Bulletin of Insectology ***56*** *(1): 41-50, 2003*
ISSN 1721-8861

Analytical determination of imidacloprid and relevant metabolite residues by LC MS/MS

Ralf SCHÖNING, Richard SCHMUCK
Bayer CropScience AG, Monheim, Germany

Abstract

A new method is described for the analytical determination of imidacloprid residues by LC MS/MS. This method allows the concurrent determination of the parent compound imidacloprid and two toxicologically relevant plant metabolites of this systemic insecticide. The method was successfully applied to examine field residue levels of these contaminants in rape and sunflower crop plants which had been seed-treated with imidacloprid. The analyzed samples included pollen, flowers, leaves, nectar, honey, wax and bees.

Residues of imidacloprid and its metabolites 5-hydroxy-imidacloprid and olefin-imidacloprid are extracted with methanol/water. The extracts are subjected to liquid-liquid partition on a column filled with diatomaceous earth and subsequent solid phase extraction on a silica gel column. Quantification is performed by reversed phase HPLC with electrospray MS/MS-detection. External bracketing standards in matrix are used to compensate possible matrix effects in the ion source.

The overall recoveries for imidacloprid, 5-hydroxy-imidacloprid and olefin-imidacloprid from plant matrices were between 91 and 97% with a relative standard deviation between 6.0 and 8.5%. The detector linearity and the repeatability of the method proved to be very precise. The limits of quantification were 0.005 and 0.01 mg/kg for imidacloprid and 5-hydroxy-imidacloprid, and for the olefin-metabolite, respectively.

Field residue studies were conducted on seed-treated rape and sunflower crops. Small bee colonies were caged on flowering sunflower and rape plots and served as collecting devices for nectar and pollen. Flower petals, leaves, honey bees and the collected pollen and nectar samples were analysed by the new HPLC-MS/MS method. The analytical results showed no quantifiable residues in any sample material.

Accordingly, honey bees will exposed to only negligible residue levels of imidacloprid when foraging on flowering seed-treated crop plants.

Key words: imidacloprid, residues, analytical method, HPLC-MS/MS, sunflower, rape, corn.

Introduction

Imidacloprid belongs to a new chemical class of active ingredients, the chloronicotinyls, (neonicotinoids). It has a new mode of action, outstanding biological efficacy, a broad spectrum of activities, low toxicity to warm-blooded animals and a good plant compatibility. Because of its excellent systemic properties imidacloprid is used as a seed dressing as well as for foliar, soil and stem treatment (Ishaaya and Degheele, 1998). The metabolism of imidacloprid in plants was investigated in various crops (Sur and Stork, 2003). All metabolites identified in plants after treatment with imidacloprid contained the 6-chloropicolyl moiety. Therefore, an analytical method was developed by Placke and Weber (1993) for the determination of imidacloprid and the total residues in plants including all compounds containing the 6-chloropicolyl moiety. The present method allows the determination of the parent compound imidacloprid and the two toxicologically relevant (Schmuck *et al*., 2003) metabolites 5-hydroxy-imidacloprid and olefin-imidacloprid (figure 1) using HPLC-MS/MS as a particularly sensitive and selective analytical technique.

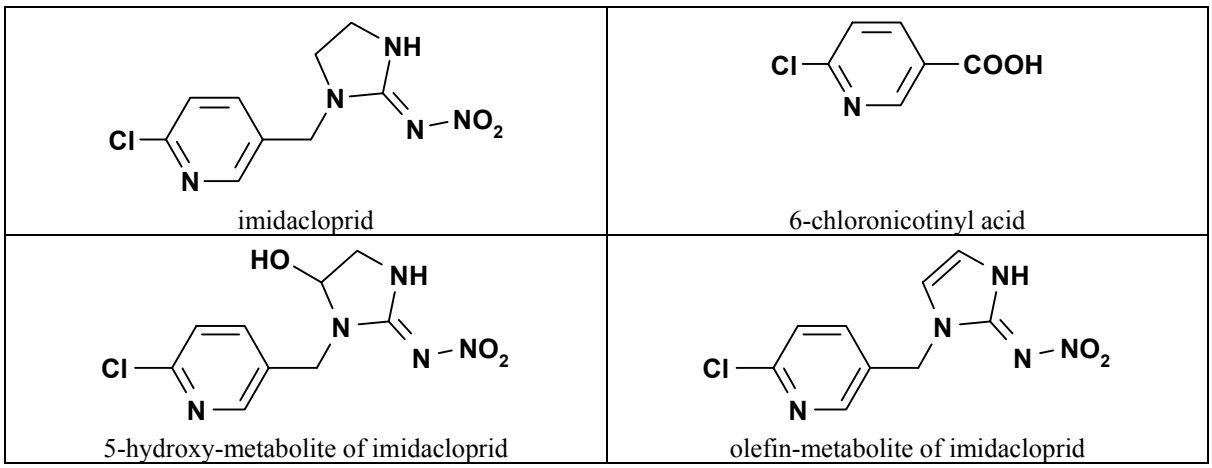


Figure 1. Structural formulas of imidacloprid and some plant metabolites.

Material and methods

Technical equipment and reagents

- HPLC-MS/MS system, such as:
  Agilent 1100 liquid chromatograph or equivalent
  Gilson 232 XL autosampler or equivalent
  Applied Biosystems API 300, 365 or 3000 tandem mass spectrometer or equivalent
  Applied Biosystems LC-MS/MS workstation data system or equivalent
- HPLC column, reversed phase, Phenomenex, Luna C18 (2), 15 cm length x 4.6 mm i.d. or equivalent
- ChemElut CE 1020 cartridges, fitted with a disposable steel needle, e.g. Varian GmbH, Alsfelder Straße 6, 64289 Darmstadt, Germany, Part No.: 1219-8008
- Silica gel (SiOH) column, e.g. Varian GmbH, Alsfelder Straße 6, 64289 Darmstadt, Germany, Part No.: 1210-2037
- Mobile phase A: 0.01% (v/v) acetic acid in water. Add 0.1 mL acetic acid and dilute to 1 litre with water.
- Mobile phase B: 0.01% (v/v) acetic acid in acetonitrile. Add 0.1 mL acetic acid and dilute to 1 litre with acetonitrile.
- Laboratory Glassware

Reference samples of Imidacloprid, 5-Hydroxy-Imidacloprid and Olefin-Imidacloprid were purchased from Bayer CropScience, Monheim.

