1. SUMMARY

This report details a method of analysis for residues of Carbaryl in surface and ground water.

Residues of the test substance are extracted by a one-step solid-phase extraction (SPE) using C18 SPE cartridges. The sample is then quantitated by daughter-ion detection using liquid chromatography/mass spectrometry (LC/MS/MS) analysis. A reverse-phase high pressure liquid chromatography (HPLC) column is used to separate the compounds which are then quantified using the multiple reaction monitoring (MRM) mode. A turbo-ion interface (atmospheric pressure ionization) is used to introduce the HPLC eluant into the mass spectrometer.

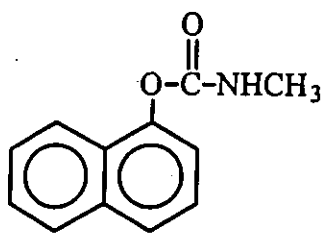
The proposed limit of quantitation (LOQ; the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for this method is 30 ppt or parts-per-trillion (0.03 ppb).

The smallest standard amount injected during the chromatographic run was equivalent to 10 ppt (0.01 ppb). The theoretical limit of detection (LOD), will be based on the signal to noise ratio and will be at least greater than 3 times level of noise.

2. EXPERIMENTAL COMPOUNDS

Molecular Structure of Carbaryl is given below.

CARBARYL (RPA 007744)



1-naphthyl methylcarbamate

CAS No. = 63-25-2
IUPAC Name = 1-naphthyl methylcarbamate

3.CHEMICALS AND SUPPLIES

3.1. CHEMICALS

Chemical	Grade	Source
Acetonitrile (ACN)	HPLC	J. T. Baker
Formic Acid, GR (98%)	Reagent	EM Science
Methanol (MeOH)	HPLC	J. T. Baker

Type I Water (in-house) from a Labconco® WaterPro™ work station (Type I water = electrical resistivity, minimum of 16.67 MΩ/cm at 25°C)

3.2. STANDARDS

Standard	Grade	Source	Catalog/model No.
RPA 007744	Analytical	RPAC	

3.3. EQUIPMENT AND SUPPLIES

Balance	Mettler	AT261& PG2002
Mega Bond Elut C18 (1g, 6 mL)	Varian	1225-6001
Vacuum Pump	Buchi	Gilford Instrument
Vacuum Manifold	Supelco	57250-U
Disposable borosilicate tubes(kimax 51 16X100mm)	VWR	60825-618

Standard Lab Equipment (graduated cylinders, pipettes, test tubes, volumetric flasks, etc.)

LC/MS/MS and HPLC systems are described in section 4.2.

Note: Equivalent materials may be substituted for those specified in this method if they can be shown to produce satisfactory results. However, the use of J.T. Baker HPLC grade Acetonitrile for the HPLC mobile phase is strongly recommended.

3.4. SOLUTIONS

(1) 0.1% formic acid in water is made by adding 1 mL formic acid to 999 mL type I water.

(2) 0.1% formic acid in acetonitrile is made by adding 1 mL formic acid to 999 mL of acetonitrile.

(3) 25% methanol is made by measuring 250 mL methanol and adjusting the volume to 1 L with type I water.

(4) 50% methanol is made by measuring 500 mL methanol and adjusting the volume to 1 L with type I water.

(5) 75% methanol is made by measuring 750 mL methanol and adjusting the volume to 1 L with type I water.

(6) 35% methanol is made by measuring 350 mL methanol and adjusting the volume to 1 L with type I water.

Note: The aforementioned examples are provided for guidance, alternative volumes may be prepared as long as the ratios of the solvent to solute ratios are maintained.

3.5. PREPARATION OF STANDARDS AND FORTIFICATION SOLUTIONS

Analytical standards are used for fortifying untreated samples to determine analytical recovery and to calibrate the response of the detector used in the analysis.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained.

3.5.1. Stock solution

To prepare a stock solution of 100 µg/mL, weigh out 10 mg of analytical standard (corrected for purity) and bring up to 100 mL with methanol in 100 mL volumetric flask. Store this stock solution in a freezer at ≤–10° C for a maximum period of 3 months from the date of preparation.

3.5.2. Fortification Solutions

To prepare a standard of 1 µg/mL, add 1 mL of the 100 µg/mL solution to a 100-mL volumetric flask and bring up to volume with 50% methanol in acidic water (0.1% formic acid in water). To make a 0.1 µg/mL standard, 10 mL of the 1 µg/mL solution is diluted to 100 mL with 50% methanol in acidic water (0.1% formic acid in water) in a volumetric flask. Seventy five microliters of this solution spiked into 25 mL of water sample is equivalent to 0.3 ppb (300 ppt). Similarly a 10-fold serial dilution of this standard can be made to yield a 0.01 ug/mL (10 ng/mL) solution. Seventy five microliters of the 10 ng/mL solution spiked in 25 mL of water is equivalent to 30 ppt (0.03 ppb).

Store all fortification standard solutions in a refrigerator at 2 to 6°C for a maximum period of 3 months from the date of preparation.

