Determination of Carbamate Pesticides In Water Samples

1.0 Scope and Application

1.1 This is a modified EPA Method 632 and describes the sample preparation and quantitative analysis of trace level carbamate pesticides in surface, municipal and wastewater using liquid-liquid extraction and a liquid chromatography quadrapole system (LC-MSD) coupled to a diode array UV-Vis detector (DAD).

1.2 The estimated detection limit for each analyte is listed in Table 1. The actual MDL may differ from those listed, depending upon the nature of interferences in the sample matrix. Validation of the target analytes produced recoveries greater than 65 percent for most analytes.

Table 1. Carbamate pesticides analyzed, their Minimum Detection Limits (MDL) and Reporting Limits (RL) in water.

Target Analytes	MDL (µg/L)	RL (µg/L)
Aldicarb	0.010	0.020
Captan	0.050	0.100
Carbaryl	0.010	0.020
Carbofuran	0.010	0.020
Diuron	0.002	0.005
Linuron	0.002	0.005
Methiocarb	0.150	0.250
Methomyl	0.010	0.020

2.0 Summary of Method

2.1 A measured volume of sample (1000 ml) is extracted with methylene chloride (DCM) using a separatory funnel. The DCM extract is dried with sodium sulfate, concentrated and solvent exchanged by rotary evaporation and adjusted to 2.0 ml with acetonitrile. The extracts are analyzed by liquid chromatography using conditions which permit the separation and measurement of the target analytes in the extracts by MSD detection.

2.2 Interferences in analyses may be encountered in very dirty samples and cleanup may be needed to aid in the elimination or reduction of these interferences. Gel Permeation Chromatography (GPC) cleanup procedures will be followed.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause LC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4.

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination. Na_2SO_4 can be solvent rinsed to eliminate contaminants.

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. GPC cleanup procedure can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4.0 Apparatus and Laboratory Supplies

4.1 Separatory funnel. 2000-ml, with TFE-fluorocarbon stopcock, ground glass or TEF stopper.

4.2 Automatic rotating extractor designed to accommodate 2 liter separatory funnels with rpm, timer and emergency stop controls.

4.3 Beakers. Borosilicate glass, 400 mL

4.4 Graduated cylinder. 1000 ml, 250 mL and 100 mL.

4.5 Glass wool. Pyrex - solvent washed prior to use.

4.6 Evapotec rotary film evaporator.

4.7 GC vials. GC autosampler vials, borosilicate glass, 2 mL with PTFE-lined screw cap.

4.8 Analytical balance. Capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Disposable Pasteur Pipettes. 2 mL, rinsed with solvents before use.

4.11 Glass filter funnel. Fluted, 75 mm or larger.

4.12 Culture tubes. 13 x 100 mm with PTFE lined screw cap.

4.13 Analytical systems

4.13.1 LC-MSD chromatograph. Analysis was performed using an Agilent 1100 series LC-MS quadrapole system coupled to an Agilent 1100 series LC system consisting of a binary pump, diode array UV-Vis detector (DAD), autosampler, thermostated column compartment and vacuum degasser. The DAD was used to assist with method development, confirmation and troubleshooting. The MS was operated with atmospheric pressure electrospray ionization (API-ES) source in positive ion mode. Section 9 describes the acquisition and analysis procedures while Table 2 lists the operating parameters.

4.13.2 Data System. Hewlett-Packard, to collect and record GC data, generates reports, computes and records response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards and calculating in external standard mode.

Table 2 Operating parameters for Agilent 1100 LC-MSD

Chromatographic Conditions
- Column: Agilent Zorbax C-18 column, 15cm x 4.6mm i.d. x 5μm (or equivalent)
- Mobile phase A: water (5mM formic acid)
- Mobile phase B: acetonitrile (5mM formic acid)
- Pump parameters: gradient from 5% to 100% Acetonitrile in 25 min.
- Flow rate: 1.0 ml/min
- Run time: 35 minutes
- Column temperature: 38ºC
- Injection volume: 20 μL
- Diode array detector (DAD):

Signal, Bw (nm)		Reference, Bw (nm)	
254	16	400	8
245	16	400	8

MS Conditions: API-ES in positive ion mode
- Drying gas flow: 12 L/min
- Drying gas temperature: 350ºC
- Nebulizer gas pressure: 40 psig
- Capillary voltage: 3000
- Fragmentor voltage: 140
- Selected ion monitoring (SIM): multi-ions (see analytical method for details)
- Scan: m/z 100-350
- Threshold: 150 counts
- Gain: 2
- Step size: 0.1 amu
- Peak width: 0.1 min
- Time filter: On

5.0 Reagents, materials, gases and standards

5.1 Reagent water is defined as water in which an interferent is not observed at method detection limit of each parameter of interest. Deionized (DI) water was used for method validation and as method blank.

5.2 Petroleum ether (PE), acetone, acetonitrile, methylene chloride (DCM), diethyl ether, isooctane. Pesticide residue quality or equivalent.

5.3 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.4 Liquid Nitrogen. 230 psi or higher.

5.5 Stock standards. Individual stock standards (100 μg/ml) are purchased as certified solutions from Chem Service.

6.0 Sample Collection, Preservation, and Storage

6.1 Samples are collected in one liter amber glass bottles and iced or refrigerated at 4 °C from time of collection until extraction.

6.2 All samples must be extracted within 7 days of collection and analyzed within 40 days of extraction.

7.0 Sample Extraction

7.1 Remove water samples from refrigerator and transfer contents to a pre-cleaned 2-liter separatory funnel. For laboratory control spike (LCS) and matrix spikes (MS/MSD) add 1.0 ml of 200 ppb carbamate spiking solution.

7.2 Add 60 ml of methylene chloride (DCM) to the empty bottle, replace the cap and rinse the bottle. Pour the DCM into the separatory funnel and repeat with another 60 mL aliquot of DCM. Extract the sample for 5 minutes on the rotating extractor with periodic venting to release excess pressure. Allow organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 400 ml beaker.

7.3 Add a second 120 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the beaker.

7.4 Set up and label pre-cleaned round bottom flasks. Add 0.5 ml acetonitrile as “keeper”. Place a filter funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the round bottom flask. Add about two inches of solvent rinsed sodium sulfate to the funnel.

7.5 Pour the combined extracts from the beaker through sodium sulfate into the round bottom flask. Rinse the beaker with about 10 mL of DCM and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~10-20 mL).

7.6 Concentrate to almost dry with rotary evaporator in water bath (36ºC). Bring to final volume of 2 mL acetonitrile. Filter with 0.45 μm Gelman filter into vial.

8.0 Cleanup Procedure

8.1 Cleanup of dirty samples may be necessary due to interferences in the analysis of baseline or co-elution with target analytes of the sample extract. Follow the in-house SOP for GPC cleanup, as needed.

9.0 Analytical Procedure

9.1 The final extracts are analyzed on an Agilent 1100 LC-MSD. Conditions for operating the Agilent 1100 LC-MSD are found in Table 2.