

Title: Determination of Imidacloprid and the Olefinic Imidacloprid, Guanidine, Olefinic Guanidine, Urea Metabolites in Well Water by High performance Liquid Chromatography Tandem Mass Spectrometry

1. Scope

This section method (SM) is for the analysis of the pesticide Imidacloprid and the Olefinic Imidacloprid, Guanidine, Olefinic Guanidine, Urea metabolites in well water and is to be followed by all authorized section personnel. The reporting limits of Imidacloprid and the Guanidine, Olefinic Guanidine, and Urea metabolites in well water are 0.05 ppb and that of Imidacloprid Olefin is 0.1 ppb.

2. Principle:

Residues of Imidacloprid and selected metabolites are extracted from well water using a solid phase cartridge, Focus. All five compounds are determined by the same injection of sample extract into a HPLC with Lichrospher 60 RP select B column and mass selective detector. The confirmation of compound identity is achieved simultaneously with collision-induced dissociation to produce a product ion for each of the analytes.

3. Safety:

- 3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.
- 3.2 All solvents should be handled with care in a ventilated area.

4. Interferences:

No known matrix interferences that cause quantitative problems above the established reporting level.

5. Apparatus and Equipment:

- 5.1 Rotary evaporator (Büchi/Brinkman or equivalent)
- 5.2 Nitrogen evaporator (Meyer N-EVAP Organomation Model # 112 or equivalent)
- 5.3 Vortex-vibrating mixer

- 5.4 Balance (Mettler PC 4400) or equivalent
- 5.5 Liquid Chromatograph (Thermo Finnigan Surveyor HPLC) equipped with a Mass selective detector (Finnigan TSQ, San Jose, Ca 95134)
- 5.6 Supelco Visiprep Vacuum Manifold (part #57250-U) or equivalent.
- 5.7 Supelco Visiprep Vacuum Manifold teflon tube, stainless steel weight and adaptor (part #57276,57273,57278) or equivalent.

6. Reagents and Supplies

- 6.3 Acetic acid, HPLC grade (Fisher #A35-500 or equivalent)
- 6.4 Acetonitrile, nanograde or equivalent pesticide grade
- 6.5 Methanol, Mass spec grade, Burdick & Jackson
- 6.6 Nitrogen, refrigerated liquid or nitrogen generator with capacity of delivering 20 liter per minute
- 6.7 Standards:
Obtain 1.0 mg/mL reference standards of Imidacloprid, Imidacloprid Olefin, Imidacloprid Urea, Imidacloprid Quanidine and Imidacloprid Quanidine Olefin from the Standard Repository, CAC, CDFA, 3292 Meadowview Road, Ca 95832. The pure standards were obtained from Bayer Corp.

Imidacloprid	CAS Number 105827-78-9, 138261-41-3
Imidacloprid Olefin	CAS Number Not found
Imidacloprid Urea	CAS Number 120868-66-8
Imidacloprid Quanidine(tautomer)	CAS Number 127202-53-3
Imidacloprid Quanidine Olefin	CAS Number Not found
- 6.8 Water, Mass spec grade, Burdick & Jackson
- 6.9 Conical tube with glass stopper, 15-mL graduated
- 6.10 Boiling flask, 500-mL
- 6.11 Funnel, 15 cm diameter
- 6.12 Disposable Pasteur pipettes, and other laboratory ware as needed
- 6.13 Analytical column: Lichrospher 60 RP select B 5 μ 125 x 3 mm (Phenomenex part #CHO-5308, Supplier refer #1.50158 may be obtained from various suppliers).
- 6.14 Guard column: Lichrospher 60 RP select B (5mm) 4 x4 mm (Agilent part #79925 SB-504 may be obtained from various suppliers). Guard column: Lichrospher 60 RP select B (5mm) 4 x4 mm (Agilent part #79925 SB-504 may be obtained from various suppliers).
- 6.15 Extraction cartridge: "Focus" manufactured and supplied by Varian (part # Varian A5606050)