3.5.3. Calibration Standards

LC/MS/MS calibration standards are prepared in 35% methanol in water via dilution of the 10.0 ng/mL solution.

The following is a typical example: additional concentrations may be prepared as needed.

Initial Conc. (ng/mL)	Volume (mL)	Diluted to (mL)	Final Conc. (ng/mL)
10	10	100	1
1	50	100	0.5
0.5	50	100	0.25
1	10	100	0.1
0.5	10	100	0.05
0.25	10	100	0.025

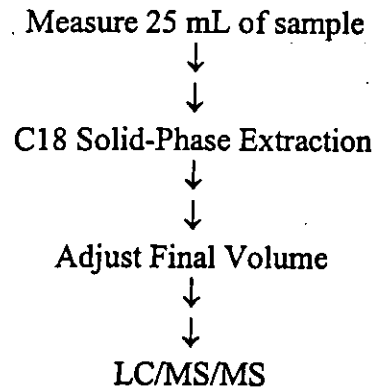
Store all calibration standard solutions in a refrigerator at 2 to 6 °C a maximum of 3 months from the date of preparation.

4. METHOD

4.1. FLOW DIAGRAM

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram



4.2. QUANTITATION

*Instrument:	PE SCIEX API 3000 Biomolecular Mass Analyzer SCIEX Turbo Ion Spray Liquid Introduction Interface Harvard infusion pump Switching Valve (VICI Valco Instruments)
	Temperature & Gas Flow Settings: Curtain gas flow (nitrogen) = ~ 1.7 L/min Nebulizer gas flow = ~ 1.5 L/min CAD gas setting = 4 Turbo Ion spray temperature = 350° C Auxillary gas flow = ~ 5.0 L/min
Computer:	Power Macintosh G3
Software:	Macintosh system 8.1 LC2Tune 1.4, Sample Control 1.4 Multiview 1.4, MacQuan 1.6
*HPLC Equipment:	Hewlett Packard Series 1100: Quatpump G1311A Vacuum Degasser G1322A Autoinjector G1313A Column Compartment G1316A
HPLC Column:	Luna C18(2), 4.6mm x 50mm, 3µ (Phenomenex)
Column Temperature:	35° C

Mobile Phase (A) : 0.1% formic acid in type I water
Mobile Phase (B) : 0.1% formic acid in acetonitrile

Time	% A	% B
0	65	35
6	30	70
6.1	0	100
7.1	0	100
7.5	65	35
9.0	65	35

Flow Rate: 1.0 mL/min
Injected Volume: 100 µL

Retention time of Carbaryl at above condition is about 3.2 min. Note that the retention time may vary from system to system. And may require optimization.

Ions monitored :

Analyte	Mode	Precursor Ion	Product Ion
Carbaryl	positive	202	145

*Note: An alternative LC/MS/MS system may be used, once demonstrated to be equivalent. The equivalent system should generate a signal to noise level of about 20:1 for a spike level at or below LOQ.

4.3. SAMPLE PROCESSING

No sample processing needed for water samples. However, samples should be allowed to reach to room temperature and mixed before being sampled for extraction. Any foreign object (eg. Leave limb, etc.) should be removed from sample before taking aliquot.

4.4. EXTRACTION

1. Measure 25 mL of sample (fortify as needed).
2. Condition Mega Bond Elut C18 SPE columns (1 g, 6 mL) by passing 10 mL methanol followed by 10 mL type I water (~ 2 drop/sec). Do not let column run dry.

NOTE: FOR THE FOLLOWING STEPS, MAINTAIN A ~1 DROP/ SEC FLOW RATE. DO NOT ALLOW THE COLUMN TO RUN DRY AT ANY TIME.

3. Load sample on conditioned C18 column. Discard eluate.
4. Wash with ~5 mL 25% methanol in water. Discard eluate.
5. Wash with ~5 mL 50% methanol in water. Discard eluate.
6. Elute with ~5 mL 75% methanol in water. Collect eluate in appropriate container.

7. Add ~ 5 mL type I water to adjust Final Volume to 10 mL. If the samples are cloudy, filter through 0.45 µm PTFE syringe filter.
8. Analyze sample using Turbo Ion Spray LC/MS/MS.

Standardization of C18 SPE columns: It may be necessary to standardize the C18 SPE columns in the following manner before analyzing samples:

A. Using a standard with a concentration between 0.01 and 0.1 µg/mL follow the elution schemes as outlined from steps 2 to 7. Collect all eluting fractions, including the pre-elution fraction, washes, as well as the target elution fraction.

B. Collect a post-elution fraction, after step 7, eluting with ~ 5 mL 100% methanol.

C. Adjust the final volume of all the fractions to 10 mL with water.

D. Analyze all the fractions by LC/MS/MS.

E. If the target fraction (fraction from step 6) contains a minimum of 85% of the respective analyte, it may be considered acceptable.

F. If the 85% recovery criterion for standard is not met, the analyst may adjust the elution scheme as follows:

For cases where the "wash" contains significant standard; either the "wash" volume or eluting solvent percentage may be decreased.

