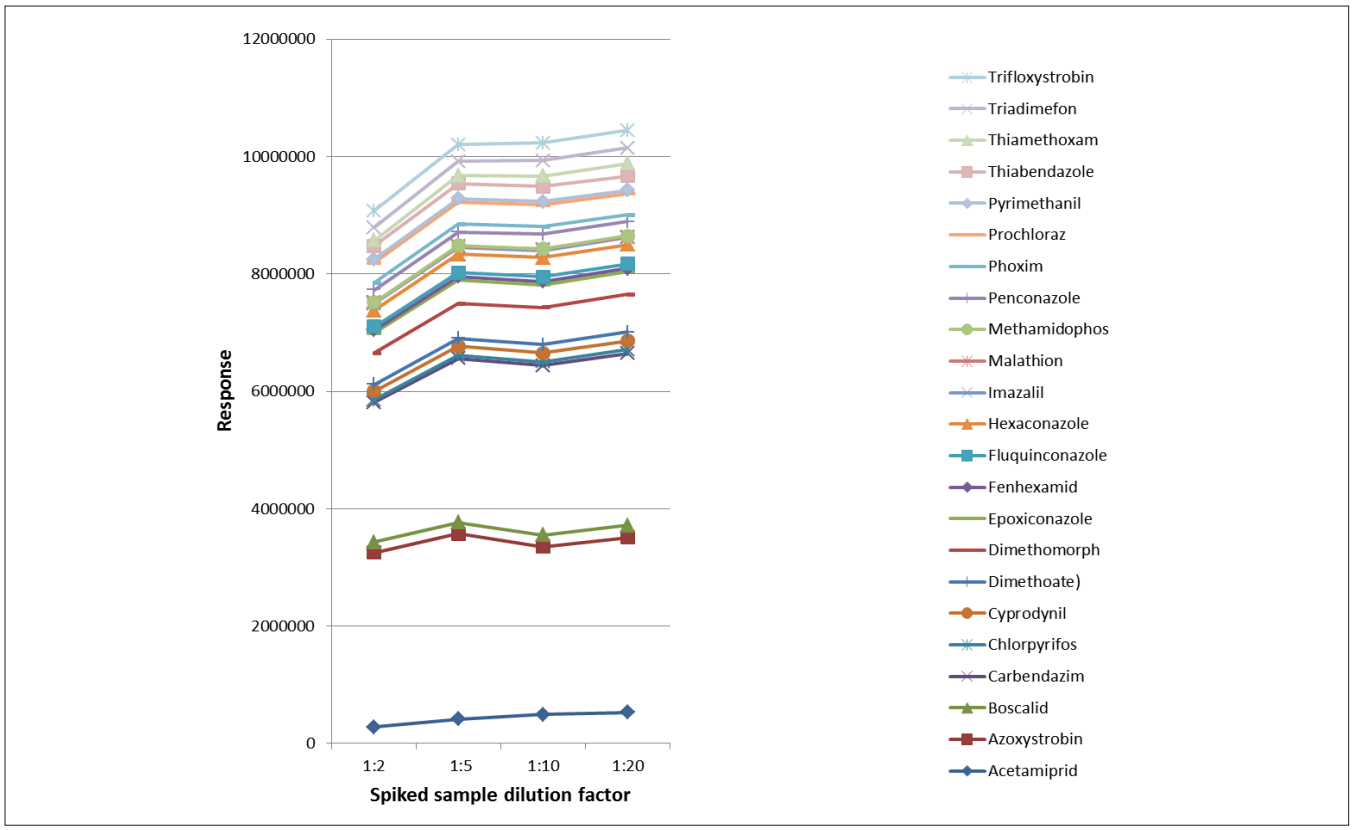

Figure 2. Result of pesticides (10µg/L) with different dilution factors.

