

2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Bayer Analytical Method CA-001-S12-01 "An Analytical Method for the Determination of Residues of Carbaryl and 1-Naphthol in Soil and Sediment Using LC-MS/MS" as performed by ADPEN Laboratories, Inc.

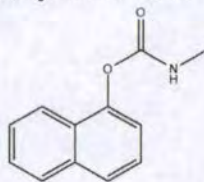
This study was designed to satisfy harmonized guideline requirements described in OCSPP 850.6100 (Data Reporting for Environmental Chemistry Methods) and Organization for Economic Co-Operation and Development (OECD), Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17. This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.⁴

3.0 MATERIALS AND METHODS

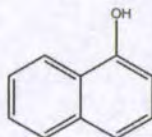
3.1 Reference Substances

The following reference substances were obtained from Bayer CropScience:

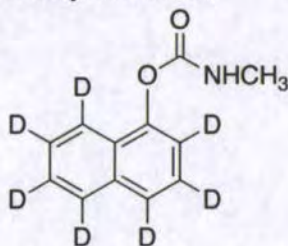
Common Name: Carbaryl
Chemical Name: 1-Naphthylenyl methylcarbamate
CAS Number: 63-25-2
Empirical Formula: C₁₂H₁₁NO₂
Standard Number: K-1748
Storage Conditions: Refrigerator
Purity: 99.8%
Expiration Date: 10/26/2019



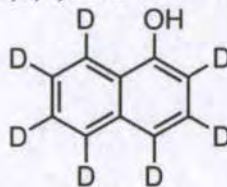
Common Name: 1-Naphthol
Chemical Name: 1-Naphtholenol
CAS Number: 90-15-3
Empirical Formula: C₁₀H₈O
Standard Number: K-1713
Storage Conditions: Freezer
Purity: 99.0%
Expiration Date: 05/14/2014



Common Name: Carbaryl-*d*₇
Chemical Name: 1-Naphthylenyl N-methylcarbamate
CAS Number: 362049-56-7
Empirical Formula: C₁₂H₄D₇NO₂
Standard Number: K-2034
Storage Conditions: Freezer
Purity: 99.0%
Expiration Date: 09/10/2015



Common Name: 1-Naphthol-*d*₇
Chemical Name: 1-Naphthol-2,3,4,5,6,7,8-D₇
CAS Number: 124251-84-9
Empirical Formula: C₁₀HD₇O
Standard Number: K-2035
Storage Conditions: Freezer
Purity: 99.0%
Expiration Date: 09/26/2015



Characterization data for the reference standards are maintained by the Sponsor, Bayer CropScience. The Certificate of Analyses is included in Appendix 3.

The reference substance (analytical standard) used in this study was procured from the Sponsor and stored as directed. All fortification and calibration solutions made from the reference substances (analytical standards) were stored according to the method.

3.2 Test System

The test system for this study was an untreated control (UTC) sandy loam soil (sample ID: 120913001-001), as it is representative of the matrix for which the method was designed.

The sample was obtained by ADPEN Laboratories, Inc. on 8/17/12. The sample was logged into LIMS and assigned a unique laboratory code, which is cross-referenced to the sample number on raw data and detailed residue reports. The sample was stored in freezer E24, which had a temperature range during storage of -27.5 to -17 °C. Sample extracts were stored in refrigerator E20 while awaiting LC/MS/MS analysis. The temperature range during the course of this study for this refrigerator was 6–8 °C.

The soil sample was characterized by AGVISE Laboratories of Northwood, North Dakota and reported on 11/3/2010. The GLP soil characterization report is presented in Appendix 4.

3.3 Analytical Procedures

Analytical Method CA-001-S12-01 was independently validated as written. The apparatus and reagents used for the method trial were as outlined in the analytical method, see Appendix 1, with equivalent apparatus or reagents substituted as necessary.

3.3.1 Fortifications

Untreated control soil samples were fortified using microliter amounts of the appropriate fortification standard at LOQ (10 ppb) and 10× LOQ (100 ppb) concentrations as per the method. Fortifications used in this method validation are below.

| Matrix | Fortification Vol. (μL) | Fortification Conc. ($\mu\text{g/mL}$) | Sample Wt. (g) | Final Conc. (ppb) | Replicates |
|--------|--------------------------------------|--|----------------|-------------------|------------|
| Soil | 100 | 0.5 | 5.0 ± 0.05 | 10 | 5 |
| | 100 | 5.0 | 5.0 ± 0.05 | 100 | 5 |