For cases where the "post-elution" fraction contains significant standard; the target elution volume may be increased or the eluting solvent percentage may be increased.

4.3 ANALYSIS BY LC/MS/MS

Inject 100 µL of each calibration standard in the range of 0.025 ng/mL up to 1 ng/mL into the LC/MS/MS (the upper standard limit may need to be set based on the linearity of the instrument response). Standard curves are generated using the MacQuan software system for each set of analysis. Injection of 5 to 6 standards is generally sufficient for the quantification of 10 to 12 samples.

Inject 100 µL of each sample/fortification/control into the LC/MS/MS. The concentration of each sample/fortification/control is determined from the standard curve, based on the peak area of each analyte.

If necessary, dilute the samples to give a response within the standard curve range.

4.6. TIME REQUIRED FOR ANALYSIS

Two sets of samples, each containing eight to ten samples, can be taken through the sample prep/extraction procedure in approximately 6 hours. Once the samples are prepared, LC/MS/MS analysis of the two sets will take approximately six hours.

5. CALCULATIONS

Calculate the amount of analyte found (in ng/mL, based on peak area) using the standard curve generated by the MacQuan software program using Equation 1.

Equation 1:

$$\text{Analyte found (ng/mL)} = \frac{\text{(peak area – intercept)}}{\text{slope}}$$

Calculate the total analyte found using equation 2.

Equation 2:

$$\text{Total analyte found (pg)} = \text{analyte found (ng/mL)} \times 1000 \times \text{total volume (mL)}$$

$$\text{Total volume (mL)} = \text{final volume (mL)} \times \text{dilution factor}$$

5.1. COMPONENT RESIDUE CONCENTRATION

Determine the component residue concentration using equation 3, as follows:

Equation 3:

$$\text{Residue found (ppt)} = \frac{\text{total analyte found (pg)}}{\text{initial sample volume (mL)}}$$

5.2. FORTIFICATION RECOVERY

For samples fortified with known amounts of analytes prior to extraction, calculate the percent recovery from Equation 4.

Equation 4:

$$\% \text{ recovery} = \frac{[\text{analyte found (ng/mL)} \times \text{total vol.(mL)}] - \text{analyte found in control (ng)}}{\text{ng spiked}} \times 100$$

OR

$$\frac{\text{analyte found (ng)} - \text{analyte found in control (ng)}}{\text{ng spiked}} \times 100 = \% \text{ Recovery}$$

6. SAFETY

There are no unusual hazards associated with this method. The analyst should read the material safety data sheets for all reagents before performing this method. Normal laboratory precautions should be taken.

Rhône-Poulenc Ag Company

Residue Study Protocol Number EC-99-451
Amendment 1

I. Study Identification

Study No.: EC-99-451
Title: Carbaryl: Validation of Method of Analysis of Carbaryl in Surface and Ground Water
Study Director: Sherry Movassaghi

II. Location of Change

1. Section 2.3, Experimental Procedure

III. Description of Change

1. Section 2.3, Experimental Procedure

Last line in this section stating "One surface water sample will be analyzed in the following steps (steps 2.3.1 through 2.3.2), will be changed to "One surface water sample will be analyzed through step 2.3.2).

Reason for Changes and Effect on Study

1. This was a typo. Section 2.3.1 only refers to analyzing reagent blanks and standard solutions.

The changes in this amendment clarify the protocol and therefore have a positive effect on the study.

I. Distribution

Study Director
Quality Assurance
Study Director Management
Enaksha Wickremesinhe, Centre Analytical Laboratories

II. Approval

Sherry Movassaghi — 3/12/99
Sherry Movassaghi
Study Director, Rhône-Poulenc Ag Company

3/12/99
Dean F. Bushey
Date
Study Director Management, Rhône-Poulenc Ag Company

3/15/99
Enaksha Wickremesinhe
Date
Central Analytical Laboratories, Inc.

Rhône-Poulenc Ag Company

Residue Study Protocol Number EC-99-451
Amendment 2

I. Study Identification

Study No.: EC-99-451
Title: Carbaryl: Validation of Method of Analysis of Carbaryl in Surface and Ground Water
Study Director: Sherry Movassaghi

II. Location of Change

1. Justification. Section.
2. Section 2.3.3: Ruggedness.

III. Description of Change

1. The test guidelines should be OPPTS 850.7100 instead of 850.700.

2. The first 2 line "Method ruggedness will be conducted by analyzing four different samples of surface water, two different samples of ground water and one sample of tap water", will change to "Method ruggedness will be conducted by analyzing four different samples of surface water, one sample of ground water and one sample of tap water (which is a ground water at the sampling location).

IV. Reason for Changes and Effect on Study

1. This was a typo.
2. Since the tap water at sampling location is a ground water, it will replace one of the ground water samples.

The changes in this amendment clarify the protocol and therefore have a positive effect on the study.

V. Distribution

Study Director
Quality Assurance
Study Director Management
Enaksha Wickremesinhe, Centre Analytical Laboratories