Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Imidacloprid

MRID #:   422563-84

Matrix: Soil

Analysis: HPLC/UV

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1.0 Summary

The method described herein is for the analysis of NTN 33893 in soil samples. It is based upon the method described in Mobay Report 99619 with modifications incorporated as a result of method development work performed at ABC Laboratories, Inc. Development and validation of this method was performed under the guidelines described in Mobay Protocol PR91004. This protocol was signed by Karen S. Cain, Study Director, on February 1, 1991.

The method involves extraction of NTN 33893 from soil matrix by refluxing and filtration, removal of co-extractives by various cleanup steps, and quantitation utilizing HPLC gradient chromatography with UV detection at 270 nm. The method as described is applicable to soil samples which are relatively free of plant organic matter such as roots. A flow diagram presenting the method is shown in Figure 1.

2.0 Experimental

2.1 Materials

2.1.1 General Glassware and Equipment

Auto sampler vials (1.5 mL)

Boiling flasks with 24/40 necks (250, 500, and 1000 mL)

Separatory funnels with Teflon® stopcocks (250 mL)

Büchner glazed porcelain filtration funnels (12.5 cm)

Graduated cylinders (10, 50, 100, 500, 1000, and 2000 mL)

Volumetric flasks (Class A; 10, 25, 100, and 1000 mL)

Volumetric pipets (Class A, various sizes as needed)

Hamilton syringe (500 μL, calibrated in 10 μL increments)

Centrifuge tubes (15 mL, graduated in 0.1 mL increments)

Glass powder funnels

Vacuum adapting tube (24/40 inner and outer joints)

Heating mantles with transformers

Magnetic stirrers

Magnetic stir bars (Teflon®, egg-shaped)

Reflux condensers (connected to cold water tap)

Filter/Degasser assembly with disposable 0.65 μ polyvinylidene difluoride filters

Rotary evaporators; equipped with a heated water bath, dry ice traps, and vacuum pump

Water aspirator

Vacuum gauge (0-30" Hg range)

Thermometer

2.1.2 Reagents and Consumable Supplies

The specific brands used for the method validation analyses are listed here. In general, while other brands having equivalent quality may be substituted, acceptable method performance would need to be demonstrated with the substituted items.

Acetonitrile, UV grade, Burdick & Jackson

Dichloromethane (DCM), High purity, Burdick & Jackson

Ethylene glycol, Baker Analyzed®, J. T. Baker

Filter disks, Acrodisc®, 13 mm PTFE, HPLC certified, 0.45μm, Gelman

Filter disks, Whatman GF/A (12.5 cm)

Filter paper, Whatman #5, 12.5 cm

In-line filter degasser, for aqueous solvents, 0.2 ,   ·tman

In-line filter degasser, for organic solvents, f·   ·nan

Methyl t-butyl ether (MTBE), High purity,   Jackson

Potassium carbonate, anhydrous, granular, Baker Analyzed®, J. T. Baker

Sodium chloride, Baker Analyzed®, J. T. Baker

Sodium phosphate, monobasic, monohydrate, crystal, Baker Analyzed®, J. T. Baker

Sodium sulfate (rinsed with dichloromethane prior to use), Baker Analyzed®, J. T. Baker

Water, reverse osmosis (prepared on site at ABC Laboratories) or bottled distilled

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Mixed reagents and solutions are prepared as described below:

20% MTBE in DCM: 200 mL of MTBE are mixed with 800 mL of DCM.

Potassium carbonate, 0.05 M: 13.82 g of K_2CO_3 are dissolved in RO water and brought to a final volume of 2 L.

Phosphate buffer mobile phase: 1.00 g of $NaH_2PO_4 \cdot H_2O$ are dissolved in 1 L of water which had previously been filtered/degassed through a 0.65 μ filter.

2.1.3 Analytical Standard

BAY NTN 33893 (1-[(6-chloro-3-pyridinyl)-methyl]-4,5-dihydro-N-nitro-$1H$-imidazol-2-amine) was provio·l by Mobay Corporation, where documentation regarding the characterization is maintained.

2.1.4 Gradient HPLC Equipment

HPLC Pumps (2), Shimadzu LC-6A pumps with high pressure gradient mixer

System controller, Shimadzu SCL-6A

Detector (UV), Shimadzu SPD-6A, set at 270 nm

Column heater, Timberline, set at 40°C

Autosampler, Shimadzu SIL-6A or SIL-6B

Guard column, 1.0 cm X 4.6 mm id LiChrospher® 60 RP - select B (5μ)

Analytical column, 12.5 cm X 4.6 mm id LiChrospher® 60 RP - select B (5μ)