3.3.2 Extraction Procedure

1. Weigh 5 g of soil into a 50-mL conical tube (fortify samples, if needed).
2. Add 20 mL of 0.2% formic acid in HPLC water/acetone (50:50, v/v).
3. Place onto a platform shaker for 10 minutes.
4. After extraction, centrifuge for 10 minutes at 2500 RPM and decant supernatant into a 50-mL centrifuge tube.
5. Add 20 mL of HPLC water/acetonitrile (50:50, v/v).
6. Place onto a shaker for 1 minute.
7. After extraction, centrifuge for 10 minutes at 2500 RPM and decant supernatant into a 50-mL graduated centrifuge tube.
8. Add 40 μL of internal standard to the centrifuge tube and dilute to 40 mL with HPLC water/acetonitrile (50:50, v/v).
9. Place 5 mL of mixture into a 5-mL centrifuge tube and place into *N*-evap at 40 °C until volume is 2.5 mL.
10. Dilute to 5 mL with acetonitrile/HPLC water (20:80, v/v).
11. Filter an aliquot with 0.45 μm Nylon filter into a 2-mL amber HPLC vial for analysis by LC-MS/MS.

3.3.3 Modifications

Other than instrument parameter optimizations, no modifications were made to the analytical procedure.

3.4 Instrumentation

| | |
|--------------|---|
| LC System: | Agilent 1290 Infinity Series |
| MS Detector: | Agilent 6490 Triple Quadrupole LC-MS/MS |

| | |
|---------------------|---|
| Flow Rate: | 0.4 mL/min |
| Column: | Zorbax Eclipse; 1.83 μm , 50 \times 2.10 mm |
| Column temperature: | 60 °C |
| Injection Volume: | 10 μL |
| Run Time: | 11 minutes |
| Retention Times: | carbaryl: 4.65 min; 1-naphthol: 4.65 min |
| Mobile Phase A: | MeOH/HPLC water (10:90, v/v), 10 mM ammonium formate + 0.00012% formic acid |
| Mobile Phase B: | MeOH/HPLC water (90:10, v/v), 10 mM ammonium formate + 0.00012% formic acid |

Mass Spectrometer Conditions

| | |
|--------------------|----------|
| Interface: | ESI |
| Polarity: | Positive |
| Curtain gas: | 14 L/min |
| Temperature: | 150 °C |
| Capillary (V): | 3000 |
| V Charging: | 1500 |
| Nebulizer (psi): | 45 |
| Sheath gas heater: | 300 |
| Sheath gas flow: | 12 |

| <u>MRM Conditions</u> | <u>Carbaryl</u> | <u>Carbaryl-<i>d</i>₇</u> | <u>1-Naphthol</u> | <u>1-Naphthol-<i>d</i>₇</u> |
|------------------------------|------------------------|---|--------------------------|---|
| MS1: | 202.09 | 209.13 | 145.08 | 152.11 |
| MS2: | 145 | 152 | 127 | 133 |
| MS1 Resolution: | Wide | Wide | Wide | Wide |
| MS2 Resolution: | Wide | Wide | Wide | Wide |
| Dwell time: | 100 | 50 | 100 | 100 |
| Frag (V): | 380 | 380 | 380 | 380 |
| Collision Energy (V): | 4 | 8 | 24 | 24 |
| Cell Acc (V): | 5 | 5 | 5 | 5 |
| Polarity: | Positive | Positive | Positive | Positive |

3.5 Data Acquisition

Peak integration and peak area count quantitation were performed by MassHunter Quantitative Analysis (version B.04.01) data handling software. A best-fit, linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of the analyte. The square of correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99.

Statistical treatment of the data including the calculation of percent recovery, means, and standard deviations were calculated using Microsoft® Office Excel. Example calculations are presented in Appendix 5.

4.1 Method Establishment/Pre-Validation Evaluation

Prior to conducting the ILV, method control was established. Instrument parameters for the mass spectrometer detector were optimized by infusing standard solutions of the target analytes. The optimized instrument parameters, analyte retention times, instrument detection limits, and linearity were established by injecting a series of calibration standards. The UTC samples and reagent blanks were analyzed to determine if interferences were present near analyte retention times. These analyses verified that the method was in control and the UTC samples were free of interferences.

