EPA # 815-B-01-002

METHOD 531.2. MEASUREMENT OF N-METHYLCARBAMOYLOXIMES AND N-METHYLCARBAMATES IN WATER BY DIRECT AQUEOUS INJECTION HPLC WITH POSTCOLUMN DERIVATIZATION

Revision 1.0

September 2001

M.V. Bassett, S.C. Wendelken, and B.V. Pepich (IT Corporation)
D.J. Munch (US EPA, Office of Ground Water and Drinking Water)
L. Henry (American Water Works Service Company)

R.L. Graves - Method 531.1, Revision 3.0 (1989)

T. Engels (Battelle Columbus Laboratories)
D.J. Munch (US EPA, Office of Water) - National Pesticide Survey Method 5, Revision 2.0 (1987)

D.L. Forest - Method 531, Revision 1.0 (1985)

TECHNICAL SUPPORT CENTER
OFFICE OF GROUND WATER AND DRINKING WATER
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

MEASUREMENT OF N-METHYLCARBAMOYLOXIMES AND N-METHYLCARBAMATES IN WATER BY DIRECT AQUEOUS INJECTION HPLC WITH POSTCOLUMN DERIVATIZATION

1. SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of certain N-methylcarbamoyloximes and N-methylcarbamates in finished drinking waters. The following compounds can be determined using this method:

Analyte	Chemical Abstracts Service (CAS) Registry Number
Aldicarb	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbaryl	63-25-2
Carbofuran	1563-66-2
3-Hydroxycarbofuran	16655-82-6
Methiocarb	2032-65-7
Methomyl	16752-77-5
1-Naphthol	90-15-3
Oxamyl	23135-22-0
Propoxur	114-26-1

1.2 Detection Limits are compound, instrument, and matrix dependent. The Detection Limit is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[1] Experimentally determined Detection Limits for the above listed analytes are provided in Section 17, Tables 2 - 4. The Detection Limit differs from, and is lower than the Minimum Reporting Level (MRL) (Sect. 3.15). The concentration range for target analytes in this method was evaluated between 0.2 ug/L and 10 ug/L. Precision and accuracy data and sample holding time data are presented in Section 17, Tables 5 - 8.

1.3 This method is restricted to use by or under the supervision of analysts skilled in HPLC analysis and the interpretation of HPLC chromatograms.

2. SUMMARY OF METHOD

2.1 A water sample is filtered. Method analytes are chromatographically separated by injecting an aliquot (up to 1000 uL) into a high performance liquid chromatographic (HPLC) system equipped with a reversed phase (C_{18}) column. After elution from the column, the analytes are hydrolyzed in a postcolumn reaction with 0.075 N sodium hydroxide (NaOH) at 80 to 100 °C to form methyl amine. The methyl amine is reacted with o-phthalaldehyde (OPA) and 2-mercaptoethanol (or N,N-dimethyl-2-mercaptoethylamine) to form a highly fluorescent isoindole which is detected by a fluorescence detector.[2] Analytes are quantitated using the external standard technique.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A set of samples prepared and analyzed on the same instrument during a 24-hour period. An analysis batch begins with a Continuing Calibration Check (CCC) at or below the MRL. Subsequent CCCs are analyzed every 10 samples, should alternate between medium and high concentrations, and must end the analysis batch. An analysis batch is limited to 20 field samples. Laboratory Reagent Blanks (LRBs), Laboratory Fortified Sample Matrices (LFSMs), Laboratory Fortified Sample Matrix Duplicates (LFSMDs), Field Duplicates (FDs), and CCCs are not counted as samples. Required batch QC samples include: LRB, CCC, LFSM, and either a FD or a LFSMD.

If a sample(s) in an analytical batch needs to be reloaded for analysis within 48 hours of the start of the original analysis batch, it may be considered part of the original analysis batch. The reanalyzed sample(s) have the same QC requirements as above. However, reanalysis of the LFSM, and FD or LFMD are not required if they were already analyzed and passed QC requirements. Any newly prepared samples would require a full set of QC samples.

3.2 SURROGATE ANALYTE (SUR) – A pure analyte, which chemically resembles target analytes and is extremely unlikely to be found in any sample. This analyte is added to a sample aliquot in known amount(s) before filtration or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample.

3.3 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all

glassware, equipment, solvents and reagents, sample preservatives, and surrogates that are used in the analysis batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.4 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation compounds are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements. For this direct injection method, a LFB is the same as a Continuing Calibration Check standard (CCC - Sect. 3.12).

3.5 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – An aliquot of a preserved field sample to which known quantities of the method analytes are added in the laboratory. The LFSM is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFSM corrected for background concentrations.

3.6 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A second aliquot of the field sample used to prepare the LFSM, fortified, processed, and analyzed identically to the LFSM. The LFSMD is used instead of the Field Duplicate to access method precision when the occurrence of target analytes are low.

3.7 LABORATORY DUPLICATES (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.8 FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances, and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as laboratory procedures.

3.9 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.10 PRIMARY DILUTION STANDARD (PDS) SOLUTION – A solution containing the analytes prepared in the laboratory from stock standard solutions

and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution and/or stock standard solution, and the surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.12 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the method analytes and surrogate(s), which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.13 QUALITY CONTROL SAMPLE (QCS) – A sample prepared using a PDS of methods analytes that is obtained from a source external to the laboratory and different from the source of calibration standards. The second source PDS and the surrogate PDS are used to fortify the QCS at a known concentration. The QCS is used to check calibration standard integrity.

3.14 DETECTION LIMIT – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination of precision (Sect. 9.2.4), and accurate quantitation is not expected at this level. [1]

3.15 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest continuing calibration standard for that analyte and can only be used if acceptable quality control criteria for this standard are met.

3.16 MATERIAL SAFETY DATA SHEET (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

4. INTERFERENCES

4.1 All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by reagent water. A final rinse with solvents may be needed. In place of a solvent rinse, non-volumetric glassware can be heated in a muffle furnace at 400 °C for 2 hours. Volumetric glassware should not be heated above 120 °C.

4.2 Method interferences may be caused by contaminants, especially amines and ammonia, in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or

elevated baselines in the chromatograms. The samples or analytical system may be contaminated from being handled with bare fingers. All items such as these must be routinely demonstrated to be free from interferences (less than $^1/_3$ the MRL for each target) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9. **Subtracting blank values from sample results is not permitted.**

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; each chemical compound should be treated as a potential health hazard, and exposure to these chemicals should be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[3-5]

5.2 Pure standard materials and stock standards of these compounds should be handled with suitable protection to skin and eyes. Care should be taken not to breathe the vapors or ingest the materials.

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Brand names and/or catalog numbers are included for illustration only.)

6.1 SAMPLE CONTAINERS – Amber glass bottles fitted with PTFE (polytetrafluoroethylene) lined screw caps.

6.2 VIALS – Screw cap or crimp top glass autosampler vials with PTFE faced septa, amber or clear.

6.3 VOLUMETRIC FLASKS – Class A, various sizes used for preparation of standards.

6.4 GRADUATED CYLINDERS – Various sizes.

6.5 MICRO SYRINGES – Various sizes.

6.6 BALANCE – Analytical, capable of accurately weighing to 0.0001 g.

6.7 DISPOSABLE SYRINGES – 5 to 30 mL (B-D Cat.#: 309603, 309650 or equivalent) size, used to filter sample extracts before analysis.