Extraction and sample clean-up

The sample materials are extracted using a mixture of methanol and water. After extraction the samples are filtered and evaporated to the aqueous remainder using a rotary evaporator. The aqueous solution is transferred onto a disposable column filled with diatomaceous earth (e.g. Varian ChemElut columns) and partitioned against cyclohexane/ethyl acetate. The eluate is evaporated to dryness and dissolved in toluene/ethyl acetate. Further sample clean-up is performed using a silica gel column. The organic solution is applied onto the column, rinsed with toluene/ethyl acetate and the residues are eluted with acetonitrile. After evaporation to dryness the samples are re-dissolved in acetonitrile/water and injected into the high performance liquid chromatograph, chromatographed under isocratic reversed phase conditions and detected by tandem mass spectrometry with electrospray ionisation. A flow chart of the method is given in figure 2.

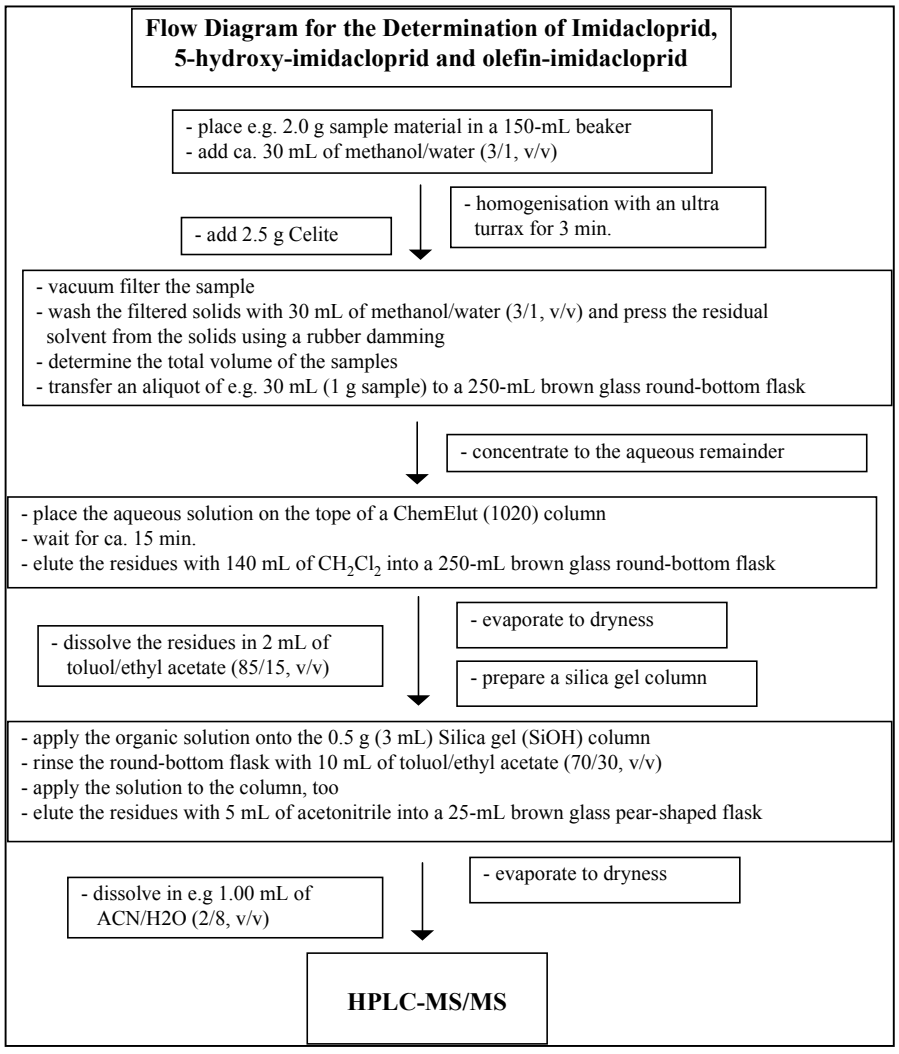


Figure 2. Flow Chart of extraction and clean-up procedure.

HPLC-MS/MS conditions

HPLC-Instrument:	Agilent 1100 with Gilson 233 XL autosampler
Column:	Phenomenex, Luna C18 (2), 5 ?m, 15 cm, 0.46 cm i.d
Injection volume:	50 µL
Oven temperature:	40 ºC
Mobile phase A:	Water + 0.1 mL acetic acid per litre
Mobile phase B:	Acetonitrile + 0.1 mL acetic acid per litre
Gradient:	0-10 min 20 % Solvent B, 11 – 15 min 90 % Solvent B, 16 – 19 min 20 % Solvent B
Detector:	Triple Quadrupole LC-MS/MS Mass Spectrometer, e-g. Applied Biosystems, API 3000, Windows NT 4.0
Interface:	Electrospray, Turbo-Ion Spray Potential: + 5000 V, Temperature: 300 ºC
Scan type:	MRM (Multiple Reaction Monitoring Mode)
Polarity:	Positive
Collision gas:	Nitrogen 5.0

The MS/MS-transitions for imidacloprid and the plant metabolites 5-hydroxy and olefine-imidacloprid are given in table 1.