7. Standards Preparation:

- 7.1 Dilute the 1.0 mg/mL standards, obtained from the CDFA/CAC Standards Repository, with a solution of methanol and water (1:1). These standards shall be prepared to cover the linear range from at least 0.0125 η g/ μ L to 1.0 η g/ μ L
 - 7.2 Keep all standards in designated refrigerator for storage.
 - 7.3 The expiration date of each mixed working standard is six months from the preparation date.
8. Sample Preservation and Storage:
- All samples and sample extracts shall be stored in the refrigerator (0-5 ° C).
9. Test Sample Preparation:
- 9.1 Sample Preparation
 - 9.1.1 Remove samples from refrigerator and allow them to reach ambient temperature.
 - 9.1.2 Weigh 250 g of the sample into a 400mL beaker.
 - 9.1.3 Connect two Varian Focus cartridges with an airtight connector. Place the cartridge assembly on an extraction vacuum manifold. Condition the cartridges by eluting with one column of methanol followed by one column of water (do not allow the cartridge to go dry).
 - 9.1.4 Fill out both of cartridges with about 2 mL of DI water. Connect with Supelco Visiprep Vacuum Manifold tube adaptor to the top cartridge. Locate the end of Teflon tubing with stainless steel weight into the beaker with sample.
 - 9.1.5 Turn on the vacuum, allow the water sample to flow through the cartridge at a rate of 5-10 mL per minute.
 - 9.1.6 After the sample has eluted, dry cartridges under very delicate vacuum for about 10 second.

- 9.1.7 Reverse the cartridges. Put a labeled 15 mL conical test tube under the outlet of each channel.
- 9.1.8 Elute the sample to the test tube with 10 mL of solution of Methanol/Acetonitrile/2%Formic Acid in Water (60/30/10) into the test tube.
- 9.1.9 Evaporate the eluant in a water bath at $40\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ with a gentle stream of nitrogen to 0.5 mL. Adjust the sample final volume to 1.0 mL with 1:1 methanol/water. Mix well and transfer the extract to an auto sampler vial for analysis.

10 Instrument Calibration:

- 10.1 Composition of calibration mixed standards are Imidacloprid, Imidacloprid Olefin, Imidacloprid Quanidine, Imidacloprid Quanidine Olefin and Imidacloprid Urea.
- 10.2 It is difficult to obtain reproducible calibration standard curves for Imidacloprid Quanidine and Imidacloprid Quanidine Olefin. The detector responses to these two compounds varied with time. Therefore we use frequent calibration for the quantification of these two compounds. We use a standard before and a standard after each sample injection. This will ensure the quantification results.

11 Analysis:

11.1 Injection Scheme

Follow the sequence of a calibration standard, a matrix blank, a standard, a matrix spike, a standard, a test sample, a standard, etc.

11.2 HPLC Instrumentation

- 11.2.1 Finnigan' Surveyor MS pump and auto-sampler with column heater and remote control through Finnigan Xcalibur system.

11.2.2 Column: Lichrospher 60 RP select B (Phenomenex part # CHO-5308).

11.2.3 Guard column: Lichrospher 60 RP select B (5mm) 4 x4 mm (Agilent part #79925 SB-504)

11.2.4 Column Temperature: 40 °C

11.2.5 Mobile Phase: Gradient
Solvent A: 0.1% formic acid in methanol
Solvent B: 0.1 % formic acid in water
Flow rate: 0.75 mL/min
Gradient:

Time(min)	Flow rate (mL/min)	A	B	C	D
0	0.50	0	90	10	0
2	0.50	0	90	10	0
15	0.50	0	10	90	0
17	0.50	0	10	90	0
19	0.50	0	90	10	0
23	0.50	0	90	10	0

11.2.6 Injection Volume: 25 µL

11.2.7 Retention Time

Imidacloprid	8.9
Imidacloprid Olefin	8.5
Imidacloprid Urea	8.7
Imidacloprid Quanidine	5.8
Imidacloprid Quanidine Olefin	5.9