6.8 FILTERS – Disposable filters used to filter samples before analysis (Millipore 0.22 um PVDF membrane, Cat. #: SLGV 013 NL or equivalent).

6.9 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) INSTRUMENTATION

6.9.1 HPLC SYSTEM – Capable of reproducibly injecting up to 1000-uL aliquots, and performing binary or ternary linear gradients at a constant flow rate near the flow rate used for development of this method, e.g., 1.5 mL/min. A ternary gradient was used to achieve the separation pictured in Figure 1. However, a binary gradient may be used as long as the minimum resolution is achieved as specified in Sect. 9.10. The use of a column heater is strongly recommended. During method development, the column was heated to 30 °C to help insure stable retention times. Column temperature may be adjusted as long as column performance is not compromised.

6.9.2 POSTCOLUMN REACTION SYSTEM – Capable of mixing reagents into the mobile phase. Reactor should be constructed using polyetheretherketone (PEEK) or PTFE tubing and equipped with two pumps capable of delivering 0.5 mL/min of each reagent; mixing tees; and two reaction coils. Postcolumn system manufacturers recommend different reaction coil temperatures for the carbamate hydrolysis reaction. Therefore, the first reaction coil temperature may range from 80 to 100 °C. Analyte signal can increase with temperature over this temperature range; however, analysts should be aware that with some systems, baseline noise can also increase with increasing temperatures. If temperatures greater than 95 °C are used, a slight backpressure must be maintained on the system to prevent boiling of the post column eluent. This may be accomplished by use of a restrictor placed in line after the detector. The second reaction takes place at ambient temperatures. Method performance data were collected with two postcolumn reaction modules: the Waters Postcolumn Carbamate System and the Pickering Model 5200 Carbamate System, both with a reactor temperature of 80 °C.

6.9.3 HPLC DETECTOR – A fluorescence detector capable of excitation at approximately 340 nm and detection of emission energy at approximately 465 nm. Optimum excitation and emission wavelengths may vary slightly for each system. For the development of this method, Waters Model 474 and Model 2475 scanning fluorescence detectors were used.

6.9.4 ANALYTICAL COLUMN – For the development of this method, an HPLC "carbamate" column (3.9 × 150 mm) packed with 4 um d_p C_{18} solid phase particles (Waters Cat. # : WAT035577) was used. Any column that

provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used.

6.9.5 HPLC DATA SYSTEM - A computerized data system is recommended for data acquisition and processing. A Waters Millennium software system was used to generate all data contained in the Section 17 tables.

7. REAGENTS AND STANDARDS

7.1 REAGENTS AND SOLVENTS – Reagent grade or better chemicals should be used. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination. Reagents of lesser purity may also affect instrument performance, such as clogging of the post column reactor.

7.1.1 REAGENT WATER – Purified water which does not contain any measurable quantities of any target analytes or interfering compounds greater than 1/3 the MRL for each compound of interest.

7.1.2 PRESERVED REAGENT WATER – Reagent water which has sample preservation reagents added in the same concentrations as the samples. To prepare 1 liter of preserved reagent water, add a sufficient amount of potassium dihydrogen citrate (Sect. 7.1.11.1) to yield a concentration of 9.2 to 9.5 g/L and sodium thiosulfate (Sect. 7.1.11.2) to yield a concentration in the range of 80 to 320 mg/L to a graduated bottle or volumetric flask. Fill to the 1 L mark with reagent water.

7.1.3 ACETONITRILE (CH_3CN, CAS#: 75-05-8) – High purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

7.1.4 METHANOL (CH_3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

7.1.5 SODIUM HYDROXIDE (NaOH, CAS#: 1310-73-2), 50% (w/w) solution (Fisher Cat. #: SS254-500 or equivalent). Due to the hygroscopic nature of NaOH pellets, a 50% (w/w) solution is used in this method for the preparation of reagents.

7.1.6 POSTCOLUMN REAGENT #1 (hydrolysis solution) – Sodium hydroxide, 0.075 N. Dilute 4 mL of 50% w/w sodium hydroxide (NaOH)

solution to 1 L with reagent water. The concentration of the hydrolysis solution can dramatically effect the analyte response. Filter and degas with helium just before use.

7.1.7 PREPARATION OF THE OPA SOLUTION (to be used in preparation of Postcolumn Reagent #2, Sect. 7.1.10) – Dissolve 100 $\pm$ 10 mg of o-phthaldehyde (OPA, CAS#: 643-79-8) in 5 - 10 mL of methanol.

7.1.8 PREPARATION OF THE OPA DILUTENT (to be used in preparation of Postcolumn Reagent #2, Sect. 7.1.10) – Use one of the following formulations in the preparation of the preliminary OPA dilutent. Performance data presented in Section 17 were collected using the sodium tetraborate formulation. Method performance was checked with the boric acid formulation and found to be equivalent. OPA dilutent is commercially available.

7.1.8.1 Prepared using sodium tetraborate : Dissolve 19.1 g of sodium tetraborate decahydrate ($Na_2B_4O_7 \bullet 10H_2O$, CAS#: 1303-96-4) in a 1 L volumetric flask. Bring the volume up to 1.0 L with reagent water to make a 0.05 M solution. The sodium borate will dissolve in less than 2 hours if a stir bar is used. Filter and degas prior to preparation of Postcolumn Reagent #2 (Sect. 7.1.10).

OR

7.1.8.2 Prepared using boric acid: Dissolve 3.0 g of boric acid (H_3BO_3, CAS#: 10043-35-3) in a 1 L volumetric flask in approximately 800 mL of reagent water. Add 1.2 mL of a 50% (w/w) NaOH solution. Bring the volume up to 1.0 L with reagent water. Filter and degas prior to preparation of Postcolumn Reagent #2 (Sect. 7.1.10).

7.1.9 PREPARATION OF THE NUCLEOPHILIC SOLUTION (to be used in preparation of Postcolumn Reagent #2, Sect. 7.1.10) – Use one of the following formulations in the preparation of the nucleophilic solution. Either one of these compounds reacts with OPA and the target methylamine to form the isoindole detected by the fluorescence detector. Both reagents have characteristic strong odors and should be handled in a fume hood.

7.1.9.1 Prepared using 2-mercaptoethanol (CAS#: 60-24-2): This compound is in liquid form and may be used directly in the preparation of the derivatization solution, Postcolumn Reagent #2 (Sect 7.1.10). Use 1 mL of 2-mercaptoethanol per liter of Postcolumn Reagent #2.

OR

7.9.1.2 Prepared using N,N-dimethyl-2-mercaptoethylamine-

hydrochloride (CAS#: 13242-44-9): Use approximately 10 mL of OPA dilutent (Sect. 7.1.8) to dissolve 2.0 ± 0.2 g of N,N-dimethyl-2-mercaptoethylamine-hydrochloride.