Field residue trials

Field residue trials were conducted in compliance with GLP and the European registration guidelines for pesticides (DFG, 1991; EC, 1991). The test plots were located in Germany (corn, sunflower and summer rape), Great Britain (summer rape), Sweden (summer rape) and France (summer rape). Control plots were placed on former grasslands to exclude any soil contamination by imidacloprid from previous croppings.

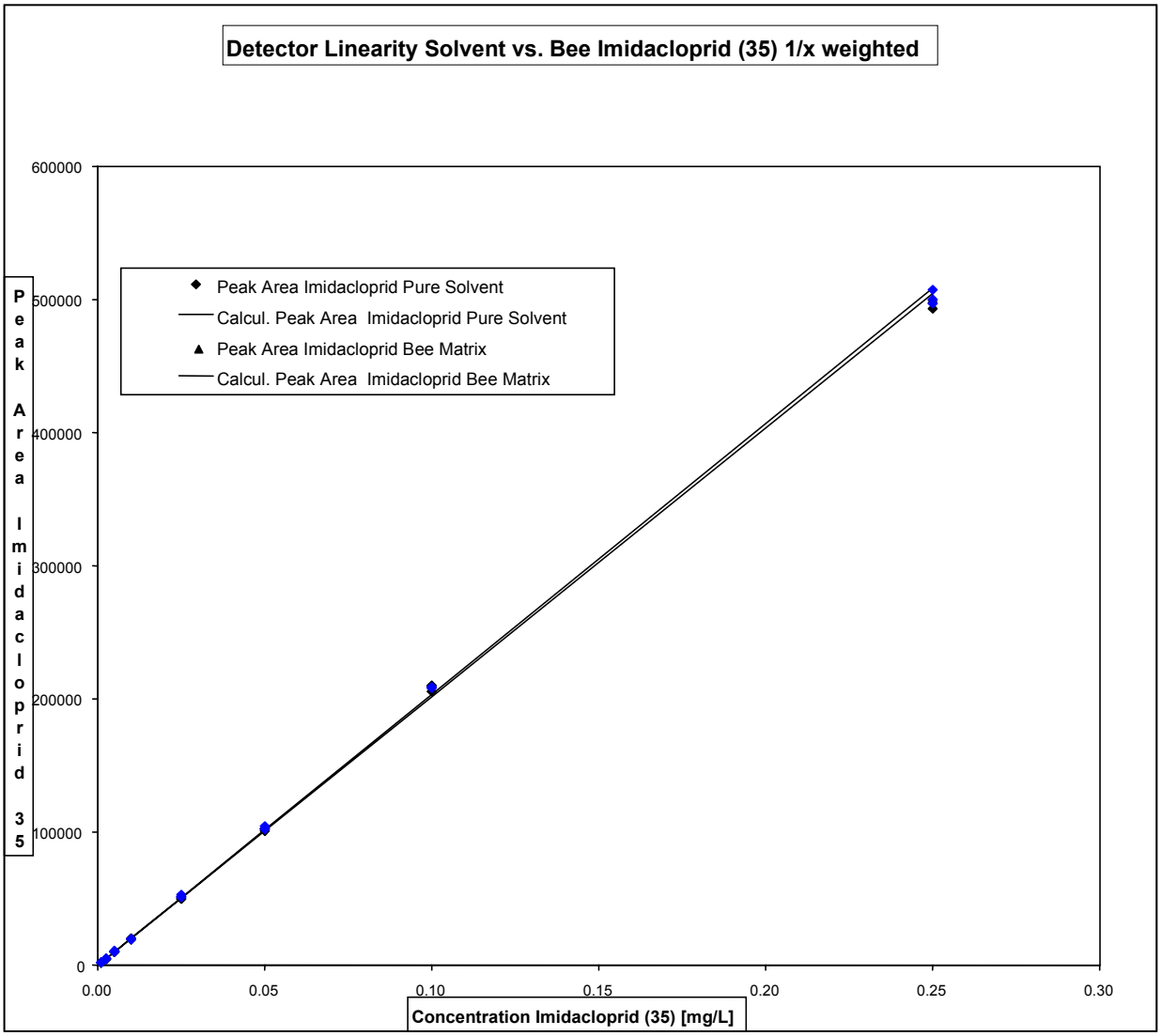
Drilling rates were 0.58 units/ha, 2 units/ha and between 3.25 and 7 kg/ha for sunflowers, corn and summer rape, respectively. The sunflower, corn and rape plants were seed-treated with imidacloprid at rates of 0.7 mg, 1.0 and 0.05 mg a.s./seed, respectively. During the flowering period small bee colonies (2000 to 3000 honeybees) were caged on the sunflower and rape plots for sampling of nectar and pollen. Pollen samples from corn plants were collected by hand. All nectar and pollen samples, flower petals and a sufficient number of honeybees, sampled from the crop plants during collection of the pollen and nectar, were subjected to a residue analysis for imidacloprid and its toxicologically relevant plant metabolites.

Table 1. MS/MS-Transitions for Imidacloprid and Metabolites ([#]the ions containing the Cl 37 isotope were detected for all analytes for confirmation).

Compound	Precursor Ion Q1 Mass (amu)	Product Ion Q3 Mass (amu)	Dwell Time (msec)	Collision Energy (eV)
Olefin-imidacloprid (35)	256[#]	236	250	-13
Olefin-imidacloprid (35)	254	205	250	-13
5-hydroxy-imidacloprid (37)	274[#]	191	250	-23
5-hydroxy-imidacloprid (35)	272	191	250	-23
Imidacloprid (37)	258[#]	211	500	-20
Imidacloprid (35)	256	209	500	-20

Table 2. Recovery values for imidacloprid and metabolites.