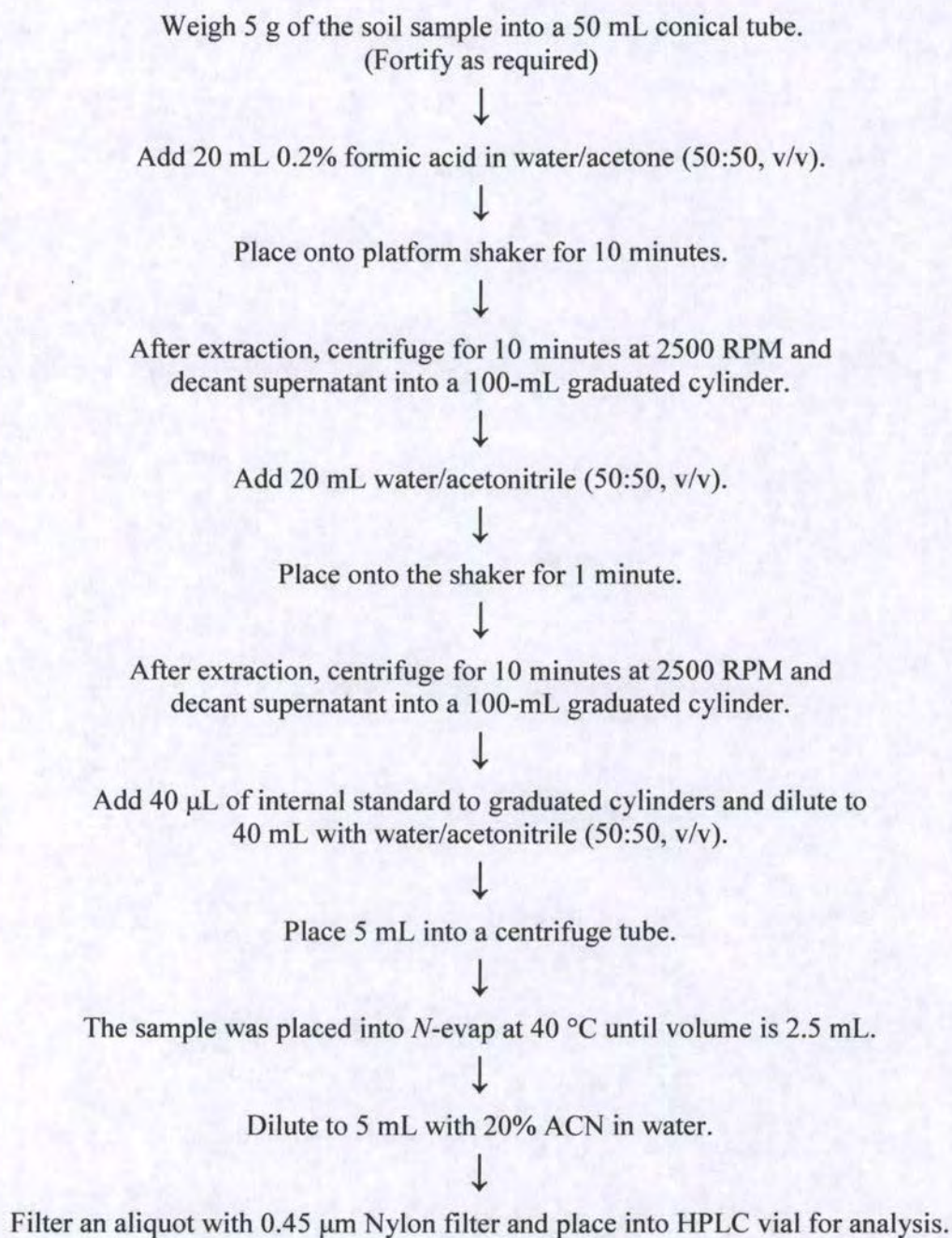
TABLE 1. Flow Diagram of the Analytical Procedure

TABLE 2. Soil Fortification Levels

| Sample Code | Fortification Level (ppb) | Number of Samples |
|---|---------------------------|-------------------|
| Analytical Set Using Sample 120913001-001 | | |
| 12112002-MB-1 | Method Blank | 1 |
| 121119001-001A, 121119001-001B | Control | 2 |
| 12112002-Recovery1-(1, 2, 3, 4, 5) | 10 | 5 |
| 12112002-Recovery2-(1, 2, 3, 4, 5) | 100 | 5 |

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1. SUMMARY

This method, which is based on analytical method R013916¹ is suitable for the determination of the residues of carbaryl and 1-naphthol in soil and sediment.

Residues of carbaryl and 1-naphthol are extracted from soil or sediment using a mixture of acetone/water (50/50 v/v) with 0.2% formic acid by shaking followed by a second extraction with acetonitrile/water (50/50 v/v). An isotopic internal standard is added to the sample which is evaporated to reduced volume and reconstituted in water. An aliquot transferred to a LC vial for analysis. The samples were analyzed for carbaryl and 1-naphthol by LC/MS/MS with quantification based on a comparison of peak areas with those of known standards.

The limit of quantitation (LOQ) for carbaryl and 1-naphthol in soil and sediment is 10ng/g. Additional recovery data was obtained on soil in study R013916¹.

2. BACKGROUND

Carbaryl is an insecticide developed by Bayer CropScience with uses in several crops.