7.1.10 FINAL PREPARATION OF POSTCOLUMN REAGENT #2 (OPA derivatization solution), – Studies have shown that this reagent made with either of the two nucleophiles listed above (Sect. 7.1.9) is stable for a period of at least 36 hours. However, individual laboratory conditions vary and daily preparation of this solution may be necessary.

7.1.10.1 Add the dissolved OPA (Sect. 7.1.7) to 1 L of either formulation of the OPA dilutent (Sect. 7.1.8.1 **or** 7.1.8.2), which has been filtered and degassed.

7.1.10.2 To the 1 liter flask, add either one of the 2 nucleophiles specified in Section 7.1.9 (7.1.9.1 **or** 7.1.9.2).

7.1.11 SAMPLE PRESERVATION REAGENTS (See also Sect. 8.1.1)

7.1.11.1 Potassium dihydrogen citrate ($C_6H_7KO_7$, CAS #: 866-83-1) – Added to adjust sample pH to ~ 3.8, which acts as a biocide to guard against potential degradation of method analytes by microorganisms and to prevent chemical degradation. Oxamyl, 3-hydroxycarbofuran, carbaryl, and methiocarb all hydrolyze in neutral or basic waters even when held at refrigerated temperatures.

7.1.11.2 Sodium thiosulfate ($Na_2S_2O_3$, CAS#: 7772-98-7) – Acts as a dechlorinating agent. Aldicarb and methiocarb are rapidly degraded in waters that are not dechlorinated.

7.2 STANDARD SOLUTIONS – Standard Solutions may be prepared from certified, commercially available solutions or from neat compounds. Compounds used to prepare solutions must be 96% pure or greater and the weight may be used without correction for purity to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of organic solvent to aqueous samples. **Even though stability times for standard solutions are suggested in the following sections, laboratories should use standard QC practices to determine when Standard Solutions described in this section need to be replaced.**

7.2.1 SURROGATE ANALYTE (SUR) STANDARD SOLUTION, 4-BROMO-3,5-DIMETHYLPHENYL N-METHYLCARBAMATE (BDMC, CAS #: 672-99-1)

7.2.1.1 SUR STOCK SOLUTION – May be purchased as certified standard or prepared from neat material. If preparing from neat material: accurately weigh approximately 25 to 35 mg of the neat SUR to the nearest 0.1 mg into a tared, 5-mL volumetric flask. Dilute to the mark with methanol. Stock solutions have been shown to be stable for 12 months when stored at -10°C or less.

7.2.1.2 SUR PRIMARY DILUTION STANDARD – Prepare the SUR Primary Dilution Standard (PDS) by adding enough of the SUR stock standard to a volumetric flask partially filled with methanol to make a final concentration near 10 ug/mL when filled to the mark with methanol. The PDS has been shown to be stable for 6 months when stored at -10 °C or less.

7.2.2 ANALYTE STANDARD SOLUTIONS – Obtain the analytes listed in the table in Section 1.1 as neat or solid standards or as commercially prepared ampulized solutions from a reputable standard manufacturer. Prepare the Analyte Stock and Primary Dilutions Standards as described below.

7.2.2.1 ANALYTE STOCK STANDARD SOLUTION – If preparing from neat material, accurately weigh approximately 25 to 35 mg of pure material to the nearest 0.1 mg into a tared, 5-mL volumetric flask. Dilute to the mark with methanol. Repeat for each target analyte.

7.2.2.2 ANALYTE PRIMARY DILUTION STANDARD (PDS) SOLUTION – Prepare the analyte PDS by dilution of the target analyte stock standards. Add enough of each target stock standard to a volumetric flask partially filled with methanol to make a final concentration near 10 ug/mL when filled to the mark with methanol. A serial dilution of this PDS to make a 1.0 ug/mL solution is useful for low level spiking. The PDSs have been shown to be stable for 6 months when stored at -10 °C or less.

7.2.3 CALIBRATION STANDARDS (CAL) – At least 5 calibration concentrations will be required to prepare the initial calibration curve (Sect. 10.2). Prepare the calibration standards over the concentration range of interest from dilutions of the Analyte PDSs in preserved reagent

water (7.1.2), filtering the CAL standards in the same manner as the samples (Sect. 11.1.4). The lowest concentration of calibration standard must be at or below the MRL, which may depend on system sensitivity. The calibration standards for the development of this method were prepared as specified below. Calibration standards must be prepared using preserved reagent water (Sect. 7.1.2). An example of the dilutions used to prepare the CALs used to collect the data in Section 17 are shown in the table below. These standards may be also be used as CCCs. If stored, the aqueous standards must be stored in amber glass and refrigerated in the same manner as the samples.

PREPARATION OF CALIBRATION (CAL) CURVE STANDARDS						
CAL Level	Analyte PDS Conc. (ug/mL)	Vol. of Analyte PDS (uL)	Vol. of 10 ug/mL SUR PDS (uL)	Final Vol. of CAL Std. (mL)	Final Conc. of CAL Std. (ug/L)	Final Conc. of SUR Std. (ug/L)
	1.0	5.0	5.0	25	0.20	2.00
	1.0	12.5	5.0	25	0.50	2.00
	1.0	25.0	5.0	25	1.00	2.00
	10.0	5.0	5.0	25	2.00	2.00
	10.0	12.5	5.0	25	5.00	2.00
	10.0	25.0	5.0	25	10.0	2.00

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE BOTTLE PREPARATION

8.1.1 Prior to shipment to the field, preservatives listed in Section 7.1.11 must be added as dry solids to each amber bottle fitted with a PTFE lined screw cap. A 40 or 60-mL sample bottle is recommended. Add a sufficient amount of potassium dihydrogen citrate to yield a concentration in the sample of 9.2 to 9.5 g/L. Potassium dihydrogen citrate buffers sample pH to ~3.8 to prevent hydrolysis of oxamyl, 3-hydroxycarbofuran, carbaryl, and methiocarb. Add sodium thiosulfate to yield a sample concentration in the range of 80 to 320 mg/L. The concentration of 80 mg/L of sodium thiosulfate is adequate for dechlorination, but difficult to measure if a small (e.g., 40-mL size) sample bottle is chosen. Method performance was tested using up to 320 mg/L without any adverse effect. Sodium thiosulfate eliminates the residual free chlorine in the samples, which

rapidly degrades aldicarb and methiocarb.

8.1.2 Grab samples must be collected in accordance with conventional sampling practices [6]. Sample bottles must not be prerinsed with sample before collection. Doing so will wash out the preservatives added to the bottles prior to shipment.

8.2 SAMPLE COLLECTION

8.2.1 When sampling from a cold water tap, remove the aerator so that no air bubbles will be trapped in the sample. Open the tap, and allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Collect samples from the flowing system.

8.2.2 When sampling from an open body of water, fill a wide-mouth bottle or beaker with sample from a representative area, and carefully fill sample bottles from the container. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

8.2.3 If sampling waters high in colloidal iron, filtration of the sample may be necessary prior to preservation in the field to help prevent the precipitation of the iron in the reactor.

8.2.4 Fill sample bottles, taking care not to flush out the sample preservation reagents. Samples do not need to be collected headspace free.