	Spiking Level (mg/kg)	Mean (%)	Range Min-Max	RSD (%)	N
Recoveries for Imidacloprid	0.005	97	68-118	6.0	53
	0.01	97	90-118	6.1	25
	0.05	97	68-111	7.5	53
	0.10	99	93-114	4.2	25
	Overall	97	68-118	6.0	156
Recoveries for 5-hydroxy-imidacloprid	0.005	92	68-101	7.6	53
	0.01	89	79-105	8.1	25
	0.05	92	65-104	6.5	53
	0.10	93	87-109	5.2	25
	Overall	92	65-109	7.0	156
Recoveries for olefin-imidacloprid	0.01	90	63-106	9.7	78
	0.10	92	65-107	7.1	78
	Overall	91	63-107	8.5	156



Data for Linearity Determination in Solvent

Standard Amount [ng]	Standard Amount [mg/L]	Peak Area Imidacloprid Solvent			Calc. Peak Area 1/x weighted
0.025	0.001	1819	1825	1959	1795
0.0625	0.0025	4527	4634	4392	4823
0.125	0.005	9795	10072	9930	9870
0.25	0.01	19160	20094	19939	19964
0.625	0.025	49724	49674	51280	50246
1.25	0.05	100714	102549	102399	100716
2.5	0.1	205637	209771	210105	201655
6.25	0.25	493231	499736	497668	504474

Data for Linearity Determination in Bee Matrix

Standard Amount [ng]	Standard Amount [mg/L]	Peak Area Imidacloprid Bee Matrix			Calc. Peak Area 1/x weighted
0.025	0.001	2014	1939	2025	1970
0.0625	0.0025	4704	4660	4991	5021
0.125	0.005	10431	10798	9914	10106
0.25	0.01	19608	19312	19713	20276
0.625	0.025	51003	51367	52915	50787
1.25	0.05	102127	104456	102091	101638
2.5	0.1	208642	209031	208290	203339
6.25	0.25	507431	497054	500221	508444

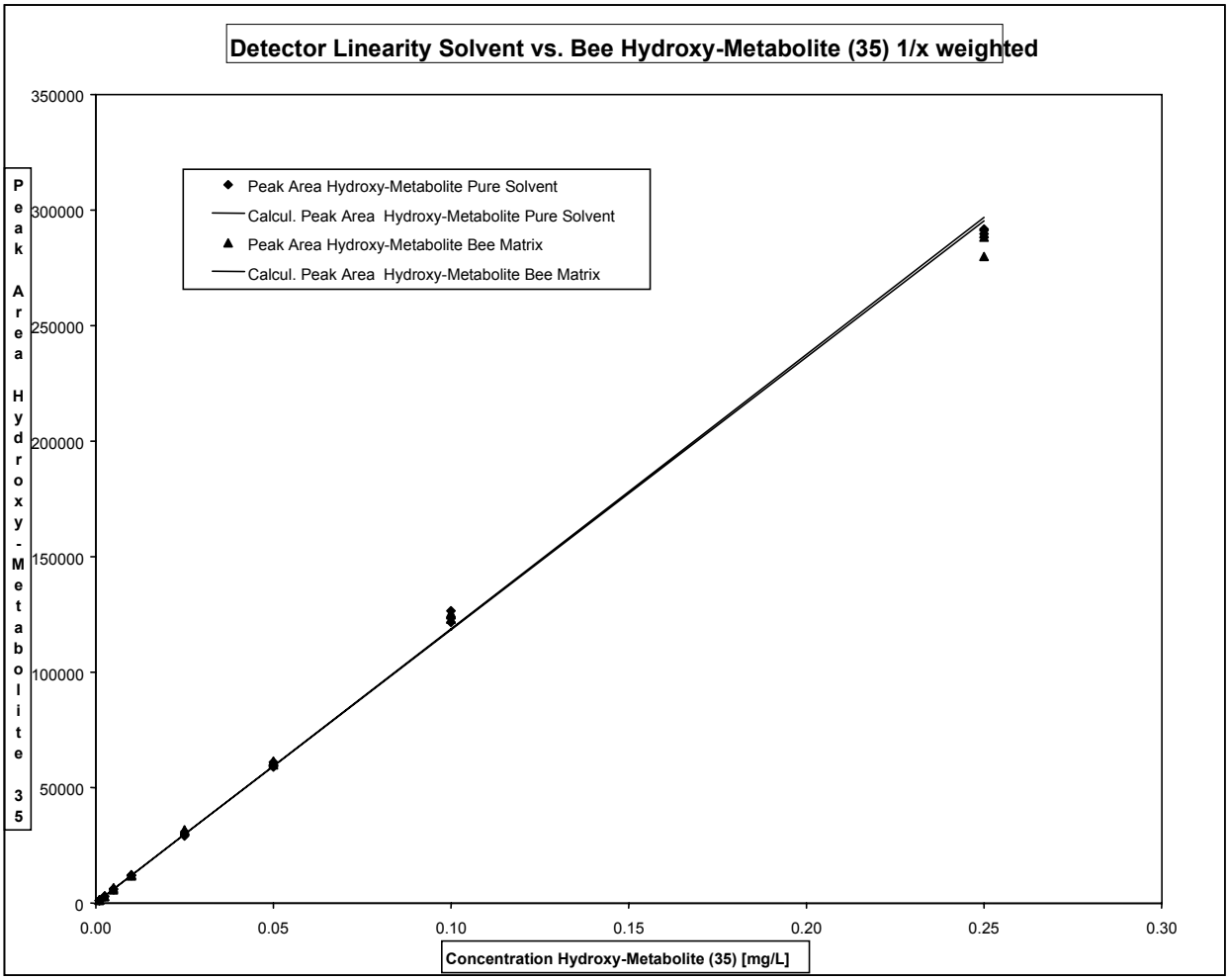
Regression parameters Standard in Solvent (Linear 1/x weighted)

slope:	2018790
y-axis intercept:	-224
correlation coefficient:	0.9997

Regression parameters Standard in Bee Matrix (Linear 1/x weighted)

slope:	2034030
y-axis intercept:	-64
correlation coefficient:	0.9998

Figure 3. Detector linearity imidacloprid in solvent vs. bee matrix.