11.3 Mass spectrometer and Operating Parameter

Model: Finnigan Model TSQ Quantum mass spectrometer
Ion Source Type: Electrospray Ionization (ESI)
Source Polarity: Positive
Capillary Temperature: 200°C
Sheath Gas: 80
Auxiliary Gas: 9
Mode of Operation: MS/MS
Precursors and Product Ions:

Analyte	Precursors	Product Ion
Imidacloprid	256	175,209
Imidacloprid Olefin	254	171,205
Imidacloprid Urea	212	128
Imidacloprid Quanidine	211	126
Imidacloprid Quanidine Olefin	209	126

11.3.1 Operating parameter detail and Tune method are listed in Appendix 1 and Appendix 2

12. Quality Control:

- 12.1 Each set of samples shall have a matrix blank and minimum of one matrix spike sample. Each set contains up to 12 samples.
- 12.2 The matrix blank shall be free of target compounds.
- 12.3 The recoveries of the matrix spike should be within the control limits.
- 12.4 The retention time shall be within ± 20 seconds of that of the standard.
- 12.5 The sample must be diluted if results fall outside the linear range of the standard curve.
- 12.6 Bracketing standard response should have a percent change less than 20 % for all five compounds.
- 12.7 Method Detection Limits (MDL)

The method detection limit refers to the lowest concentration of analyte that a method can detect reliably. To determine the MDL, 7 replicate well water samples are spiked at 0.04ppb/0.24ppb. The response of imidacloprid olefin is much less than that of the other four compounds, the spike level is therefore higher. The standard deviation from the spiked sample recoveries are used to calculate the MDL for each analyte using the follow equation:

$$\text{MDL} = tS$$

Where t is the Student t test value for the 99% confidence level with $n-1$ degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the $n=7$ replicate used to determine the MDL, $t=3.143$.

12.8 Reporting limit (RL):

The reporting limit (RL) refers to the level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. Agreed upon per client agreement, the RL is chosen in a range 1-5 times the MDL.

MDL data and the RL are tabulated in Appendix 3 and 4.

12.9 Method Validation Recovery Data and Control Limits:

12.9.1 The method validation consisted of five sample sets. Each set included six levels of fortification (0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 ppb) and a method blank. A reagent blank shall be included when a new lot of solvent is used for extraction. All spikes, method blank and reagent blank samples were processed through the entire analytical method.

12.9.2 Upper and Lower warning and control limits are set at ± 2 and ± 3 standard deviations of the average % recovery, respectively.

12.9.3 Method validation results and control limits are tabulated in appendix 4.

13. Calculations:

13.1 The quantification is based on the sum of area counts of the product ion and the precursor of the compound analyzed. The calculation is based on external standard (ESTD).

13.2 The software LCQuan in the Xcalibur system is used to establish the standard curve and to calculate the analytes in the samples. The correlation coefficient, slope, intercept of the linear regression line are calculated once the calibration standards are defined. The equation for calculating analytes is as follows:

$$y = mx + b$$

Where: y = peak response

m = slope
b = intercept
x = concentration of compound

When the unit and the dilution factor are entered correctly in the analysis sequence, the software will then correctly generate the results.

- 13.3 Results can be manually calculated by a single point standard. The unit is ppb for all water samples. This calculation is to verify the results derived from the LCQuan

The general equation is as follows:

$$\text{ppb} = \frac{(\text{sample peak area}) (\text{std. conc. ng}/\mu\text{L}) (\text{std. vol. injected}) (\text{sample final vol., (mL)}) (1000 \mu\text{L}/\text{mL})}{(\text{std. peak area}) (\text{sample vol. injected}) (\text{sample wt., g})}$$

14. Reporting Procedure:

14.1 Perform Quantification with LCQuan:

14.1.1 Create a new Processing method

Open a raw file
Select calibration options
Identify components
Define calibration settings. Or

14.1.2 Open an existing quantification method and save to an appropriate sub-directory with a new name

14.1.3 Open the sequence and review the sequence. Or

14.1.4 Go to the appropriate sub-directory and select the raw files to be used as standards. Place the standard raw files to the appropriate calibration levels. Select the unknown raw files to be calculated.