8.2.5 After collecting the sample, cap carefully to avoid spillage, and agitate by hand for 1 minute. Keep samples sealed from collection time until analysis.

8.3 SAMPLE SHIPMENT AND STORAGE – All samples should be iced during shipment and must not exceed 10 °C during the first 48 hours after collection. Samples should be confirmed to be at or below 10 °C when they are received at the laboratory. Samples stored in the lab must be held at or below 6 °C and protected from light until analysis. Samples should not be frozen.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study of all method analytes indicated that all compounds are stable for 28 days in water samples that are collected, dechlorinated, preserved, shipped and stored as described in Sections 8.1 - 8.3. Samples must be analyzed within 28 days.

9. QUALITY CONTROL

9.1 Quality control (QC) requirements include the Initial Demonstration of Capability, the determination of the Detection Limit, and subsequent analysis in each analysis batch of a Laboratory Reagent Blank (LRB), Continuing Calibration Check Standards (CCC), a Laboratory Fortified Blank (LFB), a Laboratory Fortified Sample Matrix (LFSM), and either a Laboratory Fortified Sample Matrix Duplicate (LFSMD) or a Field Duplicate Sample. This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Section 17, Tables 10 and 11. These criteria are considered the minimum acceptable QC criteria, and laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – Requirements for the Initial Demonstration of Capability are described in the following sections and summarized in Section 17, Table 10.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Before any field samples are analyzed, and any time a new set of reagents is used, it must be demonstrated that a laboratory reagent blank is reasonably free of contamination and that the criteria in Section 9.4 are met.

9.2.2 INITIAL DEMONSTRATION OF ACCURACY – Prior to the analysis of the IDC samples, verify calibration accuracy with the preparation and analysis of a mid-level QCS as defined in Section 9.11. If the analyte recovery is not $\pm$ 30% of the true value, the accuracy of the method is unacceptable. The source of the problem must be identified and corrected. After the accuracy of the calibration has been verified, prepare and analyze 4-7 replicate LFBs (or CCCs in this method) fortified at 2 ug/L, or near the mid-range of the initial calibration curve, according to the procedure described in Section 11. Sample preservatives as described in Section 8.1.1 must also be added to these samples. The average recovery of the replicate values must be within ± 20% of the true value.

9.2.3 INITIAL DEMONSTRATION OF PRECISION – Using the same set of replicate data generated for Section 9.2.2, calculate the standard deviation and percent relative standard deviation of the replicate recoveries. The relative standard deviation (%RSD) of the results of the replicate analyses must be less than 20%.

9.2.4 DETECTION LIMIT DETERMINATION – Prepare and analyze at least 7 replicate LFBs at a concentration estimated to be near the Detection Limit over at least 3 days using the procedure described in Section 11. This fortification level may be estimated by selecting a concentration with a

signal of 2 to 5 times the noise level. The appropriate concentration will be dependent upon the sensitivity of the HPLC system being used. Sample preservatives as described in Section 8.1.1 must be added to these samples. Calculate the Detection Limit using the equation

$$\text{Detection Limit} = St_{(n-1,\ 1-\ alpha\ =\ 0.99)}$$

where

$t_{(n-1,1-alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates, and
S = standard deviation of replicate analyses.

NOTE: Calculated Detection Limits need only be less than $^1/_3$ of the laboratory's MRL to be considered acceptable. Do not subtract blank values when performing Detection Limit calculations. The Detection Limit is a statistical determination of precision only.(1) If the Detection Limit replicates are fortified at a low enough concentration, it is likely that they will not meet the precision and accuracy criteria for CCCs, and may result in a calculated Detection Limit that is higher than the fortified concentration. Therefore, no precision and accuracy criteria are specified.

9.2.5 METHOD MODIFICATIONS – The analyst is permitted to modify HPLC columns and conditions prior to the postcolumn reaction. The analyst is also allowed to modify the surrogate standard. Each time such method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2).

9.3 Minimum Reporting Level (MRL) – The MRL is the threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL should not be established at an analyte concentration that is less than either three times the Detection Limit or a concentration which would yield a response less than a signal-to-noise (S/N) ratio of five. Depending upon the study's data quality objectives it may be set at a higher concentration. **Although the lowest calibration standard must be at or below the MRL, the MRL must never be established at a concentration lower than the lowest calibration standard**.

9.4 LABORATORY REAGENT BLANK (LRB) – This is a direct injection method without a conventional extraction. An LRB is required with each analysis batch (Sect. 3.1) of samples to determine any background system contamination. If within the retention time window of any analyte, the LRB produces a peak that

would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or contaminants that interfere with the measurement of method analytes must be below $^1/_3$ the MRL. If the target analytes are detected in the LRB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples in the analysis batch.

9.5 CONTINUING CALIBRATION CHECK (CCC) – A CCC is prepared in the same manner as the initial calibration solutions (Sect. 7.2.3), using preserved reagent water (Sect. 7.1.2) and filtering in the same manner as the samples (11.1.4). It is analyzed during an analysis batch at a required frequency to confirm that the instrument meets initial calibration criteria. See Section 10.3 for concentration requirements, frequency requirements, and acceptance criteria.

9.6 LABORATORY FORTIFIED BLANKS – For this direct injection method, a CCC is the same as an LFB. Consequently, the analysis of an LFB is not required.

9.7 SURROGATE RECOVERY – The surrogate standard is fortified into all samples, blanks, LRBs, and LFSMs and LFSMDs prior to sample filtration. They are also added to the calibration curve and calibration check standards. The surrogate is a means of assessing method performance from preparation and filtration to final chromatographic measurement.

9.7.1 When surrogate recovery from a sample, blank, or CCC is less than 70% or greater than 130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the sample.

9.7.2 If the reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed sample.

9.7.3 If the sample reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by reinjecting the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. If the calibration standard is acceptable, preparation (which includes spiking with surrogate and filtration) and analysis of the sample should be repeated provided the sample is still within the holding time. If this sample reanalysis also fails the recovery criterion, report all data for that sample as suspect due to surrogate recovery.

9.8 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – Analysis of an LFSM is required in each analysis batch and is used to determine that the sample matrix does not adversely affect method accuracy. Assessment of method precision is accomplished by analysis of a Field Duplicate (Sect. 9.9), however, infrequent occurrence of target analytes would hinder this assessment. If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM, or LFMSD, must be prepared and analyzed from a duplicate of the field sample. Analysis batches that contain LFSMDs will not require the analysis of a Field Duplicate. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, method performance should be established for each. Over time, LFSM data should be documented for all routine sample sources for the laboratory.

9.8.1 Within each analysis batch, a minimum of one field sample is fortified as an LFSM for every 20 samples processed. The LFSM is prepared by spiking a sample with an appropriate amount of the appropriate Analyte PDS (Sect. 7.2.2.2). Select a spiking concentration at least twice the matrix background concentration, if known. Use historical data or rotate through a range of concentrations when selecting a fortifying concentration. Selecting a duplicate bottle of a sample that has already been analyzed aids in the selection of appropriate spiking levels.

9.8.2 Calculate the percent recovery (R) for each analyte using the equation

$$R = \frac{(A - B)}{C} * 100$$

where

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample, and
C = fortification concentration.