2.1.5 Data Acquisition System

HP-1000 mini-computer, Hewlett Packard

HP-7550A graphics plotter, Hewlett Packard

Think-jet printer, Hewlett Packard

Laserjet III printer, Hewlett Packard

Computer Automated Laboratory System (CALS®) software, Beckman

Beckman Digimetry® MK-5 A-D converter

2.2   Preparation of Standards

2.2.1   Fortification Standards

The equivalent of 25.0 mg of BAY NTN 33893 (after correction for purity) is weighed into a 25-mL volumetric flask. The compound is dissolved in UV grade acetonitrile and brought to a final volume of 25.0 mL to produce a stock solution of 1000 μg/mL. The following dilutions were made to produce fortification standards of 0.400, 0.800, and 2.00 μg/mL:

Parent Soln. Conc. (μg/ml)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (μg/mL)
1000	1	25	40.0
40.0	10	100	4.00
40.0	5	100	2.00
40.0	2	100	0.800
40.0	1	100	0.400

2.2.2   Quantitation Standards

The stock 1000 μg/mL standard in acetonitrile, prepared as described previously, is used for the preparation of the 2.00 μg/mL (equivalent to 0.1 ppm, as explained in Section 2.3.3, #24) quantitation standard in water. A 2.00-mL aliquot of the 1000 μg/mL standard is transferred to a culture tube, 100 μL of ethylene glycol are added to the culture tube, and the acetonitrile is evaporated under a gentle stream of nitrogen. The contents of the culture tube are then quantitatively transferred to a 10-mL volumetric flask with reverse osmosis (RO) water and brought to volume to produce a standard of 200 μg/mL in water. One milliliter of this standard was pipetted into a 100-mL volumetric flask and brought to volume with water to produce the 2.00 μg/mL quantitation standard.

2.2.3   Linearity Curve Standards

For the linearity curve standards, an equivalent of 20.0 mg of NTN 33893 was dissolved in distilled water and brought to a final volume of 100 mL to produce a solution of 200 μg/mL. A linearity curve (solvent only) is prepared from the 200 μg/mL standard in water as follows:

Parent Soln. Conc. (µg/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (µg/mL)	Final Equivalent Conc. (ppm)
200	NA	NA	200	10.0
200	3	4	150	7.5
200	2	4	100	5.0
40.0	25	50	20.0	1.00
200	1	100	2.00	0.100
2.0	1	10	0.200	0.010
0.2	1	2	0.100	0.005

(The 40.0 µg/mL standard used for the preparation of the 20.0 µg/mL standard had been prepared previously by diluting 20 mL of the 200 µg/mL standard to a final volume of 100 mL with distilled water.)

A linearity curve in the presence of sample matrix is prepared by preparing standards in water at 2X or 4X the desired concentration and pipetting 1.00 mL of the 2X standard or 0.5 mL of the 4X standard into a control sample extract following nitrogen blow-down (see Step 18 of 2.3.2). Each sample is then brought to a final volume of 2.0 mL with RO water. A 400 µg/mL standard is prepared similarly to the 200 µg/mL standard as described in Section 2.2.2, except that a 4.0-mL, rather than 2.0-mL, aliquot is taken. Subsequent dilutions are prepared as follows:

Parent Soln. Conc. (µg/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (µg/mL)	Final Equivalent Conc. (ppm)
400	1	10	40.0	2.00
40.0	1	10	4.00	0.200
4.00	1	10	0.400	0.020

The linearity in matrix standards are prepared from the above standards as follows:

Initial Soln. Conc. (µg/mL)	Aliquot Vol. (mL)	Final Vol. (mL)	Final Conc. (µg/mL)	Final Equivalent Conc. (ppm)
40.0	1.0	2.0	20.0	1.00
4.00	1.0	2.0	2.00	0.100
4.00	0.5	2.0	1.00	0.050
0.400	1.0	2.0	0.200	0.010
0.400	0.5	2.0	0.100	0.005

2.3 Method (see Figure 1 for the flow diagram of the method)

2.3.1 Reflux Extraction

1. Accurately weigh 40.0 g of previously homogenized soil into a 500-mL boiling flask. Any standard fortifications are made at this point by pipetting an appropriate aliquot of NTN 33893 standard in acetonitrile onto the soil sample.

Care should be taken to disperse the aliquot over the surface of the homogenized soil.

2. A Teflon®-covered stir bar (egg-shaped) is added to the boiling flask, followed by 200 mL of acetonitrile. The mixture is heated to reflux and allowed to proceed for 7 hours.

3. The cooled mixture is filtered through a Büchner funnel lined with a 12.5-cm GF/A filter into a 1-L boiling flask, using water aspirator vacuum. The 500-mL boiling flask and filter cake are rinsed with 5 X 10 mL of acetonitrile.

4. The filtrate is reduced to a volume of about 5 mL on a rotary evaporator. A vacuum of 26.5" Hg and a water bath temperature raised from 25°-40°C over a period of 20 minutes was used.