14.1.5 Review the calibration

14.1.6 Review all calculated results

14.1.7 Create, review, and print peak integration report, calibration report, and summary report

14.2 Acceptance Criteria:

14.2.1 Peak retention time between standards, QC spikes and unknowns shall be within 20 seconds. If there is a known reason of retention time shifting, an explanation memo shall be included.

14.2.2 Peak response shall be within the calibration range

14.2.3 The R^2 of calibration curve or overlay calibration curves shall be greater than 0.990.

14.2.4 Recoveries of spike QC shall be within the established control range, otherwise a rerun or a re-extraction of the entire set shall be performed. If there is no sample left for extraction, an explanation memo shall be included.

14.2.5 The ratio of product ion and precursor ion between standard and unknown shall be consistent and the variation of the ratio between standard and unknown shall be within $\pm 20\%$.

14.2.6 Manual single point calculation result shall agree with the LCQuan result.

14.3 Reporting:

14.3.1 Sample results are reported out according to the client's analytical laboratory specification sheet.

14.3.2 Fill out COC, QC sheet, and control chart.

14.3.3 Prepare data package. Peer review. Report.

15 Discussion

15.1 This method is derived from the reference 16.1. A few modifications are made. One is to use the cartridge "focus" for the sample extraction. This small bed cartridge has very high capacity for both non-polar parent compounds and the polar metabolites. It is superior to the C-18 cartridge

used in the reference method. The other modification is to use external standard instead of the isotopic analytes as internal standards. Because the isotopic standards are not available to us.

- 15.2 I noticed that the responses of imidacloprid Guanidine and imidacloprid Olefin some time are not consistent. The detector response to these two compounds varies with time. The trend may be increasing or decreasing with time. It appears that the trend repeats from day to day. The reason of having this problem is unknown. My suggestion is to establish the trend by injecting combination standards continuous for two days. Then you may find a specific time frame during the day the response may increase or decrease. Pay extra attention to the data collected at the time of response change. In order to overcome this problem, a frequent calibration of the instrument response is needed. A standard before and a standard after each sample injection may be a good practice to ensure the quality of the quantification result.

16. References:

- 16.1 Billesbach, K.S.; Leimkuehler, W. M.; Widmer, S. L.; *"Analytical Method for the Determination of Imidacloprid and the Guanidine, Olefinic Guanidine, and Urea Metabolites in Groundwater by High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) "*, Bayer Corporation, Agriculture Division, Research and Development Department, P.O. Box 4913, Kansas City, Missouri 64120-001

APPENDIX I

Mass Spectrometer, TSQ Quantum, Operating Parameters

Creator: Paul Lee Last modified: 10/27/2003 by Paul Lee

MS Run Time (min): 15.00

TSQ MS Method Settings:

Segments:	1	2	3	4
Duration (min)	3.75	3.75	3.75	3.75
Scan Events	2	2	3	3

Segment 1:

Tune Method C:\Imidacloprid\Method\10-3 lower sheath gas to 10.TSQTune

Chrom filter: Not used

Q2 Gas Pressure: 1.2

Scan Events:

1: + c SRM,Source CID 6,

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
209.200	126.100	1.000	0.50	28	0.70	0.70	Tuned Value
209.200	90.200	1.000	0.50	40	0.70	0.70	Tuned Value
209.200	99.000	1.000	0.50	44	0.70	0.70	Tuned Value

2: + c SRM,Source CID 6,

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
211.200	126.200	1.000	0.50	32	0.70	0.70	Tuned Value
211.200	90.200	1.000	0.50	40	0.70	0.70	Tuned Value
211.200	99.000	1.000	0.50	46	0.70	0.70	Tuned Value

Segment 2:

Tune Method C:\Imidacloprid\Method\I-MIX TUNE 10-18-01 temp

350TSQTune.TSQTune

Chrom filter: Not used

Q2 Gas Pressure: 1.2

Scan Events:

1: + c SRM,Source CID 6,

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
209.200	126.100	1.000	0.50	28	0.70	0.70	Tuned Value
209.200	90.200	1.000	0.50	40	0.70	0.70	Tuned Value
209.200	99.000	1.000	0.50	44	0.70	0.70	Tuned Value

2: + c SRM,Source CID 6,

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
211.200	126.200	1.000	0.50	32	0.70	0.70	Tuned Value
211.200	90.200	1.000	0.50	40	0.70	0.70	Tuned Value
211.200	99.000	1.000	0.50	46	0.70	0.70	Tuned Value

Segment 3:

Tune Method C:\Imidacloprid\Method\I-MIX TUNE 10-18-01 temp
350TSQTune.TSQTune

Chrom filter: Not used

Q2 Gas Pressure: 1.2

Scan Events:

1: + c SIM Product Scan,Parent Mass 254.000,Collision Energy 30,
Center Width Time Q1 PW Q3 PW Tube Lens
205.000 1.000 0.50 1.00 1.00 Tuned Value
236.000 1.000 0.50 1.00 1.00 Tuned Value
171.000 1.000 0.50 1.00 1.00 Tuned Value

2: + c SRM,Source CID 6,
Parent Center Width Time CE Q1 PW Q3 PW Tube Lens
212.200 99.200 3.000 0.50 40 1.00 1.00 Tuned Value
212.200 128.200 3.000 0.50 26 1.00 1.00 Tuned Value

3: + c SRM,Source CID 6,
Parent Center Width Time CE Q1 PW Q3 PW Tube Lens
256.200 175.200 3.000 0.50 26 1.00 1.00 Tuned Value
256.200 209.200 3.000 0.50 26 1.00 1.00 Tuned Value

Segment 4:

Tune Method C:\Imidacloprid\Method\10-3 lower sheath gas to 10.TSQTune

Chrom filter: Not used

Q2 Gas Pressure: 1.2

Scan Events:

1: + c SIM Product Scan,Parent Mass 254.000,Collision Energy 30,
Center Width Time Q1 PW Q3 PW Tube Lens
205.000 1.000 0.50 1.00 1.00 Tuned Value
236.000 1.000 0.50 1.00 1.00 Tuned Value
171.000 1.000 0.50 1.00 1.00 Tuned Value

2: + c SRM,Source CID 6,
Parent Center Width Time CE Q1 PW Q3 PW Tube Lens
212.200 99.200 3.000 0.50 40 1.00 1.00 Tuned Value
212.200 128.200 3.000 0.50 26 1.00 1.00 Tuned Value

3: + c SRM,Source CID 6,
Parent Center Width Time CE Q1 PW Q3 PW Tube Lens
256.200 175.200 3.000 0.50 26 1.00 1.00 Tuned Value
256.200 209.200 3.000 0.50 26 1.00 1.00 Tuned Value

Syringe pump not in use

Divert Valve: in use during run

Divert Time (min) Valve State

=====

0.00	Inject \ Waste
4.00	Load \ Detector
11.31	Inject \ Waste

Appendix 2 Tune method

Two tune methods have been used. Tune method "10-3 lower sheath gas to10.TSQ Tune" is in segments 1, 4 and tune method "I-MIX TUNE 10-18-01 temp 350TSQ Tune.TSQ Tune" is in segments 2, 3. These two tune methods are identical except the Sheath Gas Pressure and Aux Gas Pressure. For "I-MIX TUNE 10-18-01 temp 350TSQ Tune.TSQ Tune" the Sheath Gas Pressure is 80 and the Aux Gas Pressure is 10. For "10-3 lower sheath gas to10.TSQ Tune" they are 1 and 0 respectively. The reason of having "10-3 lower sheath gas to10.TSQ Tune" is to conserve gas, because no data is collected in segments 1 and 4.