Data for Linearity Determination in Solvent

Standard Amount		Peak Area			Calc. Peak Area
[ng]	[mg/L]	Hydroxy-Metabolite Solvent			1/x weighted
0.025	0.001	1094	1181	1101	1187
0.0625	0.0025	2822	2951	3034	2968
0.125	0.005	6050	6235	5796	5936
0.25	0.01	11986	12014	12071	11872
0.625	0.025	29394	29048	30853	29681
1.25	0.05	59782	58991	61045	59362
2.5	0.1	121553	123468	126501	118723
6.25	0.25	289709	291205	291741	296808

Regression parameters Standard in Solvent
(Linear 1/x weighted)

slope:	1187233
y-axis intercept:	0
correlation coefficient:	0.9996

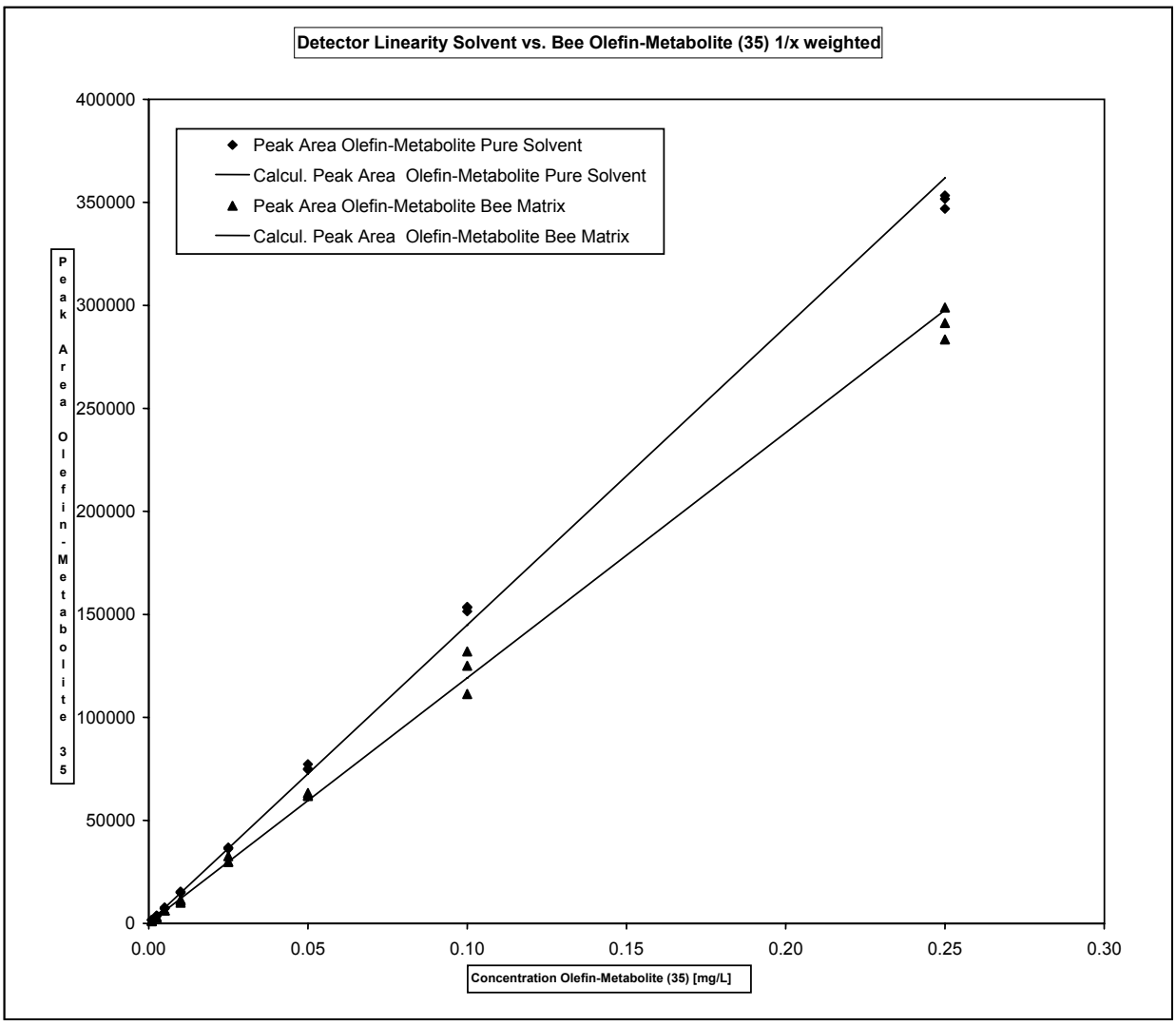
Data for Linearity Determination in Bee Matrix

Standard Amount		Peak Area			Calc. Peak Area
[ng]	[mg/L]	Hydroxy-Metabolite Matrix			1/x weighted
0.025	0.001	1319	1383	1312	1395
0.0625	0.0025	2906	2835	3054	3166
0.125	0.005	6568	5845	6248	6117
0.25	0.01	12211	12248	11782	12019
0.625	0.025	31463	31253	31731	29725
1.25	0.05	61354	59805	61089	59236
2.5	0.1	122891	124907	125539	118257
6.25	0.25	289772	279908	288292	295319

Regression parameters Standard in Bee Matrix
(Linear 1/x weighted)

slope:	1180417
y-axis intercept:	215
correlation coefficient:	0.9991

Figure 4. Detector linearity 5-hydroxy-imidacloprid in solvent vs. bee matrix.