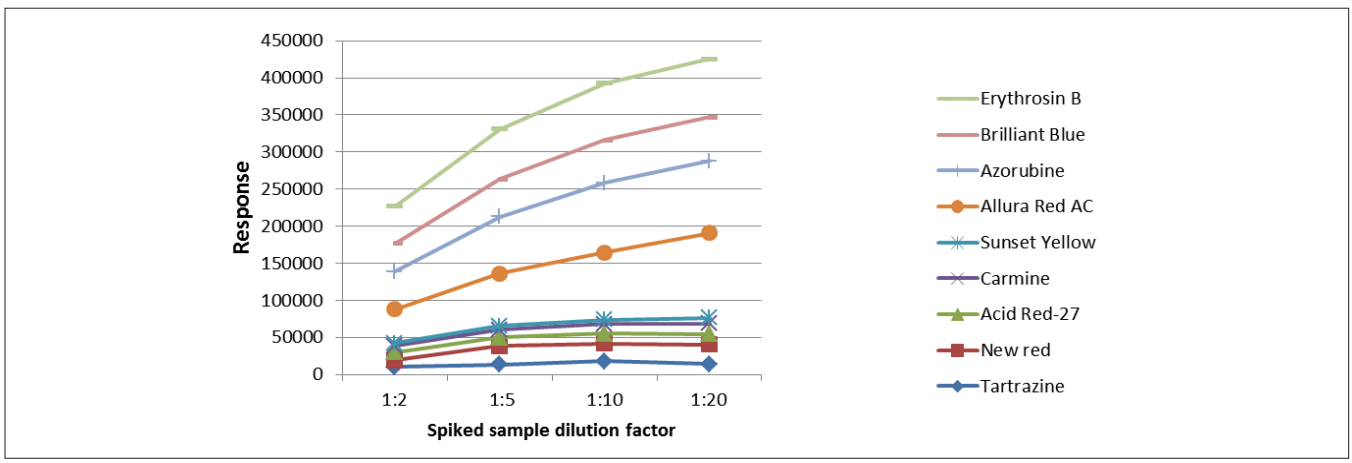

Figure 3. Result of pigments (100µg/L) with different dilution factors.

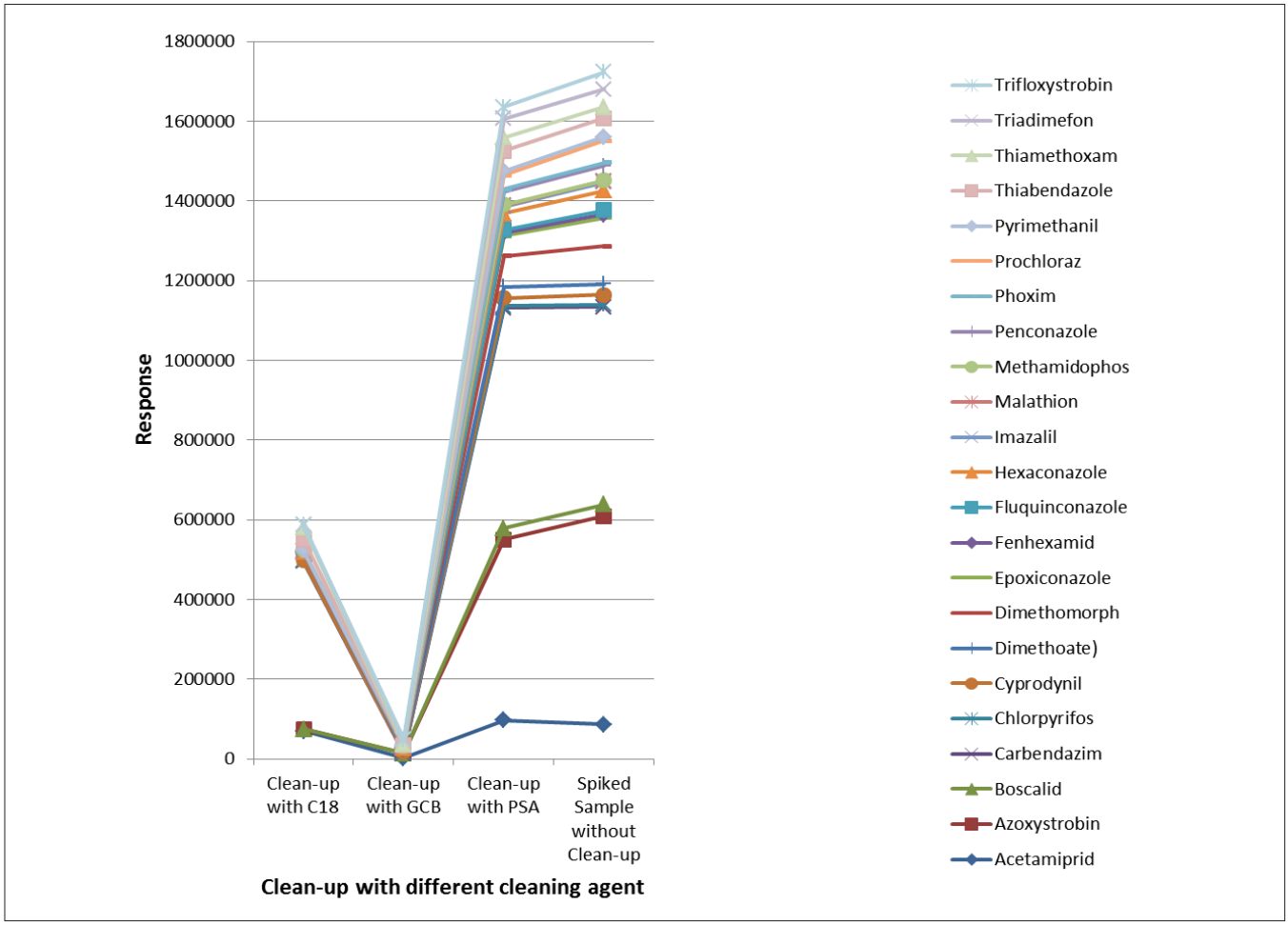

Figure 4. Effects of clean-up steps on responses of the pesticides (20µg/L).