Calibration File Name C:\Xcalibur\methods\calib_08-11-03.TSQCalib
Tune File Name C:\Imidacloprid\Method\10-3 lower sheath gas to 10.TSQ Tune

Ion Source Type ESI
Auto Peak Width Setting YES

Positive Polarity

"Q1 MS"

Spray Voltage 4000
Sheath Gas Pressure 1
Aux Gas Pressure 0
Capillary Temperature 350
Capillary Offset 35
Tube Lens Offset [1] Mass 182.000 Value 65
Tube Lens Offset [2] Mass 508.000 Value 103
Tube Lens Offset [3] Mass 997.000 Value 175
Q0 Q00 RF Amplitude Mass 180.000 Value 119; Square Root
Q00 Offset -3.0
Q0 Offset -3.1
Lens 1-1 Offset -2.0
Lens 1-2 Offset [1] Mass 182.000 Value -33.3
Lens 1-2 Offset [2] Mass 508.000 Value -56.3
Lens 1-2 Offset [3] Mass 997.000 Value -90.8
Q1 DC Offset [1] Mass 182.000 Value -2.9
Q1 DC Offset [2] Mass 508.000 Value -3.1
Q1 DC Offset [3] Mass 997.000 Value -3.7
Q1 Resolution [Man][1] Mass 182.080 Value -21.67
Q1 Resolution [Man][2] Mass 508.210 Value -23.94
Q1 Resolution [Man][3] Mass 997.400 Value -27.64
Q1 Resolution [0.10][1] Mass 182.080 Value -20.75
Q1 Resolution [0.10][2] Mass 508.210 Value -22.98
Q1 Resolution [0.10][3] Mass 997.400 Value -26.58
Q1 Resolution [1.00][1] Mass 182.080 Value -11.62
Q1 Resolution [1.00][2] Mass 508.210 Value -13.38
Q1 Resolution [1.00][3] Mass 997.400 Value -15.97
Q1 Calibration [Man][1] Mass 182.080 Value -3.73

Q1 Calibration [Man][2] Mass 508.210 Value -3.63
Q1 Calibration [Man][3] Mass 997.400 Value -3.30
Q1 Calibration [0.10][1] Mass 182.080 Value -3.73
Q1 Calibration [0.10][2] Mass 508.210 Value -3.63
Q1 Calibration [0.10][3] Mass 997.400 Value -3.30
Q1 Calibration [1.00][1] Mass 182.080 Value -4.00
Q1 Calibration [1.00][2] Mass 508.210 Value -3.88
Q1 Calibration [1.00][3] Mass 997.400 Value -3.56
Lens 2-1 Offset [1] Mass 182.000 Value -7.0
Lens 2-1 Offset [2] Mass 508.000 Value -25.9
Lens 2-1 Offset [3] Mass 997.000 Value -97.5
Lens 2-2 Offset -225.0
Lens 2-3 Offset -15.0
Q2 RF Amplitude Mass 180.000 Value 310; Square Root
Collision Pressure 1.2
Q2 Offset -10
Lens 3-1 Offset -15.0
Lens 3-2 Offset -225.0
Lens 3-3 Offset -15.0
Q3 DC Offset -8.0
Q3 RF Amplitude Mass 180.000 Value 50; Ramp
Multiplier Gain 300000
Ion Flight Time [1] Mass 182.000 Value 31.52
Ion Flight Time [2] Mass 508.000 Value 25.63
Ion Flight Time [3] Mass 997.000 Value 17.39

"Q3 MS"
Spray Voltage 4000
Sheath Gas Pressure 1
Aux Gas Pressure0
Capillary Temperature 350
Capillary Offset 35
Tube Lens Offset [1] Mass 182.000 Value 104
Tube Lens Offset [2] Mass 508.000 Value 145
Tube Lens Offset [3] Mass 997.000 Value 206
Q0 Q00 RF Amplitude Mass 180.000 Value 119; Square Root
Q00 Offset -3.0
Q0 Offset -3.1
Lens 1-1 Offset -3.5
Lens 1-2 Offset -40.0
Q1 DC Offset -5.0
Q1 RF Amplitude Mass 180.000 Value 50; Ramp
Lens 2-1 Offset -15.0
Lens 2-2 Offset -225.0
Lens 2-3 Offset -15.0
Q2 RF Amplitude Mass 180.000 Value 310; Square Root
Collision Pressure 1.2
Q2 Offset -10
Lens 3-1 Offset -15.0