9.8.3 Analyte recoveries may exhibit a matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70 - 130%. For LFSM fortification at the MRL, 50 to 150% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.9 FIELD DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX

DUPLICATE (FD or LFSMD) – Within each analysis batch, a minimum of one Field Duplicate (FD) or Laboratory Fortified Sample Matrix Duplicate (LFSMD) must be analyzed. Duplicates check the precision associated with sample collection, preservation, storage, and laboratory procedures. If target analytes are not routinely observed in field samples, an LFSMD must be analyzed rather than a FD.

9.9.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) using the equation

$$RPD = \frac{|FD1 - FD2|}{(FD1 + FD2)/2} * 100$$

9.9.2 If an LFSMD is analyzed instead of a Field Duplicate, calculate the relative percent difference (RPD) for duplicate LFSMs (LFSM and LFSMD) using the equation

$$RPD = \frac{|LFSM - LFSMD|}{(LFSM + LFSMD)/2} * 100$$

9.9.3 RPDs for FDs and duplicate LFSMs should fall in the range of ± 30% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are spiked near the MRL. At the MRL, RPDs should fall in the range of 0 to 50% for samples fortified at or above their native concentration. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects

9.10 RESOLUTION CHECK – The resolution of peaks in a calibration standard or CCC near the mid level of calibration must be monitored in each analytical batch. During the development of this method, the 2 ug/L level was monitored. Closely eluting peaks that are not baseline resolved must have a resolution (R_s) of 1.0 or greater using the equation [7]

$$R_s = \frac{1.18(t_2 - t_1)}{W_{0.5,1} + W_{0.5,2}}$$

where

t_1 and t_2 = retention times of the first and second adjacent peaks
$W_{0.5,1}$ and $W_{0.5,2}$ = widths of the adjacent peaks at half height.

Resolution must be monitored once for every 24-hour analytical batch and may be monitored at any time during the 24-hour period. It is recommended that resolution be checked prior to sample analysis, especially if the system in use has a history of resolution problems. If a resolution check fails, all samples must be reanalyzed after the problem is corrected, including the QC samples.

9.11 QUALITY CONTROL SAMPLE (QCS) – During the analysis of the IDC (Sect. 9.2), each time that new analyte standard solutions (Sects. 7.2.2.1 and 7.2.2.2) are prepared, or at least quarterly, analyze a QCS from a source different from the source of the calibration standards. The QCS is fortified into preserved reagent water (Sect. 7.1.2) and analyzed as a CCC. The acceptance criteria is the same as the CCC criteria at mid-level; the calculated amount for each analyte must be $\pm$ 30% of the true value. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

10. CALIBRATION AND STANDARDIZATION

10.1 After initial calibration is successful, a Continuing Calibration Check (CCC) is required at the beginning and end of each analysis batch, and after every tenth sample (Sect. 10.3). Initial calibration should be repeated each time a major instrument modification or maintenance is performed.

10.2 INITIAL CALIBRATION

10.2.1 Establish HPLC operating parameters equivalent to the suggested conditions in Section 17, Table 1, including the postcolumn reactor. The system is calibrated using the external standard technique. A fluorescence detector was used with the excitation and emission wavelengths optimized at 340 and 465 nm, respectively. Other HPLC conditions may be used as long as all QC requirements in Section 9 are met.

10.2.2 Prepare a set of at least 5 calibration standards as described in Section 7.2.3. The lowest concentration calibration standard must be at or below the MRL, which may depend on system sensitivity. It is recommended that at least four of the Calibration Standards are at a concentration greater than or equal to the MRL.

10.2.3 Generate a calibration curve for each analyte by plotting the peak response

(area is recommended) against analyte concentration. The instrument used during method development yielded linear curves for the target analytes over the concentration range of interest. However, data may be fit with either a linear regression (response vs. concentration) or quadratic fit (response vs. concentration). Alternately, if the ratio of the analyte peak area to concentration (or response factor) is relatively constant (%RSD < 30%) an average response factor may be used to calculate analyte concentration.

10.2.4 When quantitated using the initial calibration curve, each calibration point, except the lowest point, for each analyte should calculate to be 70-130% of its true value. The lowest calibration point should calculate to be 50-150% of its true value. Failure to meet this criteria may indicate future difficulty in meeting CCC QC requirements during the analysis batch.

10.3 CONTINUING CALIBRATION CHECK (CCC) – Minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. (In this context, a "sample" is considered to be a field sample. LRBs, CCCs, LFSMs, and LFSMDs are not counted as samples. The beginning CCC each day must be at or below the MRL in order to verify instrument sensitivity prior to any analyses. Subsequent CCCs should alternate between a medium and high concentration standard.

10.3.1 Inject an aliquot of the appropriate concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.3.2 Calculate the concentration of each analyte and surrogate in the check standard. The calculated amount for each analyte for medium and high level CCCs must be ± 30% of the true value. The calculated amount for the lowest calibration point for each analyte must be within ± 50% of the true value. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action should be taken which may require recalibration. Any field or QC samples that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored.

11. PROCEDURE

11.1 SAMPLE PREPARATION

11.1.1 Samples are preserved, collected and stored as presented in Section 8. All field and QC samples must contain the preservatives listed in Section 8.1.1. Measure a 25-mL aliquot of sample into a volumetric flask.

Alternate volumes are allowed as long as they are determined using a glass volumetric flask or pipette. When the volumes of SUR and analyte PDS added to the field and QC samples are kept to a minimum, as described below, no volume adjustment is necessary.

11.1.2 Add an aliquot of the SUR PDS (Sect. 7.2.1.2) to all samples and mix by capping and inverting the sample. If the SUR PDS volume is less than 1% of the total volume, no volume adjustment is necessary. For the development of this method, the addition of 5.0 uL of a 10 ug/mL SUR PDS to a 25-mL sample resulted in a SUR concentration of 2.0 ug/L.

11.1.3 If the sample is an LFSM, LFSMD or LFB/CCC, add the necessary amount of analyte PDS. If the PDS volume is less than 1% of the total volume, no volume adjustment is necessary. Cap and invert each sample to ensure all components are properly mixed.

11.1.4 Filter samples prior to filling appropriate autosampler vials (Filters as specified in Section 6.8 are recommended.).

11.2 ANALYSIS OF SAMPLES

11.2.1 Establish operating conditions as summarized in Table 1 of Section 17 for the HPLC system, including the postcolumn reactor. HPLC conditions and columns should be optimized prior to the initiation of the IDC. Confirm that resolution of target peaks meets the requirements of Section 9.10. Retention times are given for illustration in Table 2. Specific retention times will depend on the column and chromatographic conditions chosen and may be modified by the user as long as adequate sensitivity and peak resolution are achieved.

11.2.2 Load filled autosampler vials into the HPLC autosampler. Start the injection sequence. For the development of this method, the injection volume ranged between 200 and 1000 uL and depended on system sensitivity.

11.2.3 Determine optimal excitation and emission wavelengths (if necessary). Complete the IDC requirements described in Section 9.2.