This method is based on a previous method and validation study (Method Number R013916¹). This method includes modifications to the sample clean up and LC/MS/MS conditions. The revised method was assigned a method number of CA-001-S12-01.

The structures for these compounds are presented in Appendix 2.

3. PRINCIPLE

Residues of carbaryl and 1-naphthol are extracted from soil and sediment using an orbital shaker. A 5g sample aliquot is extracted with 50/50 acetone / water / 0.2% aqueous formic acid for 10 minutes on an orbital shaker. The sample is centrifuged for 10 minutes at ~2500 RPM and the supernatant is decanted into a graduated cylinder. An equal amount of 50/50 acetonitrile/water added to the sample, shaken on an orbital shaker for ~1 minute, and centrifuged at the same speed as above. The supernatant is decanted into the same graduated cylinder with the acetone/water. The sample is amended with an isotopic internal standard, a ~5mL aliquot is evaporated to ~2.5mL in a turbo-vap at 40°C, diluted to ~5mL with 20% ACN, and an aliquot analyzed by LC/MS/MS. Quantification of residues is based on the comparison of peak areas with those of known standards.

A flow-chart outlining the procedure summarizes the method in Appendix 4.

Additional summaries outlining the method parameters and method characteristics are presented in Tables 1 and 2.

4. APPARATUS

Use as a guide; equivalent apparatus may be substituted.

- Fisher® Brand volumetric graduated, disposable pipets (Assorted volumes)
- Rainin Microman® Classic positive-displacement pipettes (Assorted volumes)
- Assorted glass TC graduated cylinder
- VWR Pyrex® Brand volumetric flasks, glass class A (Assorted Volumes)

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- Fisher® Brand disposable Polyethylene Pasteur pipets (Cat. No. 13-711-717)
- National Scientific LC vials, Snap-Its (Cat. No.: C4011-5)
- National Scientific LC vial Snap-It Seals, (Cat. No.: C4011-55)
- BD 3ml Syringes (Cat. No. 309657)
- BD 50ml Polypropylene Conical Tube (Cat. No.: 352070)
- Gelman Nylon 0.45µm Filters (Part No. 4438)
- Phenomenex Luna™ 2.5µ C18(2) 50 x 2 mm Column (Part No.: 008-4446-BO)
- ABSciex TripleQuad 5500 LC/MS/MS System with Analyst Software Version 1.6.1 or higher
- Shimadzu LC-20AD XR pumps (two), column oven with a CBM 20A Communications Sub-Module
- CTC PAL HTC XT autosampler
- Analytical Balance with accuracy of ± 0.0001 grams for analytical standards
- HS501 IKA-Werke orbital shaker (Cat No. 2527001)
- Caliper TurboVap (Cat. No. 103198/2)
- Centrifuge tubes

5. REAGENTS

Use as a guide; equivalents or different manufactures (brands) may be substituted.

- Acetonitrile, Fisher Scientific, (Cat. No.: A996-4)
- Water Optima, Fisher Scientific, (Cat. No.: W7-4)
- Acetone Optima, Fisher Scientific, (Cat. No.: A929-4)
- Methanol, Fisher Scientific, (Cat. No.: A454-4)
- Formic Acid 99%, Acros, (Cat. No.: 14793-0010)
- Ammonium formate 99%, Fluka, (Cat. No.: 17843-50G)
- Certified analytical reference standards of carbaryl, 1-naphthol, d₇-carbaryl and d₇-1-naphthol

6. PREPARATION OF ANALYTICAL STANDARDS

NOTE: *The following procedure is an example description of how standard solutions may be prepared. Standards may be prepared as mixed solutions by dilution from individual stock solutions or prepared individually. Alternate or additional standards of appropriate weight and volume may be prepared as needed.*

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard solutions should be stored in amber glass bottles at or below 10°C when not in use. Solutions should be allowed to warm to room temperature prior to use.

6.1 Primary Stock Standard Solutions

Prepare individual 100 µg/mL stock solutions of carbaryl and 1-naphthol by placing 0.0050 grams of each analyte in separate 50 mL volumetric flasks. Dilute to volume with acetonitrile.

NOTE: Corrections for standard purities should be applied when expressing standard concentrations. For Example: If an analytical standard material has a purity of 98.5%, then 0.0102 grams (0.0100 g / 0.985) would be required to prepare a 100 µg/mL stock solution.

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The stock standard solutions are stable for a minimum of 6 months when stored in the dark at $\leq -18^{\circ}\text{C}$.

6.2 Fortification Standard Solutions

Prepare a stock 10 $\mu\text{g/mL}$ solution containing a mixture of carbaryl and 1-naphthol by taking a 5.0mL aliquot of each stock solution and diluting to 50 mL with acetonitrile.