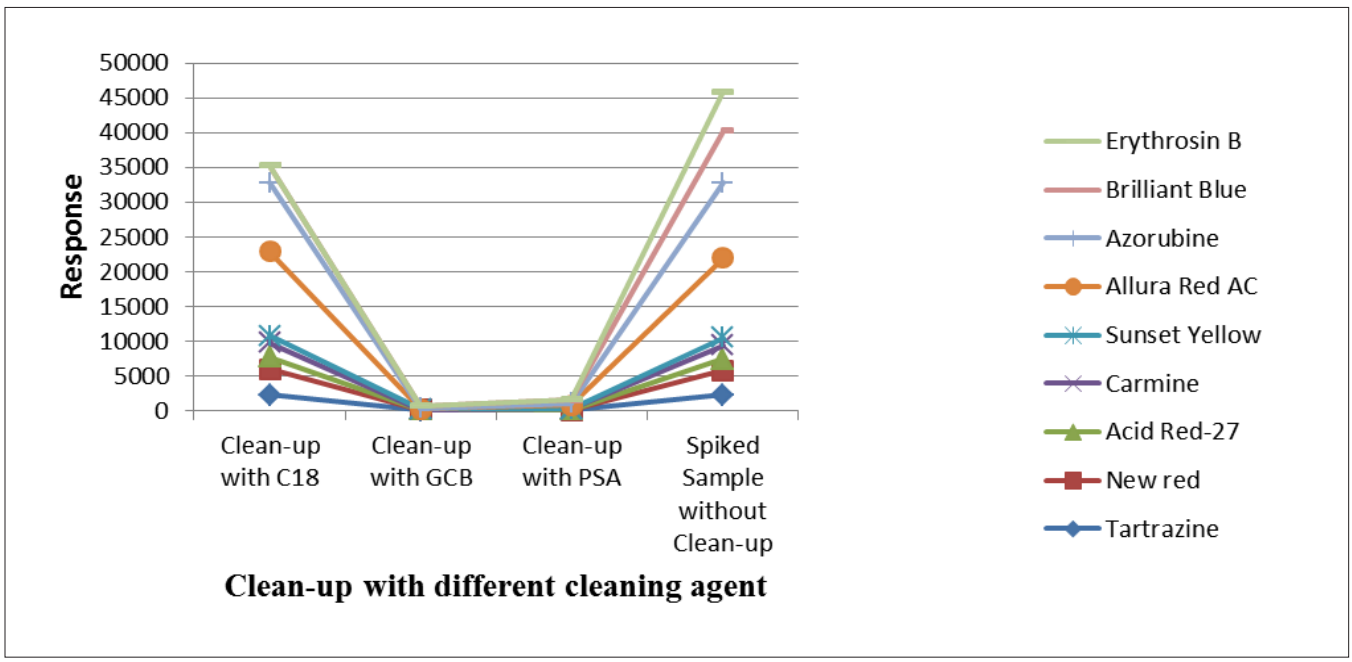

Figure 5. Effects of clean-up steps on response of pigments (200µg/L).

In this study, matrix-matched calibration curves were used for quantification. The matrix-matched calibration curves showed good linearity over three orders of magnitudes with regression coefficients (R^2) greater than 0.99, from 1 µg/L to 1000 µg/L for the nine pigments and from 0.5 µg/L to 100 µg/L for the 23 pesticides, respectively. The recoveries of the analytes were evaluated at concentrations of 50, 100 and 500 µg/L, and the mean recovery values ranging from 85.0% to 115.0% with RSD <11%. The developed method has been applied for the analysis of 10 real wine samples and the results are summarized in Table 4.

Conclusions

A rapid, sensitive and selective 'dilute-n-shot' method has been developed and validated for simultaneous determination of 23 pesticides and nine pigments in wine. The method has the advantage of analyzing pesticides and pigments in a single run using UHPLC-MS/MS method. The results demonstrated that the accuracy and precision of the method were acceptable for routine monitoring of these compounds in analytical laboratories.

Table 4. Results of the analytes determined from the 10 real wine samples (in µg/L).

Compound	Sample 01	Sample 02	Sample 03	Sample 04	Sample 05	Sample 06	Sample 07	Sample 08	Sample 09	Sample 10
Acetamiprid	-	-	4.1	10	-	-	-	-	-	-
Azoxystrobin	-	-	11.5	-	-	-	-	-	-	-
Boscalid	-	33.5	173.1	87.3	53.1	345.8	10.7	245.5	16.1	42.4
Carbendazim	2.8	-	-	-	-	-	164	16	-	-
Chlorpyrifos	3.4	-	-	-	-	-	-	-	-	-
Cyprodynil	33.9	-	-	10.8	-	82.6	-	-	-	-
Dimethoate	-	-	-	5.7	-	-	27	-	-	-
Dimethomorph	-	-	-	-	-	134.3	90.2	100.2	-	-
Fenhexamid	-	-	-	180.3	-	275.8	-	434.4	-	-
Pyrimethanil	-	-	-	43.2	-	-	115	136.4	-	-
Thiabendazole	-	-	-	6.1	-	-	-	-	-	-
Thiamethoxam	-	-	-	-	-	20.2	-	-	-	-

References

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