11.2.4 Establish an appropriate retention time window for each target and surrogate to identify them in the QC and field samples. This should be based on measurements of actual retention time variation for each compound in standard solutions analyzed on the HPLC over the course of time. Plus or minus three times the standard deviation of the retention time for each compound while establishing the initial calibration and

completing the IDC can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily on the determination of the appropriate retention window size.

11.2.5 Calibrate the system by either the analysis of a calibration curve (Sect. 10. 2) or by confirming the initial calibration is still valid by analyzing a continuing calibration check as described in Section 10.3. Begin analyzing field and QC samples at their appropriate frequency by injecting the same size aliquots under the same conditions used to analyze the initial calibration. Confirm that the system meets the resolution check criteria as described in Section 9.10 once for every 24 hour analytical batch.

11.2.6 The analyst must not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the initial calibration curve, the extract may be diluted with preserved reagent water (Sect. 7.1.2). Acceptable surrogate performance (Sect. 9.7) should be determined from the undiluted sample extract. Any dilutions will also affect analyte MRL.

12. DATA ANALYSIS AND CALCULATION

12.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspect peak to the retention time of an analyte peak in a calibration standard. Surrogate retention times should be confirmed to be within acceptance limits (Sect. 11.2.3) even if no target compounds are detected. Surrogate concentrations need to be calculated and determined to be within QC limits (Sect. 9.7).

12.2 Calculate the analyte concentrations using the initial calibration curve generated as described in Section 10.2. Quantitate only those values that fall between the MRL and the highest calibration standard. Samples with target analyte responses that exceed the highest standard require dilution and reanalysis (Sect. 11.2.5).

12.3 Adjust the calculated concentrations of the detected analytes to reflect the initial sample volume and any dilutions performed.

12.4 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration.

12.5 Analyte concentrations are reported in ug/L (usually to two significant figures).

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, AND DETECTION LIMITS – Detection Limits are presented in Section 17, Tables 3-5 and were calculated using the formula present

in Section 9.2.4. Detection Limits were evaluated using two commercially available postcolumn systems and two different fluorescence detectors. Single laboratory precision and accuracy are presented for three water matrices: reagent water (Table 6); chlorinated (finished) surface water (Table 7); and chlorinated (finished) ground water (Table 8).

13.2 SAMPLE STORAGE STABILITY STUDIES – Chlorinated (finished) ground water samples, fortified with method analytes at 2.0 ug/L, were preserved and stored as required in Section 8. The average of triplicate analyses, conducted on days 0, 2, 7, 14, and 28 days are presented in Section 17, Table 9. These data document the 28-day sample holding time.

14. POLLUTION PREVENTION

14.1 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, D.C., 20036.

15. WASTE MANAGEMENT

15.1 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.1.

16. REFERENCES

1. Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave, and W.L. Budde, "Trace Analyses for Wastewaters," Environ. Sci. Technol. 1981, 15, 1426-1435.

2. Moye, H.A., Sherrer, S.J., and St. John, P.A., "Dynamic Labeling of Pesticides for High Performance Liquid Chromatography: Detection of N-Methylcarbamates and o-Phthalaldehyde," Anal. Lett. 1977, 10, 1049.

3. "OSHA Safety and Health Standards, General Industry," (29CRF1910). Occupational Safety and Health Administration, OSHA 2206, (Revised, Jan.1976).

4. "Carcinogens-Working with Carcinogens," Publication No. 77-206, Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health, Atlanta, Georgia, August 1977.

5. "Safety In Academic Chemistry Laboratories," 3rd Edition, American Chemical Society Publication, Committee on Chemical Safety, Washington, D.C., 1979.

6. ASTM Annual Book of Standards, Part II, Volume 11.01, D3370-82, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, 1986.

7. Snyder, L.R.; Kirkland, J.J.; Glajch, J.L. Practical HPLC Method Development, 2nd ed.;Wiley: New York, 1997; pp 22-25.

17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA*

TABLE 1. INSTRUMENT METHOD CONDITIONS

Time (min)	%Water	%Methanol	%Acetonitrile
initial	88.0	12.0	0.0
5.30	88.0	12.0	0.0
5.40	68.0	16.0	16.0
14.00	68.0	16.0	16.0
16.10	50.0	25.0	25.0
20.00	50.0	25.0	25.0
22.00	88.0	12.0	0.0
30.00	88.0	12.0	0.0

Instrument Method Conditions

Column:	Waters Carbamate 3.9 × 150 mm packed with 4.0 um C_{18} stationary phase.
Postcolumn Reactor:	Reaction coil set at 80 °C, flow rate for Postcolumn Reagent #1 and #2 = 0.5 mL/min (each) for Waters unit, 0.3 mL/min for the Pickering unit.
Fluorescence Detector:	340 nm excitation, 465 nm emission with a 18 nm band width; Gain = 100; Attn. = 16; Response = Standard; 16 uL flow cell.
HPLC:	A ternary gradient comprised of water, methanol, and acetonitrile with a flow of 1.5 mL/min as shown in the table above.

*Instrumentation, when specified, does not constitute endorsement. Brand names are included for illustration only.

TABLE 2. RETENTION TIME DATA*

Analyte	Retention Time (min.)	STD DEV	%RSD
Aldicarb sulfoxide	4.36	0.0092	0.21
Aldicarb sulfone	5.07	0.0089	0.17
Oxamyl	5.74	0.0095	0.17
Methomyl	6.53	0.0077	0.12
3-Hydroxycarbofuran	9.82	0.013	0.13
Aldicarb	11.5	0.013	0.11
Propoxur	14.3	0.020	0.14
Carbofuran	14.8	0.024	0.16
Carbaryl	17.0	0.026	0.16
1-Naphthol	18.6	0.019	0.10
Methiocarb	21.8	0.015	0.07
BDMC (SUR)	22.3	0.015	0.07

*Retention time data is calculated from precision and accuracy data results presented in Table 6 and the calibration curve used to quantitate the data. Retention times may differ depending on the chromatographic conditions and columns used.

TABLE 3. DETECTION LIMITS IN REAGENT WATER USING THE WATERS POSTCOLUMN CARBAMATE SYSTEM AND THE WATERS MODEL 474 DETECTOR

Analyte	Fortification Level (ug/L)	Detection Limit[a] (ug/L)	Signal to Noise Ratio
Aldicarb sulfoxide	0.20	0.059	8:1
Aldicarb sulfone	0.10	0.057	3:1
Oxamyl	0.20	0.065	10:1
Methomyl	0.20	0.050	10:1
3-Hydroxycarbofuran	0.20	0.029	18:1
Aldicarb	0.20	0.026	9:1
Propoxur	0.20	0.037	6:1
Carbofuran	0.20	0.043	9:1
Carbaryl	0.20	0.045	13:1
1-Naphthol	0.20	0.063	10:1
Methiocarb	0.20	0.061	11:1

[a]Detection Limits were determined by analyzing 7 replicates over 3 days using the conditions outlined in Table 1 with a 1000-uL injection.