Lens 3-2 Offset -225.0
Lens 3-3 Offset [1] Mass 182.000 Value -11.7
Lens 3-3 Offset [2] Mass 508.000 Value -23.0
Lens 3-3 Offset [3] Mass 997.000 Value -96.0
Q3 DC Offset [1] Mass 182.000 Value -3.9
Q3 DC Offset [2] Mass 508.000 Value -4.9
Q3 DC Offset [3] Mass 997.000 Value -4.4
Q3 Resolution [Man][1] Mass 182.080 Value -18.89
Q3 Resolution [Man][2] Mass 508.210 Value -17.98
Q3 Resolution [Man][3] Mass 997.400 Value -16.09
Q3 Resolution [0.10][1] Mass 182.080 Value -17.97
Q3 Resolution [0.10][2] Mass 508.210 Value -17.06
Q3 Resolution [0.10][3] Mass 997.400 Value -15.09
Q3 Resolution [1.00][1] Mass 182.080 Value -8.75
Q3 Resolution [1.00][2] Mass 508.210 Value -7.87
Q3 Resolution [1.00][3] Mass 997.400 Value -5.07
Q3 Calibration [Man][1] Mass 182.080 Value -3.01
Q3 Calibration [Man][2] Mass 508.210 Value -0.84
Q3 Calibration [Man][3] Mass 997.400 Value 2.55
Q3 Calibration [0.10][1] Mass 182.080 Value -3.01
Q3 Calibration [0.10][2] Mass 508.210 Value -0.84
Q3 Calibration [0.10][3] Mass 997.400 Value 2.55
Q3 Calibration [1.00][1] Mass 182.080 Value -3.26
Q3 Calibration [1.00][2] Mass 508.210 Value -1.04
Q3 Calibration [1.00][3] Mass 997.400 Value 2.35
Multiplier Gain 300000
Ion Flight Time [1] Mass 182.000 Value 9.87
Ion Flight Time [2] Mass 508.000 Value 4.94
Ion Flight Time [3] Mass 997.000 Value 1.95

"MS/MS"

Multiplier Gain 2000000

Negative Polarity (omit)

Appendix 3

The Method Detection Limit (MDL) data

	Imidacloprid (spiked)		Imidacloprid Olefin (spiked)		Imidacloprid Urea (spiked)		Imidacloprid Quandinine (spiked)		Imidacloprid Quandinine Olefin (spiked)	
	0.04 ppb		0.24 ppb		0.04 ppb		0.04 ppb		0.04 ppb	
	ppb	%	ppb	%	ppb	%	ppb	%	ppb	%
MDL-spike1	0.034	85.2%	0.213	88.7%	0.031	77.1%	0.037	92.1%	0.026	66.1%
MDL-spike2	0.032	79.8%	0.199	82.7%	0.025	61.9%	0.031	78.5%	0.023	58.4%
MDL-spike3	0.039	98%	0.225	93.8%	0.026	64.4%	0.038	96.1%	0.029	73%
MDL-spike4	0.037	93.5%	0.211	87.8%	0.030	74.6%	0.036	89.0%	0.025	61.5%
MDL-spike5	0.037	93.0%	0.209	87.3%	0.028	71.0%	0.033	83.5%	0.024	59.5%
MDL-spike6	0.033	83.2%	0.191	79.7%	0.032	79.7%	0.030	75.6%	0.021	52.8%
MDL-spike7	0.041	101.7%	0.232	96.7%	0.033	81.7%	0.034	84.4%	0.026	64.8%
Average	0.036	90.6%	0.211	88.1%	0.029	72.9%	0.034	85.6%	0.025	62.3%
STDEV	0.0032	8.0%	0.0141	5.9%	0.0030	7.5%	0.0029	7.3%	0.0026	6.4%
MDL=3.143xSTDEV	0.0101		0.0443		0.00947		0.0092		0.0081	
RL	0.050		0.200		0.050		0.050		0.050	