Linearity in Solvent

Standard Amount		Peak Area			Calc. Peak Area
[ng]	[mg/L]	Olefin-Metabolite Solvent			1/x weighted
0.025	0.001	1698	1738	1536	1717
0.0625	0.0025	3707	3705	3783	3886
0.125	0.005	7759	7218	7232	7502
0.25	0.01	15111	14926	15413	14732
0.625	0.025	36752	36680	36149	36424
1.25	0.05	74699	75053	77271	72576
2.5	0.1	151426	153591	153222	144882
6.25	0.25	346902	353309	351678	361798

Regression Parameters Standard in Solvent (Linear 1/x weighted)

slope:	1446108
y-axis intercept:	271
correlation coefficient:	0.9992

Linearity in Bee Matrix

Standard Amount		Peak Area			Calc. Peak Area
[ng]	[mg/L]	Olefin-Metabolite Matrix			1/x weighted
0.025	0.001	1369	1023	1531	1284
0.0625	0.0025	2959	3032	2738	3070
0.125	0.005	6344	6197	6338	6046
0.25	0.01	9983	11554	10971	11999
0.625	0.025	30005	29850	32681	29855
1.25	0.05	61802	63313	62255	59617
2.5	0.1	111329	125069	131966	119139
6.25	0.25	283454	291421	298956	297707

Regression Parameters Standard in Bee Matrix (Linear 1/x weighted)

slope:	1190450
y-axis intercept:	94
correlation coefficient:	0.9985

Figure 5. Detector linearity olefin-metabolite in solvent vs. bee matrix.

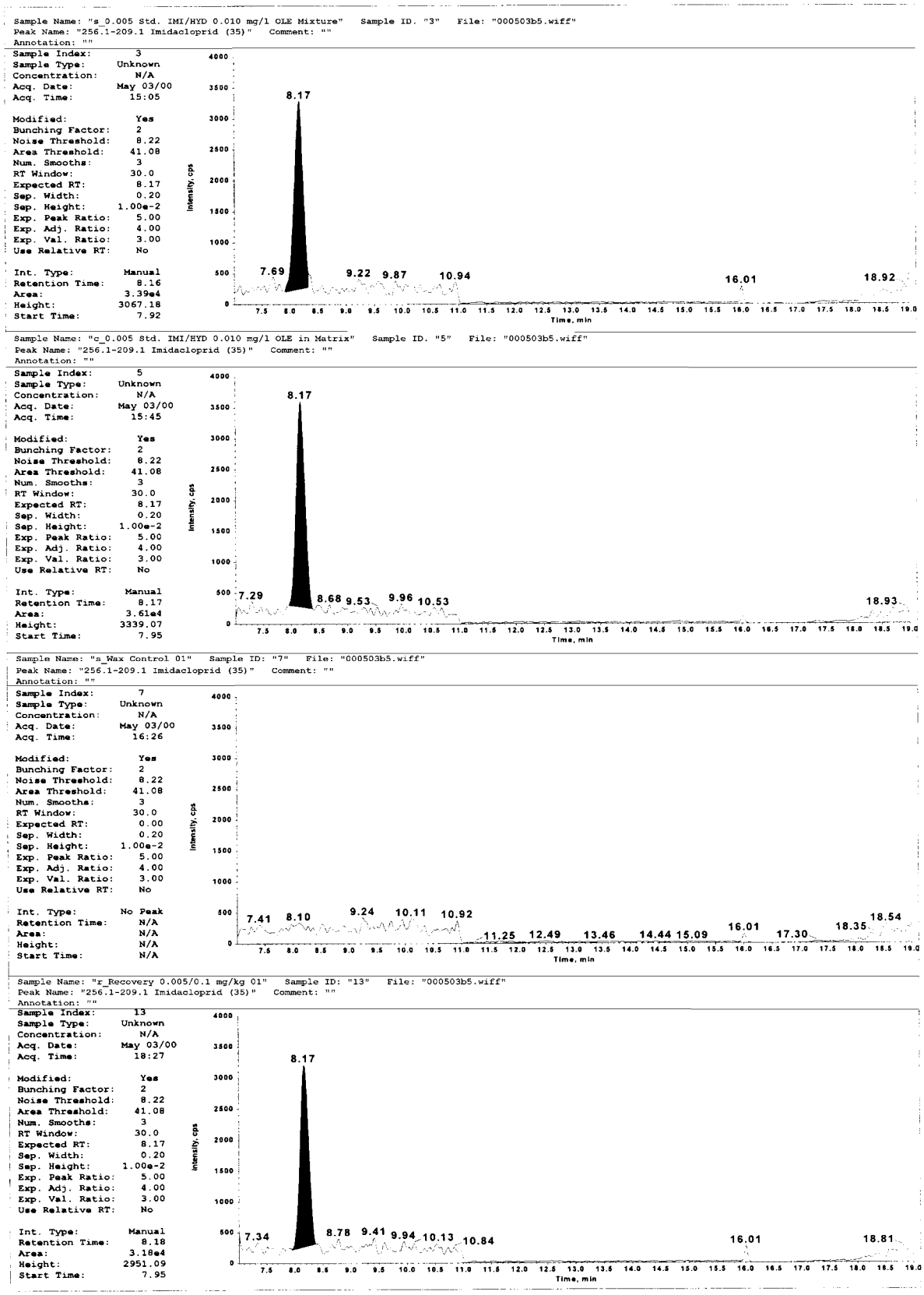


Figure 6. Representative chromatograms of imidacloprid recoveries from wax.
Top: standard imidacloprid 0.005 mg/L in Solvent
Middle 1: standard imidacloprid 0.005 mg/L in wax, control
Middle 2: wax, control
Bottom: recovery wax, fortification level 0.005 mg/kg

