

Cover Sheet for

**ENVIRONMENTAL CHEMISTRY METHOD**

***Pesticide Name:*** Imidacloprid (NTN 33893)

***MRID #:*** 106637

***Matrix:*** Water

***Analysis:*** HPLC/UV

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If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at [flynt.elizabeth@epa.gov](mailto:flynt.elizabeth@epa.gov).

106637

Report Title

Analytical Method for the Determination of NTN 33893 in Water

Data Requirement

166-1 (Supplemental):  
Prospective Ground Water Monitoring

Completion Date

November 27, 1991

Performance Laboratory

Miles Inc.  
Agriculture Division  
Research and Development Department  
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Submitting

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Laboratory Project ID

N3881401

Miles Report No.

106637

Statement of Confidentiality

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA #10(d)(1)(A), (B) or (C).

Company:

Miles Inc.  
Agriculture Division  
Research and Development Department  
Environmental Research Section

Company Agent:

D. R. Flint  
D. R. Flint, Director

Date:

11-7-94

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**106637**

**Good Laboratory Practice Certification**

**The requirements do not apply to this report.**

106637

Certification of Authenticity

Signatures

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Summary

Per request by Dennis Edwards from EPA on 11/4/94, this report is prepared to submit to the Agency. The report describes the extraction procedure for NTN 33893 from water with methylene chloride. After the solvent evaporation, the sample extract is dissolved in acetonitrile and injected into a high performance liquid chromatograph equipped with an UV-detector. The concentration is then determined by comparison peak heights or peak areas of the sample with the peak heights or peak areas of the known standard solutions.

The limit of determination (LOD) for this method is 0.1 ppb ( $\mu\text{g/L}$ )

The method validation data is available at Miles Research Park in Stilwell, Kansas.

## DETERMINATION OF NTN 33893 IN WATER

1. Scope

- 1.1 Applicable to water samples containing 0.1 ppb or higher concentrations of NTN 33893.
- 1.2 Higher concentrations can be determined after diluting the sample extracts.

2. Principle

- 2.1 Small amounts of NTN 33893 can be extracted from water with methylene chloride and determined using a high performance liquid chromatograph.
- 2.2 Peak areas or peak heights of the sample and known standards are compared.

3. Reagents

- 3.1 Acetonitrile, Burdick and Jackson "Distilled in Glass", or equivalent
- 3.2 Methylene chloride, nanograde
- 3.3 NTN 33893 analytical standard of known percentage purity (P)
- 3.4 NTN 33893 standard solution, Weigh  $0.01 \pm 0.0001$  g ( $W_s$ ) of NTN 33893 analytical standard into a 100-mL volumetric flask. Dilute to volume with acetonitrile, stopper and mix thoroughly. This solution may be kept for one week only. Correct the amount weighed to obtain a 100% basis of NTN 33893 as follows:

$$\text{NTN 33893 (100\%)} = \frac{W_s \times P}{100}$$

- 3.5 Sodium sulfate, anhydrous, ACS
- 3.6 Water, HPLC grade.

4. Equipment

- 4.1 Integration system, Hewlett-Packard LAS Model 3350 or equivalent
- 4.2 Liquid chromatograph, Shimadzu LC-6A or equivalent, equipped with a Zorbax ODS 4.6 mm x 25-cm column (DuPont P.N. 880952-702) ID # F15295 and a UV detector capable of measuring absorbances at 270 nm.
- 4.3 Rotary vacuum evaporator, Buechi RE-11, or equivalent.

## 5. Procedure

### 5.1 Preparation of the standard solutions

- 1) Weigh 0.010 - 0.011  $\pm$  0.0001 g NTN 33893 analytical standard (Book-ref. 88R11-19, purity 95.9%), into a 100-ml volumetric flask. Dilute to volume with acetonitrile and mix.
- 2) Pipet 1 mL of the standard solution from Step 1 into a 50-ml volumetric flask, dilute to volume with acetonitrile and mix. This is the 2-ppm standard solution.
- 3) Pipet 1 mL of the standard solution from Step 1 into a 100-ml volumetric flask. Dilute to volume with acetonitrile and mix. This is the 1-ppm standard solution.
- 4) Pipet 25 mL of the 1-ppm standard solution from Step 3 into a 50-ml volumetric flask. Dilute to volume with acetonitrile and mix. This is the 0.5-ppm standard solution.
- 5) Pipet 5 mL of the 1-ppm standard solution from Step 3 into a 50-ml volumetric flask. Dilute to volume with acetonitrile and mix. This is the 0.1-ppm standard solution.
- 6) Using glass syringes, filter the solutions from Step 2 through Step 5 with 0.45- $\mu$  Acrodisc filters into sample vials. These are the standard solutions for the analytical run.