Prepare a stock 100ng/mL solution containing a mixture of carbaryl and 1-naphthol by taking a 0.5 mL aliquot of 10 $\mu\text{g/mL}$ stock solution and diluting to 50mL with acetonitrile.

Prepare a 1.0ng/mL fortification solution containing a mixture of carbaryl and 1-naphthol by taking a 1.0mL aliquot of the 100ng/mL stock solution and diluting to 100mL with acetonitrile.

The fortification standard solutions are stable for a minimum of 6 months when stored in the dark at $\leq 4^{\circ}\text{C}$.

6.3 Isotopic Internal Standard Solutions

Prepare individual 100 $\mu\text{g/mL}$ stock solutions of d₇-carbaryl and d₇-1-naphthol by placing 0.0050 grams of each analyte into separate 50mL volumetric flasks. Dilute to volume with acetonitrile.

Prepare a 10 $\mu\text{g/mL}$ mixed internal standard solution containing a mixture of d₇-carbaryl and d₇-1-naphthol by taking a 0.50mL aliquot of the 100 $\mu\text{g/mL}$ stock internal standard solutions and diluting to 50mL with acetonitrile.

6.4 Calibration Standard Solutions

Prepare working calibration solutions consisting of carbaryl and 1-naphthol diluted to 50mL with 8% ACN in water.

Further calibration solutions may be prepared as needed.

| Concentration of Standard Solution used for dilution ($\mu\text{g/mL}$) | Concentration of Internal Standard Solution used for dilution ($\mu\text{g/mL}$) | Aliquot Native mix Taken (mL) | Aliquot Internal Standard Taken (mL) | Dilution Volume (mL) | Concentration of Calibration Solution (ng/mL) | Concentration of Internal Standard Solution (ng/mL) |
|---|--|-------------------------------|--------------------------------------|----------------------|---|---|
| 10.00 | 10.00 | 0.500 | 0.050 | 50 | 100.0 | 10.0 |
| 10.00 | 10.00 | 0.050 | 0.050 | 50 | 10.0 | 10.0 |
| 0.10 | 10.00 | 2.500 | 0.050 | 50 | 5.0 | 10.0 |
| 0.10 | 10.00 | 1.000 | 0.050 | 50 | 2.0 | 10.0 |
| 0.10 | 10.00 | 0.500 | 0.050 | 50 | 1.0 | 10.0 |
| 0.10 | 10.00 | 0.250 | 0.050 | 50 | 0.50 | 10.0 |
| 0.10 | 10.00 | 0.125 | 0.050 | 50 | 0.25 | 10.0 |

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7. SAMPLE PREPARATION

Note: Fortification experiments may be used for establishing and validating method efficiency as required.

7.1 Preparation of Soil Samples

- 7.1.1 Weigh 5.0 ± 0.1 g of each soil/sediment sample into a disposable 50mL conical tube. Fortify the recovery sample to the required concentration.
- 7.1.2. Add 20mL of a mixture of 50/50 acetone/water with 0.2% formic acid.
- 7.1.3. Place the conical tubes onto an orbital shaker. Shake for 10 minutes.
- 7.1.4. Centrifuge tubes for ~10 minutes at ~2500RPM
- 7.1.5. Decant supernatant into a suitably sized graduated cylinder.
- 7.1.6. Add ~20mL of a mixture of 50/50%v/v acetonitrile/water
- 7.1.7. Place the conical tubes onto orbital shaker. Shake for ~1 minute.
- 7.1.8. Centrifuge tubes for ~10 minutes at ~2500RPM
- 7.1.9. Decant supernatant into graduated cylinder.
- 7.1.10. Add 40 μ L of 10 μ g/mL mixed internal standard solution to the graduated cylinder and dilute to ~40mL with 50/50 acetonitrile/water. Stopper and mix well.
- 7.1.11. Transfer ~5mL of sample solution to centrifuge tube and dry with TurboVap at 40 °C to ~2.5mL
- 7.1.12. Add ~2.5mL of 20% ACN in water.
- 7.1.13. Transfer a portion of the sample solution into an HPLC vial for analysis. Filter with 0.45 μ m Nylon filters.

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8. LC/MS/MS ANALYSIS

8.1 Sample Analysis

Carbaryl and its metabolite 1-naphthol are analyzed by LC/MS/MS using isotopic internal standards.

Inject a 20 μ L aliquot of each test sample (or fortified sample matrix) from step 7.1.13 in Section 7.1 onto the LC/MS/MS under the conditions presented in Appendix I. Variations in equipment or sample characteristics may require different injection volumes or slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity.

It is often beneficial to make several 'priming' injections of standards and/or samples prior to starting the LC/MS/MS analysis. Typically 4 to 6 priming injections are made. The results from these injections are not included in any calculations used in residue determinations. These injections help stabilize the LC/MS/MS response prior to running the analytical set.