Appendix 4
Method Validation Data for Imidacloprid and its metabolites

Analytes		Imidacloprid		Imidacloprid Olefin		Imidacloprid Urea		Imidacloprid Quanidine		Imidacloprid Quanidine Olefin	
		Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)
Spike Level	0.1 ppb	0.100	100.0	0.110	110.0	0.100	100.0	0.130	130.0	0.080	80.0
		0.098	98.0	0.073	73.0	0.080	80.0	0.089	89.0	0.093	93.0
		0.108	108.0	0.108	108.0	0.080	80.0	0.093	93.0	0.083	83.0
		0.134	134.0	0.130	130.0	0.077	77.0	0.120	120.0	0.086	86.0
		0.088	88.0	0.098	98.0	0.074	74.0	0.061	61.0	0.070	70.0
Spike Level	0.5 ppb	0.530	106.0	0.520	104.0	0.460	92.0	0.450	90.0	0.420	84.0
		0.614	122.8	0.557	111.4	0.491	98.2	0.674	134.8	0.555	111.0
		0.509	101.8	0.474	94.8	0.392	78.4	0.447	89.4	0.364	72.8
		0.523	104.6	0.496	99.2	0.365	73.0	0.462	92.4	0.333	66.6
		0.559	111.8	0.536	107.2	0.442	88.4	0.423	84.6	0.352	70.4
Spike Level	1.0 ppb	1.110	111.0	1.100	110.0	0.960	96.0	1.050	105.0	0.890	89.0
		1.224	122.4	1.162	116.2	0.807	80.7	1.253	125.3	1.139	113.9
		1.061	106.1	0.984	98.4	0.794	79.4	0.980	98.0	0.824	82.4
		1.111	111.1	1.068	106.8	0.780	78.0	0.958	95.8	0.743	74.3
		1.046	104.6	1.028	102.8	0.827	82.7	0.842	84.2	0.713	71.3
Spike Level	2.0 ppb	1.900	95.0	1.810	90.5	1.810	90.5	1.740	87.0	1.400	70.0
		1.980	99.0	1.840	92.0	1.670	83.5	2.160	108.0	1.760	88.0
		1.770	88.5	1.680	84.0	1.580	79.0	1.750	87.5	1.450	72.5
		2.190	109.5	2.080	104.0	1.880	94.0	2.050	102.5	1.590	79.5
		1.826	91.3	1.796	89.8	1.684	84.2	1.873	93.7	1.546	77.3
Spike Level	5.0 ppb	4.880	97.6	4.720	94.4	4.730	94.6	4.340	86.8	3.580	71.6
		5.110	102.2	4.850	97.0	4.430	88.6	5.650	113.0	4.670	93.4
		5.040	100.8	4.590	91.8	4.680	93.6	4.810	96.2	4.060	81.2
		5.610	112.2	5.140	102.8	5.100	102.0	5.550	111.0	4.520	90.4
		4.333	86.7	4.226	84.5	3.943	78.9	4.661	93.2	3.846	76.9
Spike Level	10.0 ppb	11.740	117.4	10.690	106.9	10.870	108.7	10.060	100.6	8.610	86.1
		9.560	95.6	8.890	88.9	8.540	85.4	10.610	106.1	8.850	88.5
		9.490	94.9	8.760	87.6	8.180	81.8	8.930	89.3	7.390	73.9

		9.720	97.2	9.410	94.1	9.060	90.6	9.720	97.2	9.310	93.1
		9.821	98.2	9.518	95.2	9.553	95.5	12.044	120.4	10.370	103.7
Average (%)			103.9		99.1		87.0		99.5		83.1
Standard deviation (stdev)			10.9		11.3		9.0		15.7		12.0
Upper Control Limit (+ 2 stdev)			125.7		121.7		105.0		131.0		107.0
Upper Warning Limit (+ 1 stdev)			114.8		110.4		96.0		115.2		95.1
Lower Control Limit (- 2 stdev)			82.0		76.6		68.9		68.0		59.2

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12/15/2008	Changed the approval names to Stephen Siegel and Elaine Wong

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