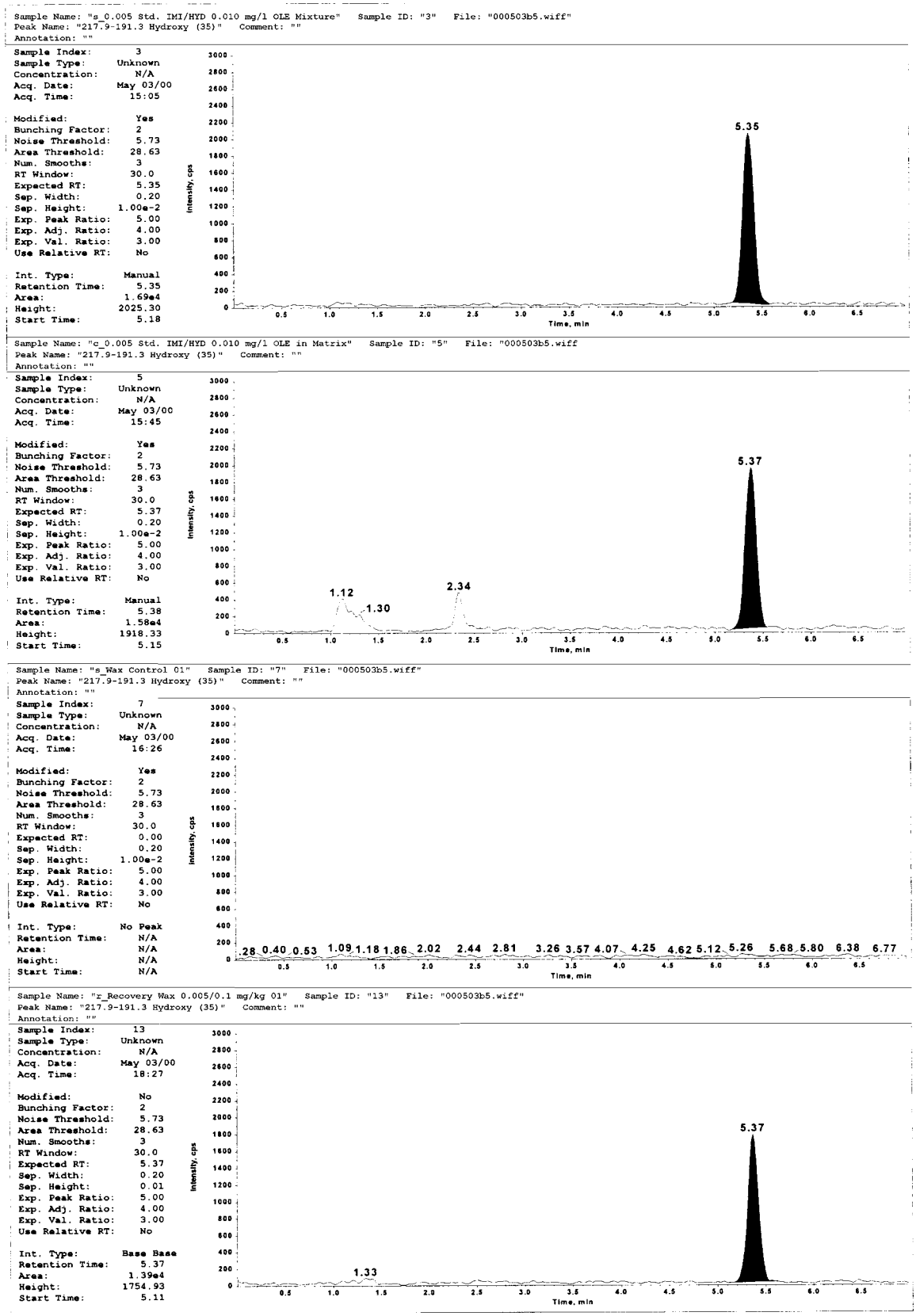


Figure 7. Representative chromatograms of 5-hydroxy-imidacloprid recoveries from wax.
Top: standard 5-hydroxy-imidacloprid 0.005 mg/L in solvent
Middle 1: standard 5-hydroxy-Imidacloprid 0.005 mg/L in wax, control
Middle 2: wax, control
Bottom: recovery wax, fortification level 0.005 mg/kg