TABLE 4. DETECTION LIMITS IN REAGENT WATER USING THE PICKERING MODEL PCX5200 POSTCOLUMN SYSTEM AND THE WATERS MODEL 474 DETECTOR

Analyte	Fortification Level (ug/L)	Detection Limit[a] (ug/L)	Signal to Noise Ratio
Aldicarb sulfoxide	0.20	0.056	13:1
Aldicarb sulfone	0.20	0.026	15:1
Oxamyl	0.20	0.045	9:1
Methomyl	0.20	0.045	11:1
3-Hydroxycarbofuran	0.20	0.041	11:1
Aldicarb	0.20	0.042	7:1
Propoxur	0.20	0.040	11:1
Carbofuran	0.20	0.058	7:1
Carbaryl	0.20	0.065	22:1
1-Naphthol	0.20	0.034	9:1
Methiocarb	0.20	0.036	5:1

[a]Detection Limits were determined by analyzing 7 replicates over 3 days using the conditions outlined in Table 1 with a 250-uL injection.

TABLE 5. DETECTION LIMITS IN REAGENT WATER USING THE WATERS POSTCOLUMN CARBAMATE ANALYSIS SYSTEM AND THE WATERS MODEL 2475 DETECTOR

Analyte	Fortification Level (ug/L)	Detection Limit[a,b] (ug/L)	Signal to Noise Ratio
Aldicarb sulfoxide	0.20	0.038	14:1
Aldicarb sulfone	0.20	0.033	9:1
Oxamyl	0.10	0.044	4:1
Methomyl	0.20	0.054	24:1
3-Hydroxycarbofuran	0.20	0.038	7:1
Aldicarb	0.20	0.049	12:1
Propoxur	0.20	0.061	10:1
Carbofuran	0.20	0.050	12:1
Carbaryl	0.10	0.043	9:1
1-Naphthol	0.20	0.115	3:1
Methiocarb	0.20	0.055	5:1

[a]Detection Limits were determined by analyzing 7 replicates over 3 days using the conditions outlined in Table 1 with a 200-uL injection.
[b]These data were collected at American Water Works Service Company.

TABLE 6. PRECISION AND ACCURACY OF LOW AND HIGH LEVEL FORTIFIED REAGENT WATER[a]

Analyte	Concentration = 0.20 ug/L (n=7)		Concentration = 10 ug/L (n=7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Aldicarb sulfoxide	112	6.2	106	1.8
Aldicarb sulfone	92	9.5	106	2.6
Oxamyl	101	8.6	106	2.2
Methomyl	101	6.5	106	2.9
3-Hydroxycarbofuran	105	6.8	108	1.2
Aldicarb	95	7.4	106	1.3
Propoxur	109	5.9	109	2.0
Carbofuran	112	6.7	110	2.2
Carbaryl	112	7.0	107	2.1
1-Naphthol	113	12.6	108	3.1
Methiocarb	105	5.9	107	1.5
BDMC (SUR)[b]	108	4.3	101	2.3

[a]Data obtained using conditions in Table 1 using a 1000-uL injection.
[b]Surrogate concentration in all samples was 2.0 ug/L.

TABLE 7. PRECISION AND ACCURACY OF LOW AND HIGH LEVEL FORTIFIED CHLORINATED SURFACE WATER[a]

Analyte	Concentration = 0.20 ug/L (n=7)		Concentration = 10 ug/L (n=7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Aldicarb sulfoxide	113	7.0	104	2.8
Aldicarb sulfone	104	5.5	106	1.4
Oxamyl	107	6.4	104	2.2
Methomyl	110	9.8	104	1.6
3-Hydroxycarbofuran	128	3.9	107	1.1
Aldicarb	123	2.7	105	1.5
Propoxur	128	6.0	106	2.1
Carbofuran	140	5.6	105	2.5
Carbaryl	112	9.7	106	0.9
1-Naphthol	113	12.1	101	1.3
Methiocarb	104	13.3	107	1.1
BDMC (SUR)[b]	108	2.1	96	3.9

[a]Data obtained using conditions in Table 1 using a 1000-uL injection.
[b]Surrogate concentration in all samples was 2.0 ug/L.

TABLE 8. PRECISION AND ACCURACY OF LOW AND HIGH LEVEL FORTIFIED CHLORINATED GROUND WATER

Analyte	Concentration = 0.20 ug/L (n=7)		Concentration = 10 ug/L (n=7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Aldicarb sulfoxide	111	7.3	106	1.1
Aldicarb sulfone	98	9.2	106	0.9
Oxamyl	99	8.4	105	1.2
Methomyl	99	10.2	105	1.4
3-Hydroxycarbofuran	107	3.0	108	0.4
Aldicarb	100	6.3	105	0.6
Propoxur	112	6.1	107	0.8
Carbofuran	112	4.1	107	1.6
Carbaryl	119	5.1	108	1.3
1-Naphthol	109	8.2	109	1.2
Methiocarb	105	3.9	107	1.0
BDMC (SUR)[b]	109	2.0	97	4.3

[a]Data obtained using conditions in Table 1 using a 1000-uL injection.
[b]Surrogate concentration in all samples was 2.0 ug/L.

TABLE 9. SAMPLE HOLDING TIME DATA FOR CHLORINATED GROUND WATER SAMPLES FORTIFIED WITH METHOD ANALYTES AT 2.0 ug/L

Analyte	% Recovery for Samples Fortified at 2.0 ug/L				
	Day 0	Day 2	Day 8	Day 15	Day 28
Aldicarb sulfoxide	94	97	93	93	96
Aldicarb sulfone	93	99	97	98	98
Oxamyl	97	97	101	103	101
Methomyl	96	95	99	98	97
3-Hydroxycarbofuran	96	99	96	95	98
Aldicarb	95	100	96	92	93
Propoxur	95	98	97	98	99
Carbofuran	96	99	97	97	100
Carbaryl	96	97	98	94	100
1-Naphthol	96	97	99	95	98
Methiocarb	95	98	96	94	97
BDMC (SUR)[b]	99	105	100	99	99

[a]Data obtained using conditions in Table 1 using a 500-uL injection.
[b]Surrogate concentration in all samples was 2.0 ug/L.