Notes:

a. If the water bath is too warm (i.e. warmer than 25°C) when the filtrates are first placed on the rotary evaporators, excessive bumping may occur.

2.3.2 Liquid-Liquid Partition Cleanup Steps

5. The contents of the flask from Step 4 are quantitatively transferred with 2 X 20 mL of reverse osmosis (RO) water to a 250-mL separatory funnel.

6. Ten milliliters of saturated NaCl solution are added to the separatory funnel. The 1-liter flask is further rinsed with 2 X 25 mL of 20% methyl t-butyl ether/dichloromethane (MTBE/DCM), which are added to the separatory funnel.

7. The separatory funnel and contents are shaken for one minute and the phases are allowed to separate.

8. The lower organic phase from Step 7 is drained through a powder funnel, containing about a teaspoon of anhydrous sodium sulfate in a fluted Whatman #5 filter paper, into a 500-mL boiling flask.

9. The sodium sulfate is rinsed with 2 X 10 mL of 20% MTBE/DCM. The aqueous phase is discarded.

10. Ethylene glycol (100 μL) is added to the organic extract from Step 9. The contents are swirled to mix.

11. The mixture from Step 10 is rotary evaporated to the ethylene glycol keeper at 25°C and 20-22" of Hg vacuum.

12. The residue from Step 11 is transferred to a 250-mL separatory funnel with 2 X 50 mL of DCM.

13. The DCM from Step 12 is partitioned against 50 mL of 0.05 M potassium carbonate by shaking for one minute.

14. The lower DCM layer is drained into a 250-mL boiling flask through 15-20 g of prewashed anhydrous sodium sulfate supported in a powder funnel by a fluted Whatman #5 filter paper.

15. The sodium sulfate from Step 14 is rinsed with 2 X 10 mL of DCM. The aqueous phase is discarded.

16. Ethylene glycol (100 μL) is added to the 250-mL boiling flask and the contents swirled to mix.

17. The organic mixture in the 250-mL boiling flask is rotary evaporated to approximately 5 mL at 25°C and 20-22" Hg vacuum.

18. The DCM/ethylene glycol from Step 17 is quantitatively transferred to a 15-mL graduated centrifuge tube with 2 X 3 mL DCM rinses. The DCM is evaporated under a gentle stream of nitrogen until only the ethylene glycol remains.

19. The extract from Step 18 is brought to a final volume of 2 mL with RO water and mixed.

20. The extract from Step 19 is placed in a disposable syringe and forced through a 0.45 μ PTFE filter directly into 1.5-mL autosampler vial.

Note: It has been observed that soil extracts which are yellow in color are less colored after filtration and that some of the color is left in the filter. It appears that some of the co-extractives are partitioning onto the PTFE. Use of filters made of other materials could potentially result in less removal of co-extractives and hence "dirtier" chromatography.

2.3.3 HPLC Quantitation and Calculations

21. Sample extracts from Step 16 are analyzed by gradient HPLC under the following conditions:

Mobile Phase Gradient Program

A = filtered reverse osmosis water with 1.0 g NaH_2PO_4/liter
Pump A - Shimadzu LC-6A

B = filtered UV - grade acetonitrile\Pump B - Shimadzu LC-6A

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Flow rate:	1.5 ml/min throughout
Initial:	90%A / 10%B
Gradient 1:	linear from 0 to 20 min. to 75%A / 25%B
Gradient 2:	linear from 20 to 25 min. to 10%A / 90%B
Plateau:	from 25 to 39 min. at 10%A / 90%B
Gradient 3:	linear from 39 to 43 min. to 90%A / 10%B
Re-equilibrate:	from 43 to 50 min. at 90%A / 10%B
Autosampler:	Shimadzu SIL-6A
Controller:	Shimadzu SCL-6A
Injection Volume:	25 µl
Guard Column:	1.0 cm x 4.6 mm ID LiChrospher® 60 RP - select B (5µm)
Column:	12.5 cm x 4.6 mm ID LiChrospher® 60 RP - select B (5 µm)
Column Heater:	Timberline Temperature: 40 °C
Detector:	Shimadzu SPD-6A UV absorbance at 270 nm Response: standard 0.08 AUFS; 0.8 V/AU
Data Acquisition:	Beckman Computer Automated Laboratory System (CALS®) software via a Beckman Digimetry® MK-5 A-D converter and a Hewlett-Packard HP mini-computer.

Notes:

a. Under the conditions described above, the retention time for NTN 33893 is 8-10 minutes.

b. Peak broadening is usually an indication that the guard cartridge should be replaced.

c. In the event of peaks from co-extractives which are not resolved from the NTN 33893 peak of interest, a change in the column temperature should be the first modification employed in an attempt to resolve the peaks, assuming that the overlap is not due to peak broadening as described above in Note b.

d. Parameters other than temperature may be modified in order to achieve optimum chromatographic response and separation.

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22. Linearity of response is assessed by injecting a series of standards, beginning with the low concentrations and proceeding to the high concentrations. Data is acquired via Beckman Computer Automated Laboratory System (CALS®) software onto a Hewlett Packard HP-1000 mini-computer. Baselines are assigned by CALS® according to operator-specified parameters (see Appendix II for a detailed description of the data acquisition system and the parameter coding available). Computer-assigned baselines are examined visually by the operator and assessed for correctness. In the case of poorly-drawn baselines, appropriate parameters are changed and the chromatographic data re-evaluated. After achieving acceptable baselines, CALS® is used to determine peak heights and to plot peak height response vs. concentration. The linear regression coefficient, r, is calculated by CALS® for the series of standards injected. A correlation of 0.995 is considered acceptable linearity. However, visual inspection of the plotted curve may yield additional information. Linearity is assessed both in solvent and in matrix extract. Preparation of these standards and the concentrations used have been described previously in 2.2.3.

23. The injection sequence followed for sample analyses is injection of a standard (0.01 or 0.05 ppm equivalent for the method validation analyses and 0.10 ppm equivalent for treated sample analyses), followed by the injection of a sample, then injection of a standard, then a sample, and so on (the injection run is always started and ended with a standard injection).

24. Calculations:

   a. Ppm Residue Values

$$sample\ ppm = \frac{peak\ height\ (sample)}{Avg.\ peak\ height\ of\ bracketing\ 0.1\ ppm\ standards} \times 0.1\ ppm$$

   Example: ABC ID 39273-009, CA control + 0.05 ppm
   (Since this was a method validation analysis, the bracketing quantitation standards were equivalent to a sample concentration of 0.05 ppm.)

$$sample\ ppm = \frac{8.362}{(10.748 + 10.849)/2} \times 0.05\ ppm$$

   Sample ppm = 0.039

   A 2.0 µg/mL standard is equivalent to 0.1 ppm in the sample, as shown below:

$$0.1\ \mu g/g\ (ppm) \times \frac{40\ g}{2\ mL} = 2.0\ \mu g/ml$$

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b.   Recovery values

$$\text{\% Recovery} = \frac{ppm\ found - ppm\ in\ control}{ppm\ added} \times 100\%$$

Example: ABC ID 39272-009, MN control + 0.05 ppm

$$\text{\% recovery} = \frac{0.039 - 0.001\ ppm}{0.05\ ppm} \times 100\%$$

% Recovery = 76%

3.0   Method Validation

3.1   Method Validation Recoveries

Recoveries from method validation analyses of Georgia soil samples are summarized in Table I.   Recoveries ranged from 70-90%, with an average recovery of 84.0 ± 9.52%. Chromatograms for these analyses are located in Appendix I.   Recoveries from method validation analyses of Minnesota soil samples are summarized in Table II. Recoveries ranged from 70-90% with an average recovery of 77.8 ± 8.58%. Chromatograms for these analyses are located in Appendix II. Recoveries from method validation of California soil samples are summarized in Table III. Recoveries ranged from 60-88%, with an average recovery of 78.3 ± 12.6%. Chromatograms for these analyses are located in Appendix III.

3.2   Limit of Determination

Residues of NTN 33893 detected in the control soil samples used for the method validation analyses ranged from non-detectable to 0.001 ppm. The lowest fortification level for which recovery was demonstrated was 0.01 ppm. Therefore the limit of determination for this method is 0.01 ppm.

3.3   Linearity Checks

Linearity of response for NTN 33893 under the instrumental conditions described in 2.3.3 was determined in solvent (water) and sample extract matrix. Preparation of the linearity check standards is described in 2.2.3. Response in solvent was linear from 0.005 ppm to 10 ppm, with a correlation of 0.99986. The curve is presented graphically in Figure 2. Chromatograms are

located in Appendix IV. Response appeared to be linear from 0.005-10.0 ppm in soil extract matrix from all three sites. Because of the difficulty in evaluating linearity at the lower end of a linear regression curve when a relatively high (10.0 ppm) point is included, regression curves were also generated using only the lower five points. For the Georgia soil extracts, a correlation of 0.99997 was obtained with all six points and a correlation of 0.99995 was obtained using the lower five points (see Figure 3). For the Minnesota soil extracts, a correlation of 0.99982 was obtained with all six points and a correlation of 0.99995 was obtained using the lower five points (see Figure 4). For the California soil extracts, a correlation of 0.99991 was obtained with all six points and a correlation of 0.99989 was obtained using the lower five points (see Figure 5). Chromatograms for the Georgia, Minnesota, and California soil linearity injections are located in Appendices VI, VII, and VIII, respectively.

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