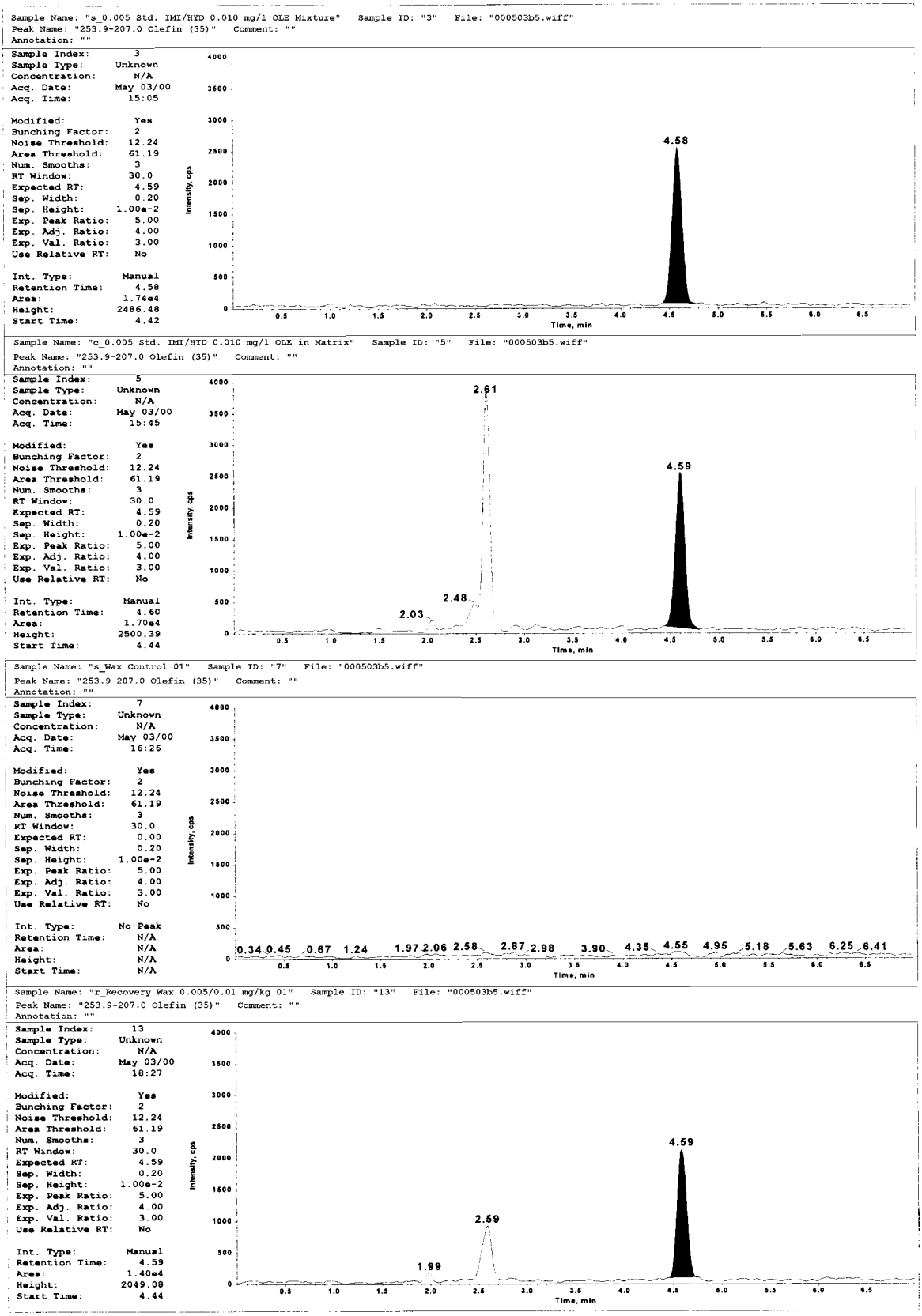


Figure 8. Representative chromatograms of Olefin-imidacloprid recoveries from wax.
Top: standard Olefin-imidacloprid 0.01 mg/L in solvent
Middle 1: standard Olefin-imidacloprid 0.01 mg/L in wax, control
Middle 2: wax, control
Bottom: recovery wax, fortification level 0.01 mg/kg

Results

Method validation

The linearity of the detector response was tested for imidacloprid, 5-hydroxy-imidacloprid and olefin-imidacloprid in solvent and in matrix over the range of 0.001 to 0.25 mg/L. A very precise linear relation between the injected amount and the resulting peak area was observed over the entire range with correlation coefficients between 0.9946 and 0.9998.

The stability of imidacloprid and the two metabolites in analytical solutions of bees, honey and pollen was tested over a period of 4 weeks under refrigerator conditions. The results demonstrate that the compounds in the analytical solutions are stable for at least 4 weeks.

The accuracy and precision of the method was evaluated on the basis of the recoveries obtained for fortified samples. Recovery experiments were performed for rape and sunflower (flowers, pollen, leaves), corn (pollen, leaves), honey, nectar, wax and bees. Control samples were fortified with a mixture of imidacloprid and the two metabolites at fortification levels of 0.005 to 0.1 mg/kg. The limit of quantitation (LOQ) was 0.005 mg/kg for imidacloprid and 5-hydroxy-imidacloprid and 0.01 mg/kg for olefin-imidacloprid. The limit of detection (LOD) was 0.0015 mg/kg for imidacloprid and 5-hydroxy-imidacloprid and 0.003 mg/kg for olefin-imidacloprid. Recoveries for imidacloprid ranged from 68 to 118% (mean: 97%, relative standard deviation (RSD): 6.0%, n=156) for 5-hydroxy-imidacloprid from 65 to 109% (mean: 92%, RSD: 7.0%, n=156) and for olefin-imidacloprid from 63 to 107% (mean: 91%, RSD: 8.5%, n=165). A summary of the obtained recovery values is given in table 2.

The repeatability of the method was determined for each analyte by running a set of five recoveries each at two different fortification levels for selected matrices. The resulting mean recovery rates ranged from 79 to 104% with relative standard deviations between 0.8 and 15.3%. These data demonstrate the excellent sensitivity, selectivity and precision of the method.

Field residue studies

About 80 samples originating from rape, 54 samples originating from sunflower and 18 samples originating from corn were collected at 9 different test sites and analysed for residues of imidacloprid and its metabolites 5-hydroxy-imidacloprid and olefin-imidacloprid.

Residue levels in nectar and pollen samples were all below the limit of quantitation (LOQ, 0.005 and 0.01 mg/kg, respectively). No residues above the LOQ were found either in flower and honeybees samples. In the youngest leaves residue levels of imidacloprid and 5-hydroxy-imidacloprid were about the limit quantitation.

Acknowledgements

The authors wish to thank Gaby Braune and Agnes Gnielka for their excellent technical assistance in performing the analytical work.

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Corresponding author: Ralf SCHÖNING, Bayer CropScience AG, Alfred Nobel Strasse 50, D- 40789 Monheim, Germany. E-mail: ralf.schoening@bayercropscience.com