8.2 LC/MS/MS Standard Calibration

Standardize the LC/MS/MS response under the conditions outlined in Appendix 1 by injecting an aliquot of each LC/MS/MS calibration solution both before and after the sample solutions.

Carbaryl and its metabolite 1-naphthol are quantified using internal standard linear regression analysis. A separate calibration curve is produced for each set of samples analyzed on the LC/MS/MS. A calibration curve is generated by 1/x weighted linear regression of the ratio of standard peak/internal standard peak areas versus the standard concentrations in ng/mL using ABSciex Analyst Software (Version 1.6.1), a computer-programmed data capturing system. The Analyst Software uses the MS/MS standard responses to calculate the regression coefficients M and B, respectively called slope and intercept, for each analytical set.

The standards were fit to the linear equation: $Y = MX + B$

where: X is the concentration of the reference standard in ng/mL
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area:isotopic peak area ratio

The equation shown below is for the calculation of carbaryl residues, 1-naphthol residues are calculated in a similar fashion.

After regression coefficients were calculated, the residue in parts per billion was determined. The ng/mL (or ppb) of carbaryl in soil was calculated using the following equation,

$$\text{Carbaryl (ng/g)} = \frac{(Y - B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume (40mL)}}{\text{Initial sample wt.(5g)}} \times \frac{\text{Aliquot taken (5mL)}}{\text{Final volume (5mL)}}$$

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Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation:

$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where: R = ppb of target analyte found in fortified sample
S = ppb of target analyte found in control sample, real or apparent
T = theoretical ppb in fortified sample

Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ of 10ng/g in soil or other appropriate level with fortification solutions. Calculate the final residue R for the control (S) and fortified control (R) samples.

9. DISCUSSION

9.1 Time Considerations

A set of thirteen samples can be prepared for analysis in 3 to 4 hours. The samples are analyzed overnight and the data processed the following working day.

10. REFERENCES

1. Analytical Method R013916 for the Determination of Carbaryl in Soil Method Designation: Carbaryl – HPLC/Fluorescence soil, Union Carbide Agricultural Products Company, RTP, North Carolina, C.W. Davis and S.J. Thomas, August 23, 1985

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Table 1 Analytical Method Summary Parameters

| Analyte(s) | Carbaryl and 1-Naphthol |
|---------------------------------|--|
| Extraction solvent / Technique | -50/50 Acetone/Water -50/50 Acetonitrile/Water -Orbital shaker |
| Cleanup Strategies | None |
| Instrument Detector Column | -Two Shimadzu LC-20AD XR (High Pressure Mixer) pumps, a Shimadzu CBM-20A communications module and a CTC PAL HTC-XT autosampler - ABSciex Triple Quad 5500 MS/MS - Phenomenex Luna 2.5 μ C18(2) 50 x 2.00 mm Column |
| Standardization Method | Multi point calibration curve (Internal standard) |
| Stability of Standard Solutions | Stock standard solutions are stable for a minimum of 3 months when stored in the dark at $\leq -18^{\circ}\text{C}$ Fortification and calibration standard solutions are stable for a minimum of 3 months when stored in the dark at $\leq 6^{\circ}\text{C}$ |
| Retention times | Carbaryl (~4.7 minutes) and 1-Naphthol (~4.7 minutes) |

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Table 2 Characteristics for The Analytical Method Used For The Quantitation Of Carbaryl And 1-Naphthol Residues In Soil.

| Analyte | Carbaryl and 1-Naphthol |
|---------------------------------|--|
| Equipment ID | ABSciex Triple Quad 5500 LC/MS/MS, Shimadzu LC and CTC PAL autosampler |
| Limit of quantitation (LOQ) | 10 ppb |
| MDL - Method Detection Limit | 3.0 ppb |
| Reliability of the method (ILV) | Pending |
| Linearity | Detector response was linear within the range of 0.25 – 100.0ng/mL for both compounds. |
| Specificity | The analytical method employs a highly specific and selective detector (LC/MS/MS) |

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Appendix 1 Instrument Conditions

Equipment with equivalent or better sensitivity and performance may be substituted.

LC/MS/MS Parameters

NOTE: As the LC/MS/MS system is used over time, system components slowly and gradually become contaminated which in turn decreases system performance. The chromatographic response and/or peak shape of one or more of the analytical targets may be gradually affected over time. Therefore, the given LC/MS/MS parameters listed below are guidelines of where to start. Each instrument has its own unique personality. Variations in equipment or sample characteristics may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. These parameters should be optimized for the instrument and column actually used. Instrument parameters and mobile phase may be adjusted to improve separation from interfering peaks.