TABLE 10. INITIAL DEMONSTRATION OF CAPABILITY (IDC) REQUIREMENTS

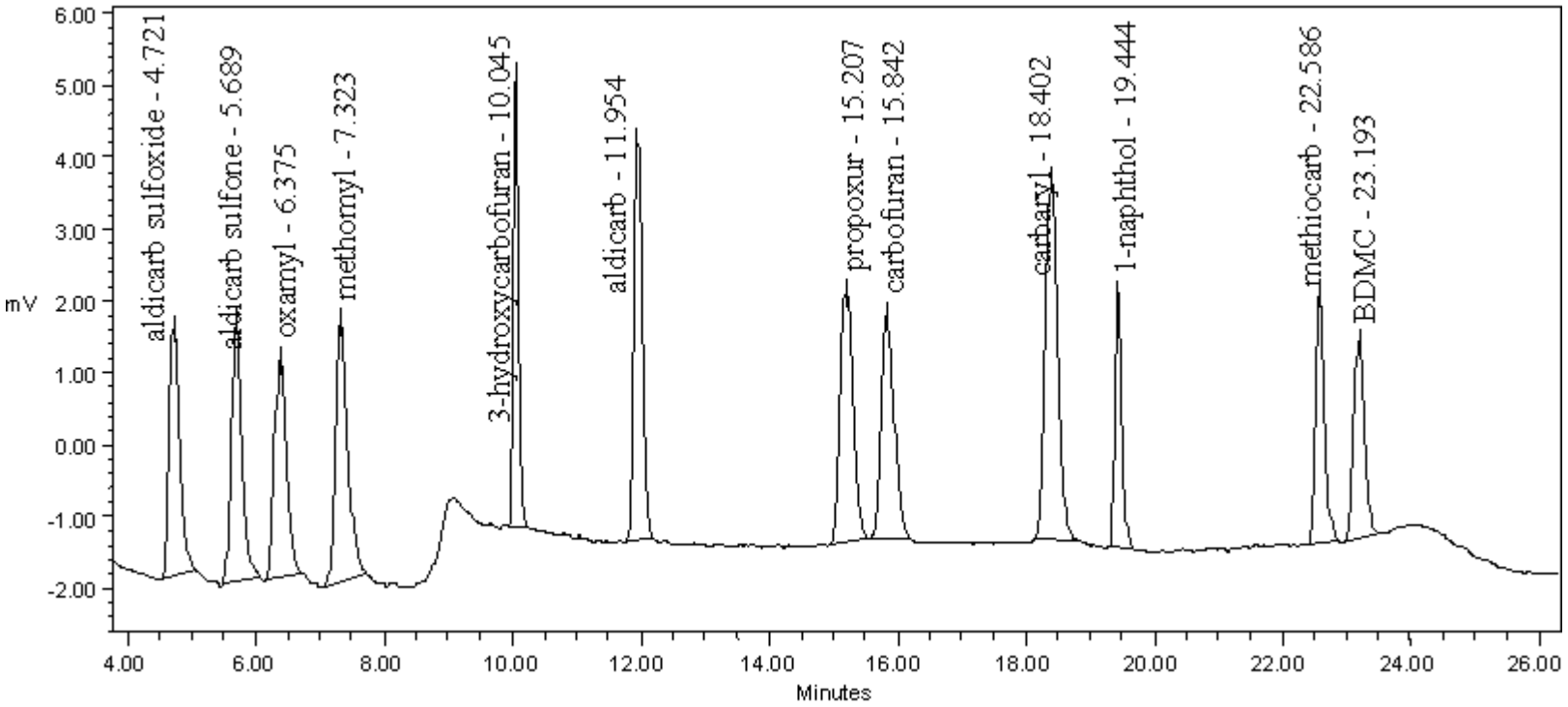
Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.1	Initial Demonstration of Low System Background	Analyze LRB prior to any other IDC steps.	Demonstrate that all target analytes are below 1/3 the intended MRL or lowest CAL standard, and that possible interference from reagents and glassware do not prevent the identification and quantitation of method analytes.
Sect. 9.2.2	Quality Control Sample (QCS)	Second source standard, used to fortify preserved reagent water (Sect. 7.1.2). Analyze as a CCC after initial calibration but prior to the analysis of the IDA samples.	Verifies initial calibration accuracy, recovery must be within $\pm$ 30% of true value.
Sect. 9.2.2	Initial Demonstration of Accuracy (IDA)	Analyze 4-7 replicate LFBs/CCCs fortified at midrange concentration.	Mean recovery $\pm$ 20% of true value.
Sect. 9.2.3	Initial Demonstration of Precision (IDP)	Calculate average recovery for replicates used in IDA.	%RSD must be $\leq$ 20%.
Sect. 9.2.4	Detection Limit Determination	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the Detection Limit. Analyze the replicates through all steps of the analysis. Calculate the Detection Limit using the equation in Sect. 9.2.4.	Data from Detection Limit replicates are not required to meet method precision and accuracy criteria. If the Detection Limit replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

TABLE 11. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.3 and Sect. 8.4	Sample and Extract Holding Times	Properly preserved samples must be shipped at or below 10 ^{0}C and may be held in the lab at or below 6 ^{0}C for 28 days. Samples should not be frozen.	Do not report data for samples that have not been properly preserved or stored, or that have exceeded their holding time.
Sect. 9.4	Laboratory Reagent Blank (LRB)	Include a LRB with each analysis batch (up to 20 samples). Analyze prior to analyzing samples and determine to be free from interferences.	Demonstrate that all target analytes are below 1/3 the intended MRL, and that possible interference from reagents and glassware do not prevent the identification and quantitation of method analytes. If targets exceed 1/3 the MRL, results for all subject analytes in the analytical batch are invalid.
Sect. 9.5 and Sect. 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level (at the MRL or below) CCC prior to analyzing samples. CCCs are then injected after every 10 samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each analyte must be 70-130% of the true value for all but the lowest level of calibration. The lowest calibration level CCC must be 50-150% of the true value. Results that are not bracketed by acceptable CCCs are invalid.
Sect. 9.7	Surrogate Standards	The surrogate, 4-bromo-3,5-dimithylphenyl –methylcarbamate is added to all field and QC samples.	Surrogate recovery must be 70-130% of the true value. Samples that fail criteria, must be reported as suspect due to surrogate recovery.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.8	Laboratory Fortified Sample Matrix (LFSM) and Laboratory Fortified Sample Matrix Duplicate (LFSMD)	With each analysis batch (Sect. 3.1), a minimum of one LFSM is extracted and analyzed. A duplicate LFSM, or LFSMD, should be extracted when occurrence of target analytes is low. Field Duplicate analysis is not required for analysis batches containing an LFSMD.	Recoveries not within 70-130% (50-150% at the MRL) of the fortified amount may indicate a matrix effect. If an LFSMD is analyzed instead of a Laboratory Duplicate , target RPDs should be within $\pm$ 30%. If all CCCs meet acceptance criteria, and LFSM or LFSMD do not, sample is designated suspect/matrix.
Sect. 9.9	Field Duplicates	Analyze at least one duplicate with each analysis batch (20 samples or less). A Laboratory Fortified Sample Matrix Duplicate may be substituted for a Field Duplicate when the occurrence of target analytes is low.	RPDs should be within $\pm$ 30%. If all CCCs meet acceptance criteria, and Field Duplicates do not, sample is designated suspect/matrix.
Sect 9.10	Resolution Check	Monitor once for every 24 hour analysis period.	Closely eluting peaks that are not baseline resolved must have a resolution of $\geq$ 1.0 or greater using the equation in Sect. 9.10
Sect. 9.11	Quality Control Sample	Analyze at least quarterly or when preparing new standards, as well as during the IDC.	Same acceptance criteria as a mid-level CCC.
Sect. 10.2	Initial Calibration	Use external standard calibration technique to generate a calibration curve with at least five standards.	When each calibration standard is calculated using the calibration curve, the results should be 70-130% of the true value for all but the lowest standard. The lowest standard should be 50-150% of the true value. The lowest CAL standard concentration must be as low or lower than the intended MRL.

FIGURE 1. SAMPLE CHROMATOGRAM OF METHOD 531.2 ANALYTES



Reagent water, sample preservatives added, fortified at 2.0 ug/L.