### 5.2 Preparation of the samples

- 1) Mix the sample and measure 500-ml into a 1-L separatory funnel.
- 2) Extract three times by vigorously shaking for 1 minute each time with 75-ml portions of methylene chloride.
- 3) Drain the methylene chloride layer into a 500-ml boiling flask through a funnel containing 4 to 5 grams of anhydrous sodium sulfate.
- 4) Strip off the methylene chloride on a roto evaporator using a water bath at room temperature. Leave 1 to 2 mL of methylene chloride in the flask.
- 5) Transfer the methylene chloride from the boiling flask into a 1/2-oz. glass bottle using a disposable pipet.
- 6) Add 2 to 3 mL of methylene chloride to the boiling flask and swirl to rinse the inner wall of the flask, then transfer into the same bottle as in Step 5.



## 2 Preparation of the samples (continued)

- 7) Remove the methylene chloride from the glass vial using a stream of nitrogen gas.
- 8) Pipet 0.5 mL of acetonitrile into the glass bottle, then seal with a polyseal cap. Rotate to dissolve residue.

## 5.3 Spiking procedure for concurrent recoveries

- 1) Weigh  $0.010 - 0.011 \pm 0.0001$  g of NTN 33893 analytical standard (Book-ref. 88R11-19, purity 95.9%), into a 100-mL volumetric flask. Dilute to volume with acetonitrile and mix.
- 2) Pipet 1 mL of standard solution from Step 1 into a 100 mL volumetric flask, dilute to volume with acetonitrile. Stopper and mix thoroughly.
- 3) Pipet 1 mL of standard solution from Step 2 into a 10 mL volumetric flask, dilute to volume with acetonitrile. Stopper and mix thoroughly.
- 4) Pipet 2.5 mL of spiking solution from Step 3 into a 500 mL volumetric flask, dilute to volume with HPLC water and mix to produce 0.5 ppb spike.
- 5) Concentrate and analyze the spiked samples with the method used for the samples.

## 5.4 Instrument

Set the following conditions on the instrument:

1. Absorbance, AUFS	0.002
2. Column Pressure, atm (approx)	220
3. Column Temperature, °C	Ambient
4. Flow, mL/min.	1.5
5. Injection Volume, $\mu$ L	20
6. Lamp	UV
7. Mobile phase acetonitrile/water	40:60
8. Wavelength, nm	270

## 6. Calculation

- 6.1 Use least squares curve fitting to generate the "best" line which can be used to calculate the corresponding concentration for a given peak height or peak area.
- 6.2 Determine the concentration (C<sub>ppm</sub>) corresponding to each sample peak area from the standard curve.
- 6.3 Calculate the amount of NTN 33893 in the sample:  
NTN 33893,  $\mu$ g/L =  $1000 \times \text{C}_{\text{ppm}} \times \text{Dilution factor}$

**7. Method Validation Summary**

- 7.1 Before each analysis, standard solutions of 0.1, 0.5, 1.0 and 2.0 ppm were injected to establish linearity and response.
- 7.2 The correlation coefficients for the standard curves were 0.999 or better.
- 7.3 The average recovery for fifteen samples spiked at 0.1, 0.2 and 0.5-ppb concentrations was 105 % with a relative standard deviation of 8.6 %.

WRITTEN BY: \_\_\_\_\_

Chemist II

APPROVED BY: \_\_\_\_\_

Manager, EAS

REASON TO ISSUE: New TM.

Ref.: 91-R-146 Pages 39-78

DATE: \_\_\_\_\_

Supersedes: None

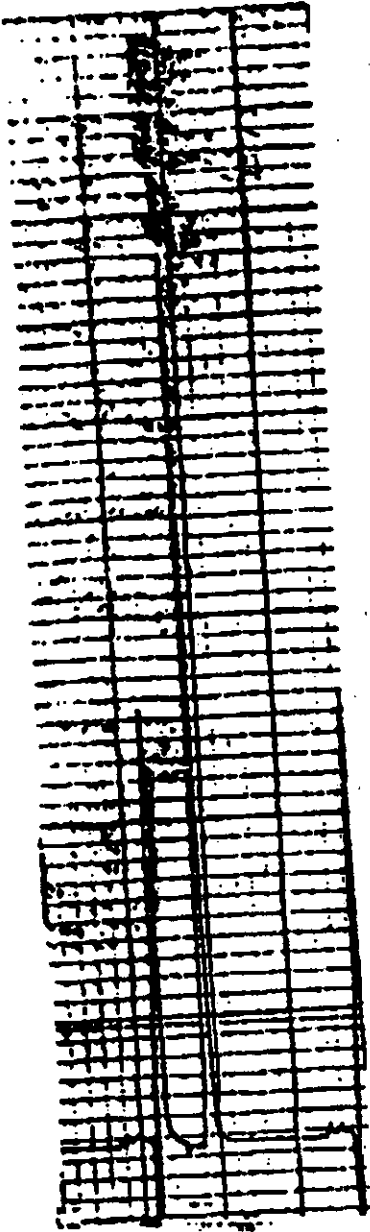


Figure 1: Chromatogram of a 1.0-ppm standard injection.

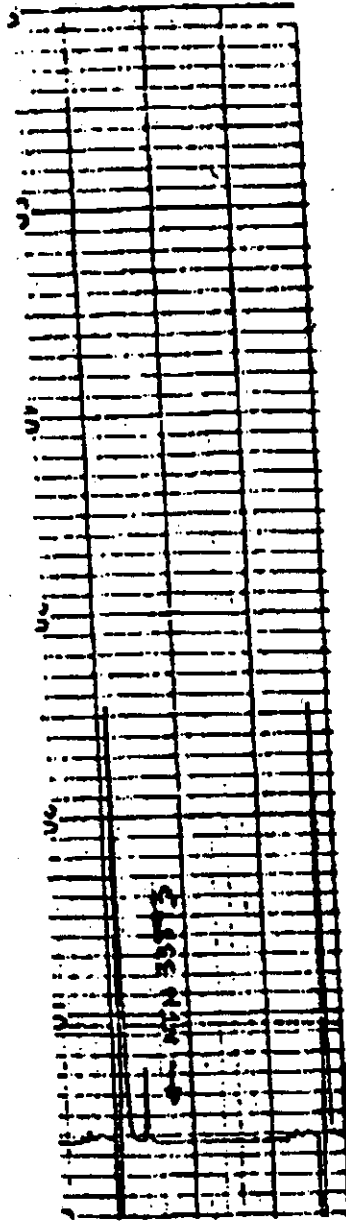


Figure 2: Chromatogram of a Blank.

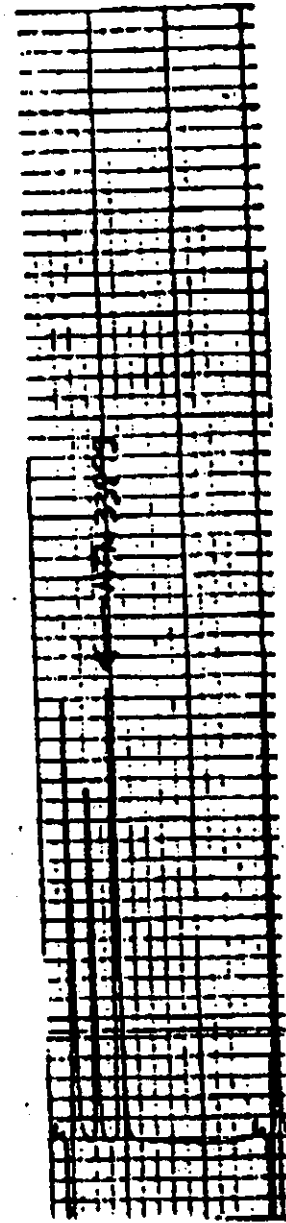


Figure 3: Chromatogram of a Sample.

AD40  
GENIE  
ESTD  
NO

106637

COMMENT & PLOT FIELDS

Comment 1 :  
Comment 2 :  
Comment 3 :  
Comment 4 :  
Start Time :  
Min Area :  
Enlarge :  
First/Last :

Stop Time :  
Max Area :  
Rt/PkNames :  
PrinterCode :

DATA INPUT

Run Time : 7.00  
Delay Start : 0.00  
Minimum Area : 500.0  
Shoulders : NO  
Solvent Slope : 1.0E+05  
# of Plates : 0.00

Number Of Peaks : 35  
Threshold : 2  
Peak Width : .04000  
Extended Peaks : NO  
Split Solvent : 2.0E+04

DATA ANALYSIS

Area Calc : YES

USER PROGRAMS

Post Run :  
Dig Prog :  
Para File :  
Overlap : YES

PEAK IDENTIFICATION

Abs Ref Wdw : .300  
Abs Non-Ref : 0.000  
Resp Factor Unkn : 1.00000  
Minimum Id Level : 0.0  
Update Ret Times : YES  
Use New Times : NO

% Ref Wdw : 0.000  
% Non-Ref : 5.000  
Suppress Unknown : NO  
Unknown Standard :  
% Update Times : 25.00  
Dead Volume Time : 0.00

TIMED EVENTS

	Time	Event	Value	S/ECH	Relay #	Update
1.	.01	NI				NO
2.	2.60	NI-				NO

CALIBRATION FILE

File Name :

CALIBRATION DATE

Date :

Count : 0

CALIB TABLE (PART 1)

	Time	Amount	Peak Name	Typ	Std	Grp	Id	Wdw
1.	2.96	1.000000	NTN33893					R

RESPONSE FACTORS

	Peak#	Peak Name	Factor
1.	1	NTN33893	1.00000

REPORT SPECIFICATION

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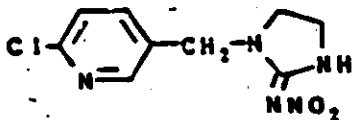
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Rpt File : /AGW/FDRMNT/NTN.FMT  
Rpt Units : AMOUNT

Appendix 4. Preparation of standard solutions.

MILES INCORPORATED  
 AGRICULTURE DIVISION  
 Pesticide Analysis Laboratory  
 PRIMARY STANDARDS CALCULATIONS INFORMATION SHEET

ALL STANDARDS MADE IN PARENT WITH 25000 EQUIVALENTS  
 PERFORMED TO ELIMINATE CORRECTION FACTORS TO BE APPLIED FOR METABOLITE RECOVERIES  
 BOTH FORTIFICATION AND INSTRUMENTAL STANDARDS DILUTED IN SAME MANNER

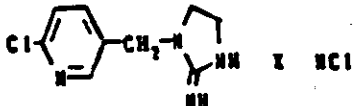
FORMULA WEIGHT PERCENT PURITY



WTK 33893 (IMIDACLOPRID)  
 K-325 (PT1701/88)  
 MILES INC., AGRICULTURE DIVISION

233.6 95.9

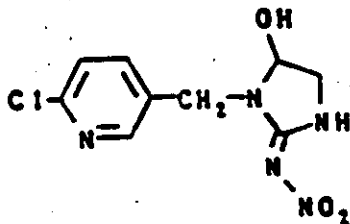
STANDARD DILUTION SOLVENT ACETONITRILE



WAK 4148--HYDROCHLORIDE (DEINITRO METABOLITE)  
 K-327 (REFERENCE SUBSTANCE NO. 888912/88)  
 BAYER PFLANZENSCHUTZ ZENTRUM

247.3 88.0

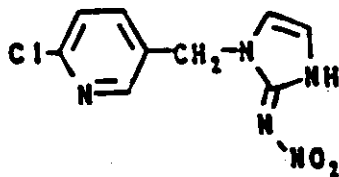
PRIMARY STANDARD DILUTION SOLVENT 90% WATER IN ACETONITRILE  
 SECONDARY STANDARD DILUTION SOLVENT ACETONITRILE



WAK 4103 (5--HYDROXY METABOLITE)  
 K-484 (REFERENCE SUBSTANCE NO. 829131/88)  
 BAYER PFLANZENSCHUTZ ZENTRUM

271.7 94.0

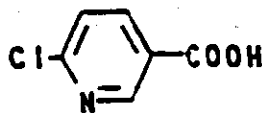
STANDARD DILUTION SOLVENT ACETONITRILE



WAK 3743 (OLEFIN METABOLITE)  
 K-385 (REFERENCE SUBSTANCE NO. 810302/88)  
 BAYER PFLANZENSCHUTZ ZENTRUM

233.7 97.8

STANDARD DILUTION SOLVENT ACETONITRILE



4-CHLOROPYRIDINE ACID  
 B-200 (LOT #0151301)  
 ALDRICH CHEMICAL CO.

157.8 98.0

STANDARD DILUTION SOLVENT ACETONITRILE

CALCULATION TO BE UTILIZED FOR AMOUNT OF NEAT STANDARD TO BE WEIGHED WHEN FINAL NOMINAL CONCENTRATION IS KNOWN:

$$G = \frac{A \times B \times X \times \frac{C}{233.6 \text{ GMOL}} \times \frac{100\%}{D} \times \frac{1 \text{ G}}{100000 \text{ UG}}}{\text{OR } \frac{A \times B \times X \times C}{D \times 25000 \text{ UGMOLS}}}$$

WHERE G = AMOUNT OF NEAT STANDARD TO BE WEIGHED OUT (G)  
 A = FINAL NEEDED SOLUTION CONCENTRATION IN PARENT EQUIVALENTS (UG/ML)  
 B = FINAL VOLUME OF SOLUTION (ML)  
 C = P.P. FORMULA WEIGHT OF NEAT STANDARD (GMOL)  
 D = PERCENT PURITY OF NEAT STANDARD (%)

EXAMPLE CALCULATION:  
 FOR DEINITRO STANDARD NEEDED TO BE 250 UG/ML, PARENT EQUIVALENTS WITH A 100 ML FINAL VOLUME.

$$G = \frac{250 \text{ UG/ML} \times 100 \text{ ML} \times 233.6 \text{ GMOL}}{95.9\% \times 25000 \text{ UGMOLS}} = 0.027108$$