Acquisition Parameters

Note: The analyst should optimize chromatographic conditions to obtain satisfactory chromatography. The following recommended LC conditions were used:

HPLC System: Two Shimadzu LC-20AD XR (High Pressure Mixer) pumps with a Shimadzu CBM-20A communications module

Autosampler: CTC PAL HTC-XT autosampler

Solvents:

A) 10:90 Methanol/Water with 10 mM Ammonium Formate and 120 uL per 1000mL formic acid

B) 90:10 Methanol/Water with 10 mM Ammonium Formate and 120 uL per 1000mL formic acid

Oven temperature 60 °C

HPLC column: Phenomenex Luna C18 (2) 50 x 2mm 2.5µm particle size

Injection volume: 20µL

HPLC gradient program:

| Time (min) | % Solvent A | % Solvent B | Flow rate µL/min |
|------------|-------------------|-------------|------------------|
| 0.02 | 90 | 10 | 400 |
| 6.50 | 30 | 70 | 400 |
| 6.60 | 10 | 90 | 400 |
| 9.00 | 10 | 90 | 400 |
| 9.01 | 90 | 10 | 400 |
| 11.00 | System Controller | Stop | |

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Mass Spectroscopy – Instrument Parameters and Conditions

Note: The analyst should optimize the mass spectrometer conditions to obtain satisfactory system response. The following recommended conditions were used on an ABSciex 5500 with a Turbo Ion Spray (ESI).

| | |
|---------------------------|------------------------------------|
| Scanning mode: | Multiple Reaction Monitoring (MRM) |
| Mode: | Positive ion mode |
| Source temperature (TEM): | 300°C |
| Curtain Gas(CUR): | 35 psi |
| Ion Source Gas 1 (GS1): | 20 psi |
| Ion Source Gas 2 (GS2): | 15 psi |
| Ion Spray Voltage (IS): | 5500 |
| Interface Heater (ihe): | On |
| Collision Gas (CAD): | 7 psi |

MRM Table:

| <u>Compound</u> | <u>Q1 Mass (amu)</u> | <u>Q3 Mass (amu)</u> | <u>Dwell (msec)</u> | <u>Declustering Potential (DP)</u> | <u>Entrance Potential (EP)</u> | <u>Collision Energy (CE)</u> | <u>Collision Cell Exit Potential (CXP)</u> |
|--------------------------------|------------------------------|------------------------------|-------------------------|--|--|--------------------------------------|--|
| Carbaryl | 202 | 145 | 100 | 106 | 10 | 13 | 12 |
| Carbaryl Confirmatory MRM | 202 | 127 | 100 | 120 | 10 | 39 | 14 |
| d ₇ -Carbaryl | 209 | 152 | 100 | 106 | 10 | 13 | 12 |
| 1-Naphthol | 145 | 127 | 100 | 230 | 10 | 27 | 6 |
| 1-Naphthol Confirmatory MRM | 145 | 91 | 100 | 120 | 10 | 37 | 14 |
| d ₇ -1-Naphthol | 152 | 133 | 100 | 230 | 10 | 27 | 6 |

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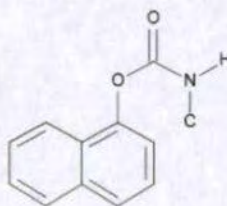
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Appendix 2 Structures

The structures for carbaryl, its metabolite 1-naphthol and the associated isotopic internal standards are presented below:

Carbaryl :

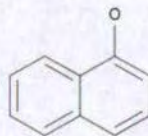
Structural formula:



| | |
|--------------------|--|
| Common name: | Carbaryl |
| CAS No.: | 63-25-2 |
| Chemical name: | 1-Naphthyl methylcarbamate |
| Empirical formula: | C ₁₂ H ₁₁ N O ₂ |
| Molecular weight: | 201.2 g/mol |

1-Naphthol:

Structural formula:



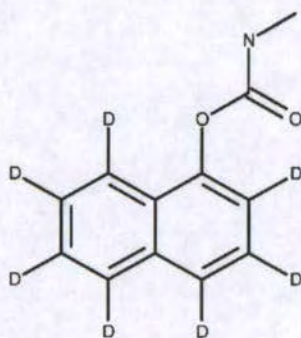
| | |
|--------------------|----------------------------------|
| Common Name: | 1-Naphthol |
| CAS No.: | 90-15-3 |
| Chemical name: | 1-Naphtholenol |
| Empirical formula: | C ₁₀ H ₈ O |
| Molecular weight: | 144.2 g/mol |

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d₇-Carbaryl:

Structural formula:



Common name:

d₇-Carbaryl

CAS No.:

362049-56-7

Chemical name:

1-Naphthyl N-methylcarbamate

Empirical formula:

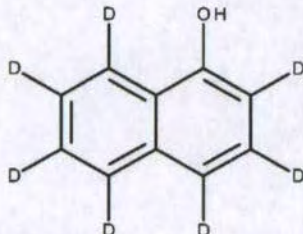
C₁₂ H₄ D₇ N O₂

Molecular weight:

208.26 g/mol

d₇-1-Naphthol:

Structural formula:



Common Name:

d₇-1-Naphthol

CAS No.:

124251-84-9

Chemical name:

1-NAPHTHOL-2,3,4,5,6,7,8-D7

Empirical formula:

C₁₀ H D₇ O

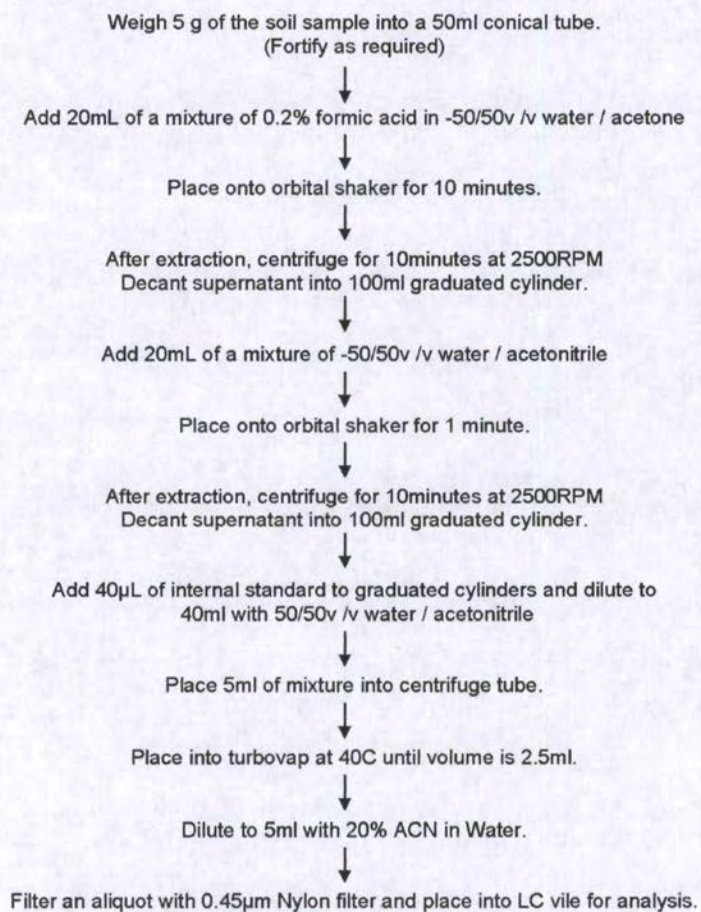
Molecular weight:

151.22 g/mol

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Appendix 4 Method Flow Chart



APPENDIX 5. Example Calculations

Residue results are calculated by comparison to the standard curves obtained from a linear regression analysis of the data found by the data system. The equation for the fit of the standard curve was used to calculate intercept and slope of the linear regression curve. The intercept and the slope were used in the equation used for quantitation. Excel is used to calculate the ppb and percent recovery and to present the data in a report format. The following equations were used for quantitation:

The following equations are used for residue calculations within Analyst:

a) Calibration curve: $y = mx + b$ Solving for x: $x = \frac{y - b}{m}$

Where,
 $m =$ slope
 $b =$ y intercept
 $x =$ Analyte found (pg)
 $y =$ Peak Area

The following equations are used for residue and recovery calculations within Excel:

b) $\text{mg of sample injected} = \frac{\text{injection size } (\mu\text{L})}{\text{final volume (mL)}} \times \text{sample wt (g)} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}}$

c) $\text{ppb} = \frac{\text{pg found}}{\text{mg injected}}$

d) $\text{Percent recovery} = \frac{(\text{ppbin the sample} - \text{ppbin the control})}{\text{ppbadded}} \times 100$

As an example, calculations to obtain the percent recovery in a control soil sample fortified with carbaryl in sample number 12112002-Recovery1-1. The calculations are shown below:

a) $\text{pg found} = \frac{18003 - (-0.003242)}{0.090332} = 12.98912 \text{ pg}$

b) $\text{mg of sample injected} = \frac{10 \mu\text{L}}{40 \text{ mL}} \times 5.04 \text{ g} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} = 1.26 \text{ mg}$

c) $\text{ppb} = \frac{12.98912 \text{ pg}}{1.26 \text{ mg}} = 10.308800 \text{ ppb}$

d) $\text{Percent recovery} = \frac{(10.308800 - 0.822195)}{10} \times 100 